DEPARTMENT OF THE ARMY
US ARMY INSTITUTE OF PUBLIC HEALTH
5158 BLACKHAWK ROAD
ABERDEEN PROVING GROUND MARYLAND 21010-5403

26 August 2014

MCHB-IP-TEP

MEMORANDUM FOR Environmental Acquisition and Logistics Sustainment Program (AMSRD-MSF/Kimberly A. Watts), U.S. Army Research Development and Engineering Command, 3072 Aberdeen Boulevard, Aberdeen Proving Ground, MD 21005

SUBJECT: Toxicology Study No. 87-XC-0CKC-11, Protocol No. 0CKC-35-10-08-01, Acute and Four-Week Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke (PVA), October – December 2010

1. An electronic copy of the subject report has been provided.

2. Please contact us if this report or any of our services did not meet your expectations.

3. The U.S. Army Public Health Command point of contact is Mr. Arthur O'Neill, Toxicology Portfolio, Toxicity Evaluation Program. He may be contacted at DSN 584-3980, Commercial 410-436-3980, or by email at usarmy.apg.medcom-phc.mbx.tox-info@mail.mil.

FOR THE DIRECTOR:

Encl

MARK S. JOHNSON
Portfolio Director, Toxicology

U.S. ARMY PUBLIC HEALTH COMMAND
5158 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5403

Toxicology Study No. 87-XC-0CKC-11

Acute and Four-Week Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke (PVA)

Prepared by: Arthur J. O'Neill and Lee C.B. Crouse

Toxicity Evaluation Program
Toxicology Portfolio
Army Institute of Public Health

Approved for public release; distribution unlimited.

Specialty: 500C, Toxicity Study

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Study Title

Toxicology Study No. 87-XC-0CKC-11
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke (PVA)

Data Requirements

U.S. EPA Health Effects Test Guidelines
OPPTS 870.3465 (1998)

OECD Guideline for the Testing of Chemicals
Section 4: Health Effects, 412 (1981)

Authors

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Study Completed On

June 2014

Performing Laboratory

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Laboratory Project ID

Protocol No. 0CKC-35-10-08-01

Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. The statistical analyses of the data were conducted by the U.S. Army Public Health Command statisticians. It is not known if these analyses were conducted in accordance with Good Laboratory Practice Standards.

2. The undessiminated black smoke pellets were evaluated on an "as received" basis. The manufacturer provided an ingredient composition list but the actual composition was not confirmed by a GLP analysis. However, the ingredient composition supplied with the pellets was considered sufficient characterization of the test substance for the purposes of this study.

Arthur J. O'Neill
Study Director
Toxicity Evaluation Program

25 AUG 2014
Date

Table of Contents

Appendices

Figures

Tables

TOXICOLOGY STUDY NO. 87-XC-0CKC-11
PROTOCOL NO. 0CKC-35-10-08-01
ACUTE AND FOUR-WEEK INHALATION TOXICITY STUDY IN RATS EXPOSED TO PYROTECHNICALLY DISSEMINATED BLACK SMOKE (PVA)
OCTOBER - DECEMBER 2010

1 Summary

1.1 Purpose

This study was conducted to evaluate the acute and repeated-dose toxicity of pyrotechnically disseminated black smoke (PVA) when administered by inhalation to male and female rats. Toxicity data in a mammalian system needed to be generated to assess acute and repeated-dose inhalation toxicity health hazards associated with the material to assure its safety before use by military personnel and to determine an occupational exposure level for workers.

1.2 Conclusions

For the acute inhalation study, all ten rats exposed to a single exposure of 2500 mg/m^3 black smoke (PVA) particulate survived the exposure and recovery periods. No significant body weight losses or adverse clinical signs were noted in the rats during the acute study. The 30-minute inhalation median lethal concentration (LC_{50}) of black smoke (PVA) in rats is greater than 2500 mg/m^3.

For the 4-week main study, there were no mortalities in rats exposed to black smoke (PVA) during the exposure and recovery periods. In addition, no adverse compound-related effects were observed in rats exposed to the test compound for clinical signs, ophthalmological parameters, or clinical pathology findings. Compound-related effects were observed in rats exposed to 2100 mg/m^3 black smoke (PVA) for body weights, body weight gains, food consumption, organ weights, and histopathological findings. Based on the incidence and potentially adverse impact of these various findings, the 2100 mg/m^3 concentration level is considered to be an adverse effect level in rats exposed by inhalation to black smoke (PVA) on a repeated-dose manner. Rats exposed to 670 mg/m^3 also had compound-related effects for organ weights and histopathological findings, and rats exposed to 130 mg/m^3 had histopathological findings, however, the effects observed in rats exposed to both 130 and 670 mg/m^3 were substantially less pronounced than the effects observed in rats exposed at 2100 mg/m^3.

The lung and nasal cavity were the primary target organs in rats subjected to repeated inhalation exposures of black smoke (PVA). Pulmonary alveolar histiocytosis and nasal epithelial hyperplasia were observed in a dose-dependent manner in all treatment groups (130, 670, and 2100 mg/m^3). Pulmonary alveolar histiocytosis, an accumulation of macrophages in the alveolar spaces, is an expected response of the respiratory tract to an inhaled particulate. During this study, the black smoke particulate within the alveoli would have been phagocytosed by macrophages and then cleared by the mucociliary apparatus; this would account for the increased number of macrophages observed. This is not necessarily an adverse effect unless the mucociliary apparatus becomes overwhelmed. However, inert particulate materials can induce an accumulation of alveolar macrophages which can result in irritation, hyperplasia, and hypertrophy (Boorman, 1990). Nasal epithelial hyperplasia, simply an increase in the number of cells in the tissue, as noted above was

also observed during this study. While the presence of an increased number of cells is not adverse, one of the most common causes of hyperplasia is chronic irritation. Since the histopathological findings in the lungs and nasal cavities were not directly associated with pathological changes that would necessarily be considered adverse (e.g., inflammation, necrosis), it is not clear if the findings were adverse or merely an adaptive response to the particulate nature of the test material that the rats were exposed to. Furthermore, based on the results of the histopathologic examinations following a one-month recovery period, it would appear that rats exposed to 2100 mg/m^3 begin to significantly recover from these findings upon cessation of the exposure to the test material.

Even though the severity of the impact of the histopathological findings in the lung and nasal cavity is not definitive, the fact that there were numerous findings in multiple endpoints (e.g., body weights, histopathology) for rats exposed to 2100 mg/m3, this level is considered to be a clear adverse effect level during this study. Effects observed in rats exposed to 670 mg/m^3 were less severe and consistent with those described for rats exposed to a higher concentration and suggest that 670 mg/m^3 is the borderline level for potentially adverse effects. The effects observed in rats exposed to 130 mg/m^3 were not considered to be adverse since they were only observed in a small number of rats and were significantly diminished in their degree of severity compared to rats exposed to higher concentration levels of the test substance.

The no-observed-adverse-effect level (NOAEL) for this study is defined as the highest dose at which toxicologically important (or "toxicologically relevant" or "adverse") effects attributable to the test substance were not detected. Due to the lack of significant toxicologically adverse findings in rats exposed to 130 mg/m^3, the NOAEL for repeated inhalation exposure to black smoke (PVA) is considered to be 130 mg/m^3.

For purposes of the benchmark determination, the most consistent and sensitive observation associated with exposure to black smoke (PVA) in the course of this study was the incidence of alveolar histiocytosis. Analysis of the incidence of alveolar histiocytosis using benchmark dose software resulted in a benchmark dose (BMD) of 539.6 mg/m^3 for male rats and 390.6 mg/m^3 for female rats and a 95 percent lower confidence limit for 10 percent effect level ($BMDL_{10}$) of 144 mg/m^3 for male rats and 81.8 mg/m^3 for female rats. A Human Equivalent Concentration (HEC) of 19.6 mg/m^3 was derived based on the BMD and $BMDL_{10}$ values for female rats.

2 References

See Appendix A for a list of references.

3 Authority

Military Interdepartmental Purchase Request (MIPR) No. MIPR1JDATHR142. This toxicology study addresses, in part, the environmental safety and occupational health requirements outlined in Army Regulations (AR) 200-1, AR 40-5, and AR 70-1; Department of Defense Instruction 4715.4; and Army Environmental Requirements and Technology Assessments (Department of the Army (DA), 2007a and b; DA, 2003; Department of Defense (DOD), 1996; and U.S. Army Environmental Command (USAEC), 2009). It was performed as part of an on-going effort by the U.S. Army Environmental Quality Technology (EQT), Ordnance Environmental Program Pollution Prevention Team, to produce safer ordnance. This program is under the direction of the U.S. Army Research,

Development, and Engineering Command Environmental Acquisition Logistics & Sustainment Program and EQT Pollution Prevention.

4 Background

The U.S. Military uses colored smokes in a variety of ways, including identification of potential targets and friendly troops, simulation of battlefield events, and as a means of communication. Previously used smoke formulations, including black smoke, were developed strictly based on their ability to produce the desired color for a specified period of time. Recent changes made to the smoke formulations and dissemination systems have focused on soldier safety during training and deployed scenarios as well as preservation of the land on which they train and fight. Disperse Red 9 and Solvent Green 3 are the primary dye components contained in the current formulation being tested. Pyrotechnic black smoke systems are used by the military to simulate battlefield effects for force-on-force and force-on-target training simulations. Although it is imperative that soldiers are trained in a similar manner in which they fight, these training exercises often result in soldiers and training instructors being repeatedly exposed to materials used to simulate battlefield scenarios. Toxicity data in a mammalian system needed to be generated to assess acute and repeated-dose inhalation toxicity health hazards associated with the material to assure its safety before use by military personnel and to determine an occupational exposure level for workers and training instructors potentially exposed on a daily basis.

Table 1. Critical Study Events

Critical Event	Date of Event
Animal Use Protocol Approved	August 25, 2010
Animals Received for Acute Exposure	October 6, 2010
Acute Exposure Conducted	October 13, 2010
Acute Exposure Recovery Phase Started	October 13, 2010
Acute Exposure Recovery Phase Completed	October 27, 2010
Acute Exposure Necropsy	October 27, 2010
Animals Received for Main and Pilot Study	October 13, 2010
Pilot Study Exposures Started	October 18, 2010
Pilot Study Exposures Completed	October 20, 2010
Main Study Exposure Phase Started	October 21, 2010
Main Study Exposure Phase Completed	November 18, 2010
Exposure Phase Necropsies	November 18 and 19, 2010
Main Study Recovery Phase Started	November 18, 2010
Main Study Recovery Phase Completed	December 17, 2010
Recovery Phase Necropsies	December 16 and 17, 2010
Study Completion	June 2014

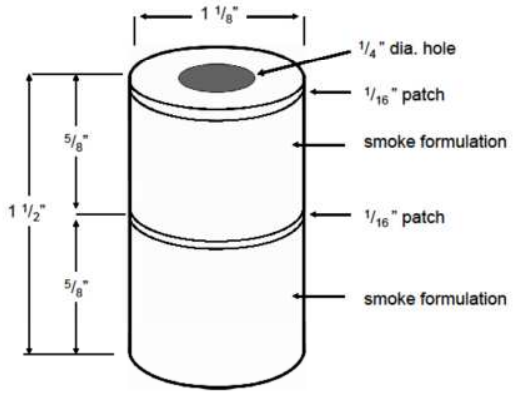
5 Materials

5.1 Test Substance

The black smoke formulation used for this study utilized polyvinyl acetate (PVA) as the binder/base and is therefore referred to as black smoke (PVA). Black smoke (PVA) is a dye-based formulation developed using an organic green and red dye mix with a sugar and potassium chlorate

pyrotechnic base (see Table 2). Initial method development work for this project was conducted with a black smoke formulation that used vinyl alcohol acetate resin (VAAR) as the binder/base, however, based on the expected use of the product, it was decided that all of the actual animal testing would be conducted with a black smoke formulation that used polyvinyl acetate (PVA) as the binder/base. The black smoke (PVA) formulation was pressed into a pellet design on August 18, 2010 and an igniter patch was applied to the bottom surface and in the middle of each pellet. The igniter consisted of a mixture of potassium nitrate, charcoal, and gum Arabic binder and was bonded to a felt cloth patch. In order to improve black smoke quantity, two pellets were joined together in a two-pellet design (see Figure 1) and were referred to as a "pellet unit" during this study (Chen et.al., 2006).

Figure 1. Black Smoke (PVA) Pellet Unit



All of the black smoke pellets used to conduct this toxicity study were supplied by the Pyrotechnics Division, Signals and Simulators Branch, U.S. Army Picatinny Arsenal, Picatinny, NJ. Pellets were stored in a desiccator located in a temperature- and humidity-controlled laboratory at the testing facility. Temperatures in the desiccator ranged from 20 to 23 degrees Celsius (ºC) and relative humidity ranged from 11 to 40 percent, except for an isolated period of 2 days in which the relative humidity range ranged from 42 to 55 percent. Information concerning the composition of the test substance was supplied by the sponsor (see Table 2), but no further attempt to was made to characterize the undesiminated test substance by the test facility.

Table 2. Composition of Black Smoke (PVA) Formulation

Component	Approximate Percentage (%)	Function / Use
Disperse Red 9	12.7	Color of Smoke
Solvent Green 3	24.8	Color of Smoke
Sugar (Sucrose)	19.2	Fuel
Potassium Chlorate	28.1	Oxidizer
Magnesium Carbonate	13.2	Coolant
Stearic Acid	1.0	Lubricant / Mixing Aid
Polyvinyl Alcohol (PVA)	5.0	Binder / Base

5.2 Animals[*†]

All studies were conducted using young adult male and female Sprague-Dawley rats obtained from Charles River Laboratories, Wilmington, Massachusetts. A total of 5 male and 5 female rats, approximately 8-weeks old at test initiation, were selected for the acute study. A total of 3 male and 3 female rats, approximately 7-weeks old at test initiation, were selected for the pilot study. A total of 36 male and 36 female rats, approximately 7-weeks old at test initiation, were selected for the main study. The attending veterinarian examined the animals and found them to be in acceptable health. The animals were quarantined/acclimated for a minimum 5-day period following their arrival to this testing facility. All rats were maintained in a temperature-, relative humidity-, and light-controlled room. The animal room environmental conditions for the main study were 69 to 72 degrees Fahrenheit (°F), 32 to 68 percent relative humidity, and a 12-hour light/dark cycle. A certified pesticide-free rodent chow (Harlan Teklad®, 8728C Certified Rodent Diet) and drinking quality water were available ad libitum except during the 10-minute exposure period. Rats were housed singly in solid bottom polycarbonate boxes with Harlan Sani-Chip® bedding and suspended on a cage rack equipped with an automatic water-nipple system. Each rat was uniquely identified by number using cage cards. In addition, an animal identification number was recorded on the tail of each rat with a water-insoluble marker prior to exposure so that individual rats could be identified after exposure. (Teklad® Certified Rat Diet is a registered trademark of Harlan, Teklad. Sani-Chip® is a registered trademark with P.J. Murphy Forest Products Corporation.)

5.3 Quality Assurance

The USAPHC Quality Systems Office audited critical phases of this study. Appendix B provides the dates of these audits along with the audited phase.

[*] Research was conducted in compliance with DoD and federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, Washington, D.C. 1996.

[†] The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

5.4 Study Personnel

Appendix C contains the names of persons contributing to the performance of this study.

6 Methods

6.1 General Description

6.1.1 Acute Study

The acute toxicity associated with a single, high-concentration exposure to black smoke (PVA) was determined by exposing 5 male and 5 female rats for 30 minutes to an atmospheric concentration of the test material targeted to at least 2000 mg/m^3. Following the exposure, the rats were held for a 14-day recovery period and monitored for morbidity/mortality, body weight changes, and/or clinical signs of toxicity. Following the recovery period, all of the rats were euthanized by CO_2 and necropsied. All rats received a gross necropsy with limited histopathology (respiratory tract).

6.1.2 Pilot Study

The initial concentration for the high level exposure chamber for the 4-week main study was targeted to be 2000 mg/m^3 black smoke (PVA) particulate. Prior to conducting the main study, a pilot study was conducted in an attempt to ensure that 2000 mg/m^3 was an appropriate design concentration for this exposure level. Although an acute toxicity study with black smoke (PVA) demonstrated that rats exposed to 2500 mg/m^3 black smoke (PVA) for 30 minutes showed no mortality or significant signs of adverse toxicity, the pilot study was an attempt to ensure that there would not be unexpected cumulative toxicity effects (i.e., mortality, body weight loss) resulting from repeated exposure to the test material. Six rats (3 male and 3 female) were exposed 30 minutes/day for 3 consecutive days to test atmospheres targeted to 2000 mg/m^3 black smoke (PVA) particulate. Rats were weighed and observed daily during the exposure period and euthanized on the day following the last exposure.

6.1.3 4-Week Main Study

Four groups of 12 rats each (6 rats/sex/group) were exposed to concentrations of black smoke (PVA) particulate targeted to 0, 100, 700, or 2000 mg/m^3. In addition, the control and high concentration level groups exposed additional rats (6 rats/sex/group) to be retained following the exposure for a 4-week recovery period. Exposure schedules of the rats were staggered over 2 days to compensate for the limited number of animals able to be necropsied on a given day by this facility. Rats were exposed 30 minutes per day, 5 days per week over a 5-week period (weekends excluded). Due to the staggered start, rats received a partial week of exposures during the first and last weeks of the study, however, all rats received a total of 20 exposures. All rats were weighed at least once per week and were observed daily for clinical signs. The amount of food consumed by each rat was determined weekly during the study. Ophthalmological examinations were performed prior to study start, at the end of the exposure period, and following the one-month recovery period. Blood samples were collected from all rats just prior to necropsy for clinical pathology analyses. At the end of the exposure period, 12 rats/sex/group were euthanized, necropsied, and examined for gross and microscopic pathological changes. Following a one-month recovery period, 6

rats/sex/group were euthanized, necropsied, and examined for gross and microscopic pathological changes.

The experimental design of the 4-week main study was modeled primarily on the U.S. Environmental Protection Agency (USEPA) Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Health Effects Test Guidelines, OPPTS 870.3465, 90-Day Inhalation Toxicity (USEPA, 1998). Changes to the 90-day study guidelines were made to appropriately reflect a 4-week exposure study as well as accurately mimic a typical daily exposure duration (e.g., 30 minutes). The Organisation for Economic Co-Operation and Development (OECD) Guideline for Testing of Chemicals, No. 412, Repeated Dose Inhalation Toxicity: 28-Day or 14-Day Study (OECD, 1981) was used as a secondary guide for experimental design. In addition, the general procedures related to the exposure chamber generation and analytical systems of this study were conducted under the appropriate USAPHC Portfolio of Toxicology Standing Operating Procedures (PTOX SOP) for conducting inhalation toxicity studies.

6.2 Selection of Exposure Chamber Design Concentration

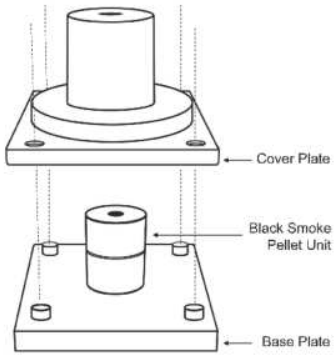
The initial concentration for the high level exposure chamber for the 4-week main study was targeted to be 2000 mg/m^3 black smoke (PVA) particulate. Prior to conducting the main study, a pilot study was conducted in an attempt to ensure that 2000 mg/m^3 was an appropriate design concentration for this exposure level. The 6 rats in the pilot study were exposed 30 minutes per day for 3 consecutive days to an overall mean concentration of 2300 ± 340 mg/m^3 black smoke (PVA) particulate. No significant body weight losses or adverse clinical observations were observed during the pilot study, and therefore, it was decided that 2000 mg/m^3 was an appropriate level to serve for the high concentration group. The low concentration level (100 mg/m^3) was 20-fold lower than the high concentration and was expected to be without adverse toxicological effects. The intermediate concentration (700 mg/m^3) represented an order of magnitude reduction from the high concentration and was expected to produce some degree of toxicity.

6.3 Inhalation Exposure System (Figure 3)

6.3.1 Test Atmosphere Generation

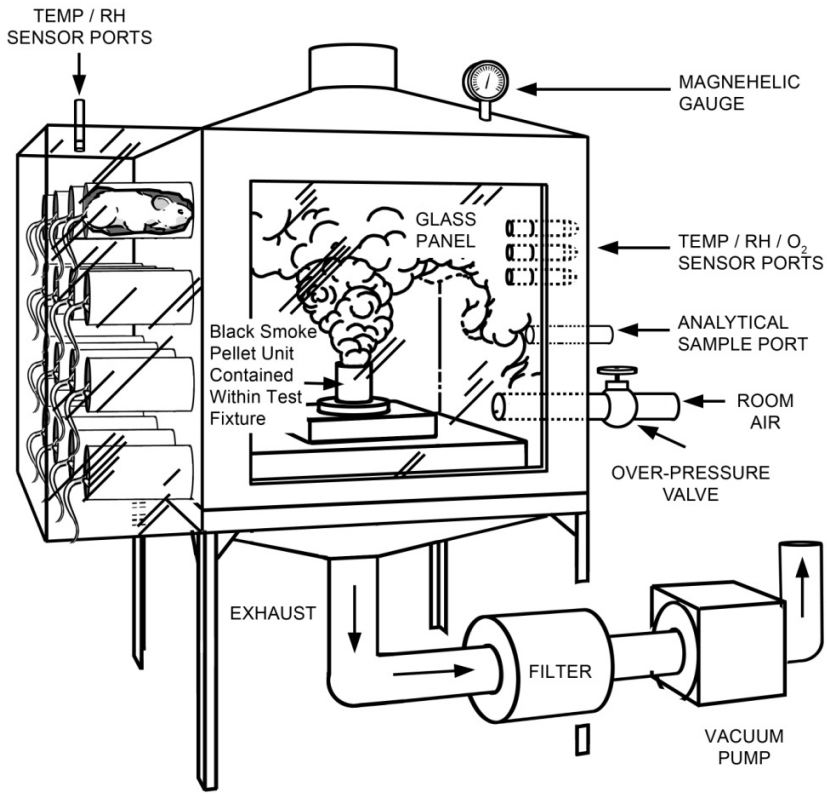
Test atmospheres were generated in the exposure chamber by pyrotechnic dissemination of black smoke (PVA) pellet units (see Table 2 and Figure 1 for detailed description of a pellet unit). A single pellet unit was used for the acute study exposure. For the 4-week inhalation study, a single pellet unit produced adequate test atmosphere concentration levels for all 3 test groups (design concentration levels of 100, 700 and 2000 mg/m^3). In order to facilitate the generation of test atmospheres, pellet units were placed in a metal smoke test fixture attached to the floor of the exposure chamber and ignited with an electric match (see Figure 2)

Figure 2. Metal Smoke Test Fixture



The burn time for each pellet unit was approximately 10 seconds. The exposure chamber was operated under semi-static conditions. During the initial ignition of the pellet, all of the ports in the faceplates attached to the wall of the exposure chamber were stoppered, a dilution air valve positioned on the wall of the chamber was opened, and the exhaust pump to the chamber was turned on. The faceplate was stoppered in order to prevent the test atmosphere from leaking uncontrollably out of the chamber, the dilution air valve was opened to permit room air to enter the chamber and prevent over pressurization of the chamber interior, and the exhaust pump evacuated unwanted test atmosphere from the chamber. The operation of the exhaust pump was considered to be the primary component for controlling the concentration of the test atmosphere in the exposure chamber. Once the concentration of particulate in the exposure chamber was considered to be within the acceptable range for the animal exposure, the exhaust pump was shut off and the dilution air valve was closed to prevent room air from entering the exposure chamber. At this point, the stoppers in the faceplate were removed and the rats were placed in the faceplate and their 30-minute exposure was initiated. In an attempt to minimize loss of the test atmosphere from the exposure chamber, the stoppers in the faceplate were removed one at a time and an exposure cylinder with a rat contained in it were placed into the faceplate immediately after removing the stopper. Once all of the rats were positioned within the faceplate, a cover was placed over the faceplate to prevent leakage of the test atmosphere into the laboratory where the exposure was being conducted.

Figure 3. Generation/Exhaust/Exposure System



6.3.2 Exposure Chamber

The exposure chamber was constructed of stainless steel and glass with a nominal internal volume of approximately 1200 liters. A different but identical chamber was used for the exposure of the control rats. The exposure chambers were New York University (NYU) style with cubical midsections and square-pyramidal inlets and outlets (Drew, 1978). The chamber distribution of black smoke (PVA) particulate was determined prior to animal exposure. Analyses of the distribution data indicated that the distribution of particulate test atmosphere was sufficiently homogenous for inhalation toxicology testing (USAPHC, 2010a).

6.3.3 Exposure Mode

Animals were exposed to test atmospheres of black smoke (PVA) via the nose-only (head-only) route of exposure. The nose-only exposure mode was selected for this study in an attempt to minimize deposition of the black smoke (PVA) particulate onto the fur of the exposed rats, and

therefore, minimizing inadvertent dermal and oral exposure of the test substance to the rats. Rats were individually restrained during exposures in perforated, stainless steel cylinders with conical nosepieces.

6.3.4 Exposure Duration

Rats were exposed for 30 minutes to the test atmosphere during each exposure in an attempt to accurately mimic a potential human exposure scenario. Acute study rats were exposed to a single exposure. The pilot study rats were exposed for 3 consecutive days. The 4-week study rats were exposed 5 days/week, over a 5-week period (weekends excluded) for a total of 20 exposures. Control rats were exposed to air only in a separate chamber for the same daily duration as the test level rats.

6.4 Characterization of Exposure Chamber Atmosphere

6.4.1 Test Substance Atmospheric Concentration

The atmospheric concentration of black smoke (PVA) particulate in the exposure chamber was determined by gravimetric analysis at regular intervals (e.g., beginning, middle, end) during each 30-minute exposure. Known volumes of chamber atmosphere were drawn from a sampling port in the exposure chamber representative of the animals' breathing zone. Samples were drawn through a 25-mm filter cassette that contained a pre-weighed Gelman glass fiber (Type A/E) filter. All filters were weighed on a Cahn model C-30 Microbalance. The atmospheric concentration of black smoke (PVA) particulate was calculated from the difference in the pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled. The vapor/gas component of the test atmosphere was also characterized, however the analytical report for this analysis was not available at the time of this report.

6.4.2 Particle Size Analysis

Samples to determine atmospheric particle size distribution (mass median aerodynamic diameter) of the black smoke (PVA) particulate were collected 3 different times during the study from the low-, intermediate-, and high-concentration level exposure chambers. Samples were collected with a Sierra® Series 210 8-Stage Cascade Impactor fitted with a Cyclone Preseparator and Anderson model SE113 Constant Flow Air Sampler. Particle size sample data were analyzed by log normal regression of particle size versus cumulative relative mass (Sierra Instruments, Inc., 1979 and USAPHC, 2010b). (Sierra® is a trademark of Sierra Instruments Inc.).

6.4.3 Environmental Monitoring

Chamber temperature was targeted at 22 ± 2 ºC and chamber humidity was targeted between 30 and 70 percent. Chamber temperature and humidity were monitored continually with an Omega model RH411 Digital Thermo-Hygrometer and recorded 1-2 times during each exposure. The exposure system was a static system so chamber airflow was not monitored during this study. However, to ensure that adequate oxygen was available to the rats while they were being exposed, the exposure chamber atmosphere was monitored for oxygen content during each exposure. Minimum oxygen concentrations were targeted at 19%. Chamber oxygen concentrations were

measured with a Teledyne Instruments model GB300 Oxygen Analyzer and recorded 1-2 times during each exposure.

6.5 Body Weights and Clinical Observations

Acute and pilot study rats were weighed and individually observed for clinical signs multiple times during the exposure/recovery period. The 4-week main study rats were weighed and individually observed for clinical signs of toxicity on a weekly basis (just prior to exposure). In addition, rats were observed for mortality and clinical signs of toxicity while they were being exposed in the chambers during each exposure and upon removal from the chambers immediately following each exposure.

6.6 Food Consumption (4-Week Main Study Only)

The amount of food consumed by individual rats was determined throughout the study on a weekly basis.

6.7 Ophthalmological Evaluations (4-Week Main Study Only)

Three ophthalmological examinations were conducted by the Attending Veterinarian for the rats assigned to the 4-week main study. Prior to initiation of exposure to black smoke (PVA), all of the 72 rats to be used for the 4-week main study had both of their eyes examined. Just prior to completion of the exposure period, the 48 rats exposed to 0 and 2100 mg/m^3 (12 rats/sex/group) had both eyes examined. Following a one-month recovery, the 24 remaining rats exposed to 0 and 2100 mg/m^3 (6 rats/sex/group) had both eyes examined. Based on the lack of any adverse findings observed during any of the ophthalmological evaluations performed on the control and high-concentration rats, rats exposed to the low and intermediate concentration levels of black smoke (PVA) did not have their eyes examined.

6.8 Clinical Pathology Evaluations (4-Week Main Study Only)

Clinical chemistry and hematology evaluations were conducted on 12 rats/sex/group immediately following the exposure period. Following a one-month recovery period, the remaining rats in the control and high concentration levels (6 rats/sex/group) received the same clinical chemistry and hematology evaluations. All rats were fasted overnight prior to collection of blood samples. Blood samples were collected just prior to necropsy via a cardiac puncture following anesthesia with carbon dioxide. A portion of each blood sample was transferred to an EDTA microtube and evaluated with an Abbott Laboratories Cell-Dyn 3700 Hematology Analyzer for the following hematology measurements: white blood cell count (WBC), WBC differential (% neutrophils (NEU %N), % lymphocytes (LYM %L), % monocytes (MONO %M), % eosinophils (EOS %E), % basophils (BASO %B)), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV). The remainder of each blood sample was transferred to a serum-gel microtube and evaluated with an IDEXX Laboratories VetTest 8008 Chemistry Analyzer and VetLyte Na, K, Cl Analyzer for the following clinical chemistry measurements: albumin (ALB), alkaline phosphatase (ALK P), alanine aminotransferase (ALT), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine

(CREA), glucose (non-fasting) (GLU), globulin (GLOB), lactate dehydrogenase (LDH), phosphorus (PHOS), total bilirubin (TBIL), total protein (TP), sodium (Na), potassium (K), and chlorine (Cl).

Coagulation parameters, including average prothrombin time (AVG PT) and average activated prothrombin time (AVG APTT), were determined (MCA 210 Microsample Coagulation Analyzer, BioData Corporation, 155 Centennial Plaza, P.O. Box 347, Horsham, PA 19044) on all valid samples.

6.9 Necropsy and Histopathology Evaluations (Acute and 4-Week Main Studies)

For the acute study, 10 rats (5 male and 5 female) were euthanized with carbon dioxide and necropsied following a 14-day recovery period. Gross examinations were performed on all rats and the lungs were removed and weighed. In addition, representative samples of the respiratory tract (lungs, trachea, pharynx, larynx, and nose) were removed and preserved in a suitable medium for future histopathological examination. For the 4-week main study, 12 rats/sex/group were euthanized with carbon dioxide and necropsied following the exposure period. Following a one-month recovery period, the remaining rats in the control and high concentration levels (6 rats/sex/group) were also euthanized with carbon dioxide and necropsied. All rats were fasted overnight prior to the necropsy. Gross examinations were performed on all rats and the following tissues were removed and weighed: adrenals, brain, heart, kidneys, liver, lungs, spleen, thymus, epididymides, testes, ovaries, and uterus. The following organs and tissues, or representative samples, were removed and preserved in a suitable medium for future histopathological examination: all gross lesions; brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex); pituitary; thyroid/parathyroid; thymus; lungs; trachea; pharynx; larynx; nose; heart; bone marrow (either femur, sternum, or rib at the costochondral junction); salivary glands; liver; spleen; kidney; adrenals; pancreas; testes; epididymides; seminal vesicles; prostate; ovaries; uterus; aorta; esophagus; stomach; duodenum; jejunum; ileum; caecum; colon; rectum; urinary bladder; representative lymph node; peripheral nerve; sternum with bone marrow; mammary gland; thigh musculature; eyes; femur (including articular surface); spinal cord at three levels (cervical, midthoracic, and lumbar); exorbital lachrymal glands; skin. All collected tissues from rats in the control group and high concentration group that were necropsied immediately following the exposure period were processed and received a full histopathological examination. Lungs, trachea, pharynx/larynx, nose, liver, heart, spleen, adrenals, and eyes were processed and examined from rats in the low concentration and intermediate concentration groups that were necropsied immediately following the exposure period. Lungs, trachea, pharynx/larynx, and nose, were processed and examined from rats in the control and high concentration groups that were necropsied following a one-month recovery period.

6.10 Statistical Analysis of Data

For variables that were measured only at the end of the exposure (week 4) and at the end of the recovery period (week 8), the dose groups and sexes were compared using a two-factor analysis of variance (ANOVA). Organ to brain and organ to body weight ratios were calculated and analyzed similarly to the other parameters measured at weeks 4 and 8. If a significant dose group by sex interaction was observed, then a one factor ANOVA was used to compare dose groups for each sex. If the dose group effect was significant, comparison of dose groups were made using a Tukey's multiple comparison test if the variance of the groups were similar and a Dunnett's T3 test

if the variances were unequal. Variance equality was determined by a Levene's test. For absolute organ weights, comparison of the dose groups and sexes was made using a two-factor analysis of covariance (ANCOVA) and body weight at the end of the exposure period was the covariate used. Even though the dose groups were assigned at Day 0 to keep the average weight for each dose group similar, the weights can change during the study dependent on the dose group. The ANCOVA adjusted for any differences in body weights among the dose groups at the end of the study, because heavier animals would tend to have heavier organs. If the dose group effect was significant, a least significant differences post hoc test was used to compare pairs of doses. Sexes and dose groups were also compared with respect to absolute body weights, as well as weekly changes in body weight and net weight changes using a two-factor ANOVA. Sexes and dose groups were also compared with respect to weekly food consumption and net food consumption for the study using a two-factor ANOVA. If the ANOVA was significant, a comparison of dose groups was made using a Tukey's multiple comparison test if the variance of the groups were similar and a Dunnett's T3 test if the variance were unequal. Variance equality was determined by a Levene's test. SPSS 16.0 was used to perform all analyses and statistical significance was defined as $p \leq .05$ for all tests. In addition, descriptive statistics (e.g., mean, standard deviation) were used to summarize experimental data (e.g., atmospheric concentrations).

6.11 Benchmark Dose and Human Equivalent Dose Determination

Benchmark Dose (BMD) is an alternative approach to the No-Observed Adverse Effect Level (NOAEL) / Lowest Observed Adverse Effect Level (LOAEL) approach in the development of a Point of Departure (POD) that has been used for many years in dose–response assessment. The BMD method gained favor with the risk assessment community because it incorporates and conveys more information than the NOAEL/LOAEL method. The dose–response assessment is a 2-step process, one defining a POD and the other is extrapolating from POD for relevance to environmental exposure. The BMD and its 95% lower confidence limit for 10% effect levels ($BMDL_{10}$) were determined from the dose–response relationship of the incidence of alveolar histiocytosis following 4 weeks of exposure, which was the most sensitive dose-responsive adverse event in this study. The BMD analysis used Benchmark Dose Software (BMDS, version 2.2) to calculate the POD using the standard suite of models (Gamma, Logistic, Log Logistic, Log Probit, Multistage, Probit, Weibull, and Quantal-Linear models) for dichotomous data (Davis et al., 2011, Gephart, et al., 2001 and USEPA, 2011a). Of the models that yielded acceptable data fits, those with large-scaled residual were excluded. The mean of the $BMDL_{10}$ of the remaining models was used as the POD for low-dose extrapolation. A dosimetric adjustment factor (DAF) was calculated and used to derive the HEC (USEPA, 2002 and USEPA, 2011b).

7 Results and Discussion

7.1 General

7.1.1 Purity and Stability of Black Smoke (PVA)

A Good Laboratory Practice (GLP)-analysis of the test substance was not performed prior to initiation of the study. However, the test substance composition information supplied by the sponsor (see Table 2) is considered to be sufficient and the black smoke (PVA) used in this study was considered to be stable and within acceptable limits for conduct of this study.

7.1.2 Generation Method Development

Prior to the initiation of the test exposures, pre-test trials were conducted to determine the most suitable method of generating test atmospheres of black smoke (PVA). The goal of this preliminary work was to achieve reasonably stable atmospheric concentrations at the targeted levels of test particulate for the acute study (approximately 2000 mg/m^3), the pilot study (approximately 2000 mg/m^3), and the 4-week main study (approximately 100, 700, and 2000 mg/m^3). The generation system used for the animal exposures was selected based on its ability to generate relatively stable atmospheres of black smoke (PVA) at the targeted concentrations.

7.1.3 Chamber Distribution of Test Atmosphere

Prior to initiation of the test exposures, a study of the chamber distribution of the particulate concentration of black smoke (PVA) was performed in the exposure chamber. A series of gravimetric sample sets were collected at various times following the ignition of a single black smoke (PVA) pellet unit in the chamber. Each sample set consisted of 4 individual samples, with 3 of the samples collected at the same time from different spatial regions of the chamber faceplate and 1 sample collected from the chamber sample port. No significant differences were observed from gravimetric samples collected at 6 different spatial locations in the exposure chamber at different time intervals, and therefore, the test substance was considered to be homogenously distributed throughout the area of the exposure chamber where the rats were being exposed. Gravimetric samples collected from the chamber sampling port during this pretest trial also indicated that the chamber concentration was similar at the exposure chamber sampling port when compared to samples collected from the chamber faceplate in the area where the rats were to be positioned during exposure. Therefore, all gravimetric chamber samples were collected from the chamber sample port during the animal exposure and this data was considered to be representative of the breathing zone of the rats. Chamber distribution data are presented in Appendix D.

7.2 Acute Study

7.2.1 Exposure Chamber Concentration and Particle Size Data

One test exposure with 10 rats (5 male and 5 female) was conducted. Rats were exposed nose-only to a single, 30-minute exposure. The mean atmospheric concentration of black smoke (PVA) particulate in the exposure chamber was determined to be 2500 ± 360 mg/m^3. The particle size distribution of the test atmosphere during the exposure was characterized by measurement of the mass median aerodynamic diameter (MMAD). The MMAD of the test atmosphere generated was 2.2 µm, the geometric standard deviation (GSD) was 1.5, with 3% of the particles less than 1 µm, 93% of the particles less than 4 µm, and 100% of the particles less than 10 µm. Exposure concentration data are summarized in Table 3.

Table 3. Acute Study: Summary of Black Smoke (PVA) Particulate Chamber Concentration and Particle Size Distribution

Atmospheric Concentration (mg/m^3)				Mass Median Aerodynamic Diameter (µm)[a]	Geometric Standard Deviation[a]	% Particles by Mass[a]		
Mean	S.D.	Range	n			<1 µm	<4 µm	<10 µm
2500	360	2000 – 2900	5	2.2	1.5	3	93	100

Note:
[a] One particle size sample collected during the 30-minute exposure.

7.2.2 Exposure Chamber Environmental Conditions

Chamber temperature remained constant at 20°C, chamber relative humidity ranged from 53% to 57%, and oxygen remained constant at 21%. The environmental conditions within the exposure chamber were considered to be acceptable for the conduct of this study.

7.2.3 Body Weights of Rats

Rats were weighed on test days 1, 2, 3, 6, 9, and 15. Slight body weight losses were observed in some rats on the day following exposure, however, all rats exhibited normal weight gain patterns following post-exposure day 1 and experienced an overall weight gain by the end of the 14-day recovery period.

7.2.4 Clinical Observation of Rats

Immediately following exposure, clinical signs observed in rats included black/gray-stained fur (primarily on the head), red nasal discharge, and salivation. The nasal discharge and salivation had resolved by test day 2. The black/gray-stained fur was observed in some rats up to test day 3. No clinical signs were observed in rats following test day 3.

7.2.5 Gross Necropsy and Histopathology

All ten rats received a gross necropsy with limited histopathology of the respiratory tract following a 14-day recovery period. Alveolar histiocytosis with hemoglobin crystals was noted in one male rat, however, this lesion was considered to be an incidental finding and not related to exposure to the test material.

7.2.6 Mortality and LC_{50} Determination

All ten rats exposed to 2500 mg/m^3 black smoke (PVA) particulate survived the exposure and recovery period. Therefore, the 30-minute, inhalation median lethal concentration (LC_{50}) of black smoke (PVA) in rats is greater than 2500 mg/m^3.

7.3 4-Week Main Study

7.3.1 Atmospheric Concentration of Black Smoke Particulate

A total of 21 animal exposures were conducted. In order to accommodate a reasonable necropsy schedule at the end of the exposure period, the initial exposure for the male and female rats was staggered by one day. Male rats were exposed from exposure #1 through #20 and female rats were exposed from exposure #2 through #21. All rats received a total of 20 exposures. Although slight differences in the chamber concentrations of black smoke were noted between the male and female rats (Table 4), the slight differences between them has no toxicologically-relevant consequences in the interpretation of the data. Therefore, the combined exposure concentration data for black smoke will be reported. The analytically determined overall (combined) mean concentrations ± standard deviation of black smoke in the exposure chambers targeted to 100, 700, or 2000 mg/m^3 were 130 ± 55, 670 ± 180, or 2100 ± 570 mg/m^3, respectively. Combined chamber concentrations for black smoke were calculated by including all chamber concentration data collected over the 21 exposures conducted. The overall mean concentrations were 130, 96, or 105 percent of the targeted concentrations of 100, 700, or 2000 mg/m^3, respectively. The daily mean concentrations ranged from 61-190, 63-150, or 78-140 percent of the overall mean concentrations of 130, 670, or 2100 mg/m^3, respectively. The difference between the overall actual mean concentrations and the targeted concentrations, the range of daily mean concentrations, and the standard deviations (daily and overall) were greater than what is typically desired (e.g., <10 percent), however, the difficulties associated with maintaining consistent particulate atmospheres are to be expected with the type of generation system used during this study. The mean concentrations for black smoke particulate were as consistent as can be reasonably expected for generating test atmospheres with pyrotechnically disseminated pellets, and therefore, were considered acceptable for evaluating the toxicity of the test substance in this study. Exposure concentration data are presented in Appendix E and summarized in Table 4.

Table 4. 4-Week Main Study: Summary of Chamber Concentrations of Black Smoke Particulate

DESIGN CONCENTRATION (mg/m^3)	GROUP IDENTIFICATION	MEASURED CONCENTRATION (mg/m^3)			
		MEAN	S.D.	RANGE	N
0	Male Rats Female Rats ***Combined***	0 0 ***0***	N/A N/A ***N/A***	0 – 0 0 – 0 ***0 – 0***	20 20 ***21***
100	Male Rats Female Rats ***Combined***	130 130 ***130***	56 56 ***55***	66 – 300 66 – 300 ***66 – 300***	59 59 ***62***
700	Male Rats Female Rats ***Combined***	680 670 ***670***	180 190 ***180***	320 – 1200 320 – 1200 ***320 – 1200***	61 61 ***64***
2000	Male Rats Female Rats ***Combined***	2100 2100 ***2100***	580 580 ***570***	940 – 3800 940 – 3800 ***940 – 3800***	62 62 ***65***

Legend:
mg/m^3 = milligrams per cubic meter
S.D. = standard deviation
N = number of samples collected
N/A = not applicable

7.3.2 Nominal Concentration of Test Substance

The generation system used during this study utilized a single black smoke (PVA) pellet unit to generate test atmospheres for all 3 dose concentrations. Since the amount of material that was disseminated for each dose concentration could not be accurately determined, the generation process used for this study did not lend itself to the calculation of a nominal concentration. In addition, since the actual exposure chamber concentrations of black smoke (PVA) particulate were measured on a frequent basis for each dose concentration, the determination of nominal concentrations was not considered a useful parameter for this study, and therefore, was not calculated.

7.3.3 Particle Size Distribution of Test Substance Atmosphere

The black smoke (PVA) particulate atmospheres generated during this study were considered to be respirable in rats. The particle size distribution of black smoke (PVA) particulate atmospheres across the 3 dose concentrations was similar. The mass median aerodynamic diameter (MMAD) for the test particulate atmospheres ranged from 2.7 to 3.4 µm, the geometric standard deviation (GSD) ranged from 1.4 to 1.6 with 0.3 to 1.3 percent of the particles less than 1µm, 37 to 64

percent of the particles less than 3 µm, and 98 to >99 percent of the particles less than 10 µm. Particle size distribution data are summarized in Table 5.

Table 5. 4-Week Main Study: Summary of Chamber Atmosphere Particle Size Data

CONCENTRATION (mg/m^3)	EXPOSURE NUMBER	MMAD (µm)	GSD	% PARTICLES BY MASS		
				<1 µm	<3 µm	<10 µm
130	7	3.0	1.6	1.0	50	>99
	12	2.7	1.5	1.2	56	>99
	20	2.7	1.6	1.3	58	>99
670	5	3.1	1.4	0.30	46	>99
	10	3.4	1.6	0.90	39	98
	18	3.4	1.5	0.55	37	99
2100	2	2.7	1.4	1.0	64	>99
	8	3.1	1.6	1.1	47	99
	17	3.0	1.5	0.40	51	>99

Legend:
mg/m^3 = milligrams per cubic meter GSD = geometric standard deviation µm = micron
MMAD = mass median aerodynamic diameter % = percent

7.3.4 Exposure Chamber Environmental Conditions

Chamber environmental conditions were considered to be reasonably similar between the 3 test chambers and the control chamber. The temperatures in the 4 exposure chambers during the 21 exposures generally ranged from 19-21 ºC, with 2 isolated instances in which the chamber temperature was as low as 18ºC. Although the chamber temperatures were below the targeted range of 20-24 ºC, the lower temperatures did not appear to have adversely affected the health of the animals based on their clinical appearance. The relative humidity in the 4 exposure chambers during the 21 exposures was generally in the targeted range of 30-70 percent (%), however there were several instances when it was as low as 25% and as high as 91%. Although the chamber relative humidity exceeded the targeted range of 30-70 percent, the instances of lower/higher humidity did not appear to adversely affect the health of the animals based on their clinical appearance. In addition, even though the relative humidity in the control chamber was generally lower than the relative humidity in the 3 test chambers, the difference was considered to be within reasonable limits. The oxygen concentration in the 4 exposure chambers during the 21 exposures generally ranged from 20-21%, with a single instance in which the oxygen concentration was 22%. Overall, the environmental conditions in the exposure chambers were within acceptable comfort levels for the rats and were considered adequate for conduct of this study. Chamber environmental conditions are presented in Appendix F and summarized in Table 6.

Table 6. 4-Week Main Study: Summary of Environmental Conditions in Exposure Chamber

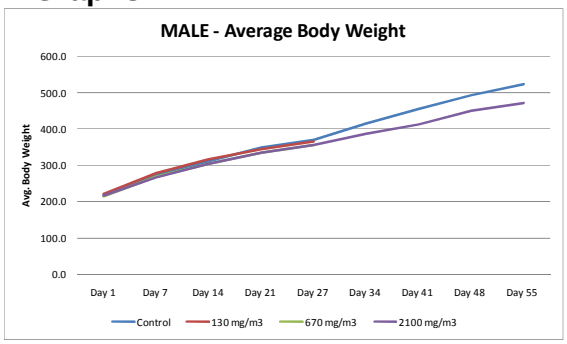
CONCENTRATION (mg/m^3)	GROUP IDENTIFICATION	TEMPERATURE (°C)		RELATIVE HUMIDITY (%)		OXYGEN (%)	
		RANGE	N	RANGE	N	RANGE	N
0	Male Rats	19-21	40	25-87	40	20-21	39
	Female Rats	19-21	40	25-87	40	20-21	39
	Combined	***19-21***	***42***	***25-87***	***42***	***20-21***	***41***
130	Male Rats	19-21	38	41-89	38	20-21	37
	Female Rats	19-21	38	41-89	38	20-21	37
	Combined	***19-21***	40	***41-89***	40	***20-21***	***39***
670	Male Rats	18-21	40	54-91	40	20-22	40
	Female Rats	18-21	39	46-91	39	20-22	39
	Combined	***18-21***	41	***46-91***	41	***20-22***	41
2100	Male Rats	18-21	40	43-89	40	20-21	40
	Female Rats	18-21	40	39-89	40	20-21	40
	Combined	***18-21***	42	***39-89***	42	***20-21***	42

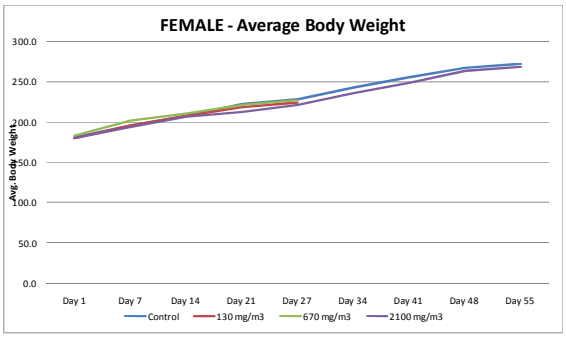
Legend:
°C = degrees Centigrade
% = percent
L/min = liters per minute
N = number of samples collected

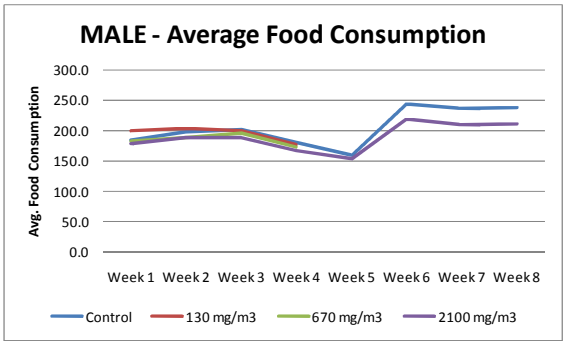
7.3.5 Body Weights of Rats and Food Consumption

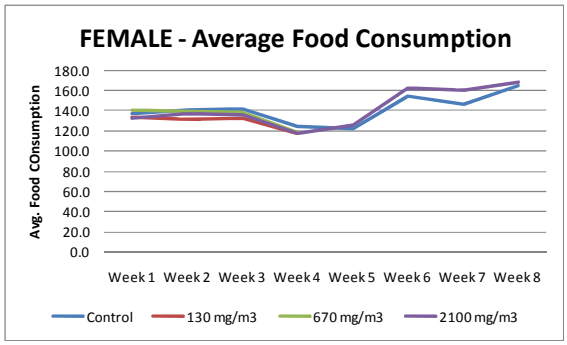
Mean body weights, body weight changes, and food consumption for rats exposed to black smoke tended to be decreased compared to controls in a generally dose-dependent manner. However, the only instances of statistically significant decrease for these indices were in male rats exposed to 2100 mg/m^3 and only occurred during the recovery period. Group and individual data for body weights, body weight gains, and food consumption are reported in Appendices G, H, and I and summarized in Figure 4. The no-observed-adverse-effect level for body weight and food consumption data in rats exposed to black smoke (PVA) was considered to be 670 mg/m^3.

Figure 4. 4-Week Main Study: Body Weight and Food Consumption Data Graphs









7.3.6 Clinical Observation of Rats

All rats from the study survived the exposure and subsequent recovery period. Rats were observed prior to each exposure, while they were in the exposure chamber, and immediately following exposure. In addition, all rats received a detailed evaluation on a weekly basis on the same day that they were weighed. No adverse clinical observations were noted in rats while they were in the exposure chambers. Immediately following exposures, clinical signs observed in rats exposed to the test material included black/red stained fur (especially on the head/face), black stained tail, black/blue stained ears, orange stained fur (primarily on forelimbs), orange stained bedding, salivation, and red nasal discharge. These signs were observed across the 3 treatment groups in an apparent dose-related manner (e.g., staining was more prevalent in 2100 mg/m^3 rats than in the rats exposed to 130 or 670 mg/m^3). Most of these clinical signs were related to the staining effect on the outward surfaces of the rats (e.g., fur, skin) resulting from exposure to the black smoke dyes and were to be expected. The presence of these signs, even in a dose-dependent manner, was not considered to be necessarily adverse in and by themselves and the presence of the test material on the fur/skin of the rats did not appear to have any immediate or obvious adverse effects. Clinical observations not necessarily related to exposure to the test material were also noted in rats during the study when they were weighed on a weekly basis or during the daily observations conducted during the recovery period (e.g., hair loss). The individual clinical signs for

rats are reported in Appendix J. The no-observed-adverse-effect level for gross clinical observations in rats exposed to black smoke (PVA) was considered to be 2100 mg/m^3.

7.3.7 Ophthalmological Evaluations

All observations in rats examined prior to study initiation, immediately following the exposure period, and following the recovery period were within normal limits. No adverse ophthalmological findings were observed in any rats examined during this study. One female rat exposed to 2100 mg/m^3 had an iris pigment effect following the one-month recovery, but this was an isolated finding and is not considered toxicologically relevant. The no-observed-adverse-effect level for ophthalmological changes in rats exposed to black smoke PVA was considered to be 2100 mg/m^3.

7.3.8 Blood Chemistry

Following the four-week exposure period, male rats exposed to 670 mg/m^3 black smoke (PVA) exhibited a decrease in cholesterol (p=0.034) but not in the 2100 mg/m^3 group (p=0.13). Cholesterol was also decreased following the four-week recovery period (p=0.039). Sodium and chloride values were respectively decreased (p=0.026) and increased (p=0.038) in rats exposed to 130 mg/m^3 following the 4-week recovery period. The only other clinical chemistry findings noted in the male rats were decreased LDH (p=0.036) and TP (p=0.013) values (following the recovery period), however, these differences were not within ranges considered to be biologically significant. Only the decreased cholesterol finding exhibited by the males exhibited a potential for a dose response relationship. Immediately following the exposure period, female rats exposed to 2100 mg/m^3 had increased ALT values (p<0.001) and female rats exposed to 130 mg/m^3 had decreased TP values (p=0.021), however, these findings were not considered biologically significant. The individual clinical chemistry data for rats are reported in Appendix K. The no-observed-adverse-effect level for clinical chemistry findings in rats exposed to black smoke (PVA) was considered to be 130 mg/m^3.

7.3.9 Hematology

Following the four-week exposure period, there were no treatment related hematology differences in male rats exposed to black smoke (PVA) compared to control rats. There were also no hematological differences noted in male rats following a four-week recovery, with the exception of an increase in RDW (p=0.019), which was not considered to be biologically significant. Female rats exhibited a greater hematological response to the test material than the male rats. Following the four-week exposure period, female rats exhibited increased NEU and NEU% values in rats exposed to 670 and 2100 mg/m^3 (p=0.028 and p=0.015; and p=0.012 and p=0.001; respectively). Decreased NEU values were also evident following the four-week recovery period (p=0.035). No other differences were observed in female rats exposed to the test material when compared to control rats following the exposure period with exception of a statistically significant increase in HCT (p=0.047) in female rats exposed to 670 mg/m^3. EOS and EOS% values were decreased (p=0.033 and p=0.028, respectively) following the recovery period, however, the differences were within normal ranges for these parameters and therefore not considered biologically meaningful. The individual hematology data for rats are reported in Appendix L. The no-observed-adverse-effect level for hematology findings in rats exposed to black smoke (PVA) was considered to be 130 mg/m^3.

7.3.10 Gross Necropsy

Multifocal black discoloration of the lungs and tracheobronchial lymph nodes were noted in all male and female rats exposed to 2100 mg/m^3 black smoke (PVA) immediately following the exposure period. This finding was also observed in rats exposed to 130 and 670 mg/m^3, but in a less frequent manner and intensity. The black discoloration was assumed to be a result of the black smoke particulate imbedded in these tissues. Although the effect from this discoloration is not clear, the mere presence of the black smoke particulate in the tissues in significant amounts has the potential to impact respiratory function, and therefore is considered to be an adverse finding. No other toxicologically important findings were observed during the gross necropsies. The individual gross necropsy data for rats are included in the Pathology Report in Appendix O. The no-observed-adverse-effect level for gross necropsy findings in rats exposed to black smoke (PVA) was considered to be 670 mg/m^3.

7.3.11 Organ Weights and Weight Ratios

Male rats had different organ weights for the adrenals, epididymides, lungs, kidneys, and thymus. Increased lung weights were observed in rats exposed to 670 mg/m^3 and 2100 mg/m^3 black smoke (PVA) compared to the control rats, however the rest of the organs only had differences at the 2100 mg/m^3 concentration compared to the control group. Female rats only showed changes in mean organ weights for kidneys, lungs, and the heart. The increased lung weights were different for both genders, and for both groups (immediately following exposure and following a 4-week recovery period). Based on organ weight data, the lungs were the primary target organ for rats exposed to high concentrations (670 and 2100 mg/m^3) of black smoke (PVA). There is evidence that the rats exposed to 2100 mg/m^3 started to recover and their organ weights appeared to be starting to return to normal weight values (equal to control group) following a 4-week recovery period. The individual organ weight and organ weight ratio data for rats are reported in Appendix N. The no-observed-adverse-effect level for organ weights and weight ratios in rats exposed to black smoke (PVA) was considered to be 130 mg/m^3.

7.3.12 Histopathology

Alveolar histiocytosis with type II pneumocyte hyperplasia was noted in all male and female rats exposed to 2100 mg/m^3 black smoke (PVA). This finding is expected as particles within the alveoli are phagocytosed by macrophages and then cleared by the mucociliary apparatus. Generally, more inert particulate materials induce an accumulation of alveolar macrophages, and depending on the relative cytotoxicity of the material, there is variable hyperplasia and hypertrophy of type II cells in the alveolar ducts and alveoli. In the 2100 mg/m^3 recovery rats, alveolar histiocytosis was significantly reduced and type II pneumocyte hyperplasia was not observed. This would suggest, based on a comparison to the lungs of the 2100 mg/m^3 rats necropsied immediately following exposure, that upon cessation of exposure to black smoke (PVA), the lung begins to recover. The remaining macrophages are likely due to the persistence of portions of the inhaled material, requiring a longer breakdown and clearance process. A longer recovery period would be required to see complete regression of this lesion. Transitional epithelial hyperplasia of the maxillary and nasoturbinates was the predominant finding in rats exposed to black smoke (PVA). Lesion severity and depth of affected nasal level decreased with the decline of test substance concentration. Male rats exposed to 2100 mg/m^3 were the most severely affected; in some rats, lesions extended from

level I, at the upper incisors, distal to level IV, the level of the first molar, with the majority extending slightly more rostral to the level of the incisive papilla. Additional findings, within this study (e.g., metaplasia /dysplasia, hyperplasia of the nasal respiratory epithelium, mucus hyperplasia, erosion and epithelial degeneration) are common, similar findings in other repeat dose exposure studies. Furthermore, based on the presence of nasal cavity lesions noted in the 2100 mg/m^3 rats necropsied immediately following exposure and the absence of lesions in the 2100 mg/m^3 rats necropsied following a 4-week recovery period, it is demonstrated that the majority of lesions can regress or recover. The Pathology Report for this study is attached as Appendix O. The no-observed-adverse-effect level for gross necropsy findings in rats exposed to black smoke (PVA) was considered to be 130 mg/m^3.

7.3.13 Benchmark Dose Determination

The incidence of moderate alveolar histiocytosis was identified as the most sensitive adverse event showing a dose-response relationship. The data is shown in Table 7 and the results from the BMDS are presented in Appendix Q. The best curve fits were obtained from the Gamma model which yielded the lowest Akaike information criterion (AIC) and scaled residual of interest. The BMD and $BMDL_{10}$ were, respectively, 539.6 mg/m^3 and 144 mg/m^3 for males, and 390.6 mg/m^3 and 81.8 mg/m^3 for females.

Table 7. Incidence of Moderate to Severe Alveolar Histiocytosis After 4 Weeks of Black Smoke Exposure

Dose mg/m^3	Male	Female
0	0/6	0/6
130	0/6	0/6
670	2/6	5/6
2100	6/6	6/6

7.3.14 Derivation of the Human Equivalent Concentration

The incidence of moderate to severe alveolar histiocytosis in female rats was the most sensitive adverse event observed in this study with a $BMDL_{10}$ of 81.8 mg/m^3. For calculation of the HEC (USEPA, 2011b), the recommended DAF for inhalation dosimetry is used. The rat/human body weight ratio raised to the ¼ power $(BW(a)/BW(h))^{0.25}$, which in the case of rats is 0.24 (DAF = $(0.25 \text{ kg}/70 \text{ kg})^{0.25}$= 0.24). The HEC was computed as HEC = $BMDL_{10}$ X DAF or HEC = 81.8 mg/m^3 X 0.24 = 19.6 mg/m^3.

8 Conclusions

For the acute inhalation study, all ten rats exposed to a single exposure of 2500 mg/m^3 black smoke (PVA) particulate survived the exposure and recovery periods. No significant body weight losses or adverse clinical signs were noted in the rats during the acute study. The 30-minute

inhalation median lethal concentration (LC_{50}) of black smoke (PVA) in rats is greater than 2500 mg/m^3.

For the 4-week main study, there were no mortalities in rats exposed to black smoke (PVA) during the exposure and recovery periods. In addition, no adverse compound-related effects were observed in rats exposed to the test compound for clinical signs, ophthalmological parameters, or clinical pathology findings. Compound-related effects were observed in rats exposed to 2100 mg/m^3 black smoke (PVA) for body weights, body weight gains, food consumption, organ weights, and histopathological findings. Based on the incidence and potentially adverse impact of these various findings, the 2100 mg/m^3 concentration level is considered to be an adverse effect level in rats exposed by inhalation to black smoke (PVA) on a repeated-dose manner. Rats exposed to 670 mg/m^3 also had compound-related effects for organ weights and histopathological findings, and rats exposed to 130 mg/m^3 had histopathological findings, however, the effects observed in rats exposed to both 130 and 670 mg/m^3 were substantially less pronounced than the effects observed in rats exposed at 2100 mg/m^3.

The lung and nasal cavity were the primary target organs in rats subjected to repeated inhalation exposures of black smoke (PVA). Pulmonary alveolar histiocytosis and nasal epithelial hyperplasia were observed in a dose-dependent manner in all treatment groups (130, 670, and 2100 mg/m^3). Pulmonary alveolar histiocytosis, an accumulation of macrophages in the alveolar spaces, is an expected response of the respiratory tract to an inhaled particulate. During this study, the black smoke particulate within the alveoli would have been phagocytosed by macrophages and then cleared by the mucociliary apparatus; this would account for the increased number of macrophages observed. This is not necessarily an adverse effect unless the mucociliary apparatus becomes overwhelmed. However, inert particulate materials can induce an accumulation of alveolar macrophages which can result in irritation, hyperplasia, and hypertrophy (Boorman, 1990). Nasal epithelial hyperplasia, simply an increase in the number of cells in the tissue, as noted above was also observed during this study. While the presence of an increased number of cells is not adverse, one of the most common causes of hyperplasia is chronic irritation due to inflammation. Since the histopathological findings in the lungs and nasal cavities were not directly associated with pathological changes that would necessarily be considered adverse (e.g., inflammation, necrosis), it is not clear if the findings were adverse or merely an adaptive response to the particulate nature of the test material that the rats were exposed to. Furthermore, based on the results of the histopathologic examinations following a one-month recovery period, it would appear that rats exposed to 2100 mg/m^3 begin to significantly recover from these findings upon cessation of the exposure to the test material.

Even though the severity of the impact of the histopathological findings in the lung and nasal cavity is not definitive, the fact that there were numerous findings in multiple endpoints (e.g., body weights, histopathology) for rats exposed to 2100 mg/m3, this level is considered to be a clear adverse effect level during this study. Effects observed in rats exposed to 670 mg/m^3 were less severe and consistent with those described for rats exposed to a higher concentration and suggest that 670 mg/m^3 is the borderline level for potentially adverse effects. The effects observed in rats exposed to 130 mg/m^3 were not considered to be adverse since they were only observed in a small number of rats and were significantly diminished in their degree of severity compared to rats exposed to higher concentration levels of the test substance.

The no-observed-adverse-effect level (NOAEL) for this study is defined as the highest dose at which toxicologically important (or "toxicologically relevant" or "adverse") effects attributable to the test substance were not detected. Due to the lack of significant toxicologically adverse findings in rats exposed to 130 mg/m^3, the NOAEL for repeated inhalation exposure to black smoke (PVA) is considered to be 130 mg/m^3.

For purposes of the benchmark determination, the most consistent and sensitive observation associated with exposure to black smoke (PVA) in the course of this study was the incidence of alveolar histiocytosis. Analysis of the incidence of alveolar histiocytosis using benchmark dose software resulted in a benchmark dose (BMD) of 539.6 mg/m^3 for male rats and 390.6 mg/m^3 for female rats and a 95 percent lower confidence limit for 10 percent effect level ($BMDL_{10}$) of 144 mg/m^3 for male rats and 81.8 mg/m^3 for female rats. A Human Equivalent Concentration (HEC) of 19.6 mg/m^3 was derived based on the BMD and $BMDL_{10}$ values for female rats.

9 Point of Contact

Questions pertaining to this report should be referred to Arthur J. O'Neill at DSN 584-3980, commercial 410-436-3980, or by e-mail: usarmy.apg.medcom-phc.mbx.tox-info@mail.mil.

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Appendix A

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Appendix B

Quality Assurance Statement

For: Toxicology Study No. 87-XC-0CKC-11, Protocol No. 0CKC-35-10-08-01, Acute and Four-Week Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke (PVA), October – December 2010", the following critical phases were audited by the Quality Systems and Regulatory Compliance Office's Quality Assurance Unit:

PRE IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Protocol Good Laboratory Practice Standards and Animal Care Review	02/18/2010	02/18/2010
Pre Acute Study - Body Weights and Observations	10/07/2010	10/15/2010
Compliance with PTOX SOPs	10/07/2010	10/15/2010
Pre Acute Study Test System Considerations	10/07/2010	10/15/2010

IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Personnel Training Records	10/13/2010	10/21/2010
Acute Test - Test Article Receipt and Control	10/13/2010	10/21/2010
Acute Test - Test System Restrainer Procedures	10/13/2010	10/21/2010
Acute Test - Exposure Duration	10/13/2010	10/21/2010
Pre Pilot Husbandry Considerations	10/15/2010	10/22/2010
Pre Pilot Maintenance and Calibration of Equipment	10/15/2010	10/22/2010
Pre Pilot Test System Identification	10/15/2010	10/22/2010
Pilot Study - Number of Rats Exposed	10/20/2010	10/28/2010
Pilot Study - Exposure Duration	10/20/2010	10/28/2010
Pilot Study - Compliance with Study Protocol and Sub-Study Endpoint Criteria Compliance	10/20/2010	10/28/2010
Pre 4 Week - Test System Refinement - Acclimation	10/21/2010	10/29/2010

APPENDIX B

QUALITY ASSURANCE STATEMENT

For: Toxicology Study No. 87-XC-0CKC-11, Protocol No. 0CKC-35-10-08-01, Acute and Four-Week Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke (PVA), October – December 2010", the following critical phases were audited by the Quality Systems and Regulatory Compliance Office's Quality Assurance Unit:

IN-LIFE PHASE OF THE STUDY (continued)

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Pre 4 Week - Ophthalmic Examinations	10/21/2010	10/29/2010
Test Article Characterization Dye Components	10/28/2010	11/02/2010
4 Week Study - Raw Data Documentation Procedures	11/04/2010	11/12/2010
4 Week Study - Compliance with PTOX SOPs	11/04/2010	11/12/2010
4 Week Study - Compliance with Study Protocol	11/04/2010	11/12/2010
Administration of Test Substance and Exposure Mode	11/16/2010	11/19/2010
4 Week Study - Analysis of Test Atmosphere	11/16/2010	11/19/2010
4 Week Study - Analysis of Combustion Gases	11/16/2010	11/19/2010
4 Week Study - VOC Sample Submission for Analysis	11/17/2010	11/23/2010
Animal Room Temperature and Humidity	11/24/2010	11/29/2010
SOP/Protocol Compliance - Husbandry Considerations	12/10/2010	12/13/2010
4 Week Study - Necropsy Procedures	12/17/2010	12/22/2010
4 Week Study - Clinical Pathology Evaluation	12/17/2010	12/22/2010
In-Life Study Endpoint Criteria	12/17/2010	12/22/2010

POST IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
MRICD Tissue Processing Support - Data Review	4/22/2011	4/27/2011

APPENDIX B

QUALITY ASSURANCE STATEMENT

For: Toxicology Study No. 87-XC-0CKC-11, Protocol No. 0CKC-35-10-08-01, Acute and Four-Week Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke (PVA), October – December 2010", the following critical phases were audited by the Quality Systems and Regulatory Compliance Office's Quality Assurance Unit:

POST IN-LIFE PHASE OF THE STUDY (Continued)

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Final Study Report Review	04/21/2014	04/21/2014
Study Raw Data Review	04/21/2014	04/21/2014

Note 1 All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2 In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QA Standard Operating Procedure.

Note 3 This report has been audited by the Quality Assurance Unit (QSARC), and is considered to be an accurate account of the data generated and of the procedures followed

Michael P. Kefauver

Michael P. Kefauver
Quality Assurance Specialist, QSARC-QAU

08/25/2014

Date

Appendix C

Archives and Study Personnel

1. ARCHIVES.

a. All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in room 1026, Building E-2100, USAPHC, for a minimum of five (5) years following submission of the final report to the Sponsor.

b. Records on animal receipt, diet, and facility environmental parameters will be archived by the Veterinary Medical Division, Toxicology Portfolio, for a minimum of five (5) years following submission of the final report to the Sponsor.

c. Some ancillary records pertaining to this study, such as instrument maintenance logs, animal room observation logs, etc., will not be archived until those logbooks have been completed. Once complete they will be archived in room 1026, Building E-2100, USAPHC.

d. Wet tissues, histology slides, and paraffin blocks are stored in building E-5158.

2. PERSONNEL.

a. Management

(1) Management (In-Life): COL Chris E. Hanson, Portfolio Director, Toxicology; Glenn J. Leach, Ph.D., Program Manager, Toxicity Evaluation Program (TEP); Dr. Mark S. Johnson, Ph.D., Program Manager, Health Effects Research Program (HERP).

(2) Management (Report): Mark S. Johnson, Portfolio Director, Toxicology; Arthur J. O'Neill, Program Manager, TEP; Dr. Michael J. Quinn, Ph.D., Program Manager, HERP.

b. Study Director: Arthur J. O'Neill, Biologist, TEP

c. Quality Assurance: Michael P. Kefauver, Quality Assurance Specialist, Quality Systems Office.

d. Veterinary Support and Animal Care: Dawn C. Fitzhugh, DVM, LTC, VC; Robert Sunderland, Animal Health Technician; Rebecca Kilby, Animal Health Technician; Jason Williams, Animal Health Technician.

e. Pathology Lab Coordinator: Patricia Beall, Biologist, TEP

f. Histopathology: Shannon M. Wallace, DVM, DACVP, LTC, VC, Pathologist, VMD

g. In-Life Support: Lee C.B. Crouse, Biologist, TEP

h. Hematology, Clinical Chemistry, Urinalysis: Matthew Bazar, Biologist, TEP; Mark Way, Biologist, TEP.

i. Archivist: Martha Thompson, Data Acquisition Specialist, TEP.

Appendix D

Chamber Distribution

Table D-1
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Chamber Distribution Summary of
Black Smoke (PVA) Particulate Atmosphere

Sample Set #1

SAMPLE ID #	TIME SAMPLE COLLECTED	SAMPLE PORT LOCATION	CONCENTRATION (mg/m^3)	% COMPARISON
1	1125	A	2692	110
2	1125	B	2290	94
3	1125	C	2359	96
			Mean = 2447	

SAMPLE ID #	TIME SAMPLE COLLECTED	SAMPLE PORT LOCATION	CONCENTRATION (mg/m^3)	% COMPARISON
4	1126	R	2477	101

Sample Set #2

SAMPLE ID #	TIME SAMPLE COLLECTED	SAMPLE PORT LOCATION	CONCENTRATION (mg/m^3)	% COMPARISON
1	1218	A	340	101
2	1218	B	280	83
3	1218	C	395	117
			Mean = 338	

SAMPLE ID #	TIME SAMPLE COLLECTED	SAMPLE PORT LOCATION	CONCENTRATION (mg/m^3)	% COMPARISON
4	1220	R	370	109

Sample Set #3

SAMPLE ID #	TIME SAMPLE COLLECTED	SAMPLE PORT LOCATION	CONCENTRATION (mg/m^3)	% COMPARISON
1	1235	D	316	106
2	1235	E	256	86
3	1235	F	322	108
			Mean = 298	

SAMPLE ID #	TIME SAMPLE COLLECTED	SAMPLE PORT LOCATION	CONCENTRATION (mg/m^3)	% COMPARISON
4	1238	R	283	95

Legend:
A = Top Left Corner of Chamber Faceplate
B, E = Center of Chamber Faceplate
C = Bottom Right Corner of Chamber Faceplate
D = Top Right Corner of Chamber Faceplate
F = Bottom Left Corner of Chamber Faceplate
R = Reference Port (Sampling Port of Exposure Chamber)
mg/m^3 = milligrams per cubic meter
% = percent

Appendix E

Exposure Chamber Atmospheric Concentration

Table E-1
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Daily Atmospheric Concentration of Black Smoke (PVA) Particulate in Exposure Chamber
0 mg/m^3 Exposure Chamber

Exposure No.	Date	Daily Mean	Chamber S.D.	Concentration Range (mg/m^3)	N
1	10/21/2010	0	-	-	1
2	10/22/2010	0	-	-	1
3	10/25/2010	0	-	-	1
4	10/26/2010	0	-	-	1
5	10/27/2010	0	-	-	1
6	10/28/2010	0	-	-	1
7	10/29/2010	0	-	-	1
8	11/1/2010	0	-	-	1
9	11/2/2010	0	-	-	1
10	11/3/2010	0	-	-	1
11	11/4/2010	0	-	-	1
12	11/5/2010	0	-	-	1
13	11/8/2010	0	-	-	1
14	11/9/2010	0	-	-	1
15	11/10/2010	0	-	-	1
16	11/11/2010	0	-	-	1
17	11/12/2010	0	-	-	1
18	11/15/2010	0	-	-	1
19	11/16/2010	0	-	-	1
20	11/17/2010	0	-	-	1
21	11/18/2010	0	-	-	1
	TOTAL	**0**	**-**	**-**	**21**

Legend: mg/m^3 = milligrams per cubic meter
S.D. = standard deviation
N = number of samples collected

Table E-2
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Daily Atmospheric Concentration of Black Smoke (PVA) Particulate in Exposure Chamber
130 mg/m^3 Exposure Chamber

Exposure No.	Date	Daily Mean	Chamber S.D.	Concentration Range (mg/m^3)	N
1	10/21/2010	100	24	83-128	3
2	10/22/2010	90	19	79-111	3
3	10/25/2010	126	41	87-168	3
4	10/26/2010	245	46	215-298	3
5	10/27/2010	174	69	116-250	3
6	10/28/2010	168	67	92-219	3
7	10/29/2010	105	60	66-175	3
8	11/1/2010	236	29	207-264	3
9	11/2/2010	138	25	111-159	3
10	11/3/2010	169	72	95-240	3
11	11/4/2010	145	16	134-164	3
12	11/5/2010	125	25	108-154	3
13	11/8/2010	79	11	70-91	3
14	11/9/2010	91	14	77-104	3
15	11/10/2010	95	15	86-111	3
16	11/11/2010	94	16	80-112	3
17	11/12/2010	117	16	105-129	2
18	11/15/2010	84	15	68-98	3
19	11/16/2010	115	20	99-138	3
20	11/17/2010	112	16	95-127	3
21	11/18/2010	86	4	82-90	3
	TOTAL[1]	**130**	**55**	**66-300**	**62**

Legend: mg/m^3 = milligrams per cubic meter
S.D. = standard deviation
N = number of samples collected

[1] = Mean, S.D., and range values rounded to 2 significant figures and determined from evaluation of all 62 data points.

Table E-3
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Daily Atmospheric Concentration of Black Smoke (PVA) Particulate in Exposure Chamber
670 mg/m^3 Exposure Chamber

Exposure No.	Date	Daily Mean	Chamber S.D.	Concentration Range (mg/m^3)	N
1	10/21/2010	707	133	580-845	3
2	10/22/2010	607	176	451-798	3
3	10/25/2010	604	112	509-728	3
4	10/26/2010	570	141	453-726	3
5	10/27/2010	558	102	448-648	3
6	10/28/2010	421	144	320-586	3
7	10/29/2010	582	134	459-725	3
8	11/1/2010	552	98	458-654	3
9	11/2/2010	653	105	585-773	3
10	11/3/2010	583	289	355-908	3
11	11/4/2010	662	181	455-785	3
12	11/5/2010	601	69	506-664	4
13	11/8/2010	834	137	978-932	3
14	11/9/2010	752	120	641-879	3
15	11/10/2010	618	156	459-772	3
16	11/11/2010	775	78	711-863	3
17	11/12/2010	865	189	685-1062	3
18	11/15/2010	878	237	676-1139	3
19	11/16/2010	994	201	806-1206	3
20	11/17/2010	741	15	724-750	3
21	11/18/2010	573	171	438-765	3
	TOTAL[1]	**670**	**180**	**320-1200**	**64**

Legend: mg/m^3 = milligrams per cubic meter
S.D. = standard deviation
N = number of samples collected

[1] = Mean, S.D., and range values rounded to 2 significant figures and determined from evaluation of all 64 data points.

Table E-4
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Daily Atmospheric Concentration of Black Smoke (PVA) Particulate in Exposure Chamber
2100 mg/m^3 Exposure Chamber

Exposure No.	Date	Daily Mean	Chamber S.D.	Concentration Range (mg/m^3)	N
1	10/21/2010	2218	356	1812-2473	3
2	10/22/2010	2535	368	2275-2796	2
3	10/25/2010	1964	399	1723-2424	3
4	10/26/2010	1919	598	1287-2475	3
5	10/27/2010	2208	1043	1236-3310	3
6	10/28/2010	2958	796	2220-3801	3
7	10/29/2010	2384	530	1881-2938	3
8	11/1/2010	1638	439	937-2052	5
9	11/2/2010	1981	664	1354-2676	3
10	11/3/2010	2457	636	1876-3136	3
11	11/4/2010	1966	615	1409-2626	3
12	11/5/2010	1835	332	1514-2301	4
13	11/8/2010	2093	506	1618-2626	3
14	11/9/2010	2213	487	1175-2738	3
15	11/10/2010	2088	744	1409-2884	3
16	11/11/2010	1906	662	1317-2623	3
17	11/12/2010	2198	714	1577-2978	3
18	11/15/2010	1972	556	1524-2594	3
19	11/16/2010	2372	728	1775-3184	3
20	11/17/2010	1918	369	1609-2327	3
21	11/18/2010	2176	530	1660-2719	3
	TOTAL[1]	**2100**	**570**	**940-3800**	**65**

Legend: mg/m^3 = milligrams per cubic meter
S.D. = standard deviation
N = number of samples collected

[1] = Mean, S.D., and range values rounded to 2 significant figures and determined from evaluation of all 65 data points.

Appendix F

Environmental Conditions

Table F-1
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Daily Environmental Data
0 mg/m^3 Exposure Chamber

		Temperature		Relative Humidity		Oxygen	
Exposure No.	Date	Range (°C)	N	Range (%)	N	Range (%)	N
1	10/21/2010	21	2	75	2	20-21	2
2	10/22/2010	19	2	33	2	21	1
3	10/25/2010	19	2	61-65	2	20-21	2
4	10/26/2010	19	2	82-85	2	21	2
5	10/27/2010	21	2	78-87	2	21	2
6	10/28/2010	19-20	2	80-84	2	21	2
7	10/29/2010	19	2	41-42	2	20-21	2
8	11/1/2010	19	2	32-33	2	21	2
9	11/2/2010	19	2	27-29	2	21	2
10	11/3/2010	19	2	29-34	2	20-21	2
11	11/4/2010	20	2	50-52	2	21	2
12	11/5/2010	21	2	40-42	2	21	2
13	11/8/2010	20	2	25-28	2	21	2
14	11/9/2010	20-21	2	29-32	2	21	2
15	11/10/2010	20	2	36-43	2	21	2
16	11/11/2010	19	2	33	2	21	2
17	11/12/2010	19	2	25-28	2	21	2
18	11/15/2010	20	2	48-49	2	21	2
19	11/16/2010	21	2	49-54	2	21	2
20	11/17/2010	21	2	53-59	2	21	2
21	11/18/2010	20	2	33	2	21	2

Legend: °C = degrees Centigrade
% = percent
N = number of samples collected

Table F-2
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Daily Environmental Data
130 mg/m^3 Exposure Chamber

		Temperature		Relative Humidity		Oxygen	
Exposure No.	Date	Range (°C)	N	Range (%)	N	Range (%)	N
1	10/21/2010	19	2	50-52	2	21	2
2	10/22/2010	19	2	45-49	2	21	1
3	10/25/2010	21	2	74-75	2	21	2
4	10/26/2010	19	2	85	2	20-21	2
5	10/27/2010	19	2	89	2	21	2
6	10/28/2010	19	2	86	2	21	2
7	10/29/2010	20	1	64	1	21	1
8	11/1/2010	19	2	59-63	2	20-21	2
9	11/2/2010	19-20	2	46-52	2	21	2
10	11/3/2010	19	2	56-59	2	21	2
11	11/4/2010	20	2	65-66	2	21	2
12	11/5/2010	21	1	55	1	21	1
13	11/8/2010	20-21	2	44-49	2	21	2
14	11/9/2010	19	2	47-51	2	21	2
15	11/10/2010	19	2	55-57	2	21	2
16	11/11/2010	19	2	47-50	2	21	2
17	11/12/2010	19	2	41-44	2	21	2
18	11/15/2010	20	2	59-63	2	21	2
19	11/16/2010	21	2	63-64	2	21	2
20	11/17/2010	21	2	58-59	2	21	2
21	11/18/2010	21	2	41-43	2	21	2

Legend: °C = degrees Centigrade
% = percent
N = number of samples collected

Table F-3
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Daily Environmental Data
670 mg/m^3 Exposure Chamber

		Temperature		Relative Humidity		Oxygen	
Exposure No.	Date	Range (°C)	N	Range (%)	N	Range (%)	N
1	10/21/2010	19	2	58-59	2	21	2
2	10/22/2010	19	2	54-56	2	21	2
3	10/25/2010	21	2	75-76	2	21	2
4	10/26/2010	19	2	86	2	21	2
5	10/27/2010	19	2	91	2	21	2
6	10/28/2010	19	2	85	2	21	2
7	10/29/2010	18-19	2	70-71	2	21	2
8	11/1/2010	19	2	66-67	2	20-21	2
9	11/2/2010	19	2	58-60	2	21	2
10	11/3/2010	19	2	59-62	2	21-22	2
11	11/4/2010	20	2	72-73	2	21	2
12	11/5/2010	21	2	65-67	2	21	2
13	11/8/2010	20	2	62-64	2	21	2
14	11/9/2010	20	2	59-63	2	21	2
15	11/10/2010	19	2	69	2	21	2
16	11/11/2010	19	2	68-70	2	21	2
17	11/12/2010	19	2	63-66	2	21	2
18	11/15/2010	20	2	74	2	21	2
19	11/16/2010	21	2	71	2	21	2
20	11/17/2010	21	2	70-71	2	21	2
21	11/18/2010	21	1	46	1	21	1

Legend: °C = degrees Centigrade
% = percent
N = number of samples collected

Table F-4
Protocol No. 0CKC-35-10-08-01
Four-Week Inhalation Toxicity Study in Rats Exposed to Black Smoke (PVA)

Daily Environmental Data
2100 mg/m^3 Exposure Chamber

		Temperature		Relative Humidity		Oxygen	
Exposure No.	Date	Range (°C)	N	Range (%)	N	Range (%)	N
1	10/21/2010	19	2	57-62	2	21	2
2	10/22/2010	19	2	43-61	2	21	2
3	10/25/2010	20-21	2	65-75	2	21	2
4	10/26/2010	19	2	80-86	2	21	2
5	10/27/2010	19	2	87-89	2	21	2
6	10/28/2010	19	2	79-85	2	21	2
7	10/29/2010	18	2	61-74	2	21	2
8	11/1/2010	19	2	58-70	2	21	2
9	11/2/2010	19	2	48-66	2	21	2
10	11/3/2010	19	2	49-64	2	21	2
11	11/4/2010	20	2	62-73	2	20-21	2
12	11/5/2010	21	2	60-70	2	21	2
13	11/8/2010	20	2	62-69	2	20-21	2
14	11/9/2010	20-21	2	51-60	2	21	2
15	11/10/2010	19	2	52-66	2	21	2
16	11/11/2010	19	2	50-66	2	21	2
17	11/12/2010	19	2	54-67	2	20-21	2
18	11/15/2010	20-21	2	60-71	2	21	2
19	11/16/2010	21	2	62-71	2	21	2
20	11/17/2010	21	2	61-71	2	21	2
21	11/18/2010	20	2	39-46	2	21	2

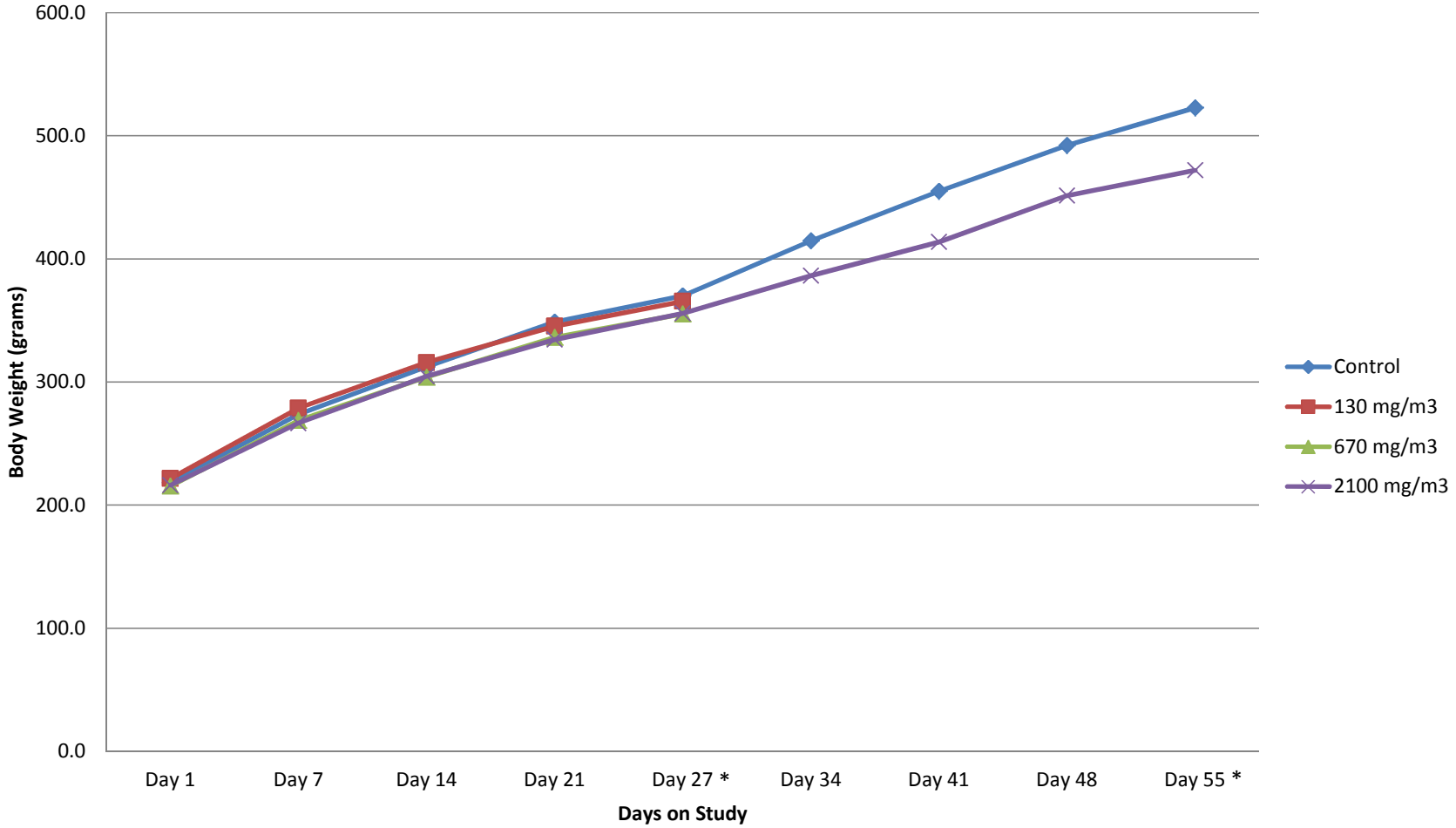
Legend: °C = degrees Centigrade
% = percent
N = number of samples collected

Appendix G

Body Weight Data

Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

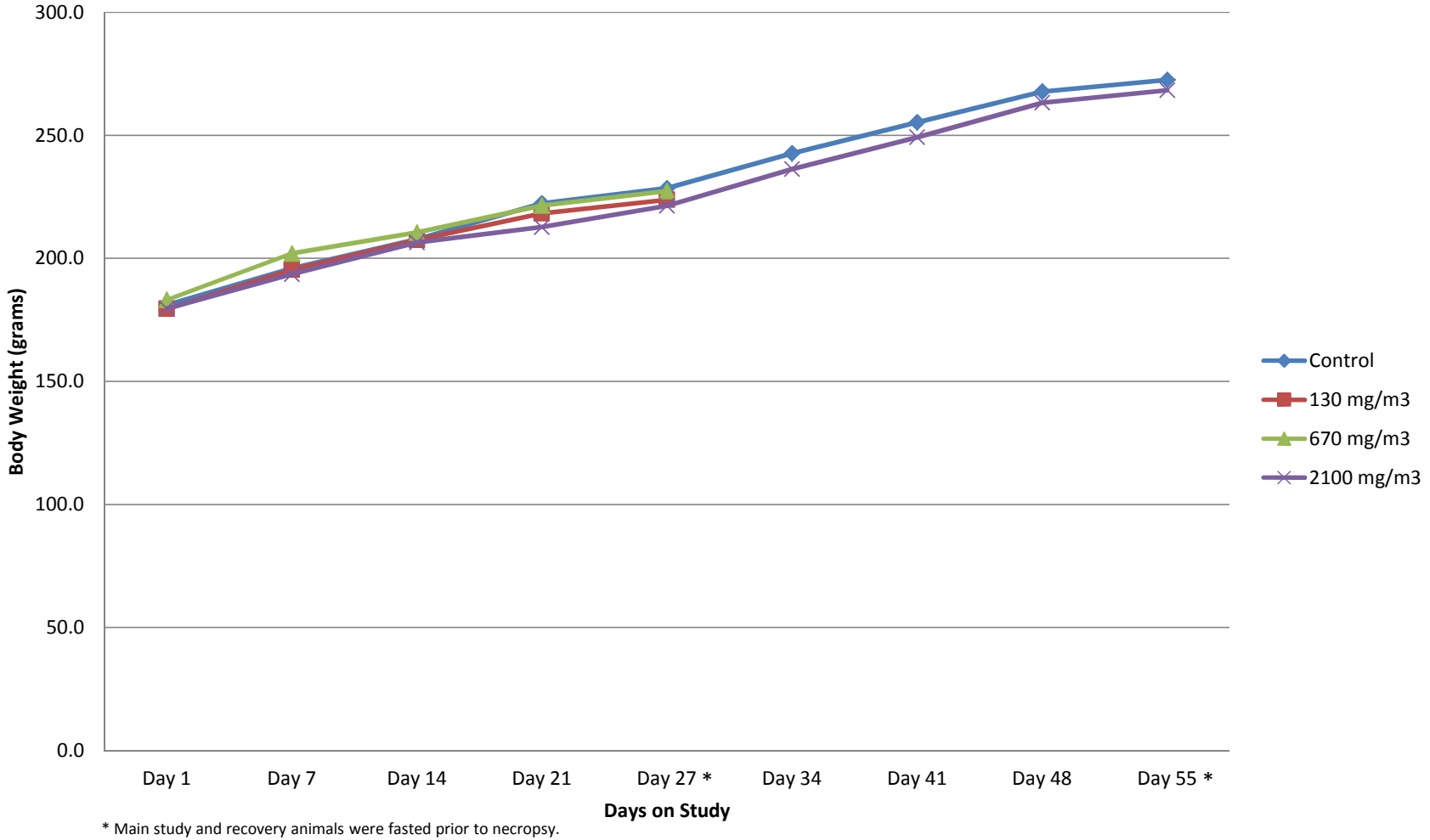
Main Study and Recovery Body Weights - Male Rats



* Main study and recovery animals were fasted prior to necropsy.

Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Main Study and Recovery Body Weights - Female Rats



* Main study and recovery animals were fasted prior to necropsy.

Table G-1
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke (PVA)

Summary of Individual Body Weights (grams)
Male Rats - Following 4-Week Exposure and One Month Recovery Periods

Period		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Day 1	Mean	218.3	221.7	215.5	216.2
	S.D.	6.36	15.27	7.66	9.15
	N	12	6	6	12
Day 7	Mean	273.6	278.8	268.8	266.6
	S.D.	9.35	24.96	13.54	14.11
	N	12	6	6	12
Day 14	Mean	312.3	315.8	304.0	304.5
	S.D.	13.37	32.79	18.79	18.07
	N	12	6	6	12
Day 21	Mean	348.7	345.3	336.2	334.5
	S.D.	19.86	38.90	20.58	20.75
	N	12	6	6	12
Day 27	Mean	370.0	365.5	355.3	355.7
	S.D.	21.77	42.59	25.52	25.66
	N	12	6	6	12
Day 34	Mean	414.7			386.3
	S.D.	15.81			33.63
	N	6	0	0	6
Day 41	Mean	455.0			413.7[a]
	S.D.	13.34			38.51
	N	6	0	0	6
Day 48	Mean	492.2			451.3
	S.D.	19.01			41.08
	N	6	0	0	6
Day 55	Mean	522.7			472.0[b]
	S.D.	21.19			46.04
	N	6	0	0	6

[a] = Significantly reduced compared to controls, p=0.009
[b] = Signficantly reduced compared to controls, p=0.034

Table G-2
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Individual Body Weights (grams)
Female Rats - Following 4-Week Exposure and One Month Recovery Period

Period		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Day 1	Mean	181.0	179.5	183.2	179.6
	S.D.	6.52	12.14	8.66	7.39
	N	12	6	6	12
Day 7	Mean	196.0	195.3	202.0	193.6
	S.D.	7.94	10.98	14.06	9.15
	N	12	6	6	12
Day 14	Mean	207.8	207.5	210.5	206.5
	S.D.	12.85	13.14	14.86	9.28
	N	12	6	6	12
Day 21	Mean	222.4	218.3	221.5	212.7
	S.D.	14.88	16.60	16.83	11.57
	N	12	6	6	12
Day 27	Mean	228.5	223.8	227.3	221.4
	S.D.	16.82	17.63	19.68	10.66
	N	12	6	6	12
Day 34	Mean	242.7			236.3
	S.D.	21.42			8.87
	N	6	0	0	6
Day 41	Mean	255.3			249.2
	S.D.	23.47			12.27
	N	6	0	0	6
Day 48	Mean	267.8			263.2
	S.D.	26.23			15.59
	N	6	0	0	6
Day 55	Mean	272.5			268.3
	S.D.	26.68			15.96
	N	6	0	0	6

Table G-3
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Body Weights (grams)
Male Rats - Following 4-Week Exposure and One Month Recovery Periods

Dose Group	Animal ID	Day 1	Day 7	Day 14	Day 21	Day 27	Day 28[1]	Day 34	Day 41	Day 48	Day 55	Day 56[2]
	11-0014	216	263	302	330	346	323					
Control	11-0016	217	257	282	309	327	308					
	11-0018	230	285	304	332	359	337					
	11-0023	221	282	327	366	384	365					
	11-0026	212	266	315	354	380	350					
	11-0037	222	272	303	332	355	338					
	11-0043	221	283	320	361	387		413	451	485	525	494
	11-0047	217	267	306	341	355		399	438	474	504	474
	11-0050	216	274	314	356	384		413	456	488	514	485
	11-0051	204	269	326	378	405		444	477	527	563	527
	11-0052	222	283	324	359	384		416	461	499	521	499
	11-0053	221	282	324	366	374		403	447	480	509	478
	Mean	**218.3**	**273.6**	**312.3**	**348.7**	**370.0**	**336.8**	**414.7**	**455.0**	**492.2**	**522.7**	**492.8**
	SD	**6.36**	**9.35**	**13.37**	**19.86**	**21.77**	**19.95**	**15.81**	**13.34**	**19.01**	**21.19**	**19.20**
	11-0017	209	248	272	292	309	285					
130 mg/m^3	11-0019	233	300	352	398	423	397					
	11-0034	243	309	348	374	401	375					
	11-0038	203	260	292	324	338	328					
	11-0048	215	263	299	324	345	320					
	11-0049	227	293	332	360	377	354					
	Mean	**221.7**	**278.8**	**315.8**	**345.3**	**365.5**	**343.2**					
	SD	**15.27**	**24.96**	**32.79**	**38.90**	**42.59**	**40.44**					
	11-0013	221	278	309	340	355	327					
670 mg/m^3	11-0021	218	276	313	352	366	342					
	11-0022	205	250	279	314	334	310					
	11-0030	213	258	291	320	339	313					
	11-0033	226	286	333	367	401	367					
	11-0036	210	265	299	324	337	318					
	Mean	**215.5**	**268.8**	**304.0**	**336.2**	**355.3**	**329.5**					
	SD	**7.66**	**13.54**	**18.79**	**20.58**	**25.52**	**21.70**					
	11-0015	219	274	310	346	376	346					
2100 mg/m^3	11-0020	221	267	298	320	338	322					
	11-0024	217	272	322	360	391	358					
	11-0025	218	264	299	322	336	317					
	11-0027	222	274	319	344	371	345					
	11-0029	218	255	279	315	329	313					
	11-0031	198	239	265	291	303		321	342	377	389	371
	11-0032	210	255	299	329	355		400	423	465	483	451
	11-0035	213	271	318	356	378		414	445	490	511	473
	11-0039	232	296	327	359	381		405	446	483	514	495
	11-0040	203	258	308	328	352		383	404	437	456	427
	11-0041	223	274	310	344	358		395	422	456	479	453
	Mean	**216.2**	**266.6**	**304.5**	**334.5**	**355.7**	**333.5**	**386.3**	**413.7**	**451.3**	**472.0**	**445.0**
	SD	**9.15**	**14.11**	**18.07**	**20.75**	**25.66**	**18.51**	**33.63**	**38.51**	**41.08**	**46.04**	**42.86**

1. Final fasted body weights of non-recovery animals.
2. Final fasted body weights of recovery animals.

Table G-4
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

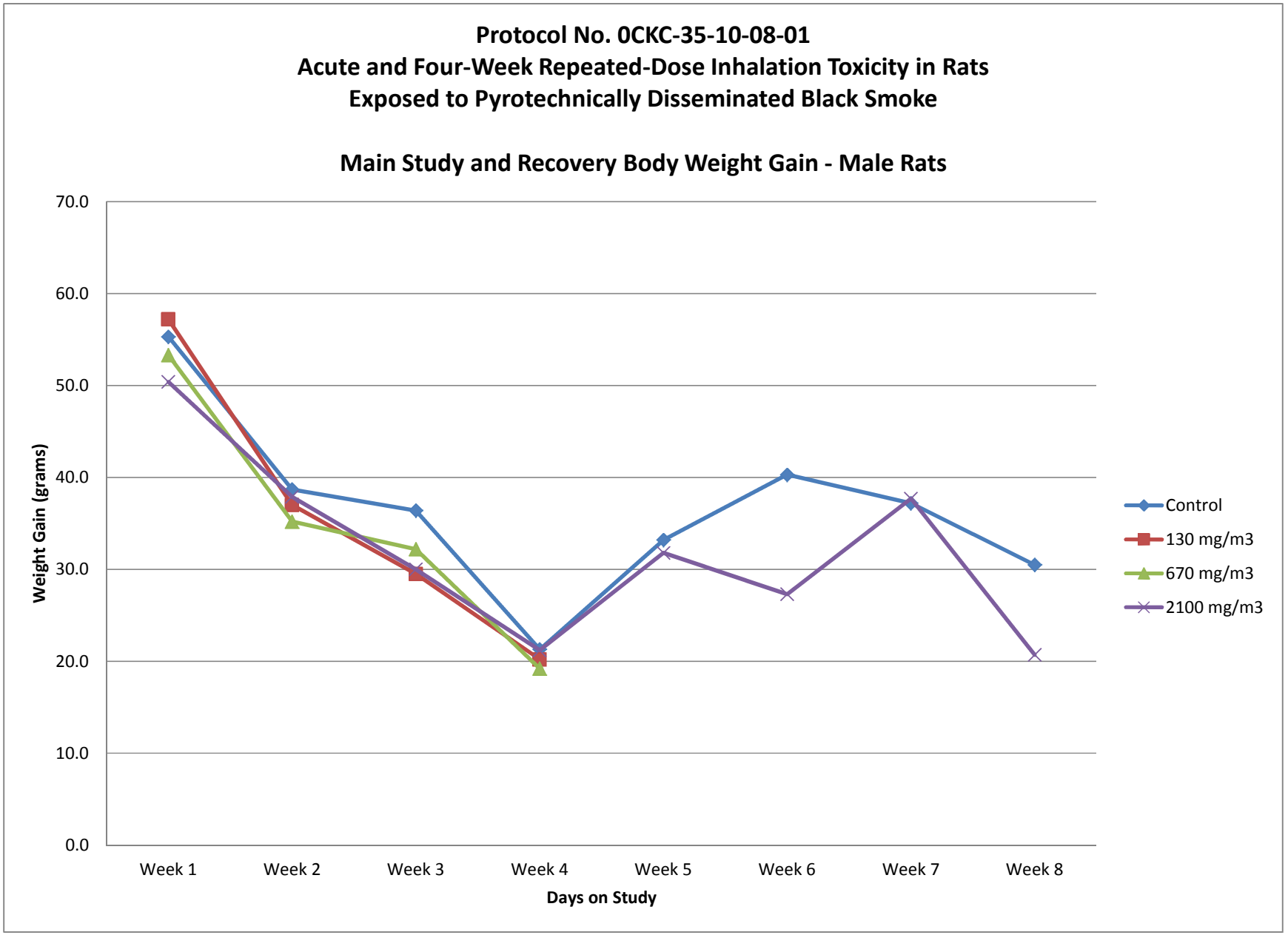
Individual Body Weights (grams)
Female Rats - Following 4-Week Exposure and One Month Recovery Period

Dose Group	Animal ID	Day 1	Day 7	Day 14	Day 21	Day 27	Day 28[1]	Day 34	Day 41	Day 48	Day 55	Day 56[2]
	11-0056	181	190	200	216	213	199					
Control	11-0059	173	189	193	198	204	190					
	11-0062	183	203	203	230	239	231					
	11-0063	184	199	213	229	231	215					
	11-0065	191	200	210	228	233	222					
	11-0075	189	203	238	240	243	230					
	11-0077	168	178	189	194	204		209	224	230	233	221
	11-0078	181	199	206	223	230		245	258	270	273	261
	11-0079	182	202	216	239	256		270	292	305	311	289
	11-0084	183	203	217	235	249		260	269	287	291	276
	11-0085	183	198	209	225	219		233	245	253	261	254
	11-0087	174	188	200	212	221		239	244	262	266	251
	Mean	**181.0**	**196.0**	**207.8**	**222.4**	**228.5**	**214.5**	**242.7**	**255.3**	**267.8**	**272.5**	**258.7**
	SD	**6.52**	**7.94**	**12.85**	**14.88**	**16.82**	**16.79**	**21.42**	**23.47**	**26.23**	**26.68**	**23.35**
	11-0058	172	189	207	222	227	219					
130 mg/m^3	11-0071	195	207	217	225	237	222					
	11-0076	166	187	195	197	202	195					
	11-0086	173	193	209	218	227	207					
	11-0091	177	185	191	204	204	194					
	11-0092	194	211	226	244	246	238					
	Mean	**179.5**	**195.3**	**207.5**	**218.3**	**223.8**	**212.5**					
	SD	**12.14**	**10.98**	**13.14**	**16.60**	**17.63**	**17.10**					
	11-0055	174	195	208	217	223	207					
670 mg/m^3	11-0060	182	193	201	209	207	200					
	11-0069	177	189	191	205	209	196					
	11-0072	184	215	227	240	244	230					
	11-0073	199	224	229	245	257	235					
	11-0094	183	196	207	213	224	205					
	Mean	**183.2**	**202.0**	**210.5**	**221.5**	**227.3**	**212.2**					
	SD	**8.66**	**14.06**	**14.86**	**16.83**	**19.68**	**16.29**					
	11-0054	173	190	200	203	212	201					
2100 mg/m^3	11-0061	183	200	207	214	220	209					
	11-0064	164	172	188	190	202	187					
	11-0066	191	203	221	228	237	219					
	11-0067	176	188	201	212	213	195					
	11-0070	180	198	214	216	229	213					
	11-0074	176	194	198	203	213		224	235	244	248	228
	11-0081	174	186	203	210	219		238	241	258	268	253
	11-0088	184	202	211	224	229		232	245	257	259	247
	11-0089	184	193	208	210	223		238	255	266	271	257
	11-0090	188	205	219	232	238		251	270	291	296	277
	11-0093	182	192	208	210	222		235	249	263	268	256
	Mean	**179.6**	**193.6**	**206.5**	**212.7**	**221.4**	**204.0**	**236.3**	**249.2**	**263.2**	**268.3**	**253.0**
	SD	**7.39**	**9.15**	**9.28**	**11.57**	**10.66**	**11.92**	**8.87**	**12.27**	**15.59**	**15.96**	**15.89**

1. Final fasted body weights of non-recovery animals.
2. Final fasted body weights of recovery animals.

Appendix H

Body Weight Change Data


Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke
Main Study and Recovery Body Weight Gain - Male Rats
Weight Gain (grams)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Days on Study
Control
130 mg/m3
670 mg/m3
2100 mg/m3

Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Main Study and Recovery Body Weight Gain - Female Rats

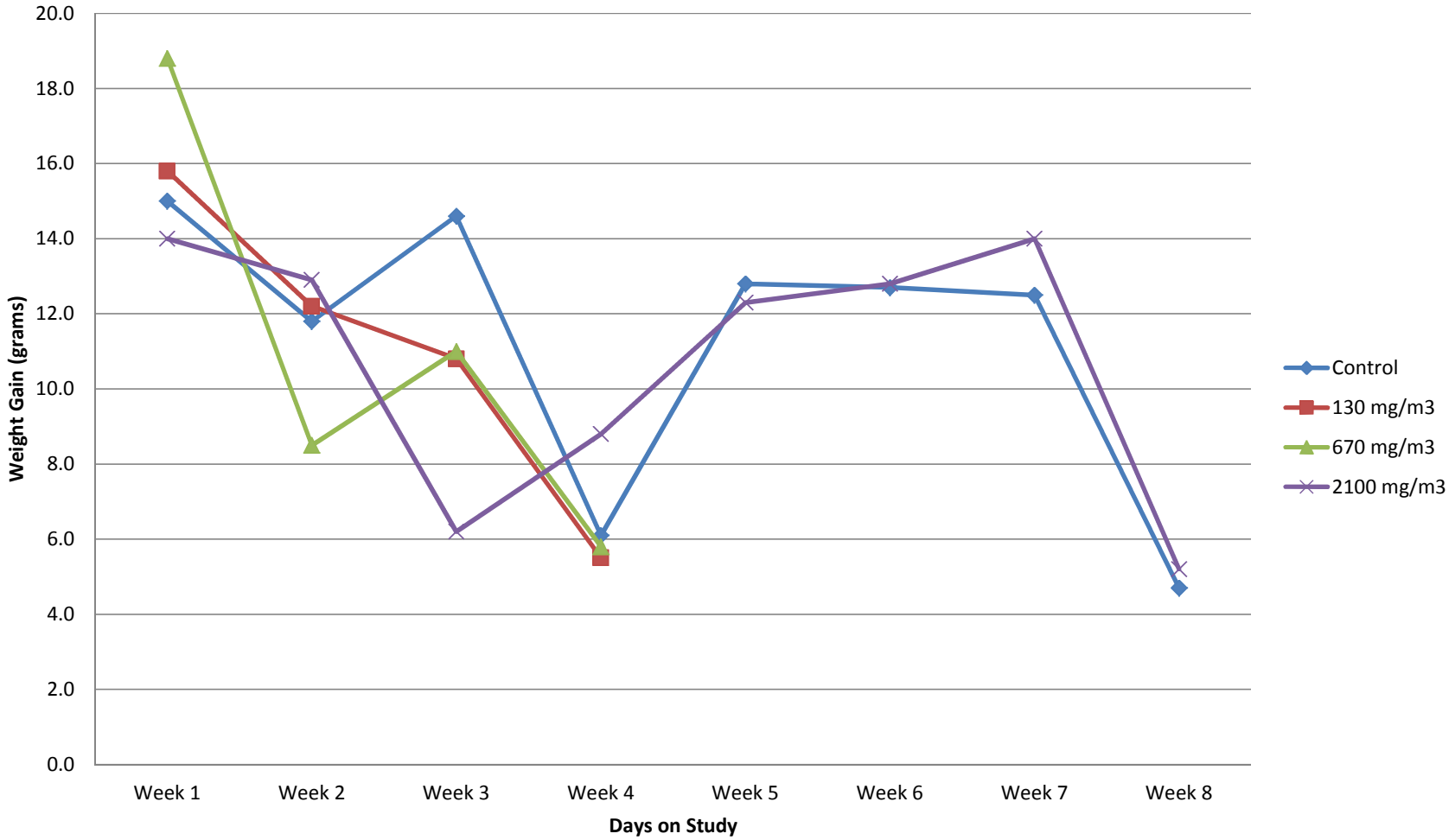


Table H-1
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Individual Body Weight Changes (grams)
Male Rats - Following 4-Week Exposure Period and One Month Recovery Period

Period		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Week 1	Mean	55.3	57.2	53.3	50.4
	S.D.	7.41	11.55	6.65	7.66
	N	12	6	6	12
Week 2	Mean	38.7	37.0	35.2	37.9
	S.D.	10.17	9.25	6.40	9.09
	N	12	6	6	12
Week 3	Mean	36.4	29.5	32.2	30.0
	S.D.	7.56	8.98	4.92	6.56
	N	12	6	6	12
Week 4	Mean	21.3	20.2	19.2	21.2
	S.D.	6.40	5.08	7.78	6.67
	N	12	6	6	12
Week 5	Mean	33.2			31.8
	S.D.	6.91			9.70
	N	6	0	0	6
Week 6	Mean	40.3			27.3[a]
	S.D.	4.55			7.74
	N	6	0	0	6
Week 7	Mean	37.2			37.7
	S.D.	6.65			4.80
	N	6	0	0	6
Week 8	Mean	30.5			20.7[b]
	S.D.	6.57			6.28
	N	6	0	0	6

[a] = Significantly reduced compared to controls (p = 0.005).
[b] = Significantly reduced compared to controls (p = 0.024).

Table H-2
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Individual Body Weight Changes (grams)
Female Rats - Following 4-Week Exposure and One Month Recovery Periods

Period		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Week 1	Mean	15.0	15.8	18.8	14.0
	S.D.	4.09	4.96	8.16	3.86
	N	12	6	6	12
Week 2	Mean	11.8	12.2	8.5	12.9
	S.D.	8.44	4.83	4.32	4.42
	N	12	6	6	12
Week 3	Mean	14.6	10.8	11.0	6.2[a]
	S.D.	7.44	5.71	3.90	4.26
	N	12	6	6	12
Week 4	Mean	6.1	5.5	5.8	8.8
	S.D.	6.54	4.42	5.15	3.67
	N	12	6	6	12
Week 5	Mean	12.8			12.3
	S.D.	4.45			5.32
	N	6	0	0	6
Week 6	Mean	12.7			12.8
	S.D.	5.75			5.60
	N	6	0	0	6
Week 7	Mean	12.5			14.0
	S.D.	4.97			4.38
	N	6	0	0	6
Week 8	Mean	4.7			5.2
	S.D.	1.97			2.64
	N	6	0	0	6

[a] = Significantly reduced compared to controls (p = 0.003).

Table H-3
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Body Weight Changes (grams)
Male Rats - Following 4-Week Exposure Period and One Month Recovery Period

Dose Group	Animal ID	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
	11-0014	47	39	28	16				
Control	11-0016	40	25	27	18				
	11-0018	55	19	28	27				
	11-0023	61	45	39	18				
	11-0026	54	49	39	26				
	11-0037	50	31	29	23				
	11-0043	62	37	41	26	26	38	34	40
	11-0047	50	39	35	14	44	39	36	30
	11-0050	58	40	42	28	29	43	32	26
	11-0051	65	57	52	27	39	33	50	36
	11-0052	61	41	35	25	32	45	38	22
	11-0053	61	42	42	8	29	44	33	29
	Mean	**55.3**	**38.7**	**36.4**	**21.3**	**33.2**	**40.3**	**37.2**	**30.5**
	SD	**7.41**	**10.17**	**7.56**	**6.40**	**6.91**	**4.55**	**6.65**	**6.57**
	11-0017	39	24	20	17				
130 mg/m^3	11-0019	67	52	46	25				
	11-0034	66	39	26	27				
	11-0038	57	32	32	14				
	11-0048	48	36	25	21				
	11-0049	66	39	28	17				
	Mean	**57.2**	**37.0**	**29.5**	**20.2**				
	SD	**11.55**	**9.25**	**8.98**	**5.08**				
	11-0013	57	31	31	15				
670 mg/m^3	11-0021	58	37	39	14				
	11-0022	45	29	35	20				
	11-0030	45	33	29	19				
	11-0033	60	47	34	34				
	11-0036	55	34	25	13				
	Mean	**53.3**	**35.2**	**32.2**	**19.2**				
	SD	**6.65**	**6.40**	**4.92**	**7.78**				
	11-0015	55	36	36	30				
2100 mg/m^3	11-0020	46	31	22	18				
	11-0024	55	50	38	31				
	11-0025	46	35	23	14				
	11-0027	52	45	25	27				
	11-0029	37	24	36	14				
	11-0031	41	26	26	12	18	21	35	12
	11-0032	45	44	30	26	45	23	42	18
	11-0035	58	47	38	22	36	31	45	21
	11-0039	64	31	32	22	24	41	37	31
	11-0040	55	50	20	24	31	21	33	19
	11-0041	51	36	34	14	37	27	34	23
	Mean	**50.4**	**37.9**	**30.0**	**21.2**	**31.8**	**27.3**	**37.7**	**20.7**
	SD	**7.66**	**9.09**	**6.56**	**6.67**	**9.70**	**7.74**	**4.80**	**6.28**

Table H-4
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Body Weight Gains (grams)
Female Rats - Following 4-Week Exposure and One Month Recovery Periods

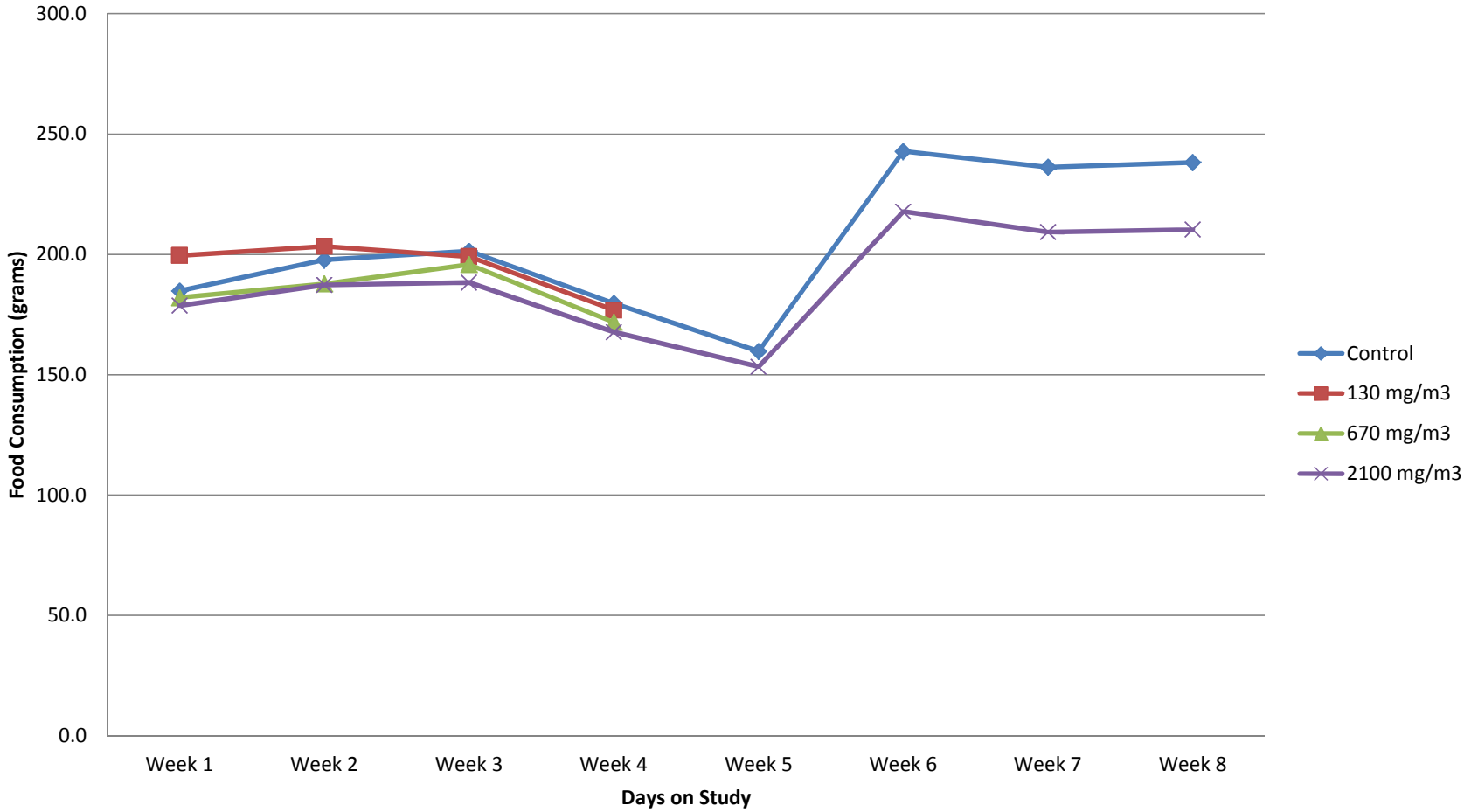
Dose Group	Animal ID	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
	11-0056	9	10	16	-3				
Control	11-0059	16	4	5	6				
	11-0062	20	0	27	9				
	11-0063	15	14	16	2				
	11-0065	9	10	18	5				
	11-0075	14	35	2	3				
	11-0077	10	11	5	10	5	15	6	3
	11-0078	18	7	17	7	15	13	12	3
	11-0079	20	14	23	17	14	22	13	6
	11-0084	20	14	18	14	11	9	18	4
	11-0085	15	11	16	-6	14	12	8	8
	11-0087	14	12	12	9	18	5	18	4
	Mean	**15.0**	**11.8**	**14.6**	**6.1**	**12.8**	**12.7**	**12.5**	**4.7**
	SD	**4.09**	**8.44**	**7.44**	**6.54**	**4.45**	**5.75**	**4.97**	**1.97**
	11-0058	17	18	15	5				
130 mg/m^3	11-0071	12	10	8	12				
	11-0076	21	8	2	5				
	11-0086	20	16	9	9				
	11-0091	8	6	13	0				
	11-0092	17	15	18	2				
	Mean	**15.8**	**12.2**	**10.8**	**5.5**				
	SD	**4.96**	**4.83**	**5.71**	**4.42**				
	11-0055	21	13	9	6				
670 mg/m^3	11-0060	11	8	8	-2				
	11-0069	12	2	14	4				
	11-0072	31	12	13	4				
	11-0073	25	5	16	12				
	11-0094	13	11	6	11				
	Mean	**18.8**	**8.5**	**11.0**	**5.8**				
	SD	**8.16**	**4.32**	**3.90**	**5.15**				
	11-0054	17	10	3	9				
2100 mg/m^3	11-0061	17	7	7	6				
	11-0064	8	16	2	12				
	11-0066	12	18	7	9				
	11-0067	12	13	11	1				
	11-0070	18	16	2	13				
	11-0074	18	4	5	10	11	11	9	4
	11-0081	12	17	7	9	19	3	17	10
	11-0088	18	9	13	5	3	13	12	2
	11-0089	9	15	2	13	15	17	11	5
	11-0090	17	14	13	6	13	19	21	5
	11-0093	10	16	2	12	13	14	14	5
	Mean	**14.0**	**12.9**	**6.2**	**8.8**	**12.3**	**12.8**	**14.0**	**5.2**
	SD	**3.86**	**4.42**	**4.26**	**3.67**	**5.32**	**5.60**	**4.38**	**2.64**

Appendix I

Food Consumption Data

Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Main Study and Recovery Food Consumption - Male Rats



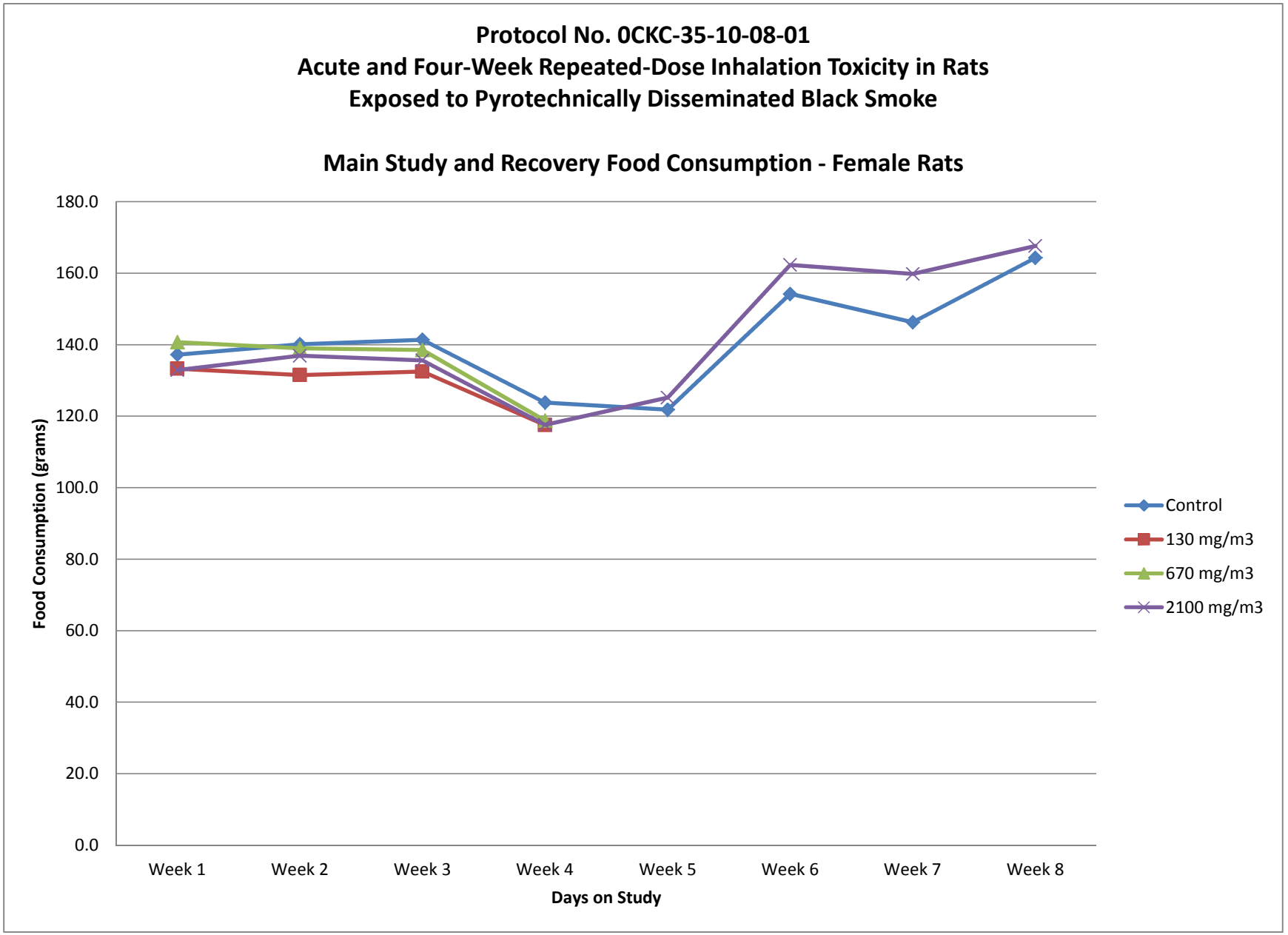

Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke
Main Study and Recovery Food Consumption - Female Rats
Food Consumption (grams)
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Days on Study
Control
130 mg/m3
670 mg/m3
2100 mg/m3

Table I-1
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Individual Food Consumption (grams)
Male Rats - Following 4-Week Exposure and One Month Recovery Periods

Period		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Week 1	Mean	184.8	199.5	182.0	178.7
	S.D.	14.00	23.36	12.70	14.27
	N	12	6	6	12
Week 2	Mean	197.7	203.3	187.8	187.3
	S.D.	14.63	23.21	12.53	11.05
	N	11	6	6	12
Week 3	Mean	201.4	199.0	195.8	188.3
	S.D.	16.89	25.63	10.11	13.51
	N	12	6	6	12
Week 4	Mean	179.6	176.8	171.8	167.7
	S.D.	12.72	22.21	10.25	10.76
	N	12	6	6	12
Week 5	Mean	159.7			153.3
	S.D.	11.79			9.69
	N	6	0	0	6
Week 6	Mean	242.8			217.8[a]
	S.D.	10.30			20.33
	N	6	0	0	6
Week 7	Mean	236.2			209.3[b]
	S.D.	13.38			21.09
	N	6	0	0	6
Week 8	Mean	238.2			210.3[c]
	S.D.	15.60			19.49
	N	6	0	0	6

[a] = Significantly reduced compared to controls (p = 0.023).
[b] = Significantly reduced compared to controls (p = 0.025).
[c] = Significantly reduced compared to controls (p = 0.021).

Table I-2
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Individual Food Consumption (grams)
Female Rats - Following 4-Week Exposure and One Month Recovery Periods

Period		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Week 1	Mean	137.2	133.3	140.7	132.9
	S.D.	11.82	8.29	12.77	10.87
	N	12	6	6	12
Week 2	Mean	140.1	131.5	139.0	136.9
	S.D.	14.66	9.50	8.99	10.61
	N	12	6	6	12
Week 3	Mean	141.4	132.5	138.5	135.6
	S.D.	14.44	12.18	11.69	13.96
	N	12	6	6	12
Week 4	Mean	123.8	117.5	118.8	117.6
	S.D.	10.93	10.50	6.46	7.29
	N	12	6	6	12
Week 5	Mean	121.8			125.2
	S.D.	12.12			5.08
	N	6	0	0	6
Week 6	Mean	154.2			162.3
	S.D.	16.01			9.03
	N	6	0	0	6
Week 7	Mean	146.3			159.8
	S.D.	16.12			20.27
	N	6	0	0	6
Week 8	Mean	164.3			167.6
	S.D.	19.39			13.22
	N	6	0	0	5

Table I-3
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Food Consumption (grams)
Male Rats - Following 4-Week Exposure and One Month Recovery Periods

Dose Group	Animal ID	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
	11-0014	179	ND	195	179				
Control	11-0016	157	166	178	152				
	11-0018	199	203	183	170				
	11-0023	181	191	193	177				
	11-0026	173	188	190	172				
	11-0037	194	198	196	177				
	11-0043	212	226	237	202	167	251	248	255
	11-0047	189	195	203	180	160	236	233	235
	11-0050	183	199	213	185	151	234	217	219
	11-0051	174	206	222	197	179	259	252	259
	11-0052	187	196	195	180	154	243	241	232
	11-0053	190	207	212	184	147	234	226	229
	Mean	**184.8**	**197.7**	**201.4**	**179.6**	**159.7**	**242.8**	**236.2**	**238.2**
	SD	**14.00**	**14.63**	**16.89**	**12.72**	**11.79**	**10.30**	**13.38**	**15.60**
	11-0017	160	167	162	144				
130 mg/m^3	11-0019	224	226	234	203				
	11-0034	221	227	219	191				
	11-0038	192	199	199	176				
	11-0048	195	189	182	158				
	11-0049	205	212	198	189				
	Mean	**199.5**	**203.3**	**199.0**	**176.8**				
	SD	**23.36**	**23.21**	**25.63**	**22.21**				
	11-0013	195	196	190	174				
670 mg/m^3	11-0021	178	182	201	170				
	11-0022	166	174	192	165				
	11-0030	170	175	184	160				
	11-0033	196	204	213	190				
	11-0036	187	196	195	172				
	Mean	**182.0**	**187.8**	**195.8**	**171.8**				
	SD	**12.70**	**12.53**	**10.11**	**10.25**				
	11-0015	184	193	194	175				
2100 mg/m^3	11-0020	175	172	175	156				
	11-0024	172	188	201	177				
	11-0025	165	175	170	148				
	11-0027	167	191	193	164				
	11-0029	183	189	180	171				
	11-0031	162	175	176	159	138	196	184	188
	11-0032	163	175	184	170	160	228	222	216
	11-0035	190	199	204	179	158	234	228	218
	11-0039	208	208	211	185	164	237	227	236
	11-0040	179	193	174	159	146	189	182	186
	11-0041	196	190	197	169	154	223	213	218
	Mean	**178.7**	**187.3**	**188.3**	**167.7**	**153.3**	**217.8**	**209.3**	**210.3**
	SD	**14.27**	**11.05**	**13.51**	**10.76**	**9.69**	**20.33**	**21.09**	**19.49**

Table I-4
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Food Consumption (grams)
Female Rats - Following 4-Week Exposure and One Month Recovery Periods

Dose Group	Animal ID	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
	11-0056	125	133	132	114				
Control	11-0059	126	123	120	110				
	11-0062	152	153	158	141				
	11-0063	142	141	148	122				
	11-0065	130	138	141	126				
	11-0075	137	164	152	127				
	11-0077	124	118	119	107	107	139	125	133
	11-0078	140	141	146	123	122	145	140	165
	11-0079	152	162	163	143	142	184	173	183
	11-0084	134	131	139	124	113	149	152	172
	11-0085	158	149	152	130	127	159	149	182
	11-0087	126	128	127	119	120	149	139	151
	Mean	**137.2**	**140.1**	**141.4**	**123.8**	**121.8**	**154.2**	**146.3**	**164.3**
	SD	**11.82**	**14.66**	**14.44**	**10.93**	**12.12**	**16.01**	**16.12**	**19.39**
	11-0058	130	138	140	126				
130 mg/m^3	11-0071	143	136	134	124				
	11-0076	130	127	116	110				
	11-0086	121	118	121	104				
	11-0091	134	126	135	111				
	11-0092	142	144	149	130				
	Mean	**133.3**	**131.5**	**132.5**	**117.5**				
	SD	**8.29**	**9.50**	**12.18**	**10.50**				
	11-0055	126	130	126	113				
670 mg/m^3	11-0060	126	129	126	110				
	11-0069	137	134	134	120				
	11-0072	154	148	151	128				
	11-0073	151	148	152	122				
	11-0094	150	145	142	120				
	Mean	**140.7**	**139.0**	**138.5**	**118.8**				
	SD	**12.77**	**8.99**	**11.69**	**6.46**				
	11-0054	125	133	125	114				
2100 mg/m^3	11-0061	130	120	116	109				
	11-0064	114	123	112	107				
	11-0066	151	156	154	129				
	11-0067	131	137	134	113				
	11-0070	128	134	127	112				
	11-0074	124	125	132	114	116	148	135	149
	11-0081	126	142	138	125	128	155	148	161
	11-0088	149	147	150	124	128	170	195	ND
	11-0089	139	140	138	117	123	165	163	168
	11-0090	136	140	150	120	130	171	164	181
	11-0093	142	146	151	127	126	165	154	179
	Mean	**132.9**	**136.9**	**135.6**	**117.6**	**125.2**	**162.3**	**159.8**	**167.6**
	SD	**10.87**	**10.61**	**13.96**	**7.29**	**5.08**	**9.03**	**20.27**	**13.22**

Appendix J

Clinical Observations

Table J-1
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Daily Clinical Observations - Pre- and Post-Exposure
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
Control	11-0014	No abnormalities observed			1	28
		Terminal Sacrifice 11/18/10				
	11-0016	No abnormalities observed			1	28
		Terminal Sacrifice 11/18/10				
	11-0018	Red color discharge	Nose		2	9
		Terminal Sacrifice 11/18/10				
	11-0023	Red color discharge	Nose		6	6
		Terminal Sacrifice 11/18/10				
	11-0026	Red color discharge	Nose		5	5
		Terminal Sacrifice 11/18/10				
	11-0037	Red color discharge	Nose		2	7
		Terminal Sacrifice 11/18/10				
	11-0043	Red color discharge	Nose		2	9
		Hair loss (barbering)	Both forelimbs		41	50
		Hair loss (barbering)	Right forelimb		51	57
		Terminal Sacrifice 12/16/10				
	11-0047	Red color discharge	Nose		5	14
		Terminal Sacrifice 12/16/10				
	11-0050	Red color discharge	Nose		6	6
		Terminal Sacrifice 12/16/10				
	11-0051	Red color discharge	Nose		13	14
		Terminal Sacrifice 12/16/10				
	11-0052	Red color discharge	Nose		6	7
		Terminal Sacrifice 12/16/10				
	11-0053	Red color discharge	Nose		2	19
		Terminal Sacrifice 12/16/10				

Note - Signs may be observed intermittently between the first and last day observed

Table J-2
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Daily Clinical Observations - Pre- and Post-Exposure
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
130 mg/m^3	11-0017	Black staining	Nose		1	28
		Salivation			1	27
		Red color discharge	Nose		5	5
		Terminal Sacrifice 11/18/10				
	11-0019	Red color discharge	Nose		5	5
		Black staining	Nose		1	28
		Salivation			8	16
		Orange-stained bedding			8	20
		Terminal Sacrifice 11/18/10				
	11-0034	Black staining	Nose		1	28
		Salivation			1	19
		Orange-stained bedding			8	20
		Red color discharge	Nose		5	5
		Terminal Sacrifice 11/18/10				
	11-0038	Black staining	Nose		1	28
		Salivation			6	28
		Orange-stained bedding			8	20
		Hair Loss	Neck		2	27
		Sore	Neck		16	27
		Terminal Sacrifice 11/18/10				
	11-0048	Black staining	Nose		1	28
		Salivation			1	21
		Orange-stained bedding			8	20
		Terminal Sacrifice 11/18/10				
	11-0049	Black staining	Nose		1	28
		Salivation			1	16
		Orange-stained bedding			19	20
		Red color discharge	Nose		5	5
		Terminal Sacrifice 11/18/10				

Note - Signs may be observed intermittently between the first and last day observed

Table J-3
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Daily Clinical Observations - Pre- and Post-Exposure
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
670 mg/m^3	11-0013	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			1	28
		Orange-stained bedding			8	26
		Red-stained fur	Face/Head	Slight	12	27
		Terminal Sacrifice 11/18/10				
	11-0021	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			1	28
		Orange-stained bedding			8	26
		Red-stained fur	Face/Head	Slight	14	27
		Terminal Sacrifice 11/18/10				
	11-0022	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			1	28
		Orange-stained bedding			8	26
		Red-stained fur	Face/Head	Slight	9	27
		Terminal Sacrifice 11/18/10				
	11-0030	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			1	28
		Orange-stained bedding			8	26
		Red-stained fur	Face/Head	Slight	9	23
		Terminal Sacrifice 11/18/10				
	11-0033	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			1	28
		Orange-stained bedding			8	26
		Red-stained fur	Face/Head	Slight	7	27
		Terminal Sacrifice 11/18/10				
	11-0036	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			1	28
		Orange-stained bedding			8	26
		Red-stained fur	Face/Head	Slight	12	27
		Terminal Sacrifice 11/18/10				

Note - Signs may be observed intermittently between the first and last day observed

Table J-4
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Daily Clinical Observations - Pre- and Post-Exposure
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
2100 mg/m³	11-0015	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	27
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	27
		Black/Blue staining	Ear		9	27
		Salivation			1	27
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	28
		Orange-stained bedding			8	26
		Terminal Sacrifice 11/18/10				
	11-0020	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	27
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	27
		Black/Blue staining	Ear		9	27
		Salivation			1	28
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	28
		Orange-stained bedding			8	26
		Terminal Sacrifice 11/18/10				
	11-0024	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	27
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Slight	12	12
		Red staining	Face/Head/Body	Moderate	9	27
		Black/Blue staining	Ear		9	27
		Salivation			1	28
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	28
		Orange-stained bedding			8	26
		Terminal Sacrifice 11/18/10				
	11-0025	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	27
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	27
		Red staining	Face/Head/Body	Slight	12	12
		Black/Blue staining	Ear		9	27
		Salivation			1	28
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	28
		Orange-stained bedding			8	26
		Terminal Sacrifice 11/18/10				
	11-0027	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	27
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	27
		Black/Blue staining	Ear		9	27
		Red staining	Face/Head/Body	Slight	12	12
		Salivation			1	28
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	28
		Orange-stained bedding			8	26
		Terminal Sacrifice 11/18/10				
	11-0029	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	27
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	27
		Black/Blue staining	Ear		9	27
		Red staining	Face/Head/Body	Slight	12	12
		Salivation			1	28
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	28
		Orange-stained bedding			8	26
		Terminal Sacrifice 11/18/10				
	11-0031	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	6	6
		Black staining	Tail		6	57
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Slight	12	57
		Red staining	Face/Head/Body	Moderate	9	34
		Black/Blue staining	Ear		9	57
		Red staining	Face/Head/Body	Slight	12	12
		Red color discharge	Nose		19	19
		Salivation			1	27
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	34
		Orange-stained bedding			8	26
		Terminal Sacrifice 12/16/10				
	11-0032	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	48
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	34
		Red staining	Face/Head/Body	Slight	12	57
		Black/Blue staining	Ear		9	44
		Salivation			1	27
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	30
		Orange-stained bedding			8	26
		Terminal Sacrifice 12/16/10				
	11-0035	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	6	6
		Red staining	Face/Head	Slight	7	7
		Black staining	Tail		6	51
		Red staining	Face/Head/Body	Slight	44	57
		Red staining	Face/Head/Body	Moderate	9	43
		Black/Blue staining	Ear		9	34
		Orange staining	Forelimbs		34	51
		Red staining	Face/Head	Moderate	12	12
		Salivation			1	28
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	12	30
		Orange-stained bedding			8	26
		Terminal Sacrifice 12/16/10				
	11-0039	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	51
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	34
		Black/Blue staining	Ear		9	44
		Red staining	Face/Head/Body	Slight	12	57
		Orange staining	Forelimbs		33	56
		Salivation			1	28
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	9	30
		Orange-stained bedding			8	26
		Terminal Sacrifice 12/16/10				
	11-0040	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	57
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	57
		Red staining	Face/Head/Body	Slight	12	12
		Black/Blue staining	Ear		9	57
		Orange staining	Forelimbs		33	57
		Salivation			5	28
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	12	30
		Orange-stained bedding			8	26
		Terminal Sacrifice 12/16/10				
	11-0041	Black staining	Face/Head	Severe	1	1
		Black staining	Face/Head	Slight	2	6
		Black staining	Tail		6	57
		Red staining	Face/Head	Slight	7	7
		Red staining	Face/Head/Body	Moderate	9	57
		Black/Blue staining	Ear		9	57
		Orange staining	Forelimbs		33	57
		Red color discharge	Left eye		41	49
		Swelling	Left eye		44	57
		Salivation			1	27
		Black staining	Face/Head/Body	Severe	2	19
		Black staining	Face/Head/Body	Moderate/Severe	12	30
		Orange-stained bedding			8	26
		Terminal Sacrifice 12/16/10				

Note - Signs may be observed intermittently between the first and last day observed

Table J-5
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Daily Clinical Observations - Pre- and Post-Exposure
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
Control	11-0056	No abnormalities observed Terminal Sacrifice 11/19/10			1	28
	11-0059	Red color discharge Terminal Sacrifice 11/19/10	Nose		1	1
	11-0062	Red color discharge Terminal Sacrifice 11/19/10	Nose		26	26
	11-0063	Red color discharge Terminal Sacrifice 11/19/10	Nose		25	25
	11-0065	No abnormalities observed Terminal Sacrifice 11/19/10			1	28
	11-0075	Red color discharge Terminal Sacrifice 11/19/10	Nose		6	26
	11-0077	No abnormalities observed Terminal Sacrifice 12/17/10			1	57
	11-0078	Red color discharge Terminal Sacrifice 12/17/10	Nose		4	27
	11-0079	No abnormalities observed Terminal Sacrifice 12/17/10			1	57
	11-0084	No abnormalities observed Terminal Sacrifice 12/17/10			1	57
	11-0085	Red color discharge Terminal Sacrifice 12/17/10	Nose		1	22
	11-0087	Red color discharge Terminal Sacrifice 12/17/10	Nose		19	27

Note - Signs may be observed intermittently between the first and last day observed

Table J-6
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Daily Clinical Observations - Pre- and Post-Exposure
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
130 mg/m^3	11-0058	Red color discharge	Nose		13	13
		Black staining	Nose		1	28
		Salivation			11	22
		Orange-stained bedding			7	14
		Terminal Sacrifice 11/19/10				
	11-0071	Black staining	Nose		1	28
		Salivation			5	5
		Orange-stained bedding			7	22
		Terminal Sacrifice 11/19/10				
	11-0076	Black staining	Nose		1	28
		Salivation			11	11
		Orange-stained bedding			13	25
		Terminal Sacrifice 11/19/10				
	11-0086	Black staining	Nose		1	28
		Orange-stained bedding			7	19
		Terminal Sacrifice 11/19/10				
	11-0091	Black staining	Nose		1	28
		Salivation			7	28
		Orange-stained bedding			7	19
		Terminal Sacrifice 11/19/10				
	11-0092	Black staining	Nose		1	28
		Salivation			1	16
		Orange-stained bedding			7	14
		Terminal Sacrifice 11/19/10				

Note - Signs may be observed intermittently between the first and last day observed

Table J-7
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Daily Clinical Observations - Pre- and Post-Exposure
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
670 mg/m^3	11-0055	Red staining	Face/Head	Slight	7	27
		Salivation			6	28
		Black staining	Face/Head	Slight/Moderate	1	28
		Orange-stained bedding			7	25
		Terminal Sacrifice 11/19/10				
	11-0060	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			6	28
		Orange-stained bedding			7	25
		Red staining	Face/Head	Slight	13	27
		Terminal Sacrifice 11/19/10				
	11-0069	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			6	28
		Orange-stained bedding			7	25
		Red staining	Face/Head	Slight	11	27
		Terminal Sacrifice 11/19/10				
	11-0072	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			13	28
		Orange-stained bedding			7	25
		Red staining	Face/Head	Slight	13	27
		Red-colored discharge	Nose		7	7
		Terminal Sacrifice 11/19/10				
	11-0073	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			4	28
		Orange-stained bedding			7	25
		Red staining	Face/Head	Slight	12	26
		Red staining	Face/Head	Slight/Moderate	20	27
		Terminal Sacrifice 11/19/10				
	11-0094	Black staining	Face/Head	Slight/Moderate	1	28
		Salivation			6	28
		Orange-stained bedding			7	25
		Red staining	Face/Head	Slight	14	27
		Red staining	Face/Head	Slight/Moderate	20	20
		Terminal Sacrifice 11/19/10				

Note - Signs may be observed intermittently between the first and last day observed

Table J-8
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Daily Clinical Observations - Pre- and Post-Exposure
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
2100 mg/m^3	11-0054	Black staining	Face/Head	Slight	5	5	11-0074	Black staining	Face/Head	Slight	4	5
		Black staining	Tail		5	27		Black staining	Tail		5	43
		Red staining	Face/Head/Body	Slight	6	11		Red staining	Face/Head/Body	Slight	6	7
		Red staining	Face/Head/Body	Moderate	12	27		Red staining	Face/Head/Body	Moderate	11	57
		Black staining	Face/Head/Body	Severe	1	28		Red staining	Face/Head/Body	Severe	34	48
		Salivation			4	28		Black staining	Face/Head/Body	Severe	1	28
		Black/Blue staining	Ear		7	27		Salivation			4	28
		Black staining	Face/Head/Body	Moderate	12	27		Black/Blue staining	Ear		7	57
		Orange-stained bedding			7	25		Orange staining	Both forelimbs		36	57
		Terminal Sacrifice 11/19/10						Black staining	Face/Head/Body	Moderate	12	29
	11-0061	Black staining	Face/Head	Slight	5	5		Orange-stained bedding			7	25
		Black staining	Tail		5	27		Terminal Sacrifice 12/17/10				
		Red staining	Face/Head/Body	Slight	6	11	11-0081	Black staining	Face/Head	Slight	5	5
		Red staining	Face/Head/Body	Moderate	12	27		Black staining	Tail		5	50
		Black staining	Face/Head/Body	Severe	1	28		Red staining	Face/Head/Body	Slight	6	57
		Salivation			1	28		Red staining	Face/Head/Body	Moderate	12	48
		Black/Blue staining	Ear		7	27		Black staining	Face/Head/Body	Severe	1	28
		Black staining	Face/Head/Body	Moderate	12	27		Salivation			4	28
		Orange-stained bedding			7	25		Black/Blue staining	Ear		7	57
		Terminal Sacrifice 11/19/10						Orange staining	Both forelimbs		36	56
	11-0064	Black staining	Face/Head	Slight	5	5		Black staining	Face/Head/Body	Moderate	12	29
		Corneal opacity*	Left eye		4	28		Orange-stained bedding			7	25
		Black staining	Tail		5	27		Terminal Sacrifice 12/17/10				
		Red staining	Face/Head/Body	Slight	6	11	11-0088	Black staining	Face/Head	Slight	4	5
		Red staining	Face/Head/Body	Moderate	12	27		Black staining	Tail		5	57
		Black staining	Face/Head/Body	Severe	1	28		Red staining	Face/Head/Body	Slight	6	11
		Salivation			4	28		Red staining	Face/Head/Body	Moderate	12	57
		Black/Blue staining	Ear		7	27		Black staining	Face/Head/Body	Severe	1	28
		Black staining	Face/Head/Body	Moderate	12	27		Salivation			4	28
		Orange-stained bedding			7	25		Black/Blue staining	Ear		7	57
		Terminal Sacrifice 11/19/10						Orange staining	Both forelimbs		36	57
	11-0066	Black staining	Face/Head	Slight	5	5		Black staining	Face/Head/Body	Moderate	12	29
		Black staining	Tail		5	27		Orange-stained bedding			7	25
		Red staining	Face/Head/Body	Slight	6	11		Terminal Sacrifice 12/17/10				
		Red staining	Face/Head/Body	Moderate	12	27	11-0089	Black staining	Face/Head	Slight	5	5
		Black staining	Face/Head/Body	Severe	1	28		Black staining	Tail		5	39
		Salivation			4	28		Red staining	Face/Head/Body	Slight	6	57
		Black/Blue staining	Ear		7	27		Red staining	Face/Head/Body	Moderate	12	48
		Black staining	Face/Head/Body	Moderate	12	27		Black staining	Face/Head/Body	Severe	1	28
		Orange-stained bedding			7	25		Salivation			6	28
		Terminal Sacrifice 11/19/10						Black/Blue staining	Ear		7	57
	11-0067	Black staining	Face/Head	Slight	4	5		Orange staining	Both forelimbs		36	57
		Black staining	Tail		5	27		Black staining	Face/Head/Body	Moderate	12	29
		Red staining	Face/Head/Body	Slight	6	11		Orange-stained bedding			7	25
		Red staining	Face/Head/Body	Moderate	12	27		Terminal Sacrifice 12/17/10				
		Black staining	Face/Head/Body	Severe	1	28	11-0090	Black staining	Face/Head	Slight	5	5
		Salivation			1	28		Black staining	Tail		5	55
		Black/Blue staining	Ear		7	27		Red staining	Face/Head/Body	Slight	6	7
		Black staining	Face/Head/Body	Moderate	12	27		Red staining	Face/Head/Body	Moderate	11	57
		Orange-stained bedding			7	25		Black staining	Face/Head/Body	Severe	1	28
		Terminal Sacrifice 11/19/10						Salivation			1	28
	11-0070	Black staining	Face/Head	Slight	5	5		Black/Blue staining	Ear		7	57
		Black staining	Tail		5	27		Orange staining	Both forelimbs		36	54
		Red staining	Face/Head/Body	Slight	6	11		Black staining	Face/Head/Body	Moderate	12	29
		Red staining	Face/Head/Body	Moderate	11	27		Orange-stained bedding			7	25
		Black staining	Face/Head/Body	Severe	1	28		Terminal Sacrifice 12/17/10				
		Salivation			4	28	11-0093	Black staining	Face/Head	Slight	5	5
		Black/Blue staining	Ear		7	27		Black staining	Tail		5	39
		Black staining	Face/Head/Body	Moderate	12	27		Red staining	Face/Head/Body	Slight	6	11
		Orange-stained bedding			7	25		Red staining	Face/Head/Body	Moderate	12	57
		Terminal Sacrifice 11/19/10						Black staining	Face/Head/Body	Severe	1	28
								Salivation			6	28
								Black/Blue staining	Ear		7	39
								Orange staining	Both forelimbs		32	57
								Black staining	Face/Head/Body	Moderate	12	29
								Orange-stained bedding			7	25
								Terminal Sacrifice 12/17/10				

Note - Signs may be observed intermittently between the first and last day observed
* - This rat pushed its head through the front of the restrainer on the first exposure day and had to be manipulated to free her most likely leading to the corneal opacity.

Table J-9
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Detailed Weekly Clinical Observations
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week* Observed
Control	11-0014	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0016	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0018	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0023	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0026	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0037	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0043	Hair loss (barbering)	Both forelimbs		3	5
		Terminal Sacrifice 12/16/10				
	11-0047	Red color discharge	Nose		1	1
		Terminal Sacrifice 12/16/10				
	11-0050	Red color discharge	Nose		4	4
		Terminal Sacrifice 12/16/10				
	11-0051	No abnormalities observed			1	5
		Terminal Sacrifice 12/16/10				
	11-0052	Red color discharge	Nose		4	4
		Terminal Sacrifice 12/16/10				
	11-0053	No abnormalities observed			1	5
		Terminal Sacrifice 12/16/10				

Note - Signs may be observed intermittently between the first and last week observed
* - Detailed weekly observations for satellite/recovery animals were taken separately for weeks 1-5. Following week 5, daily and weekly observations were combined under daily observations.

Table J-10
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Detailed Weekly Clinical Observations
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week* Observed
130 mg/m^3	11-0017	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0019	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0034	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0038	Hair Loss	Neck		1	4
		Sore	Neck		3	4
		Terminal Sacrifice 11/18/10				
	11-0048	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				
	11-0049	No abnormalities observed			1	4
		Terminal Sacrifice 11/18/10				

Note - Signs may be observed intermittently between the first and last week observed

Table J-11
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Detailed Weekly Clinical Observations
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week Observed
670 mg/m^3	11-0013	Black staining	Face/Head/Body	Slight	3	3
		Black/Blue staining	Ear		3	4
		Red staining	Face/Head/Body	Slight	3	3
		Red staining	Face/Head	Slight	1	4
		Terminal Sacrifice 11/18/10				
	11-0021	Red staining	Face/Head	Slight	1	4
		Black/Blue staining	Ear		2	2
		Terminal Sacrifice 11/18/10				
	11-0022	Red staining	Face/Head	Slight	1	4
		Black/Blue staining	Ear		2	2
		Terminal Sacrifice 11/18/10				
	11-0030	Red staining	Face/Head	Slight	1	4
		Terminal Sacrifice 11/18/10				
	11-0033	Red staining	Face/Head	Slight	1	4
		Black/Blue staining	Ear		3	4
		Terminal Sacrifice 11/18/10				
	11-0036	Red staining	Face/Head	Slight	2	4
		Black/Blue staining	Ear		4	4
		Terminal Sacrifice 11/18/10				

Note - Signs may be observed intermittently between the first and last day observed

Table J-12
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Detailed Weekly Clinical Observations
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week* Observed	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week* Observed
2100 mg/m^3	11-0015	Black staining	Tail		1	4	11-0031	Black staining	Tail		1	5
		Red staining	Face/Head/Body	Moderate	1	4		Red staining	Face/Head/Body	Moderate	1	5
		Black/Blue staining	Ear		1	4		Black/Blue staining	Ear		1	5
		Black staining	Face/Head/Body	Moderate	2	4		Black staining	Face/Head/Body	Moderate	2	4
		Terminal Sacrifice 11/18/10						Terminal Sacrifice 12/16/10				
	11-0020	Black staining	Tail		1	4	11-0032	Black staining	Tail		1	5
		Red staining	Face/Head/Body	Moderate	1	4		Red staining	Face/Head/Body	Moderate	1	5
		Black/Blue staining	Ear		1	4		Black/Blue staining	Ear		1	5
		Black staining	Face/Head/Body	Moderate	2	4		Black staining	Face/Head/Body	Moderate	2	4
		Terminal Sacrifice 11/18/10						Terminal Sacrifice 12/16/10				
	11-0024	Black staining	Tail		1	4	11-0035	Black staining	Tail		1	5
		Red staining	Face/Head/Body	Moderate	1	4		Red staining	Face/Head/Body	Moderate	1	5
		Black/Blue staining	Ear		1	4		Black/Blue staining	Ear		1	5
		Black staining	Face/Head/Body	Moderate	2	4		Black staining	Face/Head/Body	Moderate	2	4
		Terminal Sacrifice 11/18/10						Orange staining	Both forelimbs		5	5
	11-0025	Black staining	Tail		1	4		Terminal Sacrifice 12/16/10				
		Red staining	Face/Head/Body	Moderate	1	4	11-0039	Black staining	Tail		1	5
		Black/Blue staining	Ear		1	4		Red staining	Face/Head/Body	Moderate	1	5
		Black staining	Face/Head/Body	Moderate	2	4		Black/Blue staining	Ear		1	5
		Terminal Sacrifice 11/18/10						Black staining	Face/Head/Body	Moderate	2	4
	11-0027	Black staining	Tail		1	4		Orange staining	Both forelimbs		5	5
		Red staining	Face/Head/Body	Moderate	1	4		Terminal Sacrifice 12/16/10				
		Black/Blue staining	Ear		1	4	11-0040	Black staining	Tail		1	5
		Black staining	Face/Head/Body	Moderate	2	4		Red staining	Face/Head/Body	Moderate	1	5
		Terminal Sacrifice 11/18/10						Black/Blue staining	Ear		1	5
	11-0029	Black staining	Tail		1	4		Black staining	Face/Head/Body	Moderate	2	4
		Red staining	Face/Head/Body	Moderate	1	4		Orange staining	Both forelimbs		5	5
		Black/Blue staining	Ear		1	4		Terminal Sacrifice 12/16/10				
		Black staining	Face/Head/Body	Moderate	2	4	11-0041	Black staining	Tail		1	5
		Terminal Sacrifice 11/18/10						Red staining	Face/Head/Body	Moderate	1	5
								Black/Blue staining	Ear		1	5
								Black staining	Face/Head/Body	Moderate	2	4
								Orange staining	Both forelimbs		5	5
								Red-colored discharge	Left eye		5	5
								Terminal Sacrifice 12/16/10				

Note - Signs may be observed intermittently between the first and last day observed
* - Detailed weekly observations for satellite/recovery animals were taken separately for weeks 1-5. Following week 5, daily and weekly observations were combined under daily observations.

Table J-13
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Detailed Weekly Clinical Observations
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week* Observed
Control	11-0056	No abnormalities observed Terminal Sacrifice 11/19/10			1	4
	11-0059	No abnormalities observed Terminal Sacrifice 11/19/10			1	4
	11-0062	Hair loss Terminal Sacrifice 11/19/10	Both forelimbs		4	4
	11-0063	No abnormalities observed Terminal Sacrifice 11/19/10			1	4
	11-0065	No abnormalities observed Terminal Sacrifice 11/19/10			1	4
	11-0075	No abnormalities observed Terminal Sacrifice 11/19/10			1	4
	11-0077	No abnormalities observed Terminal Sacrifice 12/17/10			1	4
	11-0078	No abnormalities observed Terminal Sacrifice 12/17/10			1	4
	11-0079	No abnormalities observed Terminal Sacrifice 12/17/10			1	4
	11-0084	No abnormalities observed Terminal Sacrifice 12/17/10			1	4
	11-0085	No abnormalities observed Terminal Sacrifice 12/17/10			1	4
	11-0087	No abnormalities observed Terminal Sacrifice 12/17/10			1	4

Note - Signs may be observed intermittently between the first and last week observed
* - Detailed weekly observations for satellite/recovery animals were taken separately for weeks 1-4. Following week 4, daily and weekly observations were combined under daily observations.

Table J-14
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Detailed Weekly Clinical Observations
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week* Observed
130 mg/m^3	11-0058	No abnormalities observed			1	4
		Terminal Sacrifice 11/19/10				
	11-0071	No abnormalities observed			1	4
		Terminal Sacrifice 11/19/10				
	11-0076	No abnormalities observed			4	4
		Terminal Sacrifice 11/19/10				
	11-0086	Red staining	Face/Head	Slight	1	1
		Hair loss	Both forelimbs		2	4
		Terminal Sacrifice 11/19/10				
	11-0091	No abnormalities observed			1	4
		Terminal Sacrifice 11/19/10				
	11-0092	Red staining	Face/Head	Slight	1	4
		Terminal Sacrifice 11/19/10				

Note - Signs may be observed intermittently between the first and last week observed

Table J-15
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Detailed Weekly Clinical Observations
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week* Observed
670 mg/m^3	11-0055	Red staining	Face/Head	Slight	1	4
		Terminal Sacrifice 11/19/10				
	11-0060	Red staining	Face/Head	Slight	1	4
		Terminal Sacrifice 11/19/10				
	11-0069	Red staining	Face/Head	Slight	1	4
		Terminal Sacrifice 11/19/10				
	11-0072	Red staining	Face/Head	Slight	1	4
		Red color discharge	Nose		1	1
		Terminal Sacrifice 11/19/10				
	11-0073	Red staining	Face/Head	Slight	1	4
		Red staining	Face/Head	Moderate	4	4
		Terminal Sacrifice 11/19/10				
	11-0094	Red staining	Face/Head	Slight	1	4
		Red color discharge	Nose		1	1
		Black/Blue staining	Ears		4	4
		Terminal Sacrifice 11/19/10				

Note - Signs may be observed intermittently between the first and last week observed

Table J-16
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Detailed Weekly Clinical Observations
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week Observed	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Week Observed	Last Week Observed
2100 mg/m^3	11-0054	Black staining	Tail		1	4	11-0074	Black staining	Tail		1	4
		Red staining	Face/Head/Body	Moderate	1	4		Red staining	Face/Head/Body	Moderate	1	4
		Black/Blue staining	Ear		1	4		Black/Blue staining	Ear		1	4
		Black staining	Face/Head/Body	Moderate	1	4		Black staining	Face/Head/Body	Moderate	1	4
		Terminal Sacrifice 11/19/10						Terminal Sacrifice 12/17/10				
	11-0061	Black staining	Tail		1	4	11-0081	Black staining	Tail		1	4
		Red staining	Face/Head/Body	Moderate	1	4		Red staining	Face/Head/Body	Moderate	1	4
		Black/Blue staining	Ear		1	4		Black/Blue staining	Ear		1	4
		Black staining	Face/Head/Body	Moderate	1	4		Black staining	Face/Head/Body	Moderate	1	4
		Terminal Sacrifice 11/19/10						Terminal Sacrifice 12/17/10				
	11-0064	Black staining	Tail		1	4	11-0088	Black staining	Tail		1	4
		Red staining	Face/Head/Body	Moderate	1	4		Red staining	Face/Head/Body	Moderate	1	4
		Black/Blue staining	Ear		1	4		Black/Blue staining	Ear		1	4
		Black staining	Face/Head/Body	Moderate	1	4		Black staining	Face/Head/Body	Moderate	1	4
		Corneal opacity	Left eye		1	4		Terminal Sacrifice 12/17/10				
		Terminal Sacrifice 11/19/10					11-0089	Black staining	Tail		1	4
	11-0066	Black staining	Tail		1	4		Red staining	Face/Head/Body	Moderate	1	4
		Red staining	Face/Head/Body	Moderate	1	4		Black/Blue staining	Ear		1	4
		Black/Blue staining	Ear		1	4		Black staining	Face/Head/Body	Moderate	1	4
		Black staining	Face/Head/Body	Moderate	1	4		Terminal Sacrifice 12/17/10				
		Terminal Sacrifice 11/19/10					11-0090	Black staining	Tail		1	4
	11-0067	Black staining	Tail		1	4		Red staining	Face/Head/Body	Moderate	1	4
		Red staining	Face/Head/Body	Moderate	1	4		Black/Blue staining	Ear		1	4
		Black/Blue staining	Ear		1	4		Black staining	Face/Head/Body	Moderate	1	4
		Black staining	Face/Head/Body	Moderate	1	4		Terminal Sacrifice 12/17/10				
		Terminal Sacrifice 11/19/10					11-0093	Black staining	Tail		1	4
	11-0070	Black staining	Tail		1	4		Red staining	Face/Head/Body	Moderate	1	4
		Red staining	Face/Head/Body	Moderate	1	4		Black/Blue staining	Ear		1	4
		Black/Blue staining	Ear		1	4		Black staining	Face/Head/Body	Moderate	1	4
		Black staining	Face/Head/Body	Moderate	1	4		Terminal Sacrifice 12/17/10				
		Terminal Sacrifice 11/19/10										

Note - Signs may be observed intermittently between the first and last day observed
* - Detailed weekly observations for satellite/recovery animals were taken separately for weeks 1-4. Following week 4, daily and weekly observations were combined under daily observations.

Appendix K

Clinical Chemistry

Table K-1
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Clinical Chemistry
Male Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
ALB	Mean	3.13	2.95	3.17	3.05
(g/dL)	S.D.	0.197	0.274	0.175	0.226
	N	6	6	6	6
ALK P	Mean	227.3	219.8	241.5	178.7
(U/L)	S.D.	50.07	49.53	52.77	31.14
	N	6	6	6	6
ALT	Mean	59.8	45.7	49.0	53.0
(U/L)	S.D.	16.90	9.05	8.00	7.40
	N	6	6	6	6
BUN	Mean	18.2	17.3	18.8	16.8
(mg/dL)	S.D.	1.83	1.86	1.72	2.79
	N	6	6	6	6
CA	Mean	11.07	10.78	11.05	11.03
(mg/dL)	S.D.	0.516	0.293	0.226	0.301
	N	6	6	6	6
CHOL	Mean	67.5	44.7[a]	40.8[b]	52.7
(mg/dL)	S.D.	12.66	16.73	15.74	12.60
	N	6	6	6	6
CREA	Mean	0.75	0.72	0.72	0.75
(mg/dL)	S.D.	0.105	0.075	0.117	0.105
	N	6	6	6	6
GLOB	Mean	2.87	2.88	2.78	2.83
(g/dL)	S.D.	0.175	0.248	0.075	0.103
	N	6	6	6	6
GLU	Mean	155.3	138.5	144.8	147.8
(mg/dL)	S.D.	39.36	22.58	14.11	18.61
	N	6	6	6	6
LDH	Mean	265.2	287.2	292.8	312.8
(U/L)	S.D.	65.58	106.57	58.93	94.32
	N	6	6	6	6
PHOS	Mean	10.33	11.28	10.83	10.40
(mg/dL)	S.D.	0.920	0.811	0.625	0.707
	N	6	6	6	6
TBIL	Mean	0.10	0.10	0.10	0.10
(mg/dL)	S.D.	0.000	0.000	0.000	0.000
	N	6	6	6	6
TP	Mean	5.98	5.82	5.95	5.92
(g/dL)	S.D.	0.331	0.440	0.105	0.271
	N	6	6	6	6
Na	Mean	152.0	149.7[c]	150.0	151.2
(mmol/L)	S.D.	1.41	1.86	1.10	1.17
	N	6	6	6	6
K	Mean	8.03	9.75	9.78	8.25
(mmol/L)	S.D.	1.216	2.080	0.926	1.326
	N	6	6	6	6
Cl	Mean	101.3	103.5[d]	102.5	102.8
(mmol/L)	S.D.	1.21	1.05	1.52	0.98
	N	6	6	6	6

[a] = Signficantly reduced compared to controls (p = 0.034).
[b] = Significantly reduced compared to controls (p = 0.013).
[c] = Significantly reduced compared to controls (p = 0.026).
[d] = Significantly elevated compared to controls (p = 0.035).

Table K-2
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Clinical Chemistry
Male Rats - Following One Month Recovery Period

		Control	2100 mg/m^3
ALB	Mean	2.83	2.85
(g/dL)	S.D.	0.242	0.281
	N	6	6
ALK P	Mean	182.5	173.3
(U/L)	S.D.	56.68	56.99
	N	6	6
ALT	Mean	49.3	56.3
(U/L)	S.D.	7.76	13.63
	N	6	6
BUN	Mean	20.7	20.3
(mg/dL)	S.D.	2.25	3.67
	N	6	6
CA	Mean	10.87	10.97
(mg/dL)	S.D.	0.234	0.258
	N	6	6
CHOL	Mean	67.3	64.3[a]
(mg/dL)	S.D.	3.39	7.26
	N	6	6
CREA	Mean	0.70	0.67
(mg/dL)	S.D.	0.089	0.082
	N	6	6
GLOB	Mean	3.28	3.22
(g/dL)	S.D.	0.214	0.256
	N	6	6
GLU	Mean	157.7	160.0
(mg/dL)	S.D.	28.57	34.65
	N	6	6
LDH	Mean	299.0	220.0[b]
(U/L)	S.D.	76.04	27.00
	N	6	6
PHOS	Mean	9.75	9.37
(mg/dL)	S.D.	0.750	1.216
	N	6	6
TBIL	Mean	0.10	0.10
(mg/dL)	S.D.	0.000	0.000
	N	6	6
TP	Mean	6.10	6.07[c]
(g/dL)	S.D.	0.126	0.225
	N	6	6
Na	Mean	150.0	150.7
(mmol/L)	S.D.	1.10	1.75
	N	6	6
K	Mean	9.22	8.12
(mmol/L)	S.D.	1.059	1.639
	N	6	6
Cl	Mean	102.0	103.3
(mmol/L)	S.D.	0.89	0.82
	N	6	6

[a] = Signficantly reduced compared to controls (p = 0.039).
[b] = Signficantly reduced compared to controls (p = 0.036).
[c] = Signficantly reduced compared to controls (p = 0.013).

Table K-3
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Clinical Chemistry
Female Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
ALB	Mean	3.10	2.73	3.03	3.18
(g/dL)	S.D.	0.276	0.266	0.207	0.299
	N	6	6	6	6
ALK P	Mean	157.7	175.8	163.7	135.3
(U/L)	S.D.	30.51	29.08	58.41	23.99
	N	6	6	6	6
ALT	Mean	41.3	52.0	48.5	66.0[a]
(U/L)	S.D.	6.19	7.97	8.48	12.71
	N	6	6	6	6
BUN	Mean	16.8	18.8	20.2	18.3
(mg/dL)	S.D.	1.83	2.48	2.93	3.08
	N	6	6	6	6
CA	Mean	10.72	10.53	11.00	10.95
(mg/dL)	S.D.	0.458	0.532	0.110	0.217
	N	6	6	6	6
CHOL	Mean	55.8	45.5	46.7	54.8
(mg/dL)	S.D.	13.72	12.05	10.31	18.17
	N	6	6	6	6
CREA	Mean	0.62	0.58	0.58	0.60
(mg/dL)	S.D.	0.098	0.041	0.075	0.089
	N	6	6	6	6
GLOB	Mean	3.10	3.00	2.98	2.97
(g/dL)	S.D.	0.200	0.167	0.264	0.314
	N	6	6	6	6
GLU	Mean	128.3	116.5	126.3	122.5
(mg/dL)	S.D.	26.16	22.66	7.50	13.43
	N	6	6	6	6
LDH	Mean	247.2	311.2	289.7	1007.3
(U/L)	S.D.	65.86	116.57	65.17	1568.25
	N	6	6	6	6
PHOS	Mean	9.15	9.62	9.25	9.97
(mg/dL)	S.D.	1.120	1.765	0.677	1.363
	N	6	6	6	6
TBIL	Mean	0.12	0.10	0.10	0.12
(mg/dL)	S.D.	0.041	0.000	0.000	0.041
	N	6	6	6	6
TP	Mean	6.22	5.73[b]	6.07	6.13
(g/dL)	S.D.	0.271	0.294	0.082	0.532
	N	6	6	6	6
Na	Mean	150.2	149.7	150.2	150.0
(mmol/L)	S.D.	1.72	2.34	2.32	2.65
	N	6	6	6	5
K	Mean	7.88	8.07	8.23	8.32
(mmol/L)	S.D.	1.245	2.051	1.719	1.052
	N	6	6	6	5
Cl	Mean	102.7	103.3	102.3	102.8
(mmol/L)	S.D.	1.21	2.07	1.75	2.17
	N	6	6	6	5

[a]= Significantly elevated compared to controls ($p < 0.001$).
[b] = Significantly decreased compared to controls ($p = 0.021$).

Table K-4
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Clinical Chemistry
Female Rats - Following One Month Recovery Period

		Control	2100 mg/m^3
ALB	Mean	3.27	3.28
(g/dL)	S.D.	0.266	0.248
	N	6	6
ALK P	Mean	113.8	128.2
(U/L)	S.D.	24.47	43.48
	N	6	6
ALT	Mean	63.8	48.7
(U/L)	S.D.	10.85	13.16
	N	6	6
BUN	Mean	21.5	19.2
(mg/dL)	S.D.	3.62	3.13
	N	6	6
CA	Mean	10.83	11.12
(mg/dL)	S.D.	0.383	0.454
	N	6	6
CHOL	Mean	49.2	61.5
(mg/dL)	S.D.	14.22	9.12
	N	6	6
CREA	Mean	0.65	0.65
(mg/dL)	S.D.	0.084	0.084
	N	6	6
GLOB	Mean	3.13	3.25
(g/dL)	S.D.	0.250	0.302
	N	6	6
GLU	Mean	137.0	158.0
(mg/dL)	S.D.	20.10	22.87
	N	6	6
LDH	Mean	253.2	305.3
(U/L)	S.D.	46.77	162.44
	N	6	6
PHOS	Mean	8.18	8.30
(mg/dL)	S.D.	0.983	0.746
	N	6	6
TBIL	Mean	0.10	0.10
(mg/dL)	S.D.	0.000	0.000
	N	6	6
TP	Mean	6.38	6.55
(g/dL)	S.D.	0.232	0.226
	N	6	6
Na	Mean	148.7	150.7
(mmol/L)	S.D.	1.37	1.97
	N	6	6
K	Mean	8.47	9.18
(mmol/L)	S.D.	1.219	1.339
	N	6	6
Cl	Mean	103.5	104.3
(mmol/L)	S.D.	0.84	1.63
	N	6	6

Table K-5
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Clinical Chemistry
Male Rats - Following 4-Week Exposure Period

Group	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	(SS=short sample; ND=no data) NOTES
Control	11-0014	3.2	303	75	20	11.0	72	0.8	3.0	165	269	10.8	0.1	6.2	154	6.8	100	
	11-0016	2.8	226	80	17	10.7	54	0.8	2.6	94	176	8.9	0.1	5.4	152	9.2	102	
	11-0018	3.4	229	35	21	11.7	63	0.7	2.9	165	330	10.6	0.1	6.3	153	8.0	100	
	11-0023	3.2	232	63	17	11.5	74	0.7	3.0	208	333	10.3	0.1	6.1	151	7.2	103	
	11-0026	3.1	229	59	17	10.3	55	0.9	2.7	128	284	11.6	0.1	5.8	150	9.8	102	
	11-0037	3.1	145	47	17	11.2	87	0.6	3.0	172	199	9.8	0.1	6.1	152	7.2	101	
	Mean	**3.13**	**227.3**	**59.8**	**18.2**	**11.07**	**67.5**	**0.75**	**2.87**	**155.3**	**265.2**	**10.33**	**0.10**	**5.98**	**152.0**	**8.03**	**101.3**	
	S.D.	**0.197**	**50.07**	**16.90**	**1.83**	**0.516**	**12.66**	**0.105**	**0.175**	**39.36**	**65.58**	**0.920**	**0.000**	**0.331**	**1.41**	**1.216**	**1.21**	
130 mg/m^3	11-0017	3.0	232	47	19	10.9	38	0.8	2.7	119	422	10.5	0.1	5.6	150	8.5	104	
	11-0019	3.1	294	60	16	10.8	58	0.8	3.0	174	353	10.3	0.1	6.1	153	6.3	103	
	11-0034	3.1	241	52	20	11.0	63	0.7	3.2	155	361	12.1	0.1	6.3	150	11.5	103	
	11-0038	3.0	221	39	17	10.9	49	0.7	3.1	118	185	12.3	0.1	6.1	148	11.8	105	
	11-0048	3.1	154	38	17	10.9	44	0.7	2.6	141	243	11.2	0.1	5.7	149	9.7	102	
	11-0049	2.4	177	38	15	10.2	16	0.6	2.7	124	159	11.3	0.1	5.1	148	10.7	104	
	Mean	**2.95**	**219.8**	**45.7**	**17.3**	**10.78**	**44.7**	**0.72**	**2.88**	**138.5**	**287.2**	**11.28**	**0.10**	**5.82**	**149.7**	**9.75**	**103.5**	
	S.D.	**0.274**	**49.53**	**9.05**	**1.86**	**0.293**	**16.73**	**0.075**	**0.248**	**22.58**	**106.57**	**0.811**	**0.000**	**0.440**	**1.86**	**2.080**	**1.05**	
670 mg/m^3	11-0013	3.1	228	51	19	11.0	32	0.7	2.8	143	364	11.2	0.1	5.9	150	8.8	101	
	11-0021	2.9	238	43	18	10.7	52	0.7	2.9	140	252	10.2	0.1	5.8	150	8.8	103	
	11-0022	3.4	335	63	19	11.0	21	0.8	2.7	125	218	10.0	0.1	6.1	151	10.3	103	
	11-0030	3.3	226	41	16	11.4	53	0.6	2.7	166	357	11.2	0.1	6.0	150	9.6	101	
	11-0033	3.2	173	51	20	11.1	59	0.9	2.8	155	302	11.6	0.1	6.0	148	11.2	102	
	11-0036	3.1	249	45	21	11.1	28	0.6	2.8	140	264	10.8	0.1	5.9	151	10.0	105	
	Mean	**3.17**	**241.5**	**49.0**	**18.8**	**11.05**	**40.8**	**0.72**	**2.78**	**144.8**	**292.8**	**10.83**	**0.10**	**5.95**	**150.0**	**9.78**	**102.5**	
	S.D.	**0.175**	**52.77**	**8.00**	**1.72**	**0.226**	**15.74**	**0.117**	**0.075**	**14.11**	**58.93**	**0.625**	**0.000**	**0.105**	**1.10**	**0.926**	**1.52**	
2100 mg/m^3	11-0015	3.1	155	40	18	11.4	74	0.9	2.8	146	250	11.3	0.1	6.0	151	9.9	104	
	11-0020	3.0	153	53	16	11.0	41	0.8	2.7	148	210	10.6	0.1	5.8	150	9.5	102	
	11-0024	2.7	144	50	15	10.6	47	0.8	2.9	159	266	11.0	0.1	5.6	150	8.8	103	
	11-0025	3.0	200	61	15	10.8	41	0.7	2.8	114	292	9.4	0.1	5.8	151	7.5	104	
	11-0027	3.1	208	57	22	11.1	55	0.7	2.8	151	422	9.9	0.1	5.9	153	6.9	102	
	11-0029	3.4	212	57	15	11.3	58	0.6	3.0	169	437	10.2	0.1	6.4	152	6.9	102	
	Mean	**3.05**	**178.7**	**53.0**	**16.8**	**11.03**	**52.7**	**0.75**	**2.83**	**147.8**	**312.8**	**10.40**	**0.10**	**5.92**	**151.2**	**8.25**	**102.8**	
	S.D.	**0.226**	**31.14**	**7.40**	**2.79**	**0.301**	**12.60**	**0.105**	**0.103**	**18.61**	**94.32**	**0.707**	**0.000**	**0.271**	**1.17**	**1.326**	**0.98**	

Table K-6
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Clinical Chemistry
Male Rats - Following One-Month Recovery Period

Group	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	(SS=short sample; ND=no data) NOTES
Control	11-0043	2.8	204	59	22	10.9	68	0.8	3.5	162	252	10.3	0.1	6.3	150	10.3	101	
	11-0047	2.8	201	51	19	11.0	67	0.7	3.2	182	230	9.9	0.1	6.0	151	8.1	103	
	11-0050	2.7	116	39	17	10.8	63	0.6	3.3	135	313	8.5	0.1	6.0	150	10.3	103	
	11-0051	3.3	199	56	23	11.2	68	0.6	2.9	184	363	10.4	0.1	6.1	148	9.0	102	
	11-0052	2.8	260	42	22	10.8	73	0.7	3.4	171	410	9.2	0.1	6.2	150	7.9	102	
	11-0053	2.6	115	49	21	10.5	65	0.8	3.4	112	226	10.2	0.1	6.0	151	9.7	101	
	Mean	**2.83**	**182.5**	**49.3**	**20.7**	**10.87**	**67.3**	**0.70**	**3.28**	**157.7**	**299.0**	**9.75**	**0.10**	**6.10**	**150.0**	**9.22**	**102.0**	
	S.D.	**0.242**	**56.68**	**7.76**	**2.25**	**0.234**	**3.39**	**0.089**	**0.214**	**28.57**	**76.04**	**0.750**	**0.000**	**0.126**	**1.10**	**1.059**	**0.89**	
2100 mg/m^3	11-0031	3.4	218	72	26	11.3	72	0.6	2.9	211	190	9.9	0.1	6.2	150	8.4	104	
	11-0032	2.8	114	52	18	11.0	73	0.8	3.0	175	183	10.4	0.1	5.8	149	9.8	103	
	11-0035	2.8	140	61	23	11.2	59	0.6	3.5	156	234	7.6	0.1	6.3	154	5.3	103	
	11-0039	2.6	260	66	17	10.9	55	0.7	3.3	107	245	10.7	0.1	5.9	150	9.6	102	
	11-0040	2.7	128	33	21	10.6	61	0.7	3.1	143	225	8.3	0.1	5.9	151	7.5	104	
	11-0041	2.8	180	54	17	10.8	66	0.6	3.5	168	243	9.3	0.1	6.3	150	8.1	104	
	Mean	**2.85**	**173.3**	**56.3**	**20.3**	**10.97**	**64.3**	**0.67**	**3.22**	**160.0**	**220.0**	**9.37**	**0.10**	**6.07**	**150.7**	**8.12**	**103.3**	
	S.D.	**0.281**	**56.99**	**13.63**	**3.67**	**0.258**	**7.26**	**0.082**	**0.256**	**34.65**	**27.00**	**1.216**	**0.000**	**0.225**	**1.75**	**1.639**	**0.82**	

Table K-7
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Clinical Chemistry
Female Rats - Following 4-Week Exposure Period

Group	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	(SS=short sample; ND=no data) NOTES
Control	11-0056	3.2	140	42	16	11.1	54	0.7	3.0	146	336	9.5	0.1	6.2	150	8.9	102	
	11-0059	3.2	135	30	16	10.9	52	0.7	3.3	173	199	10.4	0.1	6.5	148	8.8	103	
	11-0062	3.0	153	45	18	10.0	72	0.5	2.8	111	289	7.7	0.1	5.8	150	6.9	104	
	11-0063	3.4	218	43	18	11.1	64	0.7	3.0	120	210	10.3	0.1	6.4	149	9.2	104	
	11-0065	3.2	155	48	14	10.9	61	0.5	3.2	104	285	8.9	0.2	6.4	151	7.3	101	
	11-0075	2.6	145	40	19	10.3	32	0.6	3.3	116	164	8.1	0.1	6.0	153	6.2	102	
	Mean	**3.10**	**157.7**	**41.3**	**16.8**	**10.72**	**55.8**	**0.62**	**3.10**	**128.3**	**247.2**	**9.15**	**0.12**	**6.22**	**150.2**	**7.88**	**102.7**	
	S.D.	**0.276**	**30.51**	**6.19**	**1.83**	**0.458**	**13.72**	**0.098**	**0.200**	**26.16**	**65.86**	**1.120**	**0.041**	**0.271**	**1.72**	**1.245**	**1.21**	
130 mg/m^3	11-0058	3.0	194	46	17	10.3	54	0.6	2.7	116	249	6.7	0.1	5.7	150	6.0	101	
	11-0071	2.9	151	51	17	11.5	57	0.6	3.2	155	365	11.9	0.1	6.1	147	10.6	103	
	11-0076	2.8	219	64	16	10.4	46	0.5	3.0	112	381	9.3	0.1	5.8	150	9.0	105	
	11-0086	2.4	188	42	20	9.9	23	0.6	3.0	114	203	9.9	0.1	5.4	147	9.7	104	
	11-0091	2.4	158	58	22	10.5	44	0.6	3.0	84	482	10.8	0.1	5.4	153	5.5	106	
	11-0092	2.9	145	51	21	10.6	49	0.6	3.1	118	187	9.1	0.1	6.0	151	7.6	101	
	Mean	**2.73**	**175.8**	**52.0**	**18.8**	**10.53**	**45.5**	**0.58**	**3.00**	**116.5**	**311.2**	**9.62**	**0.10**	**5.73**	**149.7**	**8.07**	**103.3**	
	S.D.	**0.266**	**29.08**	**7.97**	**2.48**	**0.532**	**12.05**	**0.041**	**0.167**	**22.66**	**116.57**	**1.765**	**0.000**	**0.294**	**2.34**	**2.051**	**2.07**	
670 mg/m^3	11-0055	3.1	248	63	25	11.0	47	0.6	2.8	124	349	8.6	0.1	6.0	150	7.3	101	
	11-0060	3.2	213	43	17	11.2	48	0.7	2.8	113	234	9.7	0.1	6.1	152	8.3	101	
	11-0069	2.8	102	49	20	10.9	54	0.5	3.1	126	374	8.3	0.1	6.0	153	7.5	104	
	11-0072	2.8	176	42	22	11.0	56	0.5	3.4	134	209	9.2	0.1	6.2	151	5.9	101	
	11-0073	3.0	123	53	18	10.9	27	0.6	3.1	132	264	10.0	0.1	6.1	147	9.9	102	
	11-0094	3.3	120	41	19	11.0	48	0.6	2.7	129	308	9.7	0.1	6.0	148	10.5	105	
	Mean	**3.03**	**163.7**	**48.5**	**20.2**	**11.00**	**46.7**	**0.58**	**2.98**	**126.3**	**289.7**	**9.25**	**0.10**	**6.07**	**150.2**	**8.23**	**102.3**	
	S.D.	**0.207**	**58.41**	**8.48**	**2.93**	**0.110**	**10.31**	**0.075**	**0.264**	**7.50**	**65.17**	**0.677**	**0.000**	**0.082**	**2.32**	**1.719**	**1.75**	
2100 mg/m^3	11-0054	2.8	133	83	18	10.8	32	0.5	3.0	118	223	10.7	0.1	5.8	146	9.7	104	
	11-0061	3.1	168	80	17	11.0	55	0.7	2.8	143	4170	12.2	0.1	5.9	NA	NA	NA	serum hemolyzed; 1:2 dilution LDH
	11-0064	3.3	151	61	23	10.8	45	0.6	2.6	130	240	9.0	0.1	5.9	149	9.0	104	
	11-0066	3.7	137	63	16	11.2	86	0.6	3.5	115	858	10.3	0.2	7.2	153	7.8	99	
	11-0067	3.1	97	58	21	11.2	61	0.5	3.1	125	279	8.8	0.1	6.1	151	7.0	103	
	11-0070	3.1	126	51	15	10.7	50	0.7	2.8	104	274	8.8	0.1	5.9	151	8.1	104	
	Mean	**3.18**	**135.3**	**66.0**	**18.3**	**10.95**	**54.8**	**0.60**	**2.97**	**122.5**	**1007.3**	**9.97**	**0.12**	**6.13**	**150.0**	**8.32**	**102.8**	
	S.D.	**0.299**	**23.99**	**12.71**	**3.08**	**0.217**	**18.17**	**0.089**	**0.314**	**13.43**	**1568.25**	**1.363**	**0.041**	**0.532**	**2.65**	**1.052**	**2.17**	

Table K-8
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Clinical Chemistry
Female Rats - Following One-Month Recovery Period

Group	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	(SS=short sample; ND=no data) NOTES
Control	11-0077	3.0	147	82	20	11.1	47	0.7	3.3	151	269	9.3	0.1	6.3	148	9.2	103	
	11-0078	3.6	90	68	27	10.7	55	0.7	2.7	140	302	6.7	0.1	6.3	149	7.0	104	
	11-0079	3.0	131	50	22	10.4	57	0.7	3.2	111	250	8.2	0.1	6.2	147	9.1	103	
	11-0084	3.1	115	60	24	10.4	62	0.7	3.2	134	202	9.0	0.1	6.2	148	9.4	105	
	11-0085	3.4	118	65	19	11.2	52	0.5	3.4	166	195	7.4	0.1	6.8	151	6.8	103	
	11-0087	3.5	82	58	17	11.2	22	0.6	3.0	120	301	8.5	0.1	6.5	149	9.3	103	
	Mean	**3.27**	**113.8**	**63.8**	**21.5**	**10.83**	**49.2**	**0.65**	**3.13**	**137.0**	**253.2**	**8.18**	**0.10**	**6.38**	**148.7**	**8.47**	**103.5**	
	S.D.	**0.266**	**24.47**	**10.85**	**3.62**	**0.383**	**14.22**	**0.084**	**0.250**	**20.10**	**46.77**	**0.983**	**0.000**	**0.232**	**1.37**	**1.219**	**0.84**	
2100 mg/m^3	11-0074	3.3	203	29	17	11.2	56	0.6	3.6	171	281	7.7	0.1	6.9	154	8.6	105	
	11-0081	3.6	103	39	25	11.0	76	0.6	2.9	182	632	8.0	0.1	6.6	149	9.0	104	
	11-0088	3.3	104	62	17	11.1	50	0.7	3.0	147	203	8.4	0.1	6.3	150	10.4	106	
	11-0089	3.5	107	50	19	11.0	62	0.6	3.1	156	225	8.3	0.1	6.6	149	8.0	102	
	11-0090	3.0	93	49	17	11.9	67	0.8	3.6	173	229	9.7	0.1	6.6	150	11.2	106	
	11-0093	3.0	159	63	20	10.5	58	0.6	3.3	119	262	7.7	0.1	6.3	152	7.9	103	
	Mean	**3.28**	**128.2**	**48.7**	**19.2**	**11.12**	**61.5**	**0.65**	**3.25**	**158.0**	**305.3**	**8.30**	**0.10**	**6.55**	**150.7**	**9.18**	**104.3**	
	S.D.	**0.248**	**43.48**	**13.16**	**3.13**	**0.454**	**9.12**	**0.084**	**0.302**	**22.87**	**162.44**	**0.746**	**0.000**	**0.226**	**1.97**	**1.339**	**1.63**	

Appendix L

Hematology

Table L-1
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Hematology
Male Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
WBC	Mean	13.708	11.425	14.267	12.705
(K/uL)	S.D.	5.3997	3.1900	4.6659	4.9109
	N	6	6	6	6
NEU	Mean	9.888	13.435	11.390	13.250
(%N)	S.D.	3.5594	9.1530	2.7126	2.8738
	N	6	6	6	6
LYM	Mean	82.433	77.250	81.550	77.817
(%L)	S.D.	4.8525	10.4907	3.2067	5.2312
	N	6	6	6	6
MONO	Mean	4.945	6.008	4.363	5.247
(%M)	S.D.	1.6872	1.6377	1.0439	1.5094
	N	6	6	6	6
EOS	Mean	0.828	0.884	0.701	1.352
(%E)	S.D.	0.3464	0.2590	0.2413	1.1992
	N	6	6	6	6
BASO	Mean	1.912	2.437	1.997	2.342
(%B)	S.D.	1.1206	0.9911	0.9044	0.6330
	N	6	6	6	6
RBC	Mean	7.953	7.837	8.082	8.198
(M/uL)	S.D.	0.5148	0.1781	0.4410	0.3111
	N	6	6	6	6
HGB	Mean	15.167	14.517	15.517	15.667
(g/dL)	S.D.	0.7174	0.6047	0.2317	0.5854
	N	6	6	6	6
HCT	Mean	43.67	42.93	44.68	45.27
(%)	S.D.	1.862	0.963	1.083	2.117
	N	6	6	6	6
MCV	Mean	54.95	54.82	55.38	55.20
(fL)	S.D.	1.385	0.685	1.775	2.277
	N	6	6	6	6
MCH	Mean	19.08	18.55	19.22	19.10
(pg)	S.D.	0.436	0.558	0.773	0.740
	N	6	6	6	6
MCHC	Mean	34.68	33.87	34.70	84.63
(g/dL)	S.D.	0.299	1.366	0.329	122.705
	N	6	6	6	6
RDW	Mean	16.27	16.13	15.95	16.37
(%)	S.D.	1.150	0.948	0.862	1.140
	N	6	6	6	6
PLT	Mean	1311.17	1202.67	1274.00	1210.67
(K/uL)	S.D.	135.098	197.034	181.382	126.492
	N	6	6	6	6
MPV	Mean	4.577	4.875	4.468	4.585
(fL)	S.D.	0.3132	0.4876	0.2128	0.3339
	N	6	6	6	6

Table L-2
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Hematology
Male Rats - Following One Month Recovery Period

		Control	2100 mg/m^3
WBC	Mean	9.238	10.434
(K/uL)	S.D.	1.9133	2.4979
	N	6	5
NEU	Mean	11.5483	13.3200
(%N)	S.D.	3.29317	2.65556
	N	6	5
LYM	Mean	77.4667	75.0000
(%L)	S.D.	5.71268	4.41645
	N	6	5
MONO	Mean	6.1233	6.9860
(%M)	S.D.	2.11595	2.68860
	N	6	5
EOS	Mean	1.0002	1.2258
(%E)	S.D.	0.48585	0.46261
	N	6	5
BASO	Mean	4.0417	3.4820
(%B)	S.D.	0.90568	0.76503
	N	6	5
RBC	Mean	7.892	7.912
(M/uL)	S.D.	0.2980	0.3577
	N	6	5
HGB	Mean	14.117	14.360
(g/dL)	S.D.	0.3764	0.5857
	N	6	5
HCT	Mean	41.37	41.68
(%)	S.D.	1.309	1.813
	N	6	5
MCV	Mean	52.47	52.70
(fL)	S.D.	1.408	1.617
	N	6	5
MCH	Mean	17.90	18.14
(pg)	S.D.	0.522	0.537
	N	6	5
MCHC	Mean	34.12	34.48
(g/dL)	S.D.	0.232	0.311
	N	6	5
RDW	Mean	17.23	17.66[a]
(%)	S.D.	1.633	0.862
	N	6	5
PLT	Mean	1062.00	1026.40
(K/uL)	S.D.	82.428	158.757
	N	6	5
MPV	Mean	4.392	4.320
(fL)	S.D.	0.1542	0.1956
	N	6	5

[a] = Significantly elevated compared to controls (p = 0.019).

Table L-3
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Hematology
Female Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
WBC	Mean	11.000	9.198	10.990	9.258
(K/uL)	S.D.	5.3268	2.9014	3.5484	3.4972
	N	6	5	6	6
NEU	Mean	5.7567	11.2060	12.9800[a]	15.2333[b]
(%N)	S.D.	2.43128	5.91381	4.30642	2.02452
	N	6	5	6	6
LYM	Mean	86.5333	81.5600	77.7167	77.9167
(%L)	S.D.	5.77639	6.91759	7.60642	3.61132
	N	6	5	6	6
MONO	Mean	4.7817	4.7280	6.0567	4.6200
(%M)	S.D.	2.69299	1.66371	2.31521	1.35921
	N	6	5	6	6
EOS	Mean	1.0292	0.8082	0.9830	0.7550
(%E)	S.D.	0.45101	0.27135	0.63017	0.21200
	N	6	5	6	6
BASO	Mean	1.9200	1.7026	2.2750	1.4688
(%B)	S.D.	0.54725	0.68299	0.83061	0.65124
	N	6	5	6	6
RBC	Mean	7.952	7.734	8.177	7.902
(M/uL)	S.D.	0.4876	0.1394	0.2537	0.2515
	N	6	5	6	6
HGB	Mean	14.950	14.740	15.633	15.283
(g/dL)	S.D.	0.7396	0.2702	0.5354	0.3764
	N	6	5	6	6
HCT	Mean	42.53	42.20	44.93[c]	43.02
(%)	S.D.	2.286	1.093	1.739	0.794
	N	6	5	6	6
MCV	Mean	53.52	54.56	54.97	54.47
(fL)	S.D.	0.588	1.736	1.198	1.902
	N	6	5	6	6
MCH	Mean	18.80	19.08	19.12	19.32
(pg)	S.D.	0.352	0.507	0.397	0.717
	N	6	5	6	6
MCHC	Mean	35.13	34.96	34.78	35.47
(g/dL)	S.D.	0.625	0.336	0.436	0.524
	N	6	5	6	6
RDW	Mean	15.10	14.82	15.00	15.08
(%)	S.D.	1.185	0.814	0.583	0.508
	N	6	5	6	6
PLT	Mean	1254.83	1155.80	1210.83	1289.50
(K/uL)	S.D.	51.511	43.597	106.101	176.103
	N	6	5	6	6
MPV	Mean	4.453	4.426	4.488	4.557
(fL)	S.D.	0.2679	0.1426	0.2308	0.2057
	N	6	5	6	6

[a] = Significantly elevated compared to controls (p = 0.012).
[b] = Significantly elevated compared to controls (p = 0.001).
[c] = Significantly elevated compared to controls (p = 0.047).

Table L-4
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Hematology
Female Rats - Following 4-Week Recovery Period

		Control	2100 mg/m^3
WBC	Mean	12.177	10.257
(K/uL)	S.D.	4.0849	5.5752
	N	6	6
NEU	Mean	8.2667	10.3617
(%N)	S.D.	3.09082	3.19589
	N	6	6
LYM	Mean	81.4333	79.5333
(%L)	S.D.	4.28377	5.89293
	N	6	6
MONO	Mean	4.6688	5.4167
(%M)	S.D.	2.38615	2.21322
	N	6	6
EOS	Mean	1.4708	0.8747[a]
(%E)	S.D.	0.88023	0.28816
	N	6	6
BASO	Mean	3.4167	3.8433
(%B)	S.D.	0.90760	2.86515
	N	6	6
RBC	Mean	8.025	7.837
(M/uL)	S.D.	0.4687	0.5680
	N	6	6
HGB	Mean	14.683	14.733
(g/dL)	S.D.	0.6047	0.8430
	N	6	6
HCT	Mean	42.22	42.48
(%)	S.D.	1.932	2.166
	N	6	6
MCV	Mean	52.62	54.30
(fL)	S.D.	1.486	1.915
	N	6	6
MCH	Mean	18.32	18.82
(pg)	S.D.	0.564	0.631
	N	6	6
MCHC	Mean	34.82	34.65
(g/dL)	S.D.	0.319	0.362
	N	6	6
RDW	Mean	17.53	16.83
(%)	S.D.	0.931	1.294
	N	6	6
PLT	Mean	1039.00	1065.33
(K/uL)	S.D.	102.694	124.095
	N	6	6
MPV	Mean	4.548	4.742
(fL)	S.D.	0.7251	0.5146
	N	6	6

[a] = Significantly reduced compared to controls (p = 0.028).

Table L-5
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Hematology
Male Rats - Following 4-Week Exposure Period

Group	Animal ID	WBC (K/uL)	NEU (%N)	LYM (%L)	MONO (%M)	EOS (%E)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	(ND = NO DATA) NOTES
Control	11-0014	17.20	7.850	86.400	4.230	0.773	0.722	8.13	15.40	44.1	54.2	18.9	34.8	16.4	1223.0	4.79	
	11-0016	3.75	8.880	87.700	2.470	0.493	0.462	7.13	14.00	40.5	56.8	19.7	34.6	15.0	1440.0	4.68	
	11-0018	17.50	13.200	77.200	6.550	1.290	1.800	8.04	15.60	44.4	55.2	19.4	35.1	17.7	1465.0	4.94	
	11-0023	18.00	6.730	83.000	7.140	0.671	2.460	8.70	16.10	46.2	53.1	18.5	34.8	17.3	1377.0	4.66	
	11-0026	12.50	7.270	84.400	4.730	0.523	3.100	7.99	15.00	43.3	54.2	18.8	34.6	16.3	1231.0	4.24	
	11-0037	13.30	15.400	75.900	4.550	1.220	2.930	7.73	14.90	43.5	56.2	19.2	34.2	14.9	1131.0	4.15	
	Mean	**13.708**	**9.888**	**82.433**	**4.945**	**0.828**	**1.912**	**7.953**	**15.167**	**43.67**	**54.95**	**19.08**	**34.68**	**16.27**	**1311.17**	**4.577**	
	S.D.	**5.3997**	**3.5594**	**4.8525**	**1.6872**	**0.3464**	**1.1206**	**0.5148**	**0.7174**	**1.862**	**1.385**	**0.436**	**0.299**	**1.150**	**135.098**	**0.3132**	
130 mg/m^3	11-0017	11.50	17.100	73.600	6.280	0.995	2.030	7.82	13.70	43.8	56.0	17.5	31.2	14.8	960.0	4.64	
	11-0019	15.20	9.790	80.600	5.160	0.532	3.970	8.00	14.90	43.3	54.2	18.7	34.5	15.9	1385.0	5.27	
	11-0034	10.50	5.170	86.600	4.770	0.976	2.450	7.86	15.00	43.2	54.9	19.1	34.9	15.3	1324.0	5.47	
	11-0038	15.00	30.000	57.700	8.150	1.040	3.060	7.75	14.30	42.0	54.2	18.5	34.1	16.9	1234.0	5.10	
	11-0048	9.14	12.000	81.800	4.050	1.170	1.080	8.04	15.20	43.8	54.5	18.9	34.7	16.8	1359.0	4.60	
	11-0049	7.21	6.550	83.200	7.640	0.593	2.030	7.55	14.00	41.5	55.1	18.6	33.8	17.1	954.0	4.17	
	Mean	**11.425**	**13.435**	**77.250**	**6.008**	**0.884**	**2.437**	**7.837**	**14.517**	**42.93**	**54.82**	**18.55**	**33.87**	**16.13**	**1202.67**	**4.875**	
	S.D.	**3.1900**	**9.1530**	**10.4907**	**1.6377**	**0.2590**	**0.9911**	**0.1781**	**0.6047**	**0.963**	**0.685**	**0.558**	**1.366**	**0.948**	**197.034**	**0.4876**	
670 mg/m^3	11-0013	23.00	13.300	82.800	2.820	0.428	0.723	8.63	15.80	45.8	53.1	18.3	34.5	17.2	1345.0	4.43	
	11-0021	10.50	14.100	76.000	6.000	1.010	2.910	7.90	15.40	44.3	56.1	19.5	34.7	15.4	1328.0	4.62	
	11-0022	10.80	14.100	79.400	4.100	0.506	1.830	8.63	15.80	46.1	53.4	18.3	34.4	16.8	1048.0	4.17	
	11-0030	12.70	8.900	83.500	4.330	0.813	2.410	7.96	15.30	44.2	55.5	19.2	34.5	15.8	1246.0	4.27	
	11-0033	12.90	8.450	83.300	4.850	0.537	2.900	7.62	15.30	43.2	56.8	20.0	35.3	15.0	1558.0	4.67	
	11-0036	15.70	9.490	84.300	4.080	0.910	1.210	7.75	15.50	44.5	57.4	20.0	34.8	15.5	1119.0	4.65	
	Mean	**14.267**	**11.390**	**81.550**	**4.363**	**0.701**	**1.997**	**8.082**	**15.517**	**44.68**	**55.38**	**19.22**	**34.70**	**15.95**	**1274.00**	**4.468**	
	S.D.	**4.6659**	**2.7126**	**3.2067**	**1.0439**	**0.2413**	**0.9044**	**0.4410**	**0.2317**	**1.083**	**1.775**	**0.773**	**0.329**	**0.862**	**181.382**	**0.2128**	
2100 mg/m^3	11-0015	7.95	18.100	71.300	7.190	1.160	2.200	8.37	15.70	46.8	55.9	18.7	33.5	16.8	1149.0	4.95	
	11-0020	12.70	12.700	77.000	5.760	1.530	3.010	7.99	15.50	43.7	54.7	19.4	35.5	16.8	1154.0	4.88	
	11-0024	17.20	10.800	83.300	3.230	0.473	2.220	8.17	14.90	43.3	52.9	18.3	34.5	17.9	1447.0	4.03	
	11-0025	11.10	13.000	80.100	4.050	0.445	2.400	8.52	15.70	44.7	52.4	18.4	335.1	15.4	1135.0	4.49	
	11-0027	7.58	14.700	72.200	6.510	3.650	2.950	7.70	15.50	44.3	57.5	20.1	35.0	14.7	1262.0	4.68	
	11-0029	19.70	10.200	83.000	4.740	0.856	1.270	8.44	16.70	48.8	57.8	19.7	34.2	16.6	1117.0	4.48	
	Mean	**12.705**	**13.250**	**77.817**	**5.247**	**1.352**	**2.342**	**8.198**	**15.667**	**45.27**	**55.20**	**19.10**	**84.63**	**16.37**	**1210.67**	**4.585**	
	S.D.	**4.9109**	**2.8738**	**5.2312**	**1.5094**	**1.1992**	**0.6330**	**0.3111**	**0.5854**	**2.117**	**2.277**	**0.740**	**122.705**	**1.140**	**126.492**	**0.3339**	

Table L-6
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Hematology
Male Rats - Following One Month Recovery Period

Group	Animal ID	WBC (K/uL)	NEU (%N)	LYM (%L)	MONO (%M)	EOS (%E)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	(ND = NO DATA) NOTES
Control	11-0043	11.30	13.200	75.900	5.500	0.882	4.510	7.76	14.50	42.6	54.9	18.7	34.0	16.0	1039.0	4.62	
	11-0047	10.30	9.120	81.300	5.080	0.959	3.520	8.47	14.60	43.3	51.1	17.3	33.8	18.8	1109.0	4.24	
	11-0050	7.05	7.140	82.900	6.180	0.841	3.000	7.68	13.60	39.8	51.8	17.7	34.1	15.9	1153.0	4.22	
	11-0051	10.60	14.500	69.700	9.790	1.960	5.080	7.80	14.00	40.8	52.4	17.9	34.2	15.6	1124.0	4.35	
	11-0052	9.44	15.400	72.200	6.730	0.758	4.920	7.94	13.90	40.8	51.4	17.5	34.1	19.4	1015.0	4.50	
	11-0053	6.74	9.930	82.800	3.460	0.601	3.220	7.70	14.10	40.9	53.2	18.3	34.5	17.7	932.0	4.42	
	Mean	**9.238**	**11.5483**	**77.4667**	**6.1233**	**1.0002**	**4.0417**	**7.892**	**14.117**	**41.37**	**52.47**	**17.90**	**34.12**	**17.23**	**1062.00**	**4.392**	
	S.D.	**1.9133**	**3.29317**	**5.71268**	**2.11595**	**0.48585**	**0.90568**	**0.2980**	**0.3764**	**1.309**	**1.408**	**0.522**	**0.232**	**1.633**	**82.428**	**0.1542**	
2100 mg/m^3	11-0031	12.00	10.500	76.900	8.220	1.220	3.130	8.50	15.30	44.7	52.6	18.0	34.3	18.6	1061.0	4.34	
	11-0032	12.50	12.500	78.000	4.980	0.894	3.610	7.98	13.80	40.0	50.1	17.3	34.5	17.5	1097.0	4.29	
	11-0035	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	possible clot
	11-0039	6.94	12.700	79.500	4.660	0.805	2.350	7.80	14.50	41.6	53.3	18.6	35.0	17.8	893.0	4.59	
	11-0040	8.63	13.200	69.600	11.100	1.980	4.160	7.62	14.20	41.5	54.5	18.6	34.2	16.3	845.0	4.04	
	11-0041	12.10	17.700	71.000	5.970	1.230	4.160	7.66	14.00	40.6	53.0	18.2	34.4	18.1	1236.0	4.34	
	Mean	**10.434**	**13.3200**	**75.0000**	**6.9860**	**1.2258**	**3.4820**	**7.912**	**14.360**	**41.68**	**52.70**	**18.14**	**34.48**	**17.66**	**1026.40**	**4.320**	
	S.D.	**2.4979**	**2.65556**	**4.41645**	**2.68860**	**0.46261**	**0.76503**	**0.3577**	**0.5857**	**1.813**	**1.617**	**0.537**	**0.311**	**0.862**	**158.757**	**0.1956**	

Table L-7
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Hematology
Female Rats - Following 4-Week Exposure Period

Group	Animal ID	WBC (K/uL)	NEU (%N)	LYM (%L)	MONO (%M)	EOS (%E)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	(ND = NO DATA) NOTES
Control	11-0056	16.60	3.720	90.200	3.500	0.570	2.040	8.16	15.30	43.3	53.0	18.7	35.3	14.8	1284.0	4.51	
	11-0059	8.85	5.990	86.800	4.480	0.759	2.000	8.07	15.10	43.1	53.4	18.7	35.0	17.0	1316.0	4.54	
	11-0062	8.86	9.880	75.400	10.200	1.680	2.870	7.17	13.90	38.7	54.0	19.5	36.0	14.6	1172.0	4.51	
	11-0063	9.32	6.670	86.600	3.870	1.180	1.660	8.31	15.40	44.7	53.8	18.5	34.4	15.4	1277.0	3.92	
	11-0065	18.20	5.210	88.700	3.500	1.370	1.240	8.44	15.80	44.4	52.7	18.7	35.6	15.4	1261.0	4.67	
	11-0075	4.17	3.070	91.500	3.140	0.616	1.710	7.56	14.20	41.0	54.2	18.7	34.5	13.4	1219.0	4.57	
	Mean	**11.000**	**5.7567**	**86.5333**	**4.7817**	**1.0292**	**1.9200**	**7.952**	**14.950**	**42.53**	**53.52**	**18.80**	**35.13**	**15.10**	**1254.83**	**4.453**	
	S.D.	**5.3268**	**2.43128**	**5.77639**	**2.69299**	**0.45101**	**0.54725**	**0.4876**	**0.7396**	**2.286**	**0.588**	**0.352**	**0.625**	**1.185**	**51.511**	**0.2679**	
130 mg/m^3	11-0058	8.17	20.200	73.300	4.010	0.527	1.950	7.93	14.70	42.0	52.9	18.5	35.0	15.3	1143.0	4.24	
	11-0071	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	sample clotted
	11-0076	13.00	11.200	78.200	6.990	1.080	2.520	7.61	14.90	42.3	55.6	19.6	35.1	14.2	1164.0	4.37	
	11-0086	9.96	12.100	81.100	4.340	1.080	1.410	7.60	14.40	41.2	54.2	19.0	35.0	15.0	1227.0	4.63	
	11-0091	9.81	8.430	83.200	5.670	0.806	1.930	7.72	15.10	44.0	57.0	19.6	34.4	13.8	1116.0	4.47	
	11-0092	5.05	4.100	92.000	2.630	0.548	0.703	7.81	14.60	41.5	53.1	18.7	35.3	15.8	1129.0	4.42	
	Mean	**9.198**	**11.2060**	**81.5600**	**4.7280**	**0.8082**	**1.7026**	**7.734**	**14.740**	**42.20**	**54.56**	**19.08**	**34.96**	**14.82**	**1155.80**	**4.426**	
	S.D.	**2.9014**	**5.91381**	**6.91759**	**1.66371**	**0.27135**	**0.68299**	**0.1394**	**0.2702**	**1.093**	**1.736**	**0.507**	**0.336**	**0.814**	**43.597**	**0.1426**	
670 mg/m^3	11-0055	11.90	15.400	77.200	4.510	0.520	2.420	7.77	15.30	43.6	56.1	19.6	35.0	15.5	1320.0	4.24	
	11-0060	14.80	8.860	83.800	5.030	0.498	1.820	8.40	16.20	47.5	56.6	19.3	34.1	15.2	1215.0	4.44	
	11-0069	9.08	9.460	83.400	4.940	0.433	1.760	8.13	15.10	43.8	53.8	18.5	34.4	14.8	1079.0	4.51	
	11-0072	13.70	9.760	83.000	4.660	0.917	1.690	8.07	15.30	43.8	54.3	19.0	34.9	14.0	1117.0	4.24	
	11-0073	11.50	14.800	74.700	6.700	1.710	2.080	8.21	15.50	44.1	53.8	18.9	35.2	15.6	1344.0	4.79	
	11-0094	4.96	19.600	64.200	10.500	1.820	3.880	8.48	16.40	46.8	55.2	19.4	35.1	14.9	1190.0	4.71	
	Mean	**10.990**	**12.9800**	**77.7167**	**6.0567**	**0.9830**	**2.2750**	**8.177**	**15.633**	**44.93**	**54.97**	**19.12**	**34.78**	**15.00**	**1210.83**	**4.488**	
	S.D.	**3.5484**	**4.30642**	**7.60642**	**2.31521**	**0.63017**	**0.83061**	**0.2537**	**0.5354**	**1.739**	**1.198**	**0.397**	**0.436**	**0.583**	**106.101**	**0.2308**	
2100 mg/m^3	11-0054	10.80	16.000	74.200	6.300	0.927	2.550	8.08	15.40	43.1	53.3	19.0	35.7	14.9	1047.0	4.32	
	11-0061	14.00	13.100	80.500	4.150	0.712	1.510	7.70	15.60	43.0	55.9	20.2	36.2	14.6	1347.0	4.80	
	11-0064	11.10	14.400	78.400	4.770	0.952	1.440	7.85	15.10	43.4	55.2	19.2	34.7	15.8	1158.0	4.33	
	11-0066	7.45	16.800	77.800	4.250	0.477	0.653	7.67	15.50	43.8	57.1	20.2	35.4	14.6	1508.0	4.51	
	11-0067	8.30	18.000	73.600	5.790	0.926	1.680	8.32	15.50	43.3	52.1	18.6	35.7	15.6	1231.0	4.75	
	11-0070	3.90	13.100	83.000	2.460	0.536	0.980	7.79	14.60	41.5	53.2	18.7	35.1	15.0	1446.0	4.63	
	Mean	**9.258**	**15.2333**	**77.9167**	**4.6200**	**0.7550**	**1.4688**	**7.902**	**15.283**	**43.02**	**54.47**	**19.32**	**35.47**	**15.08**	**1289.50**	**4.557**	
	S.D.	**3.4972**	**2.02452**	**3.61132**	**1.35921**	**0.21200**	**0.65124**	**0.2515**	**0.3764**	**0.794**	**1.902**	**0.717**	**0.524**	**0.508**	**176.103**	**0.2057**	

Table L-8
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Hematology
Female Rats - Following 4-Week Recovery Period

Group	Animal ID	WBC (K/uL)	NEU (%N)	LYM (%L)	MONO (%M)	EOS (%E)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	(ND = NO DATA) NOTES
Control	11-0077	19.00	6.390	86.800	3.470	0.561	2.760	7.83	14.10	40.7	51.9	18.0	34.8	18.2	1179.0	5.19	
	11-0078	7.17	5.150	82.700	6.850	1.360	3.960	7.63	14.40	41.5	54.3	18.9	34.7	17.1	899.0	3.72	
	11-0079	14.20	12.500	75.500	0.503	2.510	4.550	7.67	14.00	39.7	51.7	18.3	35.4	17.1	1036.0	5.59	
	11-0084	9.89	7.720	81.000	6.700	0.404	4.130	8.84	15.50	44.8	50.7	17.5	34.6	17.1	1070.0	4.01	
	11-0085	10.60	6.140	84.900	4.990	1.630	2.330	8.32	15.10	43.8	52.7	18.2	34.5	19.1	947.0	4.62	
	11-0087	12.20	11.700	77.700	5.500	2.360	2.770	7.86	15.00	42.8	54.4	19.0	34.9	16.6	1103.0	4.16	
	Mean	**12.177**	**8.2667**	**81.4333**	**4.6688**	**1.4708**	**3.4167**	**8.025**	**14.683**	**42.22**	**52.62**	**18.32**	**34.82**	**17.53**	**1039.00**	**4.548**	
	S.D.	**4.0849**	**3.09082**	**4.28377**	**2.38615**	**0.88023**	**0.90760**	**0.4687**	**0.6047**	**1.932**	**1.486**	**0.564**	**0.319**	**0.931**	**102.694**	**0.7251**	
2100 mg/m^3	11-0074	5.97	13.800	76.800	4.330	1.380	3.720	8.22	15.70	44.9	54.6	19.1	35.0	17.4	1129.0	5.09	
	11-0081	21.10	6.090	89.500	2.470	0.493	1.490	7.35	14.10	40.3	54.9	19.2	35.0	18.3	835.0	5.52	
	11-0088	9.61	7.550	81.200	7.120	0.930	3.240	8.40	14.80	42.8	50.9	17.6	34.5	15.7	1064.0	4.86	
	11-0089	9.10	13.200	75.900	6.980	0.800	3.150	7.26	14.00	41.0	56.5	19.3	34.2	15.4	1045.0	4.18	
	11-0090	9.67	9.230	72.600	7.920	0.816	9.440	8.43	15.80	45.2	53.6	18.7	34.9	18.2	1189.0	4.27	
	11-0093	6.09	12.300	81.200	3.680	0.829	2.020	7.36	14.00	40.7	55.3	19.0	34.3	16.0	1130.0	4.53	
	Mean	**10.257**	**10.3617**	**79.5333**	**5.4167**	**0.8747**	**3.8433**	**7.837**	**14.733**	**42.48**	**54.30**	**18.82**	**34.65**	**16.83**	**1065.33**	**4.742**	
	S.D.	**5.5752**	**3.19589**	**5.89293**	**2.21322**	**0.28816**	**2.86515**	**0.5680**	**0.8430**	**2.166**	**1.915**	**0.631**	**0.362**	**1.294**	**124.095**	**0.5146**	

Appendix M

Prothrombin Time Data

Table M-1
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Average Prothrombin Time
Male and Female Rats - Following 4-Week Exposure Period

Male Rats

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Average Prothrombin Time	Mean	9.22	10.58	9.62	9.83
	S.D.	0.316	1.521	0.560	0.696
	N	6	6	6	6

Female Rats

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Average Prothrombin Time	Mean	9.14	9.45	9.23	9.40
	S.D.	0.334	0.472	0.289	0.348
	N	6	6	6	6

Table M-2
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Average Prothrombin Time
Male and Female Rats - Following One Month Recovery Period

Male Rats

		Control	2100 mg/m^3
Average Prothrombin Time	Mean	9.72	10.18
	S.D.	0.299	0.426
	N	6	6

Female Rats

		Control	2100 mg/m^3
Average Prothrombin Time	Mean	9.33	9.12
	S.D.	0.322	0.306
	N	6	6

Table M-3
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Prothrombin Times
Male Rats - Following 4-Week Exposure Period

Group	Animal ID	Average Prothrombin Time	(ND = NO DATA) NOTES
Control	11-0014	9.3	
	11-0016	9.3	
	11-0018	9.0	
	11-0023	9.0	
	11-0026	9.1	
	11-0037	9.8	
	Mean	**9.22**	
	S.D.	**0.316**	
130 mg/m^3	11-0017	13.6	
	11-0019	9.6	
	11-0034	9.7	
	11-0038	10.1	
	11-0048	10.1	
	11-0049	10.5	
	Mean	**10.58**	
	S.D.	**1.521**	
670 mg/m^3	11-0013	10.4	
	11-0021	9.1	
	11-0022	8.9	
	11-0030	10.1	
	11-0033	9.7	
	11-0036	9.7	
	Mean	**9.62**	
	S.D.	**0.560**	
2100 mg/m^3	11-0015	8.9	
	11-0020	9.8	
	11-0024	9.3	
	11-0025	10.6	
	11-0027	10.0	
	11-0029	10.6	
	Mean	**9.83**	
	S.D.	**0.696**	

Table M-4
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Prothrombin Times
Male Rats - Following One Month Recovery Period

Group	Animal ID	Average Prothrombin Time	(ND = NO DATA) NOTES
Control	11-0043	9.8	
	11-0047	10.3	
	11-0050	9.5	
	11-0051	9.5	
	11-0052	9.8	
	11-0053	9.6	
	Mean	**9.72**	
	S.D.	**0.299**	
2100 mg/m^3	11-0031	10.9	
	11-0032	10.2	
	11-0035	9.6	
	11-0039	10.4	
	11-0040	10.1	
	11-0041	10.1	
	Mean	**10.18**	
	S.D.	**0.426**	

Table M-5
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Prothrombin Times
Female Rats - Following 4-Week Exposure Period

Group	Animal ID	Average Prothrombin Time	(ND = NO DATA) NOTES
Control	11-0056	9.3	
	11-0059	8.8	
	11-0062	9.7	
	11-0063	8.8	
	11-0065	9.2	
	11-0075	9.2	
	Mean	**9.14**	
	S.D.	**0.334**	
130 mg/m^3	11-0058	9.1	
	11-0071	9.5	
	11-0076	9.1	
	11-0086	10.4	
	11-0091	9.5	
	11-0092	9.3	
	Mean	**9.45**	
	S.D.	**0.472**	
670 mg/m^3	11-0055	9.6	
	11-0060	9.4	
	11-0069	8.8	
	11-0072	9.3	
	11-0073	9.1	
	11-0094	9.3	
	Mean	**9.23**	
	S.D.	**0.289**	
2100 mg/m^3	11-0054	9.9	
	11-0061	9.5	
	11-0064	9.4	
	11-0066	9.3	
	11-0067	8.8	
	11-0070	9.6	
	Mean	**9.40**	
	S.D.	**0.348**	

Table M-6
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Individual Prothrombin Times
Female Rats - Following 4-Week Recovery Period

Group	Animal ID	Average Prothrombin Time	(ND = NO DATA) NOTES
Control	11-0077	9.4	
	11-0078	9.4	
	11-0079	9.9	
	11-0084	9.3	
	11-0085	8.9	
	11-0087	9.3	
	Mean	**9.33**	
	S.D.	**0.322**	
2100 mg/m^3	11-0074	9.3	
	11-0081	8.8	
	11-0088	8.8	
	11-0089	9.2	
	11-0090	9.3	
	11-0093	9.5	
	Mean	**9.12**	
	S.D.	**0.306**	

Appendix N

Organ Weights and Weight Ratios

Table N-1
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Absolute Organ Weights (grams)
Male Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Body Weight	Mean	336.8	343.2	329.5	333.5
	S.D.	19.95	40.44	21.70	18.51
	N	6	6	6	6
Adrenals	Mean	0.0736	0.0702	0.0732	0.0893
	S.D.	0.00559	0.01207	0.01165	0.01017
	N	5	6	6	6
Brain	Mean	2.0745	2.0098	2.0100	2.0133
	S.D.	0.05924	0.07181	0.16475	0.12189
	N	6	6	6	6
Heart	Mean	1.3472	1.3597	1.3597	1.4400
	S.D.	0.09945	0.15735	0.13929	0.12171
	N	6	6	6	6
Kidneys	Mean	2.9772	2.9757	2.8013	2.8342
	S.D.	0.32862	0.50559	0.16539	0.21739
	N	6	6	6	6
Epididymides	Mean	1.1112	1.0545	1.0072	0.9672[a]
	S.D.	0.08384	0.06951	0.07689	0.09932
	N	6	6	6	6
Liver	Mean	12.1160	12.2102	11.7955	11.6300
	S.D.	0.99775	1.83590	0.88792	1.57697
	N	6	6	6	6
Lungs	Mean	1.6707	1.7862	2.536[b]	2.5887[b]
	S.D.	0.08112	0.22152	0.32125	0.47341
	N	6	6	6	6
Spleen	Mean	0.6445	0.6402	0.6370	0.5665
	S.D.	0.08261	0.12306	0.02981	0.08500
	N	6	6	6	6
Testes	Mean	3.3608	3.3353	3.2088	3.2352
	S.D.	0.17267	0.34273	0.14660	0.23618
	N	6	6	6	6
Thymus	Mean	0.4725	0.4770	0.4653	0.4515
	S.D.	0.07465	0.12325	0.18057	0.13150
	N	6	6	6	6

[a] = Significantly reduced compared to controls (p = 0.044)
[b] = Significantly elevated compared to controls (p = 0.00)

Table N-2
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Organ to Body Weight Ratios (grams)
Male Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Adrenals	Mean	0.00022	0.00021	0.00022	0.00027[a]
	S.D.	0.000017	0.000045	0.000033	0.000017
	N	5	6	6	6
Brain	Mean	0.00617	0.00592	0.00611	0.00604
	S.D.	0.000253	0.000656	0.000536	0.000345
	N	6	6	6	6
Heart	Mean	0.00400	0.00396	0.00413	0.00432
	S.D.	0.000252	0.000090	0.000435	0.000258
	N	6	6	6	6
Kidneys	Mean	0.00885	0.00863	0.00851	0.00850
	S.D.	0.000945	0.000555	0.000374	0.000399
	N	6	6	6	6
Epididymides	Mean	0.00330	0.00312	0.00306	0.00291
	S.D.	0.000262	0.000508	0.000278	0.000390
	N	6	6	6	6
Liver	Mean	0.03602	0.03548	0.03582	0.03480
	S.D.	0.002862	0.001567	0.002054	0.003575
	N	6	6	6	6
Lungs	Mean	0.00498	0.00522	0.00771[b]	0.00775[b]
	S.D.	0.000437	0.000424	0.000980	0.001284
	N	6	6	6	6
Spleen	Mean	0.00192	0.00187	0.00194	0.00170
	S.D.	0.000299	0.000331	0.000170	0.000274
	N	6	6	6	6
Testes	Mean	0.00999	0.00979	0.00979	0.00973
	S.D.	0.000556	0.001197	0.000958	0.000993
	N	6	6	6	6
Thymus	Mean	0.00141	0.00139	0.00141	0.00135
	S.D.	0.000221	0.000302	0.000536	0.000362
	N	6	6	6	6

[a] = Significantly elevated compared to controls (p = 0.006).
[b] = Significantly elevated compared to controls (p = 0.00).

Table N-3
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Organ to Brain Weight Ratios (grams)
Male Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Adrenals	Mean	0.03538	0.03505	0.03656	0.04440[a]
	S.D.	0.002326	0.006752	0.005842	0.004517
	N	5	6	6	6
Heart	Mean	0.64928	0.67628	0.68121	0.71599
	S.D.	0.042003	0.072585	0.097285	0.053726
	N	6	6	6	6
Kidneys	Mean	1.43530	1.47736	1.40339	1.40922
	S.D.	0.152170	0.219373	0.164405	0.093434
	N	6	6	6	6
Epididymides	Mean	0.53591	0.52583	0.50266	0.48069
	S.D.	0.041327	0.047988	0.039403	0.042894
	N	6	6	6	6
Liver	Mean	5.83726	6.07254	5.89441	5.77935
	S.D.	0.389163	0.865694	0.567892	0.719328
	N	6	6	6	6
Lungs	Mean	0.80629	0.88884	1.26555[b]	1.28821[b]
	S.D.	0.053874	0.106894	0.159367	0.234879
	N	6	6	6	6
Spleen	Mean	0.31128	0.31955	0.31911	0.28192
	S.D.	0.043952	0.066741	0.035053	0.043635
	N	6	6	6	6
Testes	Mean	1.62105	1.65890	1.60669	1.61238
	S.D.	0.092297	0.153214	0.164327	0.158517
	N	6	6	6	6
Thymus	Mean	0.22729	0.23763	0.23077	0.22352
	S.D.	0.030938	0.063762	0.082814	0.059108
	N	6	6	6	6

[a] = Significantly elevated compared to controls (p = 0.047).
[b] = Significantly elevated compared to controls (p = 0.000).

Table N-4
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Absolute Organ Weights (grams)
Male Rats - Following One Month Recovery Period

		Control	2100 mg/m^3
Body Weight	Mean	492.8	445.0
	S.D.	19.20	42.86
	N	6	6
Adrenals	Mean	0.0705	0.0742
	S.D.	0.01785	0.00895
	N	6	6
Brain	Mean	2.0826	2.0602
	S.D.	0.02883	0.06469
	N	5	6
Heart	Mean	1.5900	1.4878
	S.D.	0.09417	0.15941
	N	6	6
Kidneys	Mean	3.4630	3.3605[a]
	S.D.	0.24043	0.41073
	N	6	6
Epididymides	Mean	1.4330	1.3560
	S.D.	0.06230	0.10832
	N	6	6
Liver	Mean	16.9745	14.6140
	S.D.	1.08120	1.98577
	N	6	6
Lungs	Mean	2.1735	2.4233[b]
	S.D.	0.13187	0.15765
	N	6	6
Spleen	Mean	0.8557	0.8325
	S.D.	0.10920	0.13706
	N	6	6
Testes	Mean	3.4507	3.3942
	S.D.	0.16804	0.17083
	N	6	6
Thymus	Mean	0.4893	0.5477[c]
	S.D.	0.11509	0.18757
	N	6	6

[a] = Significantly elevated compared to controls (p = 0.006).
[b] = Significantly elevated compared to controls (p = 0.004).
[c] = Significantly elevated compared to controls (p = 0.048).

Table N-5
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Organ to Body Weight Ratios (grams)
Male Rats - Following One Month Recovery Period

		Control	2100 mg/m^3
Adrenals	Mean	0.00014	0.00017
	S.D.	0.000037	0.000035
	N	6	6
Brain	Mean	0.00429	0.00466[c]
	S.D.	0.000051	0.000380
	N	5	6
Heart	Mean	0.00323	0.00335
	S.D.	0.000265	0.000216
	N	6	6
Kidneys	Mean	0.00702	0.00754[a]
	S.D.	0.000298	0.000331
	N	6	6
Epididymides	Mean	0.00291	0.00306
	S.D.	0.000204	0.000231
	N	6	6
Liver	Mean	0.03442	0.03275
	S.D.	0.001084	0.001766
	N	6	6
Lungs	Mean	0.00441	0.00547[b]
	S.D.	0.000289	0.000397
	N	6	6
Spleen	Mean	0.00173	0.00187
	S.D.	0.000172	0.000235
	N	6	6
Testes	Mean	0.00702	0.00769
	S.D.	0.000562	0.000935
	N	6	6
Thymus	Mean	0.00100	0.00121
	S.D.	0.000246	0.000327
	N	6	6

[a] = Significantly elevated compared to controls (p = 0.017).
[b] = Significantly elevated compared to controls (p = 0.000).
[c] = Significantly elevated compared to controls (p = 0.017).

Table N-6
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Organ to Brain Weight Ratios (grams)
Male Rats - Following One Month Recovery Period

		Control	2100 mg/m^3
Adrenals	Mean	0.03597	0.03604
	S.D.	0.007284	0.004658
	N	5	6
Heart	Mean	0.77082	0.72135
	S.D.	0.052731	0.064442
	N	5	6
Kidneys	Mean	1.63318	1.62807
	S.D.	0.083727	0.161723
	N	5	6
Epididymides	Mean	0.69674	0.65745[b]
	S.D.	0.021963	0.033655
	N	5	6
Liver	Mean	7.99523	7.07679[a]
	S.D.	0.311625	0.782238
	N	5	6
Lungs	Mean	1.03734	1.17656[c]
	S.D.	0.070032	0.072791
	N	5	6
Spleen	Mean	0.39541	0.40348
	S.D.	0.037288	0.061385
	N	5	6
Testes	Mean	1.68495	1.64833
	S.D.	0.059910	0.085929
	N	5	6
Thymus	Mean	0.23431	0.26423
	S.D.	0.061777	0.085656
	N	5	6

[a] = Significantly reduced compared to controls (p = 0.037).
[b] = Significantly reduced compared to controls (p = 0.052).
[c] = Significantly elevated compared to controls (p = 0.011).

Table N-7
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Absolute Organ Weights (grams)
Female Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Body Weight	Mean	214.5	212.5	212.2	204.0
	S.D.	16.79	17.10	16.29	11.92
	N	6	6	6	6
Adrenals	Mean	0.0822	0.0860	0.0905	0.0922
	S.D.	0.01078	0.00740	0.01363	0.00875
	N	6	6	6	6
Brain	Mean	1.8683	1.8110	1.8320	1.8573
	S.D.	0.08803	0.09148	0.04763	0.06938
	N	6	6	6	6
Heart	Mean	0.8793	0.9210	0.9065	0.8838
	S.D.	0.06664	0.14327	0.06152	0.07390
	N	6	6	6	6
Kidneys	Mean	1.7160	1.9033[a]	1.7902	1.7243
	S.D.	0.22482	0.25453	0.16175	0.12805
	N	6	6	6	6
Liver	Mean	7.3177	7.0718	7.4793	6.9632
	S.D.	0.89674	0.61549	0.77167	0.46836
	N	6	6	6	6
Lungs	Mean	1.4113	1.7058	2.0180[b]	2.1158[b]
	S.D.	0.14532	0.34985	0.18998	0.19308
	N	6	6	6	6
Ovaries	Mean	0.1720	0.1698	0.1652	0.1492
	S.D.	0.04373	0.04382	0.02140	0.01833
	N	5	6	6	6
Spleen	Mean	0.5292	0.5302	0.4958	0.4502
	S.D.	0.09265	0.04254	0.05167	0.05934
	N	6	6	6	6
Thymus	Mean	0.3245	0.4262	0.4468	0.3338
	S.D.	0.15129	0.13404	0.13658	0.03724
	N	6	6	6	6
Uterus	Mean	0.4476	0.4977	0.4537	0.5223
	S.D.	0.08000	0.05510	0.09878	0.08053
	N	5	6	6	6

[a] = Significantly elevated compared to controls (p = 0.05).
[b] = Significantly elevated compared to controls (p = 0.00).

Table N-8
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Organ to Body Weight Ratios (grams)
Female Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Adrenals	Mean	0.00038	0.00041	0.00043	0.00045
	S.D.	0.000052	0.000053	0.000070	0.000037
	N	6	6	6	6
Brain	Mean	0.00874	0.00855	0.00868	0.00912
	S.D.	0.000561	0.000602	0.000798	0.000447
	N	6	6	6	6
Heart	Mean	0.00412	0.00433	0.00416	0.00434
	S.D.	0.000393	0.000571	0.000128	0.000382
	N	6	6	6	6
Kidneys	Mean	0.00798	0.00894	0.00843	0.00845
	S.D.	0.000591	0.000847	0.000330	0.000338
	N	6	6	6	6
Liver	Mean	0.03404	0.03327	0.03521	0.03413
	S.D.	0.001946	0.000611	0.001537	0.000828
	N	6	6	6	6
Lungs	Mean	0.00657	0.00807	0.00951[a]	0.01037[a]
	S.D.	0.000313	0.001887	0.000526	0.000675
	N	6	6	6	6
Ovaries	Mean	0.00081	0.00079	0.00078	0.00073
	S.D.	0.000148	0.000144	0.000061	0.000082
	N	5	6	6	6
Spleen	Mean	0.00247	0.00251	0.00233	0.00221
	S.D.	0.000426	0.000279	0.000088	0.000269
	N	6	6	6	6
Thymus	Mean	0.00155	0.00201	0.00224	0.00164
	S.D.	0.000786	0.000611	0.000427	0.000175
	N	6	6	6	6
Uterus	Mean	0.00213	0.00234	0.00212	0.00256
	S.D.	0.000434	0.000209	0.000324	0.000385
	N	5	6	6	6

[a] = Significantly elevated compared to controls (p = 0.00).

Table N-9
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Organ to Brain Weight Ratios (grams)
Female Rats - Following 4-Week Exposure Period

		Control	130 mg/m^3	670 mg/m^3	2100 mg/m^3
Adrenals	Mean	0.04398	0.04757	0.04947	0.04959
	S.D.	0.005183	0.004395	0.007806	0.003809
	N	6	6	6	6
Heart	Mean	0.47220	0.50934	0.49223	0.47691
	S.D.	0.050542	0.079962	0.045222	0.048490
	N	6	6	6	6
Kidneys	Mean	0.91887	1.04819	0.97875	0.92801
	S.D.	0.116942	0.105054	0.103848	0.051839
	N	6	6	6	6
Liver	Mean	3.91781	3.90400	4.09171	3.74916
	S.D.	0.463272	0.259233	0.507955	0.208127
	N	6	6	6	6
Lungs	Mean	0.75438	0.95095	1.10325[a]	1.13899[b]
	S.D.	0.057818	0.246802	0.119749	0.090789
	N	6	6	6	6
Ovaries	Mean	0.09179	0.09358	0.09028	0.08025
	S.D.	0.022844	0.022811	0.012693	0.008803
	N	5	6	6	6
Spleen	Mean	0.28281	0.29359	0.27107	0.24298
	S.D.	0.045934	0.030561	0.032094	0.035687
	N	6	6	6	6
Thymus	Mean	0.17455	0.23639	0.26704	0.17977
	S.D.	0.083459	0.076561	0.067688	0.019417
	N	6	6	6	6
Uterus	Mean	0.23970	0.27506	0.24824	0.28194
	S.D.	0.045830	0.029663	0.057132	0.047168
	N	5	6	6	6

[a] = Significantly elevated compared to controls (p = 0.002).
[b] = Significantly elevated compared to controls (p = 0.001).

Table N-10
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Absolute Organ Weights (grams)
Female Rats Following One Month Recovery Period

		Control	2100 mg/m^3
Body Weight	Mean	258.7	253.0
	S.D.	23.35	15.89
	N	6	6
Adrenals	Mean	0.0618	0.0608
	S.D.	0.01348	0.00646
	N	5	5
Brain	Mean	1.9123	1.8780
	S.D.	0.04411	0.06179
	N	6	6
Heart	Mean	0.9355	0.9855[b]
	S.D.	0.10768	0.12148
	N	6	6
Kidneys	Mean	1.9094	1.9085
	S.D.	0.22286	0.14279
	N	5	6
Liver	Mean	8.2387	8.3090
	S.D.	1.07960	0.83976
	N	6	6
Lungs	Mean	1.5407	1.8735[a]
	S.D.	0.15816	0.25460
	N	6	6
Ovaries	Mean	0.1527	0.1388
	S.D.	0.02938	0.01098
	N	6	6
Spleen	Mean	0.5612	0.5562
	S.D.	0.10148	0.03992
	N	6	6
Thymus	Mean	0.4125	0.4050
	S.D.	0.08734	0.05216
	N	6	6
Uterus	Mean	0.5447	0.6003
	S.D.	0.10634	0.25444
	N	6	6

[a] = Significantly elevated compared to controls (p = 0.033).
[b] = Significantly elevated compared to controls (p = 0.048).

Table N-11
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Organ to Body Weight Ratios (grams)
Female Rats Following One Month Recovery Period

		Control	2100 mg/m^3
Adrenals	Mean	0.00024	0.00025
	S.D.	0.000057	0.000030
	N	5	5
Brain	Mean	0.00744	0.00745
	S.D.	0.000672	0.000583
	N	6	6
Heart	Mean	0.00361	0.00389
	S.D.	0.000125	0.000304
	N	6	6
Kidneys	Mean	0.00739	0.00755
	S.D.	0.000851	0.000364
	N	5	6
Liver	Mean	0.03177	0.03278
	S.D.	0.002104	0.001284
	N	6	6
Lungs	Mean	0.00603	0.00742[a]
	S.D.	0.001114	0.001000
	N	6	6
Ovaries	Mean	0.00059	0.00055
	S.D.	0.000099	0.000036
	N	6	6
Spleen	Mean	0.00216	0.00220
	S.D.	0.000259	0.000093
	N	6	6
Thymus	Mean	0.00160	0.00161
	S.D.	0.000332	0.000223
	N	6	6
Uterus	Mean	0.00210	0.00243
	S.D.	0.000307	0.001218
	N	6	6

[a] = Significantly elevated compared to controls (p = 0.048).

Table N-12
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats
Exposed to Pyrotechnically Disseminated Black Smoke

Summary of Organ to Brain Weight Ratios (grams)
Female Rats - Following One Month Recovery Period

		Control	2100 mg/m^3
Adrenals	Mean	0.03246	0.03214
	S.D.	0.006630	0.002617
	N	5	5
Heart	Mean	0.48929	0.52628
	S.D.	0.056517	0.076527
	N	6	6
Kidneys	Mean	1.00372	1.01784
	S.D.	0.102496	0.092915
	N	5	6
Liver	Mean	4.30486	4.43199
	S.D.	0.528782	0.514918
	N	6	6
Lungs	Mean	0.80697	0.99717[a]
	S.D.	0.095799	0.126275
	N	6	6
Ovaries	Mean	0.07972	0.07402
	S.D.	0.014571	0.006741
	N	6	6
Spleen	Mean	0.29329	0.29632
	S.D.	0.051525	0.022249
	N	6	6
Thymus	Mean	0.21628	0.21562
	S.D.	0.048630	0.026791
	N	6	6
Uterus	Mean	0.28469	0.32042
	S.D.	0.054302	0.137533
	N	6	6

[a] = Significantly elevated compared to controls (p = 0.001).

Table N-13
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Absolute Organ Weights (grams)
Male Rats - Following 4-Week Exposure Period

Dose Group	Animal ID	Body Weight	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Lungs	Spleen	Testes	Thymus
Control	11-0014	323	0.072	2.041	1.234	2.850	1.159	11.648	1.670	0.637	3.176	0.460
	11-0016	308	0.069	1.987	1.352	2.943	1.063	11.359	1.781	0.691	3.322	0.422
	11-0018	337	0.074	2.147	1.383	3.190	1.108	13.643	1.586	0.661	3.280	0.600
	11-0023	365	0.070	2.112	1.511	3.443	1.234	12.956	1.721	0.667	3.598	0.434
	11-0026	350	0.083	2.112	1.348	2.467	0.987	11.047	1.697	0.487	3.239	0.519
	11-0037	338	ND	2.048	1.255	2.970	1.116	12.043	1.569	0.724	3.550	0.400
	Mean	**336.8**	**0.0736**	**2.0745**	**1.3472**	**2.9772**	**1.1112**	**12.1160**	**1.6707**	**0.6445**	**3.3608**	**0.4725**
	SD	**19.95**	**0.00559**	**0.05924**	**0.09945**	**0.32862**	**0.08384**	**0.99775**	**0.08112**	**0.08261**	**0.17267**	**0.07465**
130 mg/m^3	11-0017	285	0.068	1.949	1.112	2.252	1.137	9.953	1.495	0.558	3.249	0.354
	11-0019	397	0.060	2.121	1.547	3.780	0.978	14.529	2.012	0.623	3.664	0.457
	11-0034	375	0.072	1.953	1.457	3.194	1.109	13.762	1.746	0.809	3.598	0.697
	11-0038	328	0.090	1.988	1.298	2.754	0.965	10.818	1.866	0.767	2.752	0.478
	11-0048	320	0.056	2.077	1.289	2.875	1.062	11.097	1.574	0.493	3.547	0.505
	11-0049	354	0.075	1.971	1.455	2.999	1.076	13.102	2.024	0.591	3.202	0.371
	Mean	**343.2**	**0.0702**	**2.0098**	**1.3597**	**2.9757**	**1.0545**	**12.2102**	**1.7862**	**0.6402**	**3.3353**	**0.4770**
	SD	**40.44**	**0.01207**	**0.07181**	**0.15735**	**0.50559**	**0.06951**	**1.83590**	**0.22152**	**0.12306**	**0.34273**	**0.12325**
670 mg/m^3	11-0013	327	0.068	1.721	1.387	2.909	0.938	11.695	2.274	0.641	3.166	0.398
	11-0021	342	0.056	2.172	1.541	2.910	0.977	13.085	3.002	0.662	3.218	0.641
	11-0022	310	0.074	2.078	1.233	2.512	0.981	11.637	2.239	0.635	3.137	0.593
	11-0030	313	0.079	2.049	1.203	2.710	1.122	10.405	2.389	0.611	3.415	0.141
	11-0033	367	0.091	2.119	1.297	2.946	1.083	12.326	2.442	0.597	2.996	0.543
	11-0036	318	0.071	1.921	1.497	2.821	0.942	11.625	2.870	0.676	3.321	0.476
	Mean	**329.5**	**0.0732**	**2.0100**	**1.3597**	**2.8013**	**1.0072**	**11.7955**	**2.5360**	**0.6370**	**3.2088**	**0.4653**
	SD	**21.70**	**0.01165**	**0.16475**	**0.13929**	**0.16539**	**0.07689**	**0.88792**	**0.32125**	**0.02981**	**0.14660**	**0.18057**
2100 mg/m^3	11-0015	346	0.096	1.977	1.471	2.713	0.864	13.433	2.309	0.406	2.978	0.416
	11-0020	322	0.083	2.021	1.271	2.780	0.941	11.420	2.011	0.589	3.245	0.428
	11-0024	358	0.101	2.088	1.498	3.115	0.947	11.484	2.857	0.661	3.497	0.672
	11-0025	317	0.077	2.079	1.409	2.612	1.151	9.821	2.293	0.589	3.457	0.532
	11-0027	345	0.098	2.126	1.626	3.097	0.990	13.511	3.312	0.567	2.934	0.319
	11-0029	313	0.081	1.789	1.365	2.688	0.910	10.111	2.750	0.587	3.300	0.342
	Mean	**333.5**	**0.0893**	**2.0133**	**1.4400**	**2.8342**	**0.9672**	**11.6300**	**2.5887**	**0.5665**	**3.2352**	**0.4515**
	SD	**18.51**	**0.01017**	**0.12189**	**0.12171**	**0.21739**	**0.09932**	**1.57697**	**0.47341**	**0.08500**	**0.23618**	**0.13150**

Table N-14
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Organ to Body Weight Ratios (grams)
Male Rats - Following 4-Week Exposure Period

Dose Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Lungs	Spleen	Testes	Thymus
	11-0014	0.0002	0.0063	0.0038	0.0088	0.0036	0.0361	0.0052	0.0020	0.0098	0.0014
Control	11-0016	0.0002	0.0065	0.0044	0.0096	0.0035	0.0369	0.0058	0.0022	0.0108	0.0014
	11-0018	0.0002	0.0064	0.0041	0.0095	0.0033	0.0405	0.0047	0.0020	0.0097	0.0018
	11-0023	0.0002	0.0058	0.0041	0.0094	0.0034	0.0355	0.0047	0.0018	0.0099	0.0012
	11-0026	0.0002	0.0060	0.0039	0.0070	0.0028	0.0316	0.0048	0.0014	0.0093	0.0015
	11-0037	ND	0.0061	0.0037	0.0088	0.0033	0.0356	0.0046	0.0021	0.0105	0.0012
	Mean	**0.00022**	**0.00617**	**0.00400**	**0.00885**	**0.00330**	**0.03602**	**0.00498**	**0.00192**	**0.00999**	**0.00141**
	SD	**0.000017**	**0.000253**	**0.000252**	**0.000945**	**0.000262**	**0.002862**	**0.000437**	**0.000299**	**0.000556**	**0.000221**
	11-0017	0.0002	0.0068	0.0039	0.0079	0.0040	0.0349	0.0052	0.0020	0.0114	0.0012
130 mg/m^3	11-0019	0.0002	0.0053	0.0039	0.0095	0.0025	0.0366	0.0051	0.0016	0.0092	0.0012
	11-0034	0.0002	0.0052	0.0039	0.0085	0.0030	0.0367	0.0047	0.0022	0.0096	0.0019
	11-0038	0.0003	0.0061	0.0040	0.0084	0.0029	0.0330	0.0057	0.0023	0.0084	0.0015
	11-0048	0.0002	0.0065	0.0040	0.0090	0.0033	0.0347	0.0049	0.0015	0.0111	0.0016
	11-0049	0.0002	0.0056	0.0041	0.0085	0.0030	0.0370	0.0057	0.0017	0.0090	0.0010
	Mean	**0.00021**	**0.00592**	**0.00396**	**0.00863**	**0.00312**	**0.03548**	**0.00522**	**0.00187**	**0.00979**	**0.00139**
	SD	**0.000045**	**0.000656**	**0.000090**	**0.000555**	**0.000508**	**0.001567**	**0.000424**	**0.000331**	**0.001197**	**0.000302**
	11-0013	0.0002	0.0053	0.0042	0.0089	0.0029	0.0358	0.0070	0.0020	0.0097	0.0012
670 mg/m^3	11-0021	0.0002	0.0064	0.0045	0.0085	0.0029	0.0383	0.0088	0.0019	0.0094	0.0019
	11-0022	0.0002	0.0067	0.0040	0.0081	0.0032	0.0375	0.0072	0.0020	0.0101	0.0019
	11-0030	0.0003	0.0065	0.0038	0.0087	0.0036	0.0332	0.0076	0.0020	0.0109	0.0005
	11-0033	0.0002	0.0058	0.0035	0.0080	0.0030	0.0336	0.0067	0.0016	0.0082	0.0015
	11-0036	0.0002	0.0060	0.0047	0.0089	0.0030	0.0366	0.0090	0.0021	0.0104	0.0015
	Mean	**0.00022**	**0.00611**	**0.00413**	**0.00851**	**0.00306**	**0.03582**	**0.00771**	**0.00194**	**0.00979**	**0.00141**
	SD	**0.000033**	**0.000536**	**0.000435**	**0.000374**	**0.000278**	**0.002054**	**0.000980**	**0.000170**	**0.000958**	**0.000536**
	11-0015	0.0003	0.0057	0.0043	0.0078	0.0025	0.0388	0.0067	0.0012	0.0086	0.0012
2100 mg/m^3	11-0020	0.0003	0.0063	0.0039	0.0086	0.0029	0.0355	0.0062	0.0018	0.0101	0.0013
	11-0024	0.0003	0.0058	0.0042	0.0087	0.0026	0.0321	0.0080	0.0018	0.0098	0.0019
	11-0025	0.0002	0.0066	0.0044	0.0082	0.0036	0.0310	0.0072	0.0019	0.0109	0.0017
	11-0027	0.0003	0.0062	0.0047	0.0090	0.0029	0.0392	0.0096	0.0016	0.0085	0.0009
	11-0029	0.0003	0.0057	0.0044	0.0086	0.0029	0.0323	0.0088	0.0019	0.0105	0.0011
	Mean	**0.00027**	**0.00604**	**0.00432**	**0.00850**	**0.00291**	**0.03480**	**0.00775**	**0.00170**	**0.00973**	**0.00135**
	SD	**0.000017**	**0.000345**	**0.000258**	**0.000399**	**0.000390**	**0.003575**	**0.001284**	**0.000274**	**0.000993**	**0.000362**

Table N-15
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Organ to Brain Weight Ratios (grams)
Male Rats - Following 4-Week Exposure Period

Dose Group	Animal ID	Adrenals	Heart	Kidneys	Epididymides	Liver	Lungs	Spleen	Testes	Thymus
	11-0014	0.0353	0.6046	1.3964	0.5679	5.7070	0.8182	0.3121	1.5561	0.2254
Control	11-0016	0.0347	0.6804	1.4811	0.5350	5.7167	0.8963	0.3478	1.6719	0.2124
	11-0018	0.0345	0.6442	1.4858	0.5161	6.3544	0.7387	0.3079	1.5277	0.2795
	11-0023	0.0331	0.7154	1.6302	0.5843	6.1345	0.8149	0.3158	1.7036	0.2055
	11-0026	0.0393	0.6383	1.1681	0.4673	5.2306	0.8035	0.2306	1.5336	0.2457
	11-0037	ND	0.6128	1.4502	0.5449	5.8804	0.7661	0.3535	1.7334	0.1953
	Mean	**0.03538**	**0.64928**	**1.43530**	**0.53591**	**5.83726**	**0.80629**	**0.31128**	**1.62105**	**0.22729**
	SD	**0.002326**	**0.042003**	**0.152170**	**0.041327**	**0.389163**	**0.053874**	**0.043952**	**0.092297**	**0.030938**
	11-0017	0.0349	0.5705	1.1555	0.5834	5.1067	0.7671	0.2863	1.6670	0.1816
130 mg/m^3	11-0019	0.0283	0.7294	1.7822	0.4611	6.8501	0.9486	0.2937	1.7275	0.2155
	11-0034	0.0369	0.7460	1.6354	0.5678	7.0466	0.8940	0.4142	1.8423	0.3569
	11-0038	0.0453	0.6529	1.3853	0.4854	5.4416	0.9386	0.3858	1.3843	0.2404
	11-0048	0.0270	0.6206	1.3842	0.5113	5.3428	0.7578	0.2374	1.7078	0.2431
	11-0049	0.0381	0.7382	1.5216	0.5459	6.6474	1.0269	0.2998	1.6246	0.1882
	Mean	**0.03505**	**0.67628**	**1.47736**	**0.52583**	**6.07254**	**0.88884**	**0.31955**	**1.65890**	**0.23763**
	SD	**0.006752**	**0.072585**	**0.219373**	**0.047988**	**0.865694**	**0.106894**	**0.066741**	**0.153214**	**0.063762**
	11-0013	0.0395	0.8059	1.6903	0.5450	6.7955	1.3213	0.3725	1.8396	0.2313
670 mg/m^3	11-0021	0.0258	0.7095	1.3398	0.4498	6.0244	1.3821	0.3048	1.4816	0.2951
	11-0022	0.0356	0.5934	1.2089	0.4721	5.6001	1.0775	0.3056	1.5096	0.2854
	11-0030	0.0386	0.5871	1.3226	0.5476	5.0781	1.1659	0.2982	1.6667	0.0688
	11-0033	0.0429	0.6121	1.3903	0.5111	5.8169	1.1524	0.2817	1.4139	0.2563
	11-0036	0.0370	0.7793	1.4685	0.4904	6.0515	1.4940	0.3519	1.7288	0.2478
	Mean	**0.03656**	**0.68121**	**1.40339**	**0.50266**	**5.89441**	**1.26555**	**0.31911**	**1.60669**	**0.23077**
	SD	**0.005842**	**0.097285**	**0.164405**	**0.039403**	**0.567892**	**0.159367**	**0.035053**	**0.164327**	**0.082814**
	11-0015	0.0486	0.7441	1.3723	0.4370	6.7946	1.1679	0.2054	1.5063	0.2104
2100 mg/m^3	11-0020	0.0411	0.6289	1.3756	0.4656	5.6507	0.9951	0.2914	1.6056	0.2118
	11-0024	0.0484	0.7174	1.4919	0.4535	5.5000	1.3683	0.3166	1.6748	0.3218
	11-0025	0.0370	0.6777	1.2564	0.5536	4.7239	1.1029	0.2833	1.6628	0.2559
	11-0027	0.0461	0.7648	1.4567	0.4657	6.3551	1.5579	0.2667	1.3801	0.1500
	11-0029	0.0453	0.7630	1.5025	0.5087	5.6518	1.5372	0.3281	1.8446	0.1912
	Mean	**0.04440**	**0.71599**	**1.40922**	**0.48069**	**5.77935**	**1.28821**	**0.28192**	**1.61238**	**0.22352**
	SD	**0.004517**	**0.053726**	**0.093434**	**0.042894**	**0.719328**	**0.234879**	**0.043635**	**0.158517**	**0.059108**

Table N-16
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Absolute Organ Weights (grams)
Male Rats - Following One Month Recovery Period

Dose Group	Animal ID	Body Weight	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Lungs	Spleen	Testes	Thymus
	11-0043	494	0.069	2.113	1.605	3.496	1.527	16.952	2.363	0.880	3.346	0.520
Control	11-0047	474	0.061	2.037	1.728	3.099	1.439	15.394	2.258	0.683	3.503	0.463
	11-0050	485	0.084	2.089	1.638	3.588	1.397	17.123	2.069	0.907	3.628	0.588
	11-0051	527	0.048	ND	1.517	3.764	1.343	18.564	2.241	1.013	3.163	0.497
	11-0052	499	0.098	2.098	1.594	3.565	1.426	17.535	2.042	0.828	3.538	0.278
	11-0053	478	0.063	2.076	1.458	3.266	1.466	16.279	2.068	0.823	3.526	0.590
	Mean	**492.8**	**0.0705**	**2.0826**	**1.5900**	**3.4630**	**1.4330**	**16.9745**	**2.1735**	**0.8557**	**3.4507**	**0.4893**
	SD	**19.20**	**0.01785**	**0.02883**	**0.09417**	**0.24043**	**0.06230**	**1.08120**	**0.13187**	**0.10920**	**0.16804**	**0.11509**
	11-0031	371	0.086	1.99	1.223	2.633	1.253	11.481	2.240	0.612	3.471	0.280
2100 mg/m^3	11-0032	451	0.060	2.045	1.565	3.439	1.345	14.274	2.640	0.956	3.154	0.540
	11-0035	473	0.079	2.091	1.667	3.703	1.330	16.461	2.520	0.948	3.227	0.797
	11-0039	495	0.070	2.116	1.465	3.639	1.468	16.439	2.518	0.759	3.554	0.704
	11-0040	427	0.072	1.983	1.409	3.137	1.239	13.285	2.320	0.790	3.395	0.415
	11-0041	453	0.078	2.136	1.598	3.612	1.501	15.744	2.302	0.930	3.564	0.550
	Mean	**445.0**	**0.0742**	**2.0602**	**1.4878**	**3.3605**	**1.3560**	**14.6140**	**2.4233**	**0.8325**	**3.3942**	**0.5477**
	SD	**42.86**	**0.00895**	**0.06469**	**0.15941**	**0.41073**	**0.10832**	**1.98577**	**0.15765**	**0.13706**	**0.17083**	**0.18757**

ND - No data

Table N-17
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Organ to Body Weight Ratios (grams)
Male Rats - Following One Month Recovery Period

Dose Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Lungs	Spleen	Testes	Thymus
Control	11-0043	0.0001	0.0043	0.0032	0.0071	0.0031	0.0343	0.0048	0.0018	0.0068	0.0011
	11-0047	0.0001	0.0043	0.0036	0.0065	0.0030	0.0325	0.0048	0.0014	0.0074	0.0010
	11-0050	0.0002	0.0043	0.0034	0.0074	0.0029	0.0353	0.0043	0.0019	0.0075	0.0012
	11-0051	0.0001	ND	0.0029	0.0071	0.0025	0.0352	0.0043	0.0019	0.0060	0.0009
	11-0052	0.0002	0.0042	0.0032	0.0071	0.0029	0.0351	0.0041	0.0017	0.0071	0.0006
	11-0053	0.0001	0.0043	0.0031	0.0068	0.0031	0.0341	0.0043	0.0017	0.0074	0.0012
	Mean	**0.00014**	**0.00429**	**0.00323**	**0.00702**	**0.00291**	**0.03442**	**0.00441**	**0.00173**	**0.00702**	**0.00100**
	SD	**0.000037**	**0.000051**	**0.000265**	**0.000298**	**0.000204**	**0.001084**	**0.000289**	**0.000172**	**0.000562**	**0.000246**
2100 mg/m^3	11-0031	0.0002	0.0054	0.0033	0.0071	0.0034	0.0309	0.0060	0.0016	0.0094	0.0008
	11-0032	0.0001	0.0045	0.0035	0.0076	0.0030	0.0316	0.0059	0.0021	0.0070	0.0012
	11-0035	0.0002	0.0044	0.0035	0.0078	0.0028	0.0348	0.0053	0.0020	0.0068	0.0017
	11-0039	0.0001	0.0043	0.0030	0.0074	0.0030	0.0332	0.0051	0.0015	0.0072	0.0014
	11-0040	0.0002	0.0046	0.0033	0.0073	0.0029	0.0311	0.0054	0.0019	0.0080	0.0010
	11-0041	0.0002	0.0047	0.0035	0.0080	0.0033	0.0348	0.0051	0.0021	0.0079	0.0012
	Mean	**0.00017**	**0.00466**	**0.00335**	**0.00754**	**0.00306**	**0.03275**	**0.00547**	**0.00187**	**0.00769**	**0.00121**
	SD	**0.000035**	**0.000380**	**0.000216**	**0.000331**	**0.000231**	**0.001766**	**0.000397**	**0.000235**	**0.000935**	**0.000327**

ND = No data

Table N-18
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Organ to Brain Weight Ratios (grams)
Male Rats - Following One Month Recovery Period

Dose Group	Animal ID	Adrenals	Heart	Kidneys	Epididymides	Liver	Lungs	Spleen	Testes	Thymus
	11-0043	0.0327	0.7596	1.6545	0.7227	8.0227	1.1183	0.4165	1.5835	0.2461
Control	11-0047	0.0299	0.8483	1.5214	0.7064	7.5572	1.1085	0.3353	1.7197	0.2273
	11-0050	0.0402	0.7841	1.7176	0.6687	8.1967	0.9904	0.4342	1.7367	0.2815
	11-0051	ND	ND	ND	ND	ND	ND	ND	ND	ND
	11-0052	0.0467	0.7598	1.6992	0.6797	8.3580	0.9733	0.3947	1.6864	0.1325
	11-0053	0.0303	0.7023	1.5732	0.7062	7.8415	0.9961	0.3964	1.6985	0.2842
	Mean	**0.03597**	**0.77082**	**1.63318**	**0.69674**	**7.99523**	**1.03734**	**0.39541**	**1.68495**	**0.23431**
	SD	**0.007284**	**0.052731**	**0.083727**	**0.021963**	**0.311625**	**0.070032**	**0.037288**	**0.059910**	**0.061777**
	11-0031	0.0432	0.6146	1.3231	0.6296	5.7693	1.1256	0.3075	1.7442	0.1407
2100 mg/m^3	11-0032	0.0293	0.7653	1.6817	0.6577	6.9800	1.2910	0.4675	1.5423	0.2641
	11-0035	0.0378	0.7972	1.7709	0.6361	7.8723	1.2052	0.4534	1.5433	0.3812
	11-0039	0.0331	0.6923	1.7198	0.6938	7.7689	1.1900	0.3587	1.6796	0.3327
	11-0040	0.0363	0.7105	1.5819	0.6248	6.6994	1.1699	0.3984	1.7121	0.2093
	11-0041	0.0365	0.7481	1.6910	0.7027	7.3708	1.0777	0.4354	1.6685	0.2575
	Mean	**0.03604**	**0.72135**	**1.62807**	**0.65745**	**7.07679**	**1.17656**	**0.40348**	**1.64833**	**0.26423**
	SD	**0.004658**	**0.064442**	**0.161723**	**0.033655**	**0.782238**	**0.072791**	**0.061385**	**0.085929**	**0.085656**

ND = No data

Table N-19
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Absolute Organ Weights (grams)
Female Rats - Following 4-Week Exposure Period

Dose Group	Animal ID	Body Weight	Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Ovaries	Spleen	Thymus	Uterus
	11-0056	199	0.081	1.845	0.808	1.596	6.412	1.346	0.142	0.633	0.520	0.345
Control	11-0059	190	0.075	1.729	0.917	1.455	6.331	1.150	0.137	0.382	0.380	0.509
	11-0062	231	0.079	1.847	0.990	2.050	8.659	1.458	ND	0.536	0.341	ND
	11-0063	215	0.099	1.973	0.837	1.537	7.369	1.457	0.159	0.468	0.358	0.538
	11-0065	222	0.069	1.954	0.839	1.852	7.180	1.503	0.177	0.609	0.289	0.392
	11-0075	230	0.090	1.862	0.885	1.806	7.955	1.554	0.245	0.547	0.059	0.454
	Mean	**214.5**	**0.0822**	**1.8683**	**0.8793**	**1.7160**	**7.3177**	**1.4113**	**0.1720**	**0.5292**	**0.3245**	**0.4476**
	SD	**16.79**	**0.01078**	**0.08803**	**0.06664**	**0.22482**	**0.89674**	**0.14532**	**0.04373**	**0.09265**	**0.15129**	**0.08000**
	11-0058	219	0.082	1.900	1.009	2.119	7.438	1.612	0.162	0.589	0.473	0.477
130 mg/m^3	11-0071	222	0.079	1.824	1.151	1.987	7.377	1.749	0.214	0.499	0.650	0.588
	11-0076	195	0.098	1.853	0.789	1.722	6.595	1.347	0.140	0.474	0.292	0.441
	11-0086	207	0.081	1.820	0.766	2.066	6.842	1.375	0.141	0.549	0.365	0.527
	11-0091	194	0.084	1.634	0.900	1.470	6.257	2.287	0.128	0.558	0.469	0.448
	11-0092	238	0.092	1.835	0.911	2.056	7.922	1.865	0.234	0.512	0.308	0.505
	Mean	**212.5**	**0.0860**	**1.8110**	**0.9210**	**1.9033**	**7.0718**	**1.7058**	**0.1698**	**0.5302**	**0.4262**	**0.4977**
	SD	**17.10**	**0.00740**	**0.09148**	**0.14327**	**0.25453**	**0.61549**	**0.34985**	**0.04382**	**0.04254**	**0.13404**	**0.05510**
	11-0055	207	0.070	1.875	0.839	1.793	6.811	2.016	0.150	0.501	0.359	0.397
670 mg/m^3	11-0060	200	0.087	1.875	0.839	1.684	6.855	1.965	0.151	0.453	0.414	0.468
	11-0069	196	0.101	1.871	0.957	1.570	7.029	1.719	0.166	0.452	0.257	0.345
	11-0072	230	0.081	1.779	0.980	1.854	8.614	2.050	0.184	0.558	0.654	0.559
	11-0073	235	0.106	1.815	0.942	2.049	8.266	2.312	0.197	0.558	0.489	0.580
	11-0094	205	0.098	1.777	0.882	1.791	7.301	2.046	0.143	0.453	0.508	0.373
	Mean	**212.2**	**0.0905**	**1.8320**	**0.9065**	**1.7902**	**7.4793**	**2.0180**	**0.1652**	**0.4958**	**0.4468**	**0.4537**
	SD	**16.29**	**0.01363**	**0.04763**	**0.06152**	**0.16175**	**0.77167**	**0.18998**	**0.02140**	**0.05167**	**0.13658**	**0.09878**
	11-0054	201	0.094	1.943	0.744	1.695	6.694	1.972	0.174	0.378	0.319	0.410
2100 mg/m^3	11-0061	209	0.100	1.837	0.939	1.771	6.940	2.211	0.150	0.549	0.291	0.589
	11-0064	187	0.087	1.803	0.874	1.652	6.460	2.096	0.120	0.439	0.350	0.564
	11-0066	219	0.104	1.943	0.940	1.920	7.755	2.412	0.143	0.434	0.369	0.526
	11-0067	195	0.087	1.783	0.922	1.541	6.697	1.856	0.146	0.417	0.296	0.440
	11-0070	213	0.081	1.835	0.884	1.767	7.233	2.148	0.162	0.484	0.378	0.605
	Mean	**204.0**	**0.0922**	**1.8573**	**0.8838**	**1.7243**	**6.9632**	**2.1158**	**0.1492**	**0.4502**	**0.3338**	**0.5223**
	SD	**11.92**	**0.00875**	**0.06938**	**0.07390**	**0.12805**	**0.46836**	**0.19308**	**0.01833**	**0.05934**	**0.03724**	**0.08053**

ND = No data

Table N-20
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Organ to Body Weight Ratios (grams)
Female Rats - Following 4-Week Exposure Period

Dose Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Ovaries	Spleen	Thymus	Uterus
	11-0056	0.0004	0.0093	0.0041	0.0080	0.0322	0.0068	0.0007	0.0032	0.0026	0.0017
Control	11-0059	0.0004	0.0091	0.0048	0.0077	0.0333	0.0061	0.0007	0.0020	0.0020	0.0027
	11-0062	0.0003	0.0080	0.0043	0.0089	0.0375	0.0063	ND	0.0023	0.0015	ND
	11-0063	0.0005	0.0092	0.0039	0.0071	0.0343	0.0068	0.0007	0.0022	0.0017	0.0025
	11-0065	0.0003	0.0088	0.0038	0.0083	0.0323	0.0068	0.0008	0.0027	0.0013	0.0018
	11-0075	0.0004	0.0081	0.0038	0.0079	0.0346	0.0068	0.0011	0.0024	0.0003	0.0020
	Mean	**0.00038**	**0.00874**	**0.00412**	**0.00798**	**0.03404**	**0.00657**	**0.00081**	**0.00247**	**0.00155**	**0.00213**
	SD	**0.000052**	**0.000561**	**0.000393**	**0.000591**	**0.001946**	**0.000313**	**0.000148**	**0.000426**	**0.000786**	**0.000434**
	11-0058	0.0004	0.0087	0.0046	0.0097	0.0340	0.0074	0.0007	0.0027	0.0022	0.0022
130 mg/m^3	11-0071	0.0004	0.0082	0.0052	0.0090	0.0332	0.0079	0.0010	0.0022	0.0029	0.0026
	11-0076	0.0005	0.0095	0.0040	0.0088	0.0338	0.0069	0.0007	0.0024	0.0015	0.0023
	11-0086	0.0004	0.0088	0.0037	0.0100	0.0331	0.0066	0.0007	0.0027	0.0018	0.0025
	11-0091	0.0004	0.0084	0.0046	0.0076	0.0323	0.0118	0.0007	0.0029	0.0024	0.0023
	11-0092	0.0004	0.0077	0.0038	0.0086	0.0333	0.0078	0.0010	0.0022	0.0013	0.0021
	Mean	**0.00041**	**0.00855**	**0.00433**	**0.00894**	**0.03327**	**0.00807**	**0.00079**	**0.00251**	**0.00201**	**0.00234**
	SD	**0.000053**	**0.000602**	**0.000571**	**0.000847**	**0.000611**	**0.001887**	**0.000144**	**0.000279**	**0.000611**	**0.000209**
	11-0055	0.0003	0.0091	0.0041	0.0087	0.0329	0.0097	0.0007	0.0024	0.0017	0.0019
670 mg/m^3	11-0060	0.0004	0.0094	0.0042	0.0084	0.0343	0.0098	0.0008	0.0023	0.0021	0.0023
	11-0069	0.0005	0.0095	0.0049	0.0080	0.0359	0.0088	0.0008	0.0023	0.0013	0.0018
	11-0072	0.0004	0.0077	0.0043	0.0081	0.0375	0.0089	0.0008	0.0024	0.0028	0.0024
	11-0073	0.0005	0.0077	0.0040	0.0087	0.0352	0.0098	0.0008	0.0024	0.0021	0.0025
	11-0094	0.0005	0.0087	0.0043	0.0087	0.0356	0.0100	0.0007	0.0022	0.0025	0.0018
	Mean	**0.00043**	**0.00868**	**0.00416**	**0.00843**	**0.03521**	**0.00951**	**0.00078**	**0.00233**	**0.00224**	**0.00212**
	SD	**0.000070**	**0.000798**	**0.000128**	**0.000330**	**0.001537**	**0.000526**	**0.000061**	**0.000088**	**0.000427**	**0.000324**
	11-0054	0.0005	0.0097	0.0037	0.0084	0.0333	0.0098	0.0009	0.0019	0.0016	0.0020
2100 mg/m^3	11-0061	0.0005	0.0088	0.0045	0.0085	0.0332	0.0106	0.0007	0.0026	0.0014	0.0028
	11-0064	0.0005	0.0096	0.0047	0.0088	0.0345	0.0112	0.0006	0.0023	0.0019	0.0030
	11-0066	0.0005	0.0089	0.0043	0.0088	0.0354	0.0110	0.0007	0.0020	0.0017	0.0024
	11-0067	0.0004	0.0091	0.0047	0.0079	0.0343	0.0095	0.0007	0.0021	0.0015	0.0023
	11-0070	0.0004	0.0086	0.0042	0.0083	0.0340	0.0101	0.0008	0.0023	0.0018	0.0028
	Mean	**0.00045**	**0.00912**	**0.00434**	**0.00845**	**0.03413**	**0.01037**	**0.00073**	**0.00221**	**0.00164**	**0.00256**
	SD	**0.000037**	**0.000447**	**0.000382**	**0.000338**	**0.000828**	**0.000675**	**0.000082**	**0.000269**	**0.000175**	**0.000385**

ND = No data

Table N-21
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Organ to Brain Weight Ratios (grams)
Female Rats - Following 4-Week Exposure Period

Dose Group	Animal ID	Adrenals	Heart	Kidneys	Liver	Lungs	Ovaries	Spleen	Thymus	Uterus
	11-0056	0.0439	0.4379	0.8650	3.4753	0.7295	0.0770	0.3431	0.2818	0.1870
Control	11-0059	0.0434	0.5304	0.8415	3.6617	0.6651	0.0792	0.2209	0.2198	0.2944
	11-0062	0.0428	0.5360	1.1099	4.6881	0.7894	ND	0.2902	0.1846	ND
	11-0063	0.0502	0.4242	0.7790	3.7349	0.7385	0.0806	0.2372	0.1814	0.2727
	11-0065	0.0353	0.4294	0.9478	3.6745	0.7692	0.0906	0.3117	0.1479	0.2006
	11-0075	0.0483	0.4753	0.9699	4.2723	0.8346	0.1316	0.2938	0.0317	0.2438
	Mean	**0.04398**	**0.47220**	**0.91887**	**3.91781**	**0.75438**	**0.09179**	**0.28281**	**0.17455**	**0.23970**
	SD	**0.005183**	**0.050542**	**0.116942**	**0.463272**	**0.057818**	**0.022844**	**0.045934**	**0.083459**	**0.045830**
	11-0058	0.0432	0.5311	1.1153	3.9147	0.8484	0.0853	0.3100	0.2489	0.2511
130 mg/m^3	11-0071	0.0433	0.6310	1.0894	4.0444	0.9589	0.1173	0.2736	0.3564	0.3224
	11-0076	0.0529	0.4258	0.9293	3.5591	0.7269	0.0756	0.2558	0.1576	0.2380
	11-0086	0.0445	0.4209	1.1352	3.7593	0.7555	0.0775	0.3016	0.2005	0.2896
	11-0091	0.0514	0.5508	0.8996	3.8293	1.3996	0.0783	0.3415	0.2870	0.2742
	11-0092	0.0501	0.4965	1.1204	4.3172	1.0163	0.1275	0.2790	0.1678	0.2752
	Mean	**0.04757**	**0.50934**	**1.04819**	**3.90400**	**0.95095**	**0.09358**	**0.29359**	**0.23639**	**0.27506**
	SD	**0.004395**	**0.079962**	**0.105054**	**0.259233**	**0.246802**	**0.022811**	**0.030561**	**0.076561**	**0.029663**
	11-0055	0.0373	0.4475	0.9563	3.6325	1.0752	0.0800	0.2672	0.1915	0.2117
670 mg/m^3	11-0060	0.0464	0.4475	0.8981	3.6560	1.0480	0.0805	0.2416	0.2208	0.2496
	11-0069	0.0540	0.5115	0.8391	3.7568	0.9188	0.0887	0.2416	0.1374	0.1844
	11-0072	0.0455	0.5509	1.0422	4.8420	1.1523	0.1034	0.3137	0.3676	0.3142
	11-0073	0.0584	0.5190	1.1289	4.5543	1.2738	0.1085	0.3074	0.2694	0.3196
	11-0094	0.0551	0.4963	1.0079	4.1086	1.1514	0.0805	0.2549	0.2859	0.2099
	Mean	**0.04947**	**0.49223**	**0.97875**	**4.09171**	**1.10325**	**0.09028**	**0.27107**	**0.26704**	**0.24824**
	SD	**0.007806**	**0.045222**	**0.103848**	**0.507955**	**0.119749**	**0.012693**	**0.032094**	**0.067688**	**0.057132**
	11-0054	0.0484	0.3829	0.8724	3.4452	1.0149	0.0896	0.1945	0.1642	0.2110
2100 mg/m^3	11-0061	0.0544	0.5112	0.9641	3.7779	1.2036	0.0817	0.2989	0.1584	0.3206
	11-0064	0.0483	0.4847	0.9163	3.5829	1.1625	0.0666	0.2435	0.1941	0.3128
	11-0066	0.0535	0.4838	0.9882	3.9913	1.2414	0.0736	0.2234	0.1899	0.2707
	11-0067	0.0488	0.5171	0.8643	3.7560	1.0409	0.0819	0.2339	0.1660	0.2468
	11-0070	0.0441	0.4817	0.9629	3.9417	1.1706	0.0883	0.2638	0.2060	0.3297
	Mean	**0.04959**	**0.47691**	**0.92801**	**3.74916**	**1.13899**	**0.08025**	**0.24298**	**0.17977**	**0.28194**
	SD	**0.003809**	**0.048490**	**0.051839**	**0.208127**	**0.090789**	**0.008803**	**0.035687**	**0.019417**	**0.047168**

ND = No data

Table N-22
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Absolute Organ Weights (grams)
Female Rats Following One Month Recovery Period

Dose Group	Animal ID	Body Weight	Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Ovaries	Spleen	Thymus	Uterus
Control	11-0077	221	0.053	1.877	0.776	1.843	6.325	1.796	0.119	0.446	0.384	0.459
	11-0078	261	0.052	1.848	0.974	1.594	8.243	1.619	0.136	0.519	0.515	0.478
	11-0079	289	0.061	1.923	1.099	2.212	9.146	1.447	0.134	0.736	0.492	0.578
	11-0084	276	0.058	1.921	0.970	1.943	9.077	1.542	0.201	0.549	0.311	0.744
	11-0085	254	ND	1.974	0.906	ND	8.880	1.509	0.161	0.505	0.320	0.486
	11-0087	251	0.085	1.931	0.888	1.955	7.761	1.331	0.165	0.612	0.453	0.523
	Mean	**258.7**	**0.0618**	**1.9123**	**0.9355**	**1.9094**	**8.2387**	**1.5407**	**0.1527**	**0.5612**	**0.4125**	**0.5447**
	SD	**23.35**	**0.01348**	**0.04411**	**0.10768**	**0.22286**	**1.07960**	**0.15816**	**0.02938**	**0.10148**	**0.08734**	**0.10634**
2100 mg/m^3	11-0074	228	0.062	1.869	0.902	1.709	7.116	1.672	0.124	0.511	0.352	1.110
	11-0081	253	0.050	1.802	0.968	2.019	8.188	1.881	0.149	0.520	0.458	0.588
	11-0088	247	0.064	1.967	0.870	1.814	7.864	2.219	0.142	0.555	0.446	0.503
	11-0089	257	0.061	1.875	1.059	1.829	8.471	1.502	0.126	0.546	0.365	0.445
	11-0090	277	ND	1.826	1.194	2.033	9.650	2.014	0.147	0.613	0.356	0.467
	11-0093	256	0.067	1.929	0.920	2.047	8.565	1.953	0.145	0.592	0.453	0.489
	Mean	**253.0**	**0.0608**	**1.8780**	**0.9855**	**1.9085**	**8.3090**	**1.8735**	**0.1388**	**0.5562**	**0.4050**	**0.6003**
	SD	**15.89**	**0.00646**	**0.06179**	**0.12148**	**0.14279**	**0.83976**	**0.25460**	**0.01098**	**0.03992**	**0.05216**	**0.25444**

ND = No data

Table N-23
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Organ to Body Weight Ratios (grams)
Female Rats Following One Month Recovery Period

Dose Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Ovaries	Spleen	Thymus	Uterus
Control	11-0077	0.0002	0.0085	0.0035	0.0083	0.0286	0.0081	0.0005	0.0020	0.0017	0.0021
	11-0078	0.0002	0.0071	0.0037	0.0061	0.0316	0.0062	0.0005	0.0020	0.0020	0.0018
	11-0079	0.0002	0.0067	0.0038	0.0077	0.0316	0.0050	0.0005	0.0025	0.0017	0.0020
	11-0084	0.0002	0.0070	0.0035	0.0070	0.0329	0.0056	0.0007	0.0020	0.0011	0.0027
	11-0085	ND	0.0078	0.0036	ND	0.0350	0.0059	0.0006	0.0020	0.0013	0.0019
	11-0087	0.0003	0.0077	0.0035	0.0078	0.0309	0.0053	0.0007	0.0024	0.0018	0.0021
	Mean	**0.00024**	**0.00744**	**0.00361**	**0.00739**	**0.03177**	**0.00603**	**0.00059**	**0.00216**	**0.00160**	**0.00210**
	SD	**0.000057**	**0.000672**	**0.000125**	**0.000851**	**0.002104**	**0.001114**	**0.000099**	**0.000259**	**0.000332**	**0.000307**
2100 mg/m^3	11-0074	0.0003	0.0082	0.0040	0.0075	0.0312	0.0073	0.0005	0.0022	0.0015	0.0049
	11-0081	0.0002	0.0071	0.0038	0.0080	0.0324	0.0074	0.0006	0.0021	0.0018	0.0023
	11-0088	0.0003	0.0080	0.0035	0.0073	0.0318	0.0090	0.0006	0.0022	0.0018	0.0020
	11-0089	0.0002	0.0073	0.0041	0.0071	0.0330	0.0058	0.0005	0.0021	0.0014	0.0017
	11-0090	ND	0.0066	0.0043	0.0073	0.0348	0.0073	0.0005	0.0022	0.0013	0.0017
	11-0093	0.0003	0.0075	0.0036	0.0080	0.0335	0.0076	0.0006	0.0023	0.0018	0.0019
	Mean	**0.00025**	**0.00745**	**0.00389**	**0.00755**	**0.03278**	**0.00742**	**0.00055**	**0.00220**	**0.00161**	**0.00243**
	SD	**0.000030**	**0.000583**	**0.000304**	**0.000364**	**0.001284**	**0.001000**	**0.000036**	**0.000093**	**0.000223**	**0.001218**

ND = No data

Table N-24
Protocol No. 0CKC-35-10-08-01
Acute and Four-Week Repeated-Dose Inhalation Toxicity in Rats Exposed to Pyrotechnically Disseminated Black Smoke

Individual Organ to Brain Weight Ratios (grams)
Female Rats - Following One Month Recovery Period

Dose Group	Animal ID	Adrenals	Heart	Kidneys	Liver	Lungs	Ovaries	Spleen	Thymus	Uterus
Control	11-0077	0.0282	0.4134	0.9819	3.3697	0.9568	0.0634	0.2376	0.2046	0.2445
	11-0078	0.0281	0.5271	0.8626	4.4605	0.8761	0.0736	0.2808	0.2787	0.2587
	11-0079	0.0317	0.5715	1.1503	4.7561	0.7525	0.0697	0.3827	0.2559	0.3006
	11-0084	0.0302	0.5049	1.0115	4.7251	0.8027	0.1046	0.2858	0.1619	0.3873
	11-0085	ND	0.4590	ND	4.4985	0.7644	0.0816	0.2558	0.1621	0.2462
	11-0087	0.0440	0.4599	1.0124	4.0192	0.6893	0.0854	0.3169	0.2346	0.2708
	Mean	**0.03246**	**0.48929**	**1.00372**	**4.30486**	**0.80697**	**0.07972**	**0.29329**	**0.21628**	**0.28469**
	SD	**0.006630**	**0.056517**	**0.102496**	**0.528782**	**0.095799**	**0.014571**	**0.051525**	**0.048630**	**0.054302**
2100 mg/m^3	11-0074	0.0332	0.4826	0.9144	3.8074	0.8946	0.0663	0.2734	0.1883	0.5939
	11-0081	0.0277	0.5372	1.1204	4.5438	1.0438	0.0827	0.2886	0.2542	0.3263
	11-0088	0.0325	0.4423	0.9222	3.9980	1.1281	0.0722	0.2822	0.2267	0.2557
	11-0089	0.0325	0.5648	0.9755	4.5179	0.8011	0.0672	0.2912	0.1947	0.2373
	11-0090	ND	0.6539	1.1134	5.2848	1.1030	0.0805	0.3357	0.1950	0.2558
	11-0093	0.0347	0.4769	1.0612	4.4401	1.0124	0.0752	0.3069	0.2348	0.2535
	Mean	**0.03214**	**0.52628**	**1.01784**	**4.43199**	**0.99717**	**0.07402**	**0.29632**	**0.21562**	**0.32042**
	SD	**0.002617**	**0.076527**	**0.092915**	**0.514918**	**0.126275**	**0.006741**	**0.022249**	**0.026791**	**0.137533**

ND = No data.

Pathology report for

0CKC-35-10-08-01

Acute and Four-Week Repeated-Dose Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke

April 01, 2013

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Appendix O

Pathology Report

TABLE OF CONTENTS

INTRODUCTION

The purpose of this study is to determine the toxicity of a pyrotechnically disseminated black smoke formulation used by the military. Nose only inhalation exposures of the test atmosphere were conducted in rats at a single dose (acute study; one exposure),repeat-dose with immediate necropsy(4-week study; 20 exposures) and repeat-dose with 4 week recovery period of the test atmosphere. In the acute study, rats were exposed to a single, 30 minute duration of the test atmosphere followed by a 14-day recovery period. At termination of this group, a complete necropsy was conducted; respiratory tract only was harvested and examined microscopically. The 4-week study rats were exposed 30 minutes per day for 20 days. Complete histopathologic examination was performed on males and females in this group. Selected tissues were examined in all remaining groups.

METHODS

Necropsies were performed at US Army Public Health Command (USAPHC), Portfolio of Toxicology (PTOX). Protocol specified tissues were collected and appropriately preserved in 10% buffered formalin, selectively trimmed and placed in cassettes labeled with protocol number, animal identification number and laboratory assigned accession number. Cassettes were placed in labeled formalin filled bottles and transported to the US Army Institute of Chemical Defense (USAMRICD) for processing. Tissues were routinely processed, to include decalcification of bone and nasal cavity, and paraffin embedded. All processed and embedded tissues were microtomed at 5*u*m thick, and automatically stained with hematoxylin and eosin and coverslipped. Nasal cavity was sectioned at four locations: 1) posterior part of the upper incisors, 2) incisive papilla 3) second palatine crest 4) first molar teeth. Two additional sections were made posterior to the 4^{th} cut to include examination of the eye in those animals it required. The pathologist examined slides for compound-induced histopathologic changes via light microscopy. The prevelance and severity of findings were graded as compared to controls. Control animals were examined for background findings, all findings were recorded. Findings, in all animals and groups, were assigned as none, minimal, mild, moderate or severe. The description and criteria of severity grades for specific organs is summarized in Appendix C.

RESULTS

4-Week Repeated Dose Study, Immediate Necropsy:

Four groups of 12 rats each (6rats/sex/group) were exposed to atmospheric concentrations of black smoke. Rats were exposed nose-only for 30 minutes per day, 2-5 days/week, for a total of 20 exposure days.

Based on incidence and severity, findings associated with repeated dose exposure to Black Smoke were noted in a dose dependent manner in the nasal cavity and lung. Alveolar histiocytosis, an accumulation of macrophages and foamy macrophages within alveolar spaces was the predominant lesion in repeat-dose, immediate necropsy males and females, increasing in incidence and severity from the 130mg/m^3 to the 670mg/m^3

and up to the 2100mg/m^3 concentrations. In all animals, regardless of group, to include recovery groups, in which alveolar histiocytosis was noted as a response to inhalation of the test material, accumulation of macrophages were centriacinar and multifocal. Alveolar histiocytosis may occur incidentally as was noted in one 0mg/m^3 repeat-dose with immediate sacrifice male (animal #11-0023) and in one 0mg/m^3 repeat-dose with recovery male (animal # 11-0052). Alveolar histiocytosis can occur incidentally in older rats or as a background lesion associated with a pigment or inert material (Boorman, 1990); in animal #11-0023, hemoglobin crystals were observed. Additionally, these lesions were isolated or minimally multifocal with no specific distribution pattern, contrasting test material exposures.

Type II pneumocyte hyperplasia was noted in the lungs of all 2100mg/m^3 4-week, immediate necropsy males and females. Type II pneumocyte hyperplasia occurs after injury to alveolar epithelium and appears as rows or "side by side" cuboidal epithelium along alveolar septa (Figure 1, Appendix A).

Mild to moderate tracheobronchial lymphoid hyperplasia was observed in all repeat-dose, immediate necropsy, exposure groups (3/4 male and 3/5 female 2100mg/m^3, 3/3 male and 3/5 female 670mg/m^3, 0/2 male and 2/4 female 130mg/m^3, and 0/4 male 0/4 female 0mg/m^3 controls). These lymph nodes were not specifically identified for harvest and examination, however, due to gross observations were subsequently harvested. This accounts for the lack of 100% examination across all groups.

Table 1. Prevalence and Severity of Test Article Related Lung Findings in 4- Week Repeated Dose (20 doses), Immediate Necropsy

Dose group	0		130mg/m^3		670mg/m^3		2100mg/m^3	
Sex	M	F	M	F	M	F	M	F
Number examined	6	6	6	6	6	6	6	6
Alveolar Histiocytosis								
None	5	6	1					
Minimal			5	4				
Mild	1			2	4	1		
Moderate					2	5	5	6
Severe							1	

Table 2. Prevalence and Severity of Test Article Related Nasal cavity Findings per Section in 4-Week Repeat Dose (20 doses), Immediate Necropsy

Dose group	0mg/m^3		130mg/m^3		670mg/m^3		2100mg/m^3	
Sex	M	F	M	F	M	F	M	F
Number examined	6	6	6	6	6	6	6	6
Section I								
None	6	6						
Minimal			6	3				
Mild				2	4	5		5

Moderate					2	1	3	1
Severe							3	
Section II								
None	6	6	6	5	2	3		4
Minimal				1	2	2		2
Mild					2	1	1	
Moderate							5	
Severe								
Section III								
None	6	6	6	6	4	5	2	5
Minimal					1			1
Mild					1	1	2	
Moderate							2	
Severe								
Section IV								
None	6	6	6	6	5	6	4	6
Minimal					1			
Mild							1	
Moderate							1	
Severe								

Note: Greatest severity lesion recorded in table per section. Only hyperplasia, dysplasia/metaplasia or loss were recorded in table.

Within the nasal cavity, the 2100mg/m^3 exposure group males and females were the most severely affected; males were affected more severely than females. Males in this group had a variety of lesions extending posteriorly within the nasal cavity all the way to level IV, at the level of the first molar teeth. Lesion severity and depth of affected nasal level decreased with the decline of particle concentration. Females, in this group, had lesions up to level III, but mostly of minimal and mild severity. Additionally, at level III only one female was affected by a minimal lesion.

In all exposure concentrations, the most common observed lesion was transitional epithelial hyperplasia of the maxillary and nasoturbinates; a more diffuse distribution to include the dorsolateral and mediolateral meatus was observed in more severely affected rats. Normal transitional epithelium, in rats, is composed of cuboidal, nonciliated cells, 1-2 cell layers thick and lines the maxillary and nasoturbinates and dorsolateral and mediolateral meatus in the anterior portion of the nose. Transitional epithelial hyperplasia, in this study, was characterized by 3-7 cell layers thick and multifocal to diffuse in distribution. In all males and females of each group, 2100mg/m^3, 670mg/m^3 and 130mg/m^3, transitional epithelial hyperplasia was observed in varying severities.

The second most common lesion was respiratory epithelial hyperplasia of the septum with or without associated erosions and neutrophilic infiltrates(5/6 males and 1/6 females 2100mg/m^3; 1/6 males and 1/6 females 670mg/m^3; 0/6 males and 0/6 females 130mg/m^3).

Additionally, 2/6 male 2100mg/m^3 exposures, had degeneration of olfactory epithelium and/or epithelial metaplasia. Normally, respiratory epithelium of the nasal septum of the rat is lined by pseudostratified columnar cells composed of 6 cell types, mucous, ciliated, nonciliated columnar, cuboidal, brush and basal. The respiratory epithelium of the nasal septum in animal #11-0015 is multifocally replaced by squamous cells, a finding termed metaplasia. Metaplasia is defined as the reversible replacement of one differentiated cell type with another differentiated cell type. Normal olfactory epithelium is composed of three cell types, but are several cell layers thick due to the numerous middle layers of nuclei of the olfactory sensory neurons. Animal #11-0015 and #11-0024 had degeneration and loss of cell layers.

Renal lymphocytic interstitial infiltrates were noted in the majority of male and female controls and all dose groups in minimal to mild amounts. These were considered background lesions and of minimal significance. One 2100mg/m^3 4- week repeat dose male, immediate necropsy (#11-0015) had interstitial fibrosis and tubular loss. This is, likely, indicative of a prior insult followed by fibrotic repair. The cause is not evident in the section examined; this finding is not considered to be test-article related.

Mononuclear infiltrates within the heart, with or without myocardial degeneration or necrosis was observed in the majority of the animals in the 130mg/m^3 and 670mg/m^3 male and female exposure groups. The severity ranged from minimal to moderate with minimal and mild severities predominating. 2/6 male, 2100mg/m^3 had minimal to mild lesions, none were observed in the females of this group. The control groups observed 1/6 males with mild lesions. Degenerative myocardial disease is common in F344 rats and other strains. (Mackenzie, 1990) The lesion is myocardial degeneration and necrosis with few mononuclear cells with no evidence of clinical disease. The cardiac findings observed in this study are likely early degenerative myocardial disease and, with the lack of a dose relationship, unrelated to test material exposure.

Ultimobranchial cysts are congenital anomalies of the thyroid gland. These cysts are found in almost every lobe when serial sections are performed (Hardisty 1990). This lesion was observed across all male and female control and exposure concentrations equally. Other findings that occurred infrequently or comparable to controls were considered to be background lesions or of minimal significance and not treatment related. These lesions were prostatic and epididymal lymphocytic infiltrates, splenic hemosiderosis and sinus plasmacytosis of the submandibular lymph node. Additional isolated lesions were identified per animal and were considered of minimal consequence for discussion, refer to Appendix B Histopathology Data, for morphologic findings per animal.

4-Week Repeated Dose, 1-Month Recovery Study
Control and high dose levels from the 4- week repeat dose study had an additional recovery group utilizing 6rats/sex/group. Rats were exposed nose-only for 30 minutes per day, 2-5 days/week, for a total of 20 exposure days and then held for a one-month period following the exposures.

Alveolar histiocytosis was noted in all male and female 2100mg/m^3 repeat-dose, 1-month recovery groups. The severity of the lesion was significantly reduced compared to the 2100mg/m^3 repeat-dose, immediate necropsy group. Additionally, type II pneumocyte hyperplasia was not demonstrated. One 0mg/m^3 male (#11-0052) had minimal alveolar histiocytosis, this was considered to be an incidental background lesion.

Within the nasal cavity, five 2100mg/m^3 males in the recovery group had nasal cavity findings. Four males had hyperplasia of the respiratory epithelium of the nasal septum in sections II or III with neutrophilic infiltrates. It is undetermined, at this time, if this is test-article related.

Histopathologic evalution was limited in the recovery group to respiratory system and gross lesions, if noted. Few additional lesions were noted. Thyroid gland ultimobranchial cysts were noted in equivalent frequency as all other control and exposure repeat-dose, immediate necropsy groups.

Table 3. Prevalence and Severity of Test Article Related Lung Findings in Repeat dose (20), 1 month recovery

Dose group	0		2100mg/m^3	
Sex	M	F	M	F
Number examined	6	6	6	6
Alveolar Histiocytosis				
None	5	6		
Minimal	1			
Mild			6	6
Moderate				
Severe				

Table 4. Prevalence and Severity of Test Article Related Nasal Cavity Findings in Repeat dose (20), 1 month recovery

Dose group	0mg/m^3	2100 mg/m^3

Sex	M	F	M	F
Number examined	6	6	6	6
Section I				
None	6	6	6	6
Minimal				
Mild				
Moderate				
Severe				
Section II				
None	6	6	5	6
Minimal			1	
Mild				
Moderate				
Severe				
Section III				
None	6	6	2	5
Minimal			1	1
Mild			2	
Moderate			1	
Severe				
Section IV				
None	6	6	6	6
Minimal				
Mild				
Moderate				
Severe				

Acute study
5 male and 5 female rats were exposed for 30 minutes to atmospheric concentrations of the test material. Following the exposure, rats were held for a 14-day observation period and monitored for morbidity/mortality, weight loss, and/or clinical signs of toxicity. Following the 14-day recovery period, rats were euthanized and necropsied.

Alveolar histiocytosis with hemoglobin crystals was noted in one 2500mg/m^3 male. This lesion and all others noted were considered incidental findings and not related to exposure to the test material.

Gross Observations (**all studies and groups)**
Multifocal black discoloration of the lungs and tracheobronchial lymph nodes were noted in all males and females of the 2100mg/m^3 4-week repeat-dose, immediate necropsy exposure groups. This was noted less frequently in the other exposure concentrations. During processing of tissues for histologic examination, processing fluids became intensely blue-black. It is likely that the majority of “color” that could have been observed free within lung sections and tracheobronchial lymph nodes, histologically, was removed during processing, therefore, pigment was not observed histologically.

Clinical chemistry and Hematology (all studies and groups)
Statistical analysis of clinical chemistry values noted that males in the 130mg/m^3 and 670mg/m^3 in the 4-week repeat dose (20 doses), immediate necropsy exposure groups and males in the 4-week repeat dose (20 doses), 4-week recovery groups exhibited a decrease in cholesterol. A literature search revealed several references to magnesium, in general, and magnesium chloride associations with reduction in plasma cholesterol and lipids or atherosclerotic vessels in mice and rabbits, respectively. (Cohen, 2002) The black smoke formulation utilized in this study contained magnesium carbonate, however, it is not known if this component is a factor in the low cholesterol values. Further investigation would be required to determine the significance of this finding.

DISCUSSION
4-week repeat dose, main study rats only
Inhalation of respiratory toxicants results in deposition of particles with an aerodynamic diameter of 5-30um in the nasopharynx by impaction due to the higher air velocity and turbulence in these regions. Particles with an aerodynamic diameter of 1-5um are deposited in the bronchial regions by sedimentation. Particles less than 1um that have reached the alveoli are deposited primarily by diffusion (Boorman, 1990). The aerodynamic diameter of particles in this study ranged from 2.7*u*m -3.4*u*m. As expected, lesions associated with the test material were concentrated in the nasal cavity and lung.

Alveolar histiocytosis with type II pneumocyte hyperplasia was noted in all male and female rats exposed to 2100mg/m^3 and necropsied immediately after exposure. This finding is expected as particles within the alveoli are phagocytosed by macrophages and then cleared by the mucociliary apparatus. Per the literature, more inert particulate materials induce primarily an accumulation of alveolar macrophages. Depending on the relative cytotoxicity of the material, there is variable hyperplasia and hypertrophy of type II cells in the alveolar ducts and alveoli. (Boorman, 1990)

In rats exposed to 2100mg/m^3 black smoke formulation and allowed to recover for 4 weeks, alveolar histiocytosis was significantly reduced and type II pneumocyte hyperplasia was not observed. This would suggest, based on comparison to the lungs of the 2100mg/m^3 rats necropsied immediately following exposure, that upon cessation of exposure, the lung begins to recover. The remaining macrophages are likely due to the persistence of portions of the inhaled material, requiring a longer breakdown and clearance process. A longer recovery period would be required to determine if further lesion regression or resolution occurs.

Transitional epithelial hyperplasia of the maxillary and nasoturbinates, was the predominant finding in all rats exposed to the Black Smoke formulation and necropsied immediately following exposure. Lesion severity and depth of affected nasal level decreased with the decline of particle concentration. Males exposed to 2100mg/m^3 were the most severely affected; in some rats in this group, lesions extended from level I, at the upper incisors, distal to level IV, the level of the first molar, with the majority

extending slightly more rostral to the level of the incisive papilla. Lesion type and location is summarized in Appendix B. In cited literature, one of the frequent sites of early morphologic evidence of injury from inhaled xenobiotics in section I is the transitional epithelium covering the distal third of the nasoturbinates and maxilloturbinates, the lateral walls, and the adjacent median septum. (Renne, 2007)

Additional findings within this study, metaplasia or dysplasia, hyperplasia of the nasal respiratory epithelium, mucus hyperplasia, erosion and epithelial degeneration are common, similar findings in other repeat dose exposure studies. Squamous metaplasia was observed in one 2100mg/m^3 male rat (Animal #11-0015). Metaplasia is the replacement of one cell type with another in response to prolonged or continued injury; generally, the replacement of a more sensitive cell type with a more resistant one. It is a rare lesion, not normally seen in healthy rats, but is prevalent in inhalation studies with irritant substances.

In most reported subchronic studies , removal of the causative agent results in regression of hyperplastic and metaplastic changes in nasal respiratory epithelium following a recovery period of a few weeks. (Renne, 2007) Degeneration with or without erosion of the olfactory epithelium of the dorsal middle meatus was noted in two male rats exposed to 2100mg/m^3 and necropsied immediately following exposure (animals #11-0015 and #11-0024). This is a common site of injury in inhaled irritants. Based on the absence of nasal cavity lesions in the majority of rats exposed to 2100mg/m^3 and allowed to recover for 4 weeks, cessation of exposure to the Black Smoke formulation demonstrates the beginning of lesion recovery/healing.

Toxicant-induced nasal lesions in laboratory animals generally exhibit characteristic, site-specific, distribution patterns. (Harkema, 2006). For example, formaldehyde induces lesions in the inspiratory airways of the nose, such as the nasal vestibule, the proximal regions of the lateral and middle medial meatus and the dorsal meatus; this pattern is attributed to airflow-driven distribution. Ozone exposure demonstrates a specific cellular distribution pattern, mucus cell metaplasia, suggestive of specific cellular susceptibility as well as airflow. The pattern of distribution in Black Smoke appears to be suggestive of an air-flow predominant driven pattern.

The presence of olfactory epithelial degeneration in two male rats exposed to 2100mg/m^3 and necropsied immediately following exposure might additionally suggest a specific cellular pattern. Olfactory tissue shows marked sensitivity to the cytotoxic effects of vinyl acetate through biotransformation pathways.(Bogdanffy MS, 2004) Polyvinyl acetate (PVA) is a minor (5%) component of the Black Smoke formulation tested. In 2- year inhalation bioassays, vinyl acetate induces nasal tumors in rats, but not in mice. However, the exposure concentrations required to produce these effects are high: 600ppm by inhalation. (Bogdanffy MS, 2004)

There are significant anatomical differences within the nasal cavity between species, rodents and dogs have more complex nasal turbinates than humans and higher primates, causing air to flow through the upper half of their nasal cavity, while airflow is

confined to the lower two-thirds in humans and primates. (Haschek, 2010) Due to the difference in target site of particular chemicals, airflow patterns may result in exposure of cell populations which differ, on the basis of morphologic and probably metabolic characteristics, between the rat and the monkey.(Morgan KT, 1990) Species differences should, therefore, be taken into account when extrapolating data from rodent inhalation studies and applying them to human exposure.

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APPENDIX A

HISTOPATHOLOGY DATA

OCKC-35-10-08-01
BLACK SMOKE (PVA) ACUTE INHALATION TOXICITY STUDY PROJECT

A. Single exposure of 2477mg/m^3 Black Smoke (+ 2 control rats) with necropsy after 14-day recovery.

Male 0mg/m^3 (control)

11-0004

1. Esophagus; trachea; larynx; thyroid gland; lung; nasal cavity; eye; intraorbital lacrimal gland; harderian gland: No significant findings.

Not examined: squamous epithelium,larynx

Gross necropsy findings: None

Male 2477mg/m^3

11-0001

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; nasal cavity; eye; harderian gland: No significant findings.

Gross necropsy findings: None

11-0002

1. Esophagus; trachea; larynx; thyroid gland; lung; tracheobronchial lymph node; nasal cavity; eye; optic nerve; harderian gland; intraorbital lacrimal gland: No significant findings.
2. Lymph node, site not specified: Sinus plasmacytosis, diffuse, moderate.

Gross necropsy findings: None

11-0003

1. Esophagus; trachea; nasal cavity; eye; harderian gland; intraorbital lacrimal gland: No significant findings.
2. Larynx, laryngeal associated lymphoid tissue: Increased lymphocytes, mild.
3. Lung, left lobe: Alveolar histiocytosis, focal, minimal with eosinophilic crystals (consistent with hemoglobin crystals).
4. Lung, BALT: Increased lymphocytes, diffuse, minimal.
5. Thyroid gland: Ultimobranchial cyst.

Not examined: Tracheobronchial lymph node.

Gross necropsy findings: None

11-0005

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; nasal cavity: No significant findings.
2. Lung, right: Lymphohistiocytic nodules, perivascular, multifocal, mild with focal subpleural nodule.

Gross necropsy findings: None

11-0006

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; trachea;.
2. Lung, BALT: Increased lymphocytes, multifocal, minimal.
3. Nasal cavity, NALT (section IV): Lymphoid hyperplasia, diffuse, mild.

Gross necropsy findings: None

Female 0mg/m^3 (control)

11-0007

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; nasal cavity: No significant findings.

Gross necropsy findings: None

Female 2477mg/m^3

11-0008

1. Esophagus; trachea; larynx; tracheobronchial lymph node; nasal cavity: No significant findings.
2. Thyroid gland: Ultimobranchial cyst, focal.
3. Lung, right: Alveolar histiocytosis, focally extensive, minimal with increased perivascular neutrophils and intrahistiocytic brown granular material.

Gross necropsy findings: None

11-0009

1. Esophagus; trachea; larynx; lung; nasal cavity: No significant findings.
2. Thyroid gland: Ultimobranchial cyst, focal.

Gross necropsy findings: None

11-0010

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tracheobronchial lymph node; nasal cavity: No significant findings.

Gross necropsy findings: None

11-0011

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tracheobronchial lymph node; nasal cavity: No significant findings.

Gross necropsy findings: None

11-0012

1. Esophagus; trachea; larynx; parathyroid gland; lung; tracheobronchial lymph node; lung; nasal cavity: No significant findings.
2. Thyroid gland: Ultimobranchial cyst, focal.

Gross necropsy findings: None

B. 20 exposures of 0 or 2100mg/m^3 Black smoke with necropsy immediately following exposure
Male 0mg/m^3 (control)

11-0014:

1. Esophagus; trachea; larynx; parathyroid gland; tracheobronchial lymph node; lung; spleen; tongue; submandibular salivary gland; thymus; cerebrum; cerebellum; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; seminiferous tubule; testes; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.
2. Liver: Lymphohistiocytic infiltrates, multifocal and random, minimal.
3. Lymph node, submandibular: Sinus plasmacytosis, diffuse, moderate.

Not examined: Mammary gland; pituitary gland

Gross necropsy findings: None

11-0016

1. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; lung; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; cerebrum; cerebellum; pituitary gland; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; seminiferous tubule; testes; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Liver: Lymphohistiocytic infiltrates, multifocal, centrilobular, minimal.
3. Submandibular lymph node, medullary sinus: Hyperplasia, plasmacytic and lymphocytic, diffuse, mild.

Not examined: parathyroid gland; rectum; adrenal medulla; optic nerve.

Gross necropsy findings: Constricted spleen; cage bedding in stomach

11-0018

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; lung; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; cecum; colon; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; harderian gland; eye: No significant findings.
2. Liver: Infiltrates, lymphohistiocytic, multifocal, random, moderate.
3. Adipose, epididymis: Infiltrates, polymorphonuclear and mononuclear, multifocal, minimal.

Not examined: Rectum; ileum; intraorbital lacrimal gland; optic nerve

Gross necropsy findings: None

11-0023

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; heart; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; testes; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung, alveoli: Infiltrates, histiocytic and neutrophilic, multifocal, mild with eosinophilic crystals , hemorrhage and erythrophagocytosis. (consistent with hemoglobin crystals).
3. Lung: Alveolar adenoma.

4. Lung, BALT: Hyperplasia, multifocal, mild.
5. Liver: Infiltrates, lymphohistiocytic, multifocal, random, mild.
6. Kidney: Infiltrates, mononuclear, interstitial, multifocal, minimal.
7. Epididymis: Infiltrates, lymphocytic, interstitial, multifocal, mild.

Not examined: optic nerve.

Gross necropsy findings: None

11-0026

1. Esophagus; trachea; larynx; parathyroid gland; lung; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; testes; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye;: No significant findings.
2. Thyroid gland: Ultimobranchial cyst, focal
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Epididymis: Infiltrates, lymphocytic, perivascular, mild.
5. Prostate gland: Infiltrates, lymphocytic, multiofocal, minimal.

Not examined: tracheobronchial lymph node, optic nerve

Gross necropsy findings: None

11-0037

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; tongue; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, focally extensive, mild
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Salivary gland, parotid: Infiltrates, lymphohistiocytic, multifocal, moderate.
5. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.

Not examined: tracheal bifurcation; adrenal medulla- only one adrenal; optic nerve; intraorbital lacrimal gland

Gross necropsy findings: None

Male 2100mg /m^3

11-0015:

1. Esophagus; trachea; larynx; liver; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; spinal cord; joint; bone; bone marrow; harderian gland; eye : No significant findings.
2. Lung: Alveolar histiocytosis, diffuse, moderate with type II pneumocyte hyperplasia, rare neutrophils and rare brown granular pigment.
3. Lung, BALT: Hyperplasia, lymphoid, multifocal, moderate.
2. Thyroid gland: Ultimobranchial cyst, focal.

4. Kidney, right: Infiltrates, lymphocytic, focal, minimal.
5. Kidney, left: Fibrosis, focally extensive, moderate with tubular loss, tubular regeneration and lymphocytic infiltrates.
6. Nasal cavity, ventral meatus, squamous epithelium (section I): Infiltrates, neutrophilic, submucosal and transmigrating with rare pustules and intraluminal neutrophils and debris.
7. Nasal cavity, maxillary and nasoturbinates, (section I and II): Transitional epithelial hyperplasia, diffuse, moderate with transmigrating neutrophils and rare microabscesses.
8. Nasal cavity, submucosa (section I, II, III, IV): Lymphocytic infiltrates, multifocal, mild.
9. Nasal cavity, olfactory epithelium, dorsomedial meatus (section II): Degeneration, focally extensive, moderate with erosions, and necrotic debris.
10. Nasal cavity, respiratory epithelium, septum (section I and II): Squamous metaplasia, multifocal, moderate with neutrophilic infiltrates.
11. Nasal cavity, respiratory epithelium, septum (section III): Erosion, multifocal, moderate with neutrophilic infiltrates, epithelial thinning and luminal debris with brown, granular material.
12. Nasal cavity, respiratory epithelium, septum (section I, II, III and IV): Epithelial hyperplasia, multifocal, moderate with mucus hyperplasia and neutrophilic infiltrates.
13. Nasal cavity, respiratory epithelium, septum (section IV): Dysplasia, focal, mild.
Not examined: parathyroid gland, optic nerve, intraorbital lacrimal gland.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; tracheobronchial lymph nodes black

<u>11-0020</u>

1. Esophagus; trachea; larynx; parathyroid gland; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; heart; kidney; adrenal gland; urinary bladder; coagulating gland; epididymis; testes; prostate; spinal cord; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Thyroid gland: Ultimobranchial cyst, focal.
3. Lung: Alveolar histiocytosis, multifocal, moderate with rare neutrophils and type II pneumocyte hyperplasia.
4. Lung, BALT: Increased lymphocytes, multifocal, minimal.
5. Liver: Infiltrates, lymphohistiocytic, multifocal, random, mild.
6. Submandibular lymph node, germinal centers: Apoptosis, increased, diffuse, mild.
7. Adipose, perigastric: Infiltrates, lymphohistiocytic, focally extensive, minimal.
8. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, diffuse, severe.
9. Nasal cavity, respiratory epithelium, septum (section I): Mucus hyperplasia, multifocal, minimal.
10. Nasal cavity, respiratory epithelium, septum (section III): Epithelial hyperplasia, multifocal, mild with rare pustules.
11. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, mediolateral meatus (section II): Epithelial hyperplasia, multifocal, moderate.
Not examined: tracheobronchial lymph node; seminal vesicle; optic nerve.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; tracheobronchial lymph nodes black

11-0024

1. Esophagus; trachea; larynx; thyroid gland; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; heart; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; spinal cord; joint; bone; bone marrow; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia, rare neutrophils and perivascular lymphocytes.
3. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild.
4. Liver: Infiltrates, lymphohistiocytic, multifocal, random, mild.
5. Kidney: Infiltrates, lymphocytic, interstitial, focal, minimal.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, dorsomedial and dorsolateral meatus (section I): Epithelial hyperplasia, diffuse, severe.
7. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, dorsomedial and dorsolateral meatus (section II): Epithelial hyperplasia, multifocal, moderate with intraluminal pyogranulomatous infiltrates.
8. Nasal cavity, respiratory epithelium, septum (section II): Epithelial hyperplasia, focal, mild with intraluminal pyogranulomatous infiltrates.
9. Nasal cavity, respiratory epithelium, septum (section III): Epithelial hyperplasia, multifocal, mild with neutrophilic infiltrates and intraluminal pyogranulomatous infiltrates.
10. Nasal cavity, olfactory epithelium, dorsomedial meatus and dorsal septum (section III): Degeneration, multifocal, moderate.
11. Nasal cavity, olfactory epithelium, 2nd and 3rd ethmoturbinate (section IV): Degeneration, multifocal, moderate with rare squamous metaplasia.
12. Heart, myocardium: Infiltrates, mononuclear, multifocal, rare.

Not examined: parathyroid gland; adrenal medulla; optic nerve; intraorbital lacrimal gland.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; tracheobronchial lymph nodes black

11-0025

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle; peripheral nerve; heart; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; spinal cord; joint; bone; bone marrow; harderian gland; eye: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphohistiocytic, diffuse, moderate.
3. Lung: Alveolar histiocytosis, multifocal, severe with type II pneumocyte hyperplasia, rare neutrophils and multinucleated giant cells.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
5. Lymph node, submandibular: Lymphoid hyperplasia, diffuse, severe.
6. Kidney: Infiltrates, lymphocytic, interstitial, focal, minimal.
7. Prostate, dorsal: Infiltrates, lymphocytic, interstitial, focal, mild.
8. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, dorsal and lateral meatus, (section I): Epithelial hyperplasia, diffuse, moderate.

9. Nasal cavity, respiratory epithelium, septum and dorsal meatus (section I): Epithelial hyperplasia, multifocal, mild with mucus hyperplasia.
10. Nasal cavity, maxillary and nasoturbinates, transitional epithelium (section II): Epithelial hyperplasia, multifocal, mild.
Not examined: mammary gland; optic nerve; intraorbital lacrimal gland; partial eye.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; tracheobronchial lymph nodes black

11-0027

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; haired skin; mammary gland; skeletal muscle; peripheral nerve; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; spinal cord; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia and few multinucleated giant cells.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Heart: Infiltrates, mononuclear, multifocal, mild with rare cardiomyocyte degeneration.
5. Epididymis: Infiltrates, lymphocytic, interstitial, focal, minimal.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, mediolateral meatus,(section I): Epithelial hyperplasia, diffuse, moderate.
7. Nasal cavity, respiratory epithelium, septum (section I): Mucus hyperplasia, multifocal, minimal.
8. Nasal cavity, transitional epithelium,maxillary and nasoturbinates, mediolateral meatus (section II): Epithelial hyperplasia, diffuse, moderate.
9. Kidney: Infiltrates, lymphocytic, interstitial, focal, minimal.
10. Nasal cavity, maxillary sinus, submucosa (section III): Infiltrates, lymphocytic, bilateral, mild.
11. Lymph node, tracheobronchial: Hyperplasia, lymphoid, mild with rare histiocytic aggregates.
12. Lung, BALT: Hyperplasia, multifocal, mild.
Not examined: adrenal medulla, rectum, optic nerve.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; tracheobronchial lymph nodes black

11-0029

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland;liver; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; kidney; adrenal gland; urinary bladder; seminal vesicle; testes; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia, rare multinucleated giant cells and lymphocytic infiltrates.
3. Lung, BALT: Hyperplasia, lymphoid, multifocal, minimal.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal with rare hepatocellular degeneration.
5. Lymph node, submandibular: Sinus plasmacytosis, diffuse, moderate.
6. Heart, myocardium: Infiltrates, histiocytic, focal, minimal with cardiomyocte degeneration.
7. Epididymis: Infiltrates, lymphocytic, interstitial, multifocal, minimal.

8. Nasal cavity, respiratory epithelium, septum (section I): Epithelial metaplasia, multifocal and bilateral, moderate.
9. Nasal cavity, transitional epithelium (section I): Epithelial hyperplasia, diffuse, severe.
10. Nasal cavity, transitional epithelium (section II): Epithelial hyperplasia, diffuse, moderate.
11. Nasal cavity, respiratory epithelium, septum (section II): Epithelial hyperplasia, bilateral, mild.
12. Nasal cavity, respiratory epithelium, septum (section III): Epithelial hyperplasia, bilateral, mild with erosion, intraepithelial abscesses and lumenal debris.
13. Nasal cavity, respiratory epithelium, septum (section I): Erosion, focal, mild with intralumenal neutrophils and debris.
14. Nasal cavity, respiratory epithelium, septum (section IV): Dysplasia, multifocal, mild.
Not examined: tracheobronchial lymph node; coagulating gland; optic nerve

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; tracheobronchial lymph nodes black; cage bedding in stomach.

Female 0mg/m^3 (control)

11-0056

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; cecum; colon; rectum; haired skin; skeletal muscle; peripheral nerve; heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye: No significant findings.
2. Lung, BALT: Increased lymphocytes, multifocal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate with rare hepatocellular degeneration.
4. Lymph node, submandibular: Plasmacytosis, medullary, diffuse, moderate.
5. Kidney: Infiltrates, lymphocytic, interstitial, focal, minimal.
6. Nasal cavity, nasoturbinate, submucosa (section I): Infiltrates, lymphocytic, multifocal, mild with rare neutrophils.
Not examined: mammary gland; ileum; intraorbital lacrimal gland; optic nerve

Gross necropsy findings:None

11-0059

1. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; lung; liver; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; uterus; ovary; spinal cord; nasal cavity; joint; bone; bone marrow; harderian gland; eye: No significant findings.
2. Salivary gland, parotid: Infiltrates, mononuclear, focal, mild.
Not examined: parathyroid gland; urinary bladder; optic nerve; intraorbital lacrimal gland.

Gross necropsy findings: None

11-0062

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; lung; spleen; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum;

haired skin; mammary gland; skeletal muscle; heart; kidney; adrenal gland; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
Not examined: pituitary gland; mammary gland; femoral nerve; optic nerve; uterus; ovary; urinary bladder

Gross necropsy findings: Cage bedding in stomach and intestine

11-0063

1. Esophagus; trachea; larynx; tracheobronchial lymph node; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; haired skin; mammary gland; skeletal muscle; peripheral nerve; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Lymph node, submandibular: Plasmacytosis, focal, minimal.
6. Heart: Infiltrates, mononuclear, focal, mild.
Not examined: parathyroid gland;rectum; adrenal medulla, 1 adrenal; minimal evaluation of urinary bladder mucosa.

Gross necropsy findings: Cage bedding in stomach and intestine

11-0065

1 Esophagus; trachea; larynx; parathyroid gland; lung; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Lymph node, submandibular: Plasmacytosis, multifocal, mild.
6. Nasolacrimal duct, submucosa (section III): Infiltrates, lymphocytic, multifocal and bilateral, mild.
Not examined:mammary gland; tracheobronchial lymph node; optic nerve.

Gross necropsy findings: Cage bedding in stomach and intestine

11-0075

1. Esophagus; trachea; larynx; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle; peripheral nerve; heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.
3. Liver: Infiltrates, lymphohistiocytic, multifocal, mild.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Lymph node, submandibular: Plasmacytosis, multifocal, minimal.
6. Kidney, right, interstitium: Infiltrates, lymphocytic, focal, minimal.
7. Kidney, glomerulus, left: Amyloid, focal, minimal.

Not examined: Tracheobronchial lymph node; mammary gland; optic nerve.

Gross necropsy findings: None

Female 2100mg/m^3

11-0054

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, moderate.
3. Lung: Alveolar histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia, rare neutrophils and multinucleated giant cells.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate with rare hepatocellular degeneration.
5. Lymph node, submandibular: Hyperplasia, lymphocytic, diffuse, moderate with sinus plasmacytosis.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, mediolateral meatus (section I): Epithelial hyperplasia, diffuse, moderate.
7. Nasal cavity, transitional epithelium, nasoturbinates (section II): Epithelial hyperplasia, multifocal, minimal.

Not examined:Intraorbital lacrimal gland.

Gross necropsy findings: Cranio-ventral right and left lung mottled black; tracheobronchial lymph nodes black; cage bedding in stomach and intestines

11-0061

1. Esophagus; trachea; larynx; parathyroid gland; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye;: No significant findings.
2. Thyroid gland: Ultimobrachial cyst.
3. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, moderate.
4. Lung: Alveolar histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia and rare neutrophils.
5. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
6. Spleen: Lymphocytic hyperplasia, diffuse, mild.
7. Lymph node, submandibular: Sinus plasmacytosis, diffuse, severe with lymphoid hyperplasia.
8. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, mediolateral meatus (section I): Epithelial hyperplasia, diffuse, mild.

Not examined: mammary gland; intraorbital lacrimal gland; optic nerve.

Gross necropsy findings: Cranio-ventral right and left lung mottled black; tracheobronchial lymph nodes black; cage bedding in stomach and intestines

11-0064

Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; haired skin; mammary gland; skeletal muscle; peripheral nerve;

heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphocytic, diffuse, moderate with rare histiocytic aggregates.
3. Lung: Histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia, few neutrophils and multinucleated giant cells.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
5. Lymph node, submandibular: Lymphoid hyperplasia, diffuse, moderate with sinus plasmacytosis.
6. Kidney: Mineral, focal, minimal.
7. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, mediolateral meatus (section I): Epithelial hyperplasia, diffuse, mild.
8. Nasal cavity, respiratory epithelium, ventral septum (section III): Epithelial hyperplasia, multifocal, minimal with focal erosion and neutrophilic infiltrates.
Not examined: rectum.

Gross necropsy findings: Cranio-ventral right and left lung mottled black; tracheobronchial lymph nodes black; cage bedding in stomach and intestines

<u>11-0066</u>
1. Esophagus; trachea; larynx; parathyroid gland; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate, with type II pneumocyte hyperplasia.
3. Liver: Infiltrates, lymphohistiocyitic, multifocal and random, mild.
4. Lymph node, submandibular: Hyperplasia, lymphocytic, diffuse, moderate.
5. Thyroid gland: Ultimobranchial cyst.
6. Kidney, right: Mineralization, multifocal, mild.
7. Kidney, left: Mineralization, corticomedullary junction, moderate.
8. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, mediolateral meatus (section I): Epithelial hyperplasia, multifocal, mild.
9. Nasal cavity, transitional epithelium (section II): Epithelial hyperplasia, multifocal, minimal
Not examined: tracheobronchial lymph node, optic nerve.

Gross necropsy findings: Cranio-ventral right and left lung mottled black; tracheobronchial lymph nodes black; cage bedding in stomach and intestines

<u>11-0067</u>
1. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Lymph node, submandibular gland: Plasmacytosis, multifocal, minimal.
5. Kidney, interstitium, left: Infiltrates, lymphocytic, focal, minimal.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.

7. Nasal cavity, ventral meatus, squamous epithelium (section III): Infiltrates, neutrophilic, focal, minimal.
Not examined: parathyroid gland; optic nerve; intraorbital lacrimal gland.

Gross necropsy findings: Cranio-ventral right and left lung mottled black; tracheobronchial lymph nodes black; cage bedding in stomach and intestines

11-0070

1. Esophagus; trachea; larynx; parathyroid gland; tracheobronchial lymph node; spleen; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.
3. Lung: Alveolar histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia and multinucleated giant cells.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
5. Lymph node, submandibular: Hyperplasia, lymphocytic, diffuse, moderate.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.
Not examined: adrenal medulla in section examined; mammary gland; optic nerve.

Gross necropsy findings: right and left lung, moderately black, cranial aspect

C. 20 exposures of 100mg or 700mg Black Smoke with necropsy immediately following exposure
Male 130mg/m^3

11-0017

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; testes; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
4. Heart: Myocardial necrosis, focal with histiocytic infiltrates.
5. Epididymes: Infiltrates, lymphocytic, multifocal, minimal.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.

Not examined: tracheobronchial lymph node; optic nerve; intraorbital lacrimal gland.

Gross necropsy findings: Right and left craniodorsal lung black mottled; tracheobronchial lymph nodes black

11-0019

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; testes; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung, BALT: Hyperplasia, multifocal, minimal.
3. Lung: Alveolar histiocytosis, multifocal, minimal.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
5. Heart: Infiltrates, mononuclear, multifocal, moderate with myocardial necrosis.
6. Epididymes: Infiltrates, lymphocytic, interstitium, multifocal, minimal.
7. Prostate: Infiltrates, lymphohistiocytic, multifocal, moderate.
8. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.

Not examined: tracheobronchial lymph node; optic nerve

Gross necropsy findings: Lungs heavy, expanded; right cranioventral lungs are mildly black mottled

11-0034

1. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; lung; spleen; heart; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Kidney, left: Infiltrates, interstitial, lymphocytic, focal, minimal.
4. Prostate: Infiltrates, interstitial, lymphocytic, multifocal, mild.
5. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.

Not examined: parathyroid gland; optic nerve

Gross necropsy findings: Cage bedding in stomach and intestines

11-0038

1. Esophagus; trachea; larynx; thyroid gland; spleen; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal with rare hepatocellular necrosis.
4. Heart: Infiltrates, mononuclear, focal, minimal with rare myocardial degeneration.
5. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.
Not examined: parathyroid gland; tracheobronchial lymph node; optic nerve

Gross necropsy findings: Right cranial and middle lobes diffusely black; left lung slightly mottled black

11-0048

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; spleen; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; epididymis; testes; prostate; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
4. Heart: Infiltrates, mononuclear, multifocal, minimal with rare myocardial necrosis.
5. Nasal cavity, transitional epithelium, nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.
Not examined: coagulating gland; intraorbital lacrimal gland; optic nerve

Gross necropsy findings: Right and left lung black mottled; slight increase in right cranial and middle lobes

11-0049

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; spleen; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; testes; prostate; harderian gland; eye; optic nerve: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.
3. Lung, BALT: Hyperplasia, lymphoid, multifocal, minimal.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
5. Heart: Infiltrates, multifocal, mild with rare myocardial necrosis.
6. Epididymes: Infiltrates, interstitial, lymphocytic, multifocal, minimal.
7. Prostate: Infiltrates, interstitial, lymphocytic, multifocal, minimal.
8. Nasal cavity, transitional epithelium, nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.
Not examined: tracheobronchial lymph node; optic nerve; intraorbital lacrimal gland.

Gross necropsy findings: Right cranial and medial lung lobes diffusely black; left lung slightly mottled black

Male 670mg/m^3

11-0013

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung, BALT: Hyperplasia, multifocal, minimal.
3. Lung: Alveolar histiocytosis, multifocal, moderate.

4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
5. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
6. Prostate: Infiltrates, lymphocytic, perivascular, multifocal, minimal.
7. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, dorsal and mediolateral meatus (section I): Epithelial hyperplasia, diffuse, mild.
Not examined: tracheobronchial lymph node; adrenal medulla in sections examined; optic nerve.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; black tracheobronchial lymph nodes.

11-0021

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; heart; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; harderian gland; eye: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, moderate.
3. Lung: Alveolar histiocytosis, multifocal, moderate with type II pneumocyte hyperplasia.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
5. Kidney, left: Tubular ectasia, focal, mild with fibrosis, lymphocytic infiltrates and rare tubular basophilia.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, dorsal and mediolateral meatus(section I): Epithelial hyperplasia, diffuse, mild.
7. Nasal cavity, respiratory epithelium, septum (section I): Mucus hyperplasia, multifocal, minimal.
8. Nasal cavity, transitional epithelium, nasoturbinates(section II): Epithelial hyperplasia, multifocal, mild.
Not examined: intraorbital lacrimal gland; optic nerve.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; black tracheobronchial lymph nodes.

11-0022

1. Esophagus; trachea; larynx; thyroid gland; spleen; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; testes; prostate; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, mild with rare type II pneumocyte hyperplasia and multinucleated giant cells.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
4. Epididymes, interstitium: Infiltrates, lymphocytic, multifocal, minimal.
5. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, moderate with subepithelial gland hyperplasia.
6. Nasal cavity, respiratory epithelium, septum (section II): Epithelial hyperplasia, focally extensive, mild with erosion and neutrophilic infiltrates.
Not examined: parathyroid gland; tracheobronchial lymhph node; optic nerve.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; black tracheobronchial lymph nodes.

11-0030

1. Esophagus; trachea; larynx; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; intraorbital lacrimal gland; harderian gland; eye: No significant findings.

2. Thyroid gland: Ultimobranchial cyst.
3. Lung: Alveolar histiocytosis, multifocal, mild with rare neutrophils, and multinucleated giant cells.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
5. Spleen: Hemosiderosis, focal, minimal.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.
7. Nasal cavity, olfactory epithelium, septum (section IV): Degeneration, focal, minimal.
8. Nasal cavity, olfactory epithelium, dorsolateral meatus (section III): Degeneration, focal, minimal.
Not examined: tracheobronchial lymph node; parathyroid gland; optic nerve.

Gross necropsy findings: Cranio-dorsal aspect of right and left lung mottled black; black tracheobronchial lymph nodes.

<u>11-0033</u>
1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; testes; prostate; intraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, moderate with few foamy macrophages.
3. Lung: Alveolar histiocytosis, multifocal, mild with rare type II pneumocyte hyperplasia.
4. Lung, BALT: Hyperplasia, multifocal, minimal.
5. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
6. Kidney, left: Tubular basophilia, focal, minimal.
7. Epididymes, interstitium: Infiltrates, lymphocytic, multifocal, minimal.
8. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, moderate.
9. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.
10. Nasal cavity, respiratory epithelium, dorsal septum (section I): Mucus cell hyperplasia, multifocal, mild.
11. Nasal cavity, respiratory epithelium, septum (section III): Erosion, focally extensive, mild with cellular debris.
12. Nasal cavity, NALT (section IV): Hyperplasia, lymphoid, diffuse, mild.

Gross necropsy findings: Right lung deep black; cranio-ventral aspect of left lung mottled black; black tracheobronchial lymph nodes.

<u>11-0036</u>
1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; testes; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild.
3. Lung: Alveolar histiocytosis, multifocal, mild with rare type II pneumocytes.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
5. Heart: Myocardial degeneration, multifocal, with lymphohistiocytic infiltrates.
6. Epididymes: Infiltrates, interstitial, lymphocytic, multifocal, mild.
7. Prostate: Infiltrates, interstitial and glandular, lymphocytic, multifocal, moderate.
8. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, dorsal meatus(section I): Epithelial hyperplasia, multifocal, moderate.
9. Nasal cavity, respiratory epithelium, septum (section I): Mucus hyperplasia, multifocal, minimal.

10. Nasal cavity, transitional epithelium, nasoturbinates (section II): Epithelial hyperplasia, multifocal, mild.
Not examined: optic nerve.

Gross necropsy findings: Cranio-ventral aspect of right and left lung mottled black

Female 130mg/m^3

11-0058

1. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; spleen; haired skin; mammary gland; heart; adrenal gland; urinary bladder; uterus; ovary; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Kidney, left: Mineral, multifocal, mild.
5. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.

Not examined: parathyroid gland; intraorbital lacrimal gland; optic nerve

Gross necropsy findings: Cage bedding in stomach and intestine.

11-0071

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; spleen; haired skin; mammary gland; heart; adrenal gland; urinary bladder; uterus; ovary; intraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.
3. Lung: Osseous metaplasia, focal, minimal.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
5. Kidney, left and right: Mineral, focal, minimal.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.

Not examined: optic nerve.

Gross necropsy findings: Mild black staining of lungs; cage bedding in stomach and intestine

11-0076

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; haired skin; mammary gland; heart; adrenal gland; urinary bladder; uterus; ovary; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
4. Spleen, white pulp: Hyperplasia, lymphoid, diffuse, minimal
5. Kidney, left and right: Mineral, multifocal, mild.
6. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.

Not examined: tracheobronchial lymph node; optic nerve

Gross necropsy findings: Slight discoloration of lungs; cage bedding in stomach and intestines

11-0086

1. Esophagus; trachea; larynx; thyroid gland; spleen; haired skin; mammary gland; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; harderian gland; eye: No significant findings.

2. Lung: Alveolar histiocytosis, multifocal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Eyelid: Pyogranuloma, focal, moderate.
5. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.
Not examined: parathyroid gland; tracheobronchial lymph node; intraorbital lacrimal gland; optic nerve

Gross necropsy findings: Slight discoloration of lungs; tracheobronchial lymph nodes black; cage bedding in stomach and intestines

11-0091

1. Esophagus; trachea;parathyroid gland; spleen; haired skin; mammary gland; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; harderian gland; eye: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.
3. Larynx, lumen: Neutrophils and macrophages, few, with mucus.
4. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild.
5. Lung: Alveolar histiocytosis, multifocal, mild.
6. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
7. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.
8. Nasal cavity, transitional epithelium , maxillary and nasoturbinates (section II): Epithelial hyperplasia, multifocal, minimal.
Not examined: intraorbital lacrimal gland; optic nerve.

Gross necropsy findings:Moderate staining of lungs; tracheobronchial lymph nodes black; cage bedding in stomach and intestine.

11-0092

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; liver; spleen; haired skin; mammary gland; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; intraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild.
3. Lung: Alveolar histiocytosis, multifocal, mild.
4. Kidney, right: Mineral, focal, minimal.
5. Nasal cavity, transitional epithelium, nasoturbinates (section I): Epithelial hyperplasia, multifocal, minimal.
Not examined: mammary gland; optic nerve.

Gross necropsy findings: Mild staining of lungs; black tracheobronchial lymph nodes.

Female 670mg/m^3

11-0055

1. Esophagus; trachea; larynx; thyroid gland; lung; liver; spleen; haired skin; mammary gland; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate with rare neutrophils and type II pneumocyte hyperplasia.
3. Spleen, white pulp: Hyperplasia, lymphoid, mild.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
5. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.
6. Lung, BALT: Hyperplasia, lymphoid, focal, mild with histiocytic aggregates.

Not examined: parathyroid gland; tracheobronchial lymph node; intraorbital lacrimal gland; optic nerve

Gross necropsy findings: Cranio-ventral aspect of right and left lung mottled black; black tracheobronchial lymph nodes

11-0060

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; spleen; haired skin; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate with rare type II pneumocyte hyperplasia.
3. Liver: Infiltrates, lymphohistiocytic, multifocal, random, mild.
4. Nasal cavity, transitional epithelium, nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.

Not examined: Mammary gland; optic nerve; only 1 adrenal gland

Gross necropsy findings: Slight lung discoloration; black tracheobronchial lymph nodes; cage bedding in stomach.

11-0069

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; spleen; haired skin; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild.
3. Lung: Alveolar histiocytosis, multifocal, moderate with rare neutrophils and multinucleated giant cells intracytoplasmic brown granular material.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
5. Nasal cavity, transitional epithelium, maxillary and nasoturbinates, mediolateral meatus (section I): Epithelial hyperplasia, multifocal, mild.

Not examined: Mammary gland; optic nerve

Gross necropsy findings: Cranio-ventral aspect of right and left lung mottled black; black tracheobronchial lymph nodes

11-0072

1. Esophagus; trachea; larynx; thyroid gland; spleen; haired skin; mammary gland; heart; adrenal gland; urinary bladder; uterus; ovary; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate with rare type II pneumocyte hyperplasia.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Kidney, right and left: Mineral, multifocal, mild.
5. Nasal cavity, transitional epithelium, nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.
6. Nasal cavity, transitional epithelium, nasoturbinates (section II): Epithelial hyperplasia, multifocal, minimal.
7. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, moderate.

Not examined: parathyroid gland; intraorbital lacrimal gland; optic nerve

Gross necropsy findings: Cranio-ventral aspect of right and left lung mottled black; black tracheobronchial lymph nodes; cage bedding in stomach and intestine

11-0073

1. Esophagus; trachea; parathyroid gland; spleen; haired skin; mammary gland; heart; adrenal gland; urinary bladder; uterus; ovary; harderian gland; eye: No significant findings.
2. Larynx: Hyperplasia, lymphocytic, focal, minimal.
3. Thyroid gland: Ultimobranchial cyst.
4. Lymph node, tracheobronchial: Hyperplasia, lymphocytic, diffuse, mild.
5. Lung: Alveolar histiocytosis, multifocal, mild.
6. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
7. Kidney, right: Mineral, focal, minimal.
8. Nasal cavity, respiratory epithelium, septum (section I): Mucus hyperplasia, multifocal, mild.
9. Nasal cavity, transitional epithelium, maxillary and nasoturbinates (section I): Epithelial hyperplasia, multifocal, mild.
10. Nasal cavity, transitional epithelium, nasoturbinates (section II): Epithelial hyperplasia, multifocal, mild.

Not examined: intraorbital lacrimal gland; optic nerve.

Gross necropsy findings: Cranio-ventral aspect of right and left lung mottled black; white raised area on lung; black tracheobronchial lymph nodes; Cage bedding in stomach, intestine, cecum

11-0094

1. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; spleen; haired skin; mammary gland; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; intraorbital lacrimal gland; harderian gland; eye: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, moderate with few neutrophils and type II pneumocyte hyperplasia.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Nasal cavity, transitional epithelium, maxillary and nasoturbinates and mediolateral meatus (section I): Epithelial hyperplasia, multifocal, moderate.
5. Nasal cavity, transitional epithelium, nasoturbinates (section II): Epithelial hyperplasia, multifocal, minimal.
6. Nasal cavity, respiratory epithelium, septum (section III): Hyperplasia, focally extensive, mild with erosin, lumenal neutrophils and brown granular debris.
7. Heart: Infiltrates, histiocytic, focal, minimal.

Not examined: parathyroid gland; optic nerve.

Gross necropsy findings: Moderate staining in lungs; black tracheobronchial lymph nodes

D. 20 exposures of 0 or 2000mg Black Smoke with necropsy after 1-month recovery period

Male 0mg/m^3

11-0043

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; nasal cavity: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.

Gross necropsy findings: None

11-0047

1. Esophagus; trachea; larynx; thyroid gland; lung; nasal cavity: No significant findings.

Not examined: parathyroid gland; tracheobroncial lymph node.

Gross necropsy findings: Cage bedding in stomach.

11-0050

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; lung; nasal cavity: No significant findings.

Gross necropsy findings:None

11-0051

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; lung; nasal cavity: No significant findings.

Gross necropsy findings: None

11-0052

1. Esophagus; trachea; larynx; thyroid gland; nasal cavity: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, minimal.

Not examined: parathyroid gland; tracheobronchial lymph node.

Gross necropsy findings: None

11-0053

1. Esophagus; trachea; larynx; parathyroid gland; tracheobronchial lymph node; nasal cavity: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.
3. Lung, BALT: Hyperplasia, lymphoid, multifocal, minimal.

Gross necropsy findings:None

Male 2100mg/m^3

11-0031

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild with few macrophage aggregates.
3. Lung: Alveolar histiocytosis, multifocal, mild.
4. Nasal cavity, respiratory epithelium, ventral septum (level III): Hyperplasia, focal, minimal with neutrophilic infiltrates.

Gross necropsy findings: Right and left lungs multifocal black mottling; cage bedding in stomach

11-0032

1. Esophagus; larynx; thyroid gland; parathyroid gland: No significant findings.
2. Trachea, lumen: Macrophages, rare with mucus.
3. Lung: Alveolar histiocytosis, multifocal, mild with rare neutrophils.
4. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild with few macrophage aggregates.
5. Nasal cavity, respiratory epithelium, septum (level II): Hyperplasia, focal, minimal with neutrophilic infiltrates.

Gross necropsy findings: Right and left lungs multifocal black mottling; Tracheobronchial lymph nodes black

11-0035

1. Esophagus; trachea; larynx; parathyroid gland; nasal cavity: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.
3. Lung: Alveolar histiocytosis, multifocal, mild.
4. Lung: Eosinophilic crystals, multifocal, minimal with neutrophils.

Not examined: tracheobronchial lymph node

Gross necropsy findings:Liver: diffuse reticular pattern.

11-0039

1. Esophagus; trachea; larynx; parathyroid gland: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.
3. Lung, BALT: Hyperplasia, multifocal, mild.
4. Lung, Alveolar histiocytosis, multifocal, mild.
5. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild with few macrophage aggregates.
6. Nasal cavity, respiratory epithelium, septum (level III): Hyperplasia, multifocal, mild with neutrophilic infiltrates and intralumenal degenerate macrophages, neutrophils and brown granular debris.

Gross necropsy findings: Right and left lungs multifocal black mottling; Tracheobronchial lymph nodes black; bent tail

11-0040

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, diffuse, mild with moderate macrophage aggregates.
3. Lung: Alveolar histiocytosis, multifocal, mild.
4. Nasal cavity, respiratory epithelium, septum (level III): Hyperplasia, multifocal, mild with neutrophilic infiltrates, intraluminal debris and degenerate neutrophils.

Gross necropsy findings: Right and left lungs multifocal black mottling (right lung worse than left); Tracheobronchial lymph nodes black

11-0041

1. Esophagus; trachea; larynx: No significant findings.
1. Lung: Alveolar histiocytosis, multifocal, mild.
2. Lung: Eosinophilic crystals, multifocal, minimal with rare neutrophils.
3. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild with few macrophage aggregates.

4. Nasal cavity, nasolacrimal duct, lumen (level II): Cellular and eosinophilic debris, rare.
5. Nasal cavity, respiratory epithelium, septum and maxillary turbinates (level III): Hyperplasia, multifocal, moderate with erosion and neutrophilic infiltrates.
6. Nasal cavity, nasolacrimal duct, lumen (level III): Cellular, eosinophilic and brown granular debris.
Not examined: thyroid, parathyroid gland

Gross necropsy findings: Right and left lungs multifocal black mottling (right lung worse than left); Tracheobronchial lymph nodes black

Female 0mg/m^3

11-0077

1. Esophagus; trachea; larynx; thyroid gland; lung; nasal cavity: No significant findings.
Not examined: tracheobronchial lymph node; parathyroid gland

Gross necropsy findings: Right and left cranial lungs red; cage bedding in gastrointestinal system; stomach black and red-tinged

11-0078

1. Esophagus; trachea; larynx; lung; nasal cavity: No significant findings.
Not examined: tracheobronchial lymph node; thyroid gland; parathyroid gland

Gross necropsy findings: Cage bedding in cecum and stomach

11-0079

1. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; lung; nasal cavity: No significant findings.
Not examined: parathyroid gland

Gross necropsy findings: Cage bedding in cecum; jejunum and colon; multifocal white raised nodules on colon

11-0084

1. Esophagus; trachea; larynx; parathyroid gland; tracheobronchial lymph node; lung; nasal cavity: No significant findings.
2. Thyroid gland: Ultimobranchial cyst.

Gross necropsy findings: Uterus: dilated and fluid filled

11-0085

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; nasal cavity: No significant findings.
Not examined: tracheobronchial lymph no*de*

Gross necropsy findings: None

11-0087

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; nasal cavity: No significant findings.
Not examined: tracheobronchial lymph node

Gross necropsy findings: None

Female 2100mg/m^3

11-0074

1. Esophagus; trachea; larynx; thyroid gland; nasal cavity; adipose: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, mild.

Not examined: parathyroid gland; tracheobronchial lymph node

Gross necropsy findings: Diffuse blue-gray tinged subcutaneous and visceral fat; Right and left lung multifocally black; tracheobronchial lymph nodes black

11-0081

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; nasal cavity; adipose: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse with histiocytic nodules.
3. Lung: Alveolar histiocytosis, multifocal, mild.

Gross necropsy findings: Diffuse blue-gray tinged subcutaneous and visceral fat; Right and left lung multifocally black; tracheobronchial lymph nodes black

11-0088

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; adipose; nasal cavity: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse with histiocytic nodules.
3. Lung: Alveolar histiocytosis, multifocal, mild.

Gross necropsy findings: Diffuse blue-gray tinged subcutaneous and visceral fat; Right and left lung multifocally black; tracheobronchial lymph nodes black; Orange/brown fluid in jejunum

11-0089

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; nasal cavity: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, mild.

No: tracheobronchial lymph node

Gross necropsy findings: Diffuse blue-gray tinged subcutaneous and visceral fat; Right and left lung multifocally black; tracheobronchial lymph nodes black; cage bedding in jejunum and cecum

11-0090

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland: No significant findings.
2. Lung: Alveolar histiocytosis, multifocal, mild.
3. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild with moderate macrophage aggregates.
4. Nasal cavity, respiratory epithelium, septum (level III): Hyperplasia, multifocal, minimal with neutrophilic infiltrates.

Gross necropsy findings: Diffuse blue-gray tinged subcutaneous and visceral fat; Right and left lung multifocally black; tracheobronchial lymph nodes black

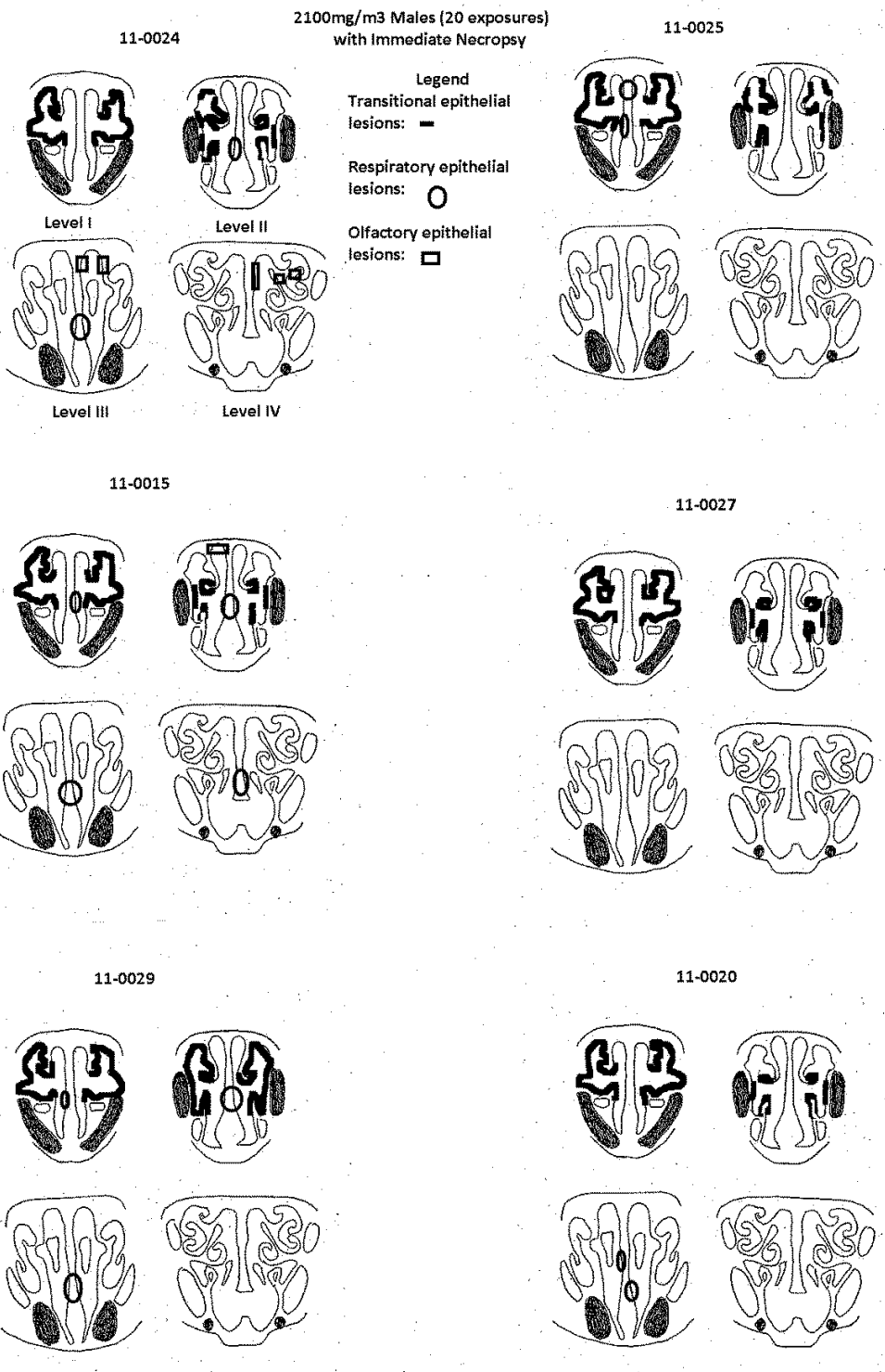
11-0093

1. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; nasal cavity: No significant findings.
2. Lymph node, tracheobronchial: Hyperplasia, lymphoid, diffuse, mild with few histiocytic aggregates.
3. Lung: Alveolar histiocytosis, multifocal, mild.

Gross necropsy findings: Diffuse blue-gray tinged subcutaneous and visceral fat; Right and left lung multifocally black; tracheobronchial lymph nodes black; decreased peri-renal fat

APPENDIX B

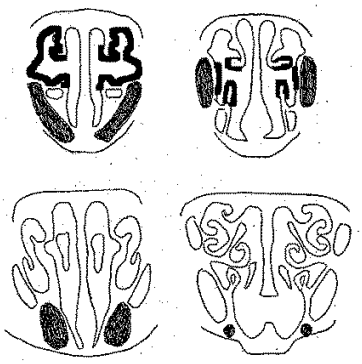
NASAL DIAGRAMS



NOTE: Basic nasal diagrams utilized in Appendix C were extracted from:
Mery S, Gross EA, Joyner DR, Godo M and Morgan KT (1994) Nasal diagrams: A tool for recording the distribution of nasal lesions in rats and mice. Toxicol Path. 22(4): 353-372

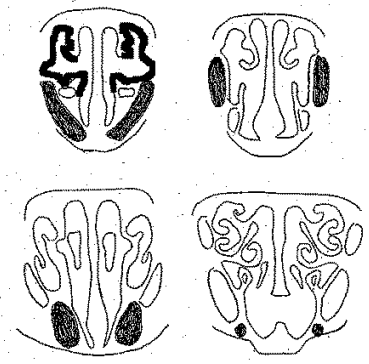
2100mg/m3 Females (20 exposures)
with Immediate Necropsy

11-0054



Legend

Transitional epithelial lesions: ■



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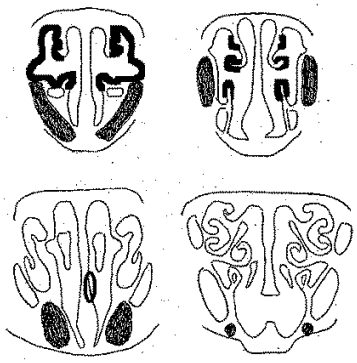

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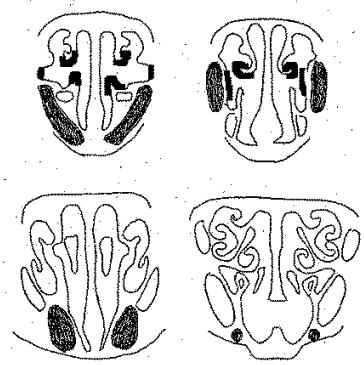

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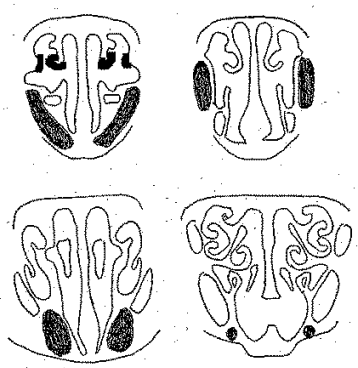
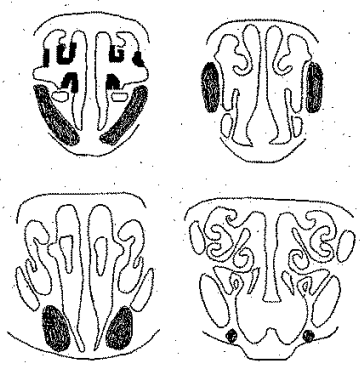


11-0070

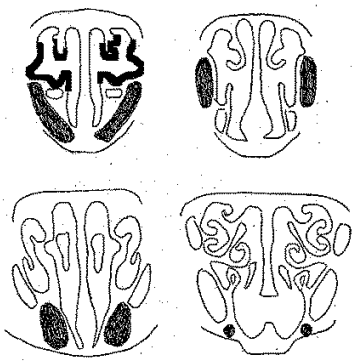


670mg/m3 Males (20 exposures)
with immediate Necropsy

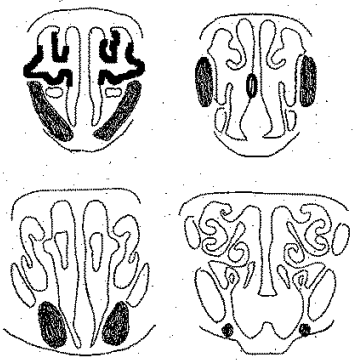
Legend

Transitional epithelial lesions: ▬

11-0013



11-0022



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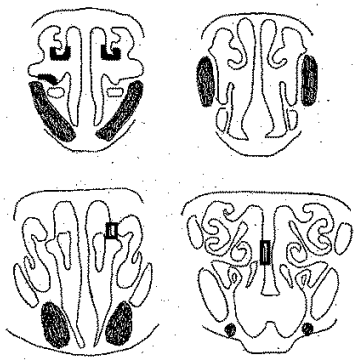

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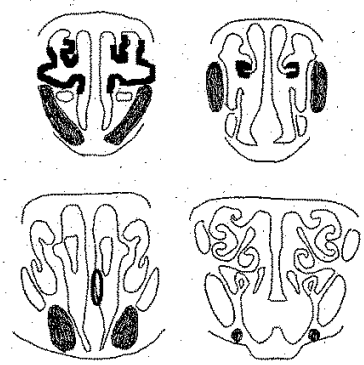

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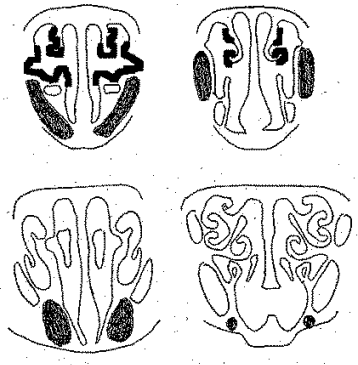

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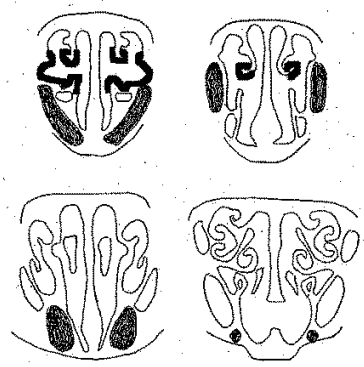
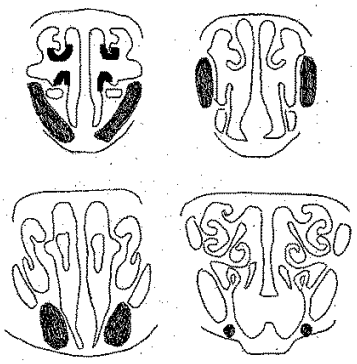


670mg/m3 Females (20 exposures)
with Immediate Necropsy

Legend

Transitional epithelial lesions: ▬

11-0055



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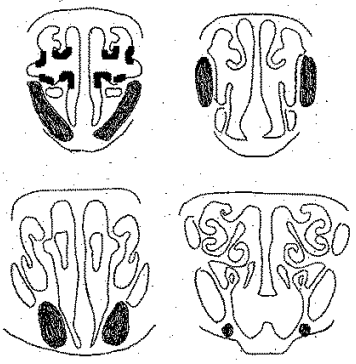

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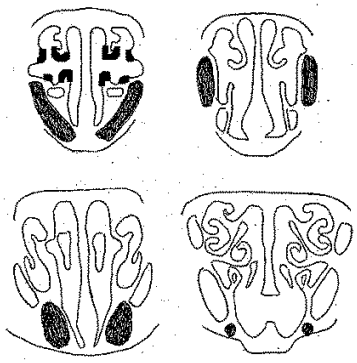

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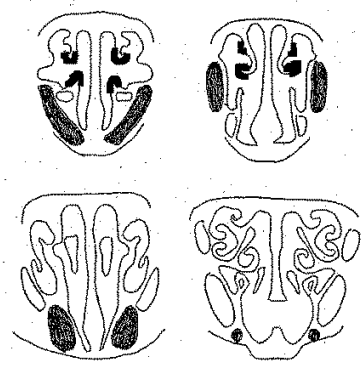

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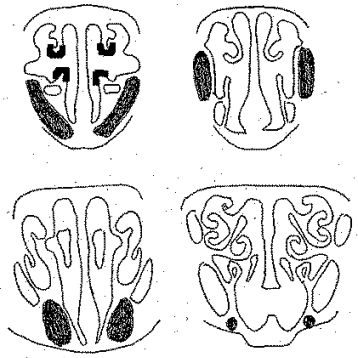

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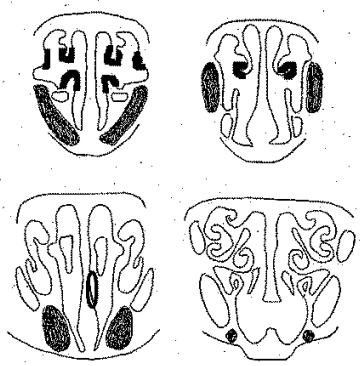
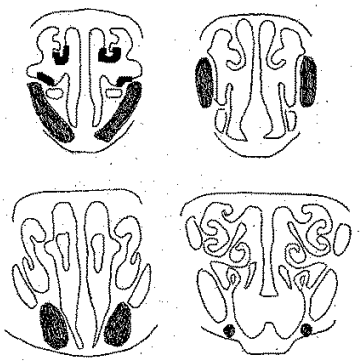


130mg/m3 Males (20 exposures)
with Immediate Necropsy

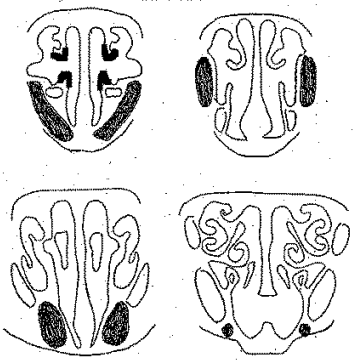
11-0017



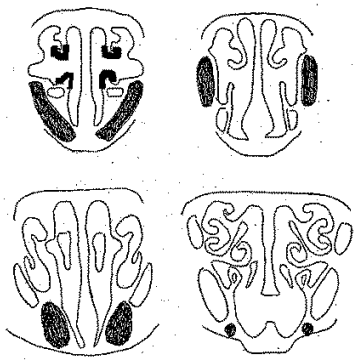
Legend

Transitonal epithelial lesions: ▬

11-0019



11-0034



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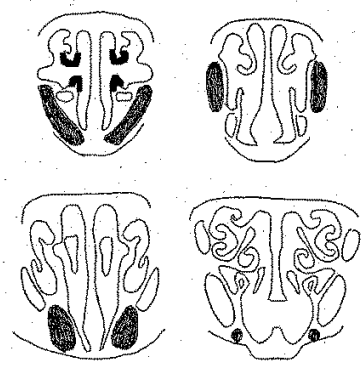

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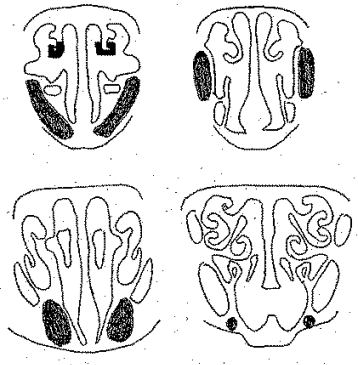

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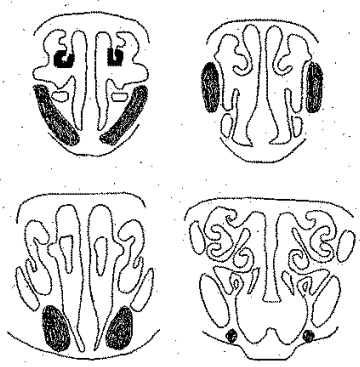
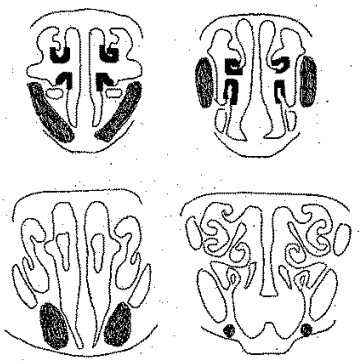


130mg/m3 Females (20 exposures)
with Immediate Necropsy

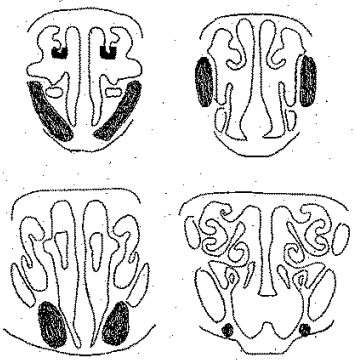
11-0091



Legend

Transitional epithelial lesions: ▬

11-0092



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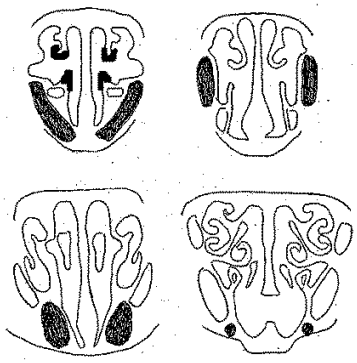

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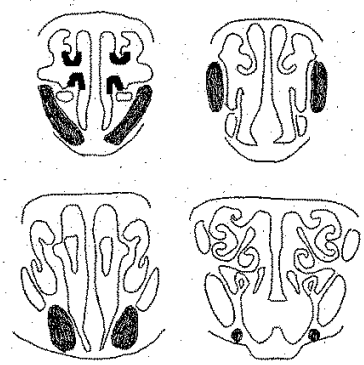

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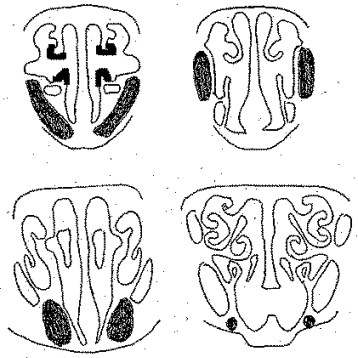

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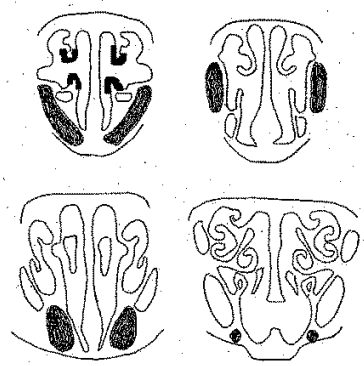


APPENDIX C

DEFINITION OF SEVERITY GRADES

DEFINITION OF SEVERITY GRADES

Lung, BALT: Hyperplasia, lymphoid
Minimal = minimal elevation of mucosa, mainstem bronchi; affecting one lung lobe
Mild = mild elevation of mucosa, mainstem bronchi with 1-2 increased bronchiolar lymphocytes; affecting more than one lung lobe.
Moderate = moderate elevation of mucosa, mainstem bronchi with increased bronchiolar lymphocytes, greater than mild criteria; affecting majority of lung lobes.
Severe = moderate to severe elevation of mucosa, mainstem bronchi and bronchiolar lymphocytes with or without follicle formation and generally affecting all lung lobes.

Liver: Infiltrates, lymphohistiocytic (microgranulomas)
Minimal = 0-4 foci
Mild = 5-14 foci
Moderate = 15-25 foci
Severe = > 25 foci

Lymph node: Plasmacytosis
Minimal = few plasma cells within sinuses with majority of cells lymphocytes and macrophages
Mild = increased numbers of plasma cells than minimal with resident lymphocytes and macrophages outnumbering plasma cells
Moderate = plasma cells filling sinuses, outnumbering resident lymphocytes and macrophages
Severe = plasma cells diffusely expanding sinuses with few resident lymphocytes and macrophages, with or without expansion into cortex

Lung: Alveolar histiocytosis
Minimal = minimal increase of alveolar macrophages from resident cells, affecting 1 or more lung lobes
Mild = mild increase of alveolar macrophages, multifocal distribution, affecting 25-50% of lung lobe, few lobes involved
Moderate = moderate increase of alveolar macrophages, multifocal distribution, affecting 50-75% of lung lobe, majority of lobes involved
Severe = severe increase of alveolar macrophages, multifocal to diffuse distribution, affecting > 75% of lung lobe, majority of lobes involved

Nasal cavity: Epithelia hyperplasia
Minimal = focal to no greater than two small, affected areas distributed within normal anatomical location, 1 cell thickness greater than normal.
Mild = focally extensive or greater than two affected areas but less than diffuse distributed within normal anatomical location, 1-2 cell thickness greater than normal.
Moderate = diffuse distribution within normal anatomical location, 1-3 cell thickness greater than normal.
Severe = diffuse distribution within normal anatomical location, 4 cells or greater thickness, with or without synechiae of turbinates

Heart: Infiltrates, mononuclear
Minimal = focal, few cells
Mild = multifocal, few cells
Moderate = focally extensive or multifocal with moderate number of cells, affecting some surrounding tissue architecture
Severe = focally extensive or multifocal with numerous cells, significantly affecting surrounding tissue architecture

APPENDIX D

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The portion of the study described in this contributing scientist report, including the tissue processing piece conducted at the United States Army Medical Research Institute of Chemical Defense's Comparative Pathology Branch, was conducted in compliance with Title 40, Code of Federal Regulations (CFR) Part 792, Good Laboratory Practice Standards.

E-Signed by WALLACE.SHANNON.MARIE.1068279042
VERIFY authenticity with ApproveIt
WALLACE.SHANNON.MARIE.1068279042

_____ ___15 Apr 2013__
Shannon M. Wallace, DVM, DACVP Date
LTC, VC
Pathologist, Toxicology

Army Institute of Public Health

Appendix P

Statistical Analysis Report

Statistical analysis.

Hematology, clinical chemistry, and coagulation statistical analyses were performed with SPSS© for Windows, Release 16.0.0, (SPSS, Chicago, IL, USA). Statistical significance was defined at the $p < 0.05$ level. The male and female data were analyzed separately. Data from necropsies immediately following the 4-week exposure were analyzed with one-factor analysis of variance (ANOVA) using dose as the between-subject factor. When differences among treatments were detected, a two-sided Dunnett's post-hoc test was performed to compare controls to treatments. Data from necropsies following the 4-week recovery period were analyzed with an independent sample T-Test since only two treatments were represented (e.g., control and high concentration). For each analysis, data for each physiological variable were combined across treatments and tested for normality using the Shapiro-Wilk test. Data identified as not normal were transformed with the natural log retested. If the data were not identified as log-normal, they were ranked for statistical analysis.

Following the four week exposure, there were no treatment related differences compared to controls for the male hematology. There were also no differences after the four week recovery with the exception of an increase in RDW (p=0.019) which was not in range considered biologically significant. Males following the four week exposure, exhibited a decrease in cholesterol in the low and mid treatments (p=0.034 and p=0.013, respectively). Cholesterol was also decreased following the four week recovery (p=0.039). Sodium and chloride were respectively decreased (p=0.026) and increased (p=0.035) in the low treatment following the four week exposure period. The only other male differences in clinical chemistry noted after the recovery period were decreased LDH (p=0.036) and TP (p=0.013) though these differences were not within ranges considered biologically significant. Only cholesterol in the males exhibited a potential dose response.

Females exhibited a greater hematological response than the males. Following the four week exposure in females, NEU and NEU% were increased in the mid and high treatments (p=0.028 and p=0.015; and p=0.012 and p=0.001; respectively). NEU were also decreased following the four week recovery (p=0.035). No other differences compared to controls were present following the exposure with exception of a borderline increase in HCT (p=0.047) in the mid treatment. EOS and EOS% were decreased (p=0.033 and p=0.028, respectively) following the recovery, though the differences were not

considered biologically significant. After the exposure, the only clinical chemistry differences observed in females were increased ALT in the high treatment ($p<0.001$) and decreased TP in the low treatment ($p=0.021$) though these were not considered biologically significant. There were no differences in female clinical chemistry observed after the recovery period.

I. PROCEDURE

I.I Introduction

A four week inhalation study of Black Smoke was performed with 36 male and 36 female rats. For both males and females, inhalation doses of 130 mg/m^3, 670 mg/m^3, and 2100 mg/m^3 were administered, as well as a control group (0 mg/m^3). The rats were dosed every day for four weeks, excluding non-work days. At the end of each week, each rat's body weight and the amount of food each rat consumed was measured. After the fourth's week weigh-in dosing stopped, and all but 12 rats were euthanized (6 – control, 6 – 2100 mg/m^3). These remaining 12 rats, called the 'recovery group', were weighed at the end of the next four weeks and then were euthanized after a total of 8 weeks in the study. In total then, for each of the first four weeks there were 12 rats per gender for the high (2100 mg/m^3) and low (control) dose groups and 6 rats for the two intermediate dosing groups. From week 5 on, there were only 6 rats per gender and they were in the high or low dose groups.

I.II Factors

Within the dataset, there were two observed independent variables for each rat: dose and gender. After looking at body weight means, it was very apparent that the males consumed more food, gained more weight and were just larger rats than females. Therefore, we decided that it would be more useful to have separate analyses for males and females. In terms of the analysis, we performed an analysis for male body weight, female body weight, male body weight gain, etc.

I.III Descriptive Data

Initially, a line graph was constructed to visually see if any trends from week 1 to week 8 were present, as well as to see if means for any dose group were different within a particular week. The x-axis represents week 1 through week 8 (day 1 – day 55), and the y-axis is either, body weight or one of the other two measured variables. For week 1 through 4, four different lines are present representing the four different dose groups. Then from week 5 to week 8, only two lines are present, representing the recovery group's data. In addition, a descriptive statistics table is given, showing the sample size (N), mean and standard deviation broken out by dose within week. Any week that is bold indicates the week is statistically significant in terms of the dose group means on the variable of interest. The maximum N is 6 or 12 depending on the dose, however a few missing observations did occur during the study. Also, since the standard deviation is a measure of the spread of the variable, the ideal result would be a small standard deviation, which would mean that all the rats within the dose group "behaved" the same in terms of gaining weight, food consumption, etc.

I.IV Statistical Methods

For analysis, weeks 1-4 and weeks 5-8 were treated differently. For weeks 1 -4, a one-factor ANOVA was run to compare the means of a dependent variable (body weight, food consumption, etc.) of the four different dose groups. If the p-value of the ANOVA test was below .05, we could conclude that at least two of the doses were significantly different from each other, with 95% confidence. To determine which doses were significantly different than the control, a Dunnetts-C post hoc test was used.

- To run the ANOVA test, three assumptions must be met. The first is independent observations, which occurred in this study. The second is homogeneity of variances within a particular week. ANOVA is particularly receptive to heterogeneous variances among groups particularly if the results indicate that the group data is only slightly heterogeneous (a p-value close to, but smaller than .05). A Levene's test with an alpha of .05 was used to determine homogeneous variances between groups. For any groups that failed the Levene's test, we still ran the AVOVA but also did a post-hoc Dunnet's T3 test to compare means, accounting for unequal variance. Lastly, the data needs to be normally distributed. A Shapiro-Wilk test was used to determine this with an alpha of .05.
- If the p-value of the Shapiro-Wilk normality test was less than .05, a non-parametric Kruskall-Wallis test was used to compare the four dose groups' medians instead of ANOVA.

Weeks 5 – 8 utilized a T-test to compare the control and 2100 mg/m^3 group at each week. If the p-value was less than .05, the two groups had significantly different means. Homogeneity of variances once again needed to be checked, because it affected some internal mechanics of the T-tests; SPSS calculated significance under both possibilities in the analysis. The data once again needed to be normally distributed, and if not, a Mann-Whitney (non-parametric) test was used.

II. Significant Results

Body weight, body weight gain and food consumption were all highly related outcome variables. All three measurements related to the size and mass of the rat. The table below shows all of the comparisons that were significantly different at the 95% confidence level. There were only 8 comparisons that were found to be significant from all three datasets (males/females and recovery/non-recovery rats). This is somewhat due to the large standard deviations for the dosed groups. Only one of those comparisons was NOT in the recovery period and that was females at week 3 for body weight gain. The significant difference noted for females at week 3 was the only significant difference for that gender for any comparison.

- The Black Smoke didn't seem to affect the rats during dosing, but after dosing, the control rats recovered much more quickly and got back to a healthier weight faster than the rats dosed at 2100 mg/m^3.
- For males in the recovery period, there was a substantial amount of evidence that the control rats were eating more and gaining more weight, when compared to the 2100 mg/m^3 rats.
- Comparing the high to low dosed group, males were much more affected by the inhalation of the Black Smoke than were the females.
- The large standard deviations indicate that the rats within each dose group reacted, in terms of body weight and food consumption, much differently from one another once they started inhaling Black Smoke, as compared to the control group.

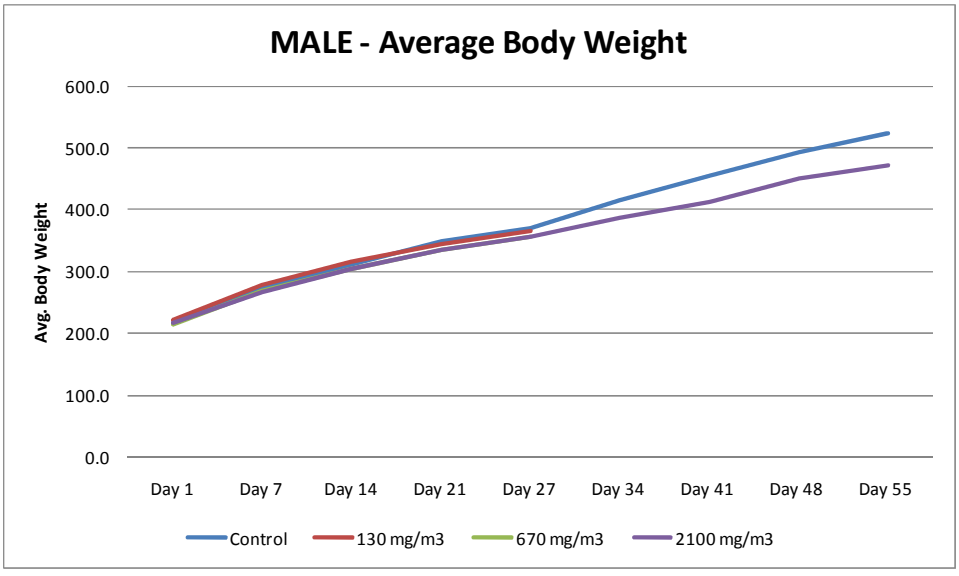
Body Weight, Body Weight Gain and Food Consumption Significance Results					
Measurement	**Gender**	**Week/Day**	**Dose Group**	**Mean**	**p-value**
Body Weight	Male	Day 41	Control	455.0	0.009
			2100 mg/m^3	413.7	
Body Weight	Male	Day 55	Control	522.7	0.034
			2100 mg/m^3	472.0	
Body Weight Gain	Male	Week 6	Control	40.3	0.005
			2100 mg/m^3	27.3	
Body Weight Gain	Male	Week 8	Control	30.5	0.024
			2100 mg/m^3	20.7	
Body Weight Gain	Female	Week 3	Control	14.6	.003*
			2100 mg/m^3	6.2	
Food Consumption	Male	Week 6	Control	242.8	0.023
			2100 mg/m^3	217.8	
Food Consumption	Male	Week 7	Control	236.2	0.025
			2100 mg/m^3	209.3	
Food Consumption	Male	Week 8	Control	238.2	0.021
			2100 mg/m^3	210.3	

* Dunnett-C post hoc test

III. BODY WEIGHT

III.I Male Body Weight

All four dose groups had almost identical trends and means through the first four weeks. As time increased, the 2100 mg/m^3 group does started to drop off, in terms of body weight, from the other three groups. During the recovery period, especially, there was a clear and growing difference between the 2100 mg/m^3 and control group.



III.I.I Male Body Weight: Day 1-27

As per the experiment design, the mean weight of each dose group was the same for the first week (Day 1). After day 1, the 2100 mg/m^3 dose group generally had the lowest body weight. From day 7 to day 27, the two highest doses generally had identical means while the control and 130 mg/m^3 dose had similar means. This shows that the 130 mg/m^3 dose may have the same effect as having no dose, while the top two doses were very similar in the reaction they caused on the rats.

Male Body Weight: Day 1-27

		N	Mean	Std. Deviation
Day 1	Control	12	218.3	6.4
	130 mg/m3	6	221.7	15.3
	670 mg/m3	6	215.5	7.7
	2100 mg/m3	12	216.2	9.2
Day 7	Control	12	273.6	9.3
	130 mg/m3	6	278.8	25.0
	670 mg/m3	6	268.8	13.5
	2100 mg/m3	12	266.6	14.1
Day 14	Control	12	312.3	13.4
	130 mg/m3	6	315.8	32.8
	670 mg/m3	6	304.0	18.8
	2100 mg/m3	12	304.5	18.1
Day 21	Control	12	348.7	19.9
	130 mg/m3	6	345.3	38.9
	670 mg/m3	6	336.2	20.6
	2100 mg/m3	12	334.5	20.8
Day 27	Control	12	370.0	21.8
	130 mg/m3	6	365.5	42.6
	670 mg/m3	6	355.3	25.5
	2100 mg/m3	12	355.7	25.7

III.I.II Male Body Weight: Day 34-55

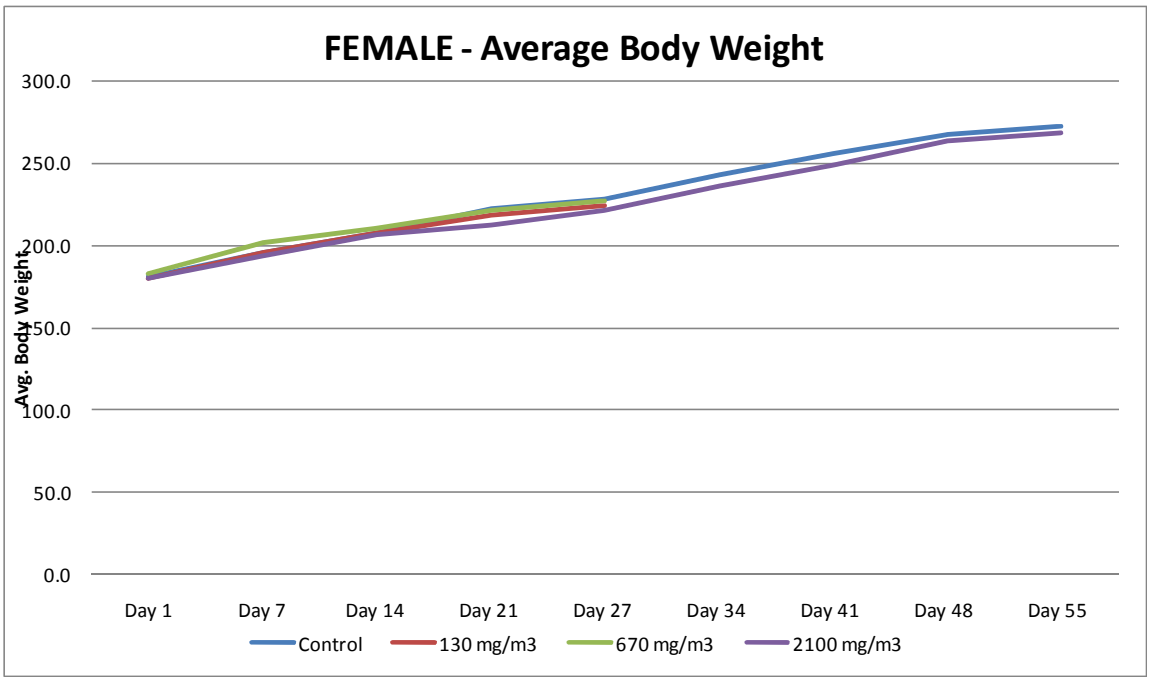
In the recovery period, there was a noticeable difference between the two groups' means. The average differences in body weights ranged from 28 grams to 50 grams. The standard deviation for the dosed group is much higher than the control group, which means the dosed group had a lot more variation in their body weights for the recovery period than did the control group. This indicated that, some dosed rats recovered better than others.

Male Body Weight: Day 34-55

	Dose	N	Mean	Std. Deviation
Day 34	Control	6	414.7	15.8
	2100 mg/m3	6	386.3	33.6
Day 41	Control	6	455.0	13.3
	2100 mg/m3	6	413.7	38.5
Day 48	Control	6	492.2	19.0
	2100 mg/m3	6	451.3	41.1
Day 55	Control	6	522.7	21.2
	2100 mg/m3	6	472.0	46.0

III.II Female Body Weight

The difference in average body weight between the doses for females was very minimal. The two groups in the recovery period followed almost an identical trend through the last four weeks. It is noted that the control group did have a higher body weight mean than the 2100 mg/m^3 group for all four recovery weeks. There appeared to be some peaks in the first four weeks and it also can be seen that the 670 mg/m^3 group had the highest body weight for the first two weeks.



III.II.I Female Body Weight: Day 1-27

There were no differences among the body weights for the three lower dose groups during the first 27 days. The three smallest doses all have very similar means for the first 27 days. It appeared that only the high dosage hindered the female rats from gaining weight.

Female Body Weight: Day 1-27

		N	Mean	Std. Deviation
Day 1	Control	12	181.0	6.5
	130 mg/m3	6	179.5	12.1
	670 mg/m3	6	183.2	8.7
	2100 mg/m3	12	179.6	7.4
Day 7	Control	12	196.0	7.9
	130 mg/m3	6	195.3	11.0
	670 mg/m3	6	202.0	14.1
	2100 mg/m3	12	193.6	9.1
Day 14	Control	12	207.8	12.9
	130 mg/m3	6	207.5	13.1
	670 mg/m3	6	210.5	14.9
	2100 mg/m3	12	206.5	9.3
Day 21	Control	12	222.4	14.9
	130 mg/m3	6	218.3	16.6
	670 mg/m3	6	221.5	16.8
	2100 mg/m3	12	212.7	11.6
Day 27	Control	12	228.5	16.8
	130 mg/m3	6	223.8	17.6
	670 mg/m3	6	227.3	19.7
	2100 mg/m3	12	221.4	10.7

III.II.II Female Body Weight: Day 34-55

The differences in mean weights for females during recovery were a lot smaller in magnitude than they were for males. The range of differences was only 4 to 6 grams. The standard deviations for the control group were a lot larger than the dosed group. This was due to Rat 11-0077 which had particularly low body weights for all measurement days compared to the other 11 rats, and was part of the control group.

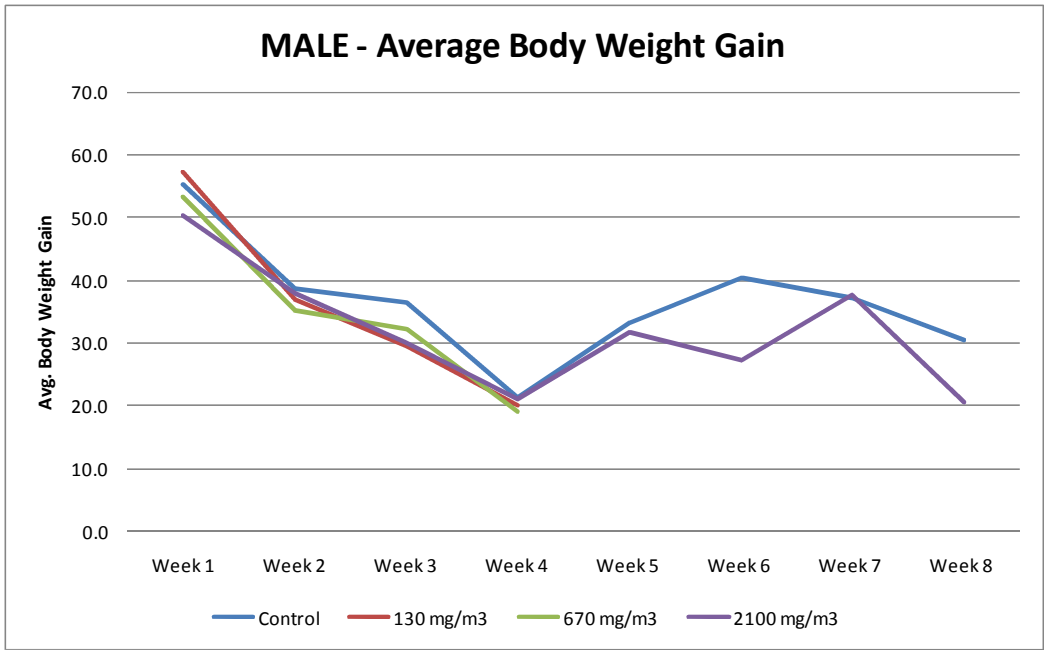
Female Body Weight: Day 34-55

	Dose	N	Mean	Std. Deviation
Day 34	Control	6	242.7	21.4
	2100 mg/m3	6	236.3	8.9
Day 41	Control	6	255.3	23.5
	2100 mg/m3	6	249.2	12.3
Day 48	Control	6	267.8	26.2
	2100 mg/m3	6	263.2	15.6
Day 55	Control	6	272.5	26.7
	2100 mg/m3	6	268.3	16.0

IV. BODY WEIGHT GAIN

IV.I Male Body Weight Gain

For weeks 1 through 4, there was no real obvious differences between the four dose groups. It was apparent that all four groups decreased in how much weight they gained to some extent all four weeks. The two recovery groups seemed to follow no true pattern for the last four weeks. However, the control group did gain more weight than the dosed group for all recovery weeks but week 7.



IV.I.I Male Body Weight Gain: Week 1-4

During the first four weeks, no dose had the highest or lowest mean for all four weeks. The range of averages for week 1 was 4.9 grams, week 2 was 3.5 grams, week 3 was 6.9 grams and week 4 was 2.2 grams. With such small ranges and a good amount of variation in the order of body weight averages, there wasn't much evidence of any dose effect occurring on the number of grams gained.

Male Body Weight Change: Week 1-4

		N	Mean	Std. Deviation
Week 1	Control	12	55.3	7.4
	130 mg/m3	6	57.2	11.5
	670 mg/m3	6	53.3	6.7
	2100 mg/m3	12	50.4	7.7
Week 2	Control	12	38.7	10.2
	130 mg/m3	6	37.0	9.3
	670 mg/m3	6	35.2	6.4
	2100 mg/m3	12	37.9	9.1
Week 3	Control	12	36.4	7.6
	130 mg/m3	6	29.5	9.0
	670 mg/m3	6	32.2	4.9
	2100 mg/m3	12	30.0	6.6
Week 4	Control	12	21.3	6.4
	130 mg/m3	6	20.2	5.1
	670 mg/m3	6	19.2	7.8
	2100 mg/m3	12	21.2	6.7

IV.I.II Male Body Weight Gain: Week 5-8

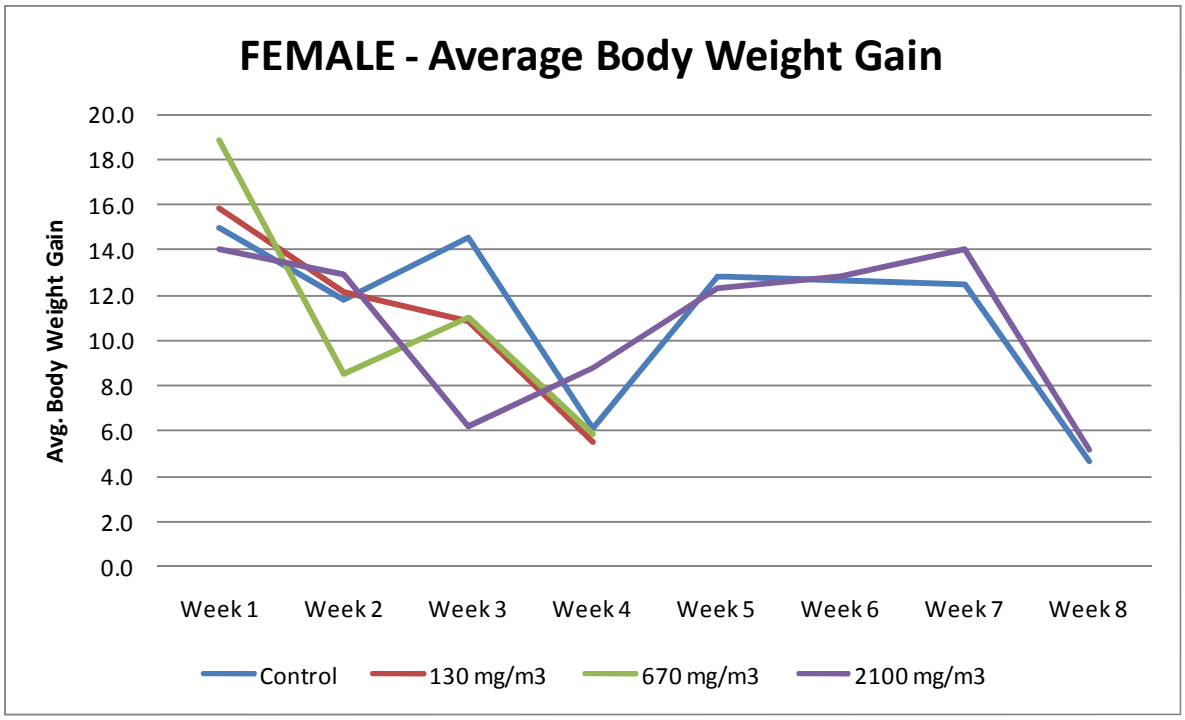
Week 6 and week 8 had large differences, 13 grams and 9.9 grams respectively. However, week 5 and week 7 had very minimal body weight gain differences.

Male Body Weight Change: Week 5-8

	Dose	N	Mean	Std. Deviation
Week 5	Control	6	33.2	6.9
	2100 mg/m3	6	31.8	9.7
Week 6	Control	6	40.3	4.5
	2100 mg/m3	6	27.3	7.7
Week 7	Control	6	37.2	6.6
	2100 mg/m3	6	37.7	4.8
Week 8	Control	6	30.5	6.6
	2100 mg/m3	6	20.7	6.3

IV.II Female Body Weight Gain

There was no inherent pattern to the body weight gain of the four female dose groups. However, during week 3, control and 670 mg/m^3 dose group actually increased their weight gain average while the 2100 mg/m^3 dose group drastically decreased its weight gain. The recovery period doses both had the same pattern; however nothing too obvious could be interpreted from the trends.



IV.II.I Female Body Weight Gain: Week 1-4

The means at week 1 were very similar, with a total range of 4.8 grams. 670 mg/m^3 had the highest mean, but also had the highest standard deviation indicating that there was a rat that had a much larger weight gain than the others in that group. All groups by week 4 decreased their weight gain by almost half of what they were gaining before, except for the 2100 mg/m^3 group which decreased their weight gain in week 3.

Female Body Weight Change: Week 1-4

		N	Mean	Std. Deviation
Week 1	Control	12	15.0	4.1
	130 mg/m3	6	15.8	5.0
	670 mg/m3	6	18.8	8.2
	2100 mg/m3	12	14.0	3.9
Week 2	Control	12	11.8	8.4
	130 mg/m3	6	12.2	4.8
	670 mg/m3	6	8.5	4.3
	2100 mg/m3	12	12.9	4.4
Week 3	Control	12	14.6	7.4
	130 mg/m3	6	10.8	5.7
	670 mg/m3	6	11.0	3.9
	2100 mg/m3	12	6.2	4.3
Week 4	Control	12	6.1	6.5
	130 mg/m3	6	5.5	4.4
	670 mg/m3	6	5.8	5.2
	2100 mg/m3	12	8.8	3.7

IV.II.II Female Body Weight Gain: Week 5-8

The means and standard deviations for the two recovery groups appeared to be almost identical for weeks 5 through 8. The largest mean difference was 1.5 grams and that was in week 7.

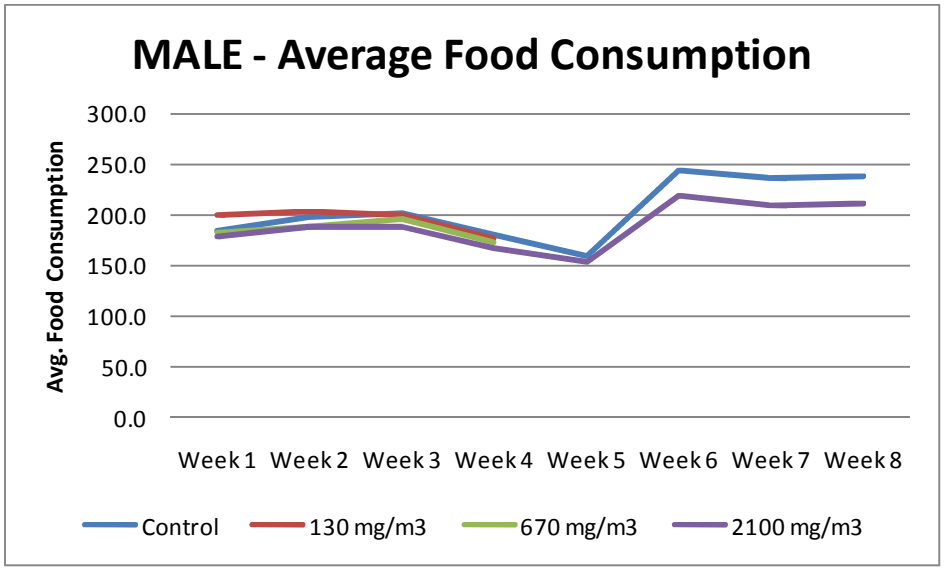
Female Body Weight Change: Week 5-8

	Dose	N	Mean	Std. Deviation
Week 5	Control	6	12.8	4.4
	2100 mg/m3	6	12.3	5.3
Week 6	Control	6	12.7	5.8
	2100 mg/m3	6	12.8	5.6
Week 7	Control	6	12.5	5.0
	2100 mg/m3	6	14.0	4.4
Week 8	Control	6	4.7	2.0
	2100 mg/m3	6	5.2	2.6

V. FOOD CONSUMPTION

V.I Male Food Consumption

All four doses followed the same trend throughout the study for food consumption. The higher two doses had lower mean food consumption compared to the control and low-intermediate dose. From week 6 on, the control group had a much higher food consumption average when compared to the dosed amount.



V.I.I Male Food Consumption: Week 1-4

Food consumption was fairly level through the first three weeks and then dropped off from week three to four. The average drop off of the three treated groups and the drop off of the control groups was almost equal, both being around 22 GRAMS???**double check – I don't know**. In weeks 3 and 4, the control group did have the highest food consumption average.

Male Food Consumption: Week 1-4

		N	Mean	Std. Deviation
Week 1	Control	12	184.8	14.0
	130 mg/m3	6	199.5	23.4
	670 mg/m3	6	182.0	12.7
	2100 mg/m3	12	178.7	14.3
Week 2	Control	11	197.7	14.6
	130 mg/m3	6	203.3	23.2
	670 mg/m3	6	187.8	12.5
	2100 mg/m3	12	187.3	11.0
Week 3	Control	12	201.4	16.9
	130 mg/m3	6	199.0	25.6
	670 mg/m3	6	195.8	10.1
	2100 mg/m3	12	188.3	13.5
Week 4	Control	12	179.6	12.7
	130 mg/m3	6	176.8	22.2
	670 mg/m3	6	171.8	10.2
	2100 mg/m3	12	167.7	10.8

V.I.II Male Food Consumption: Week 5-8

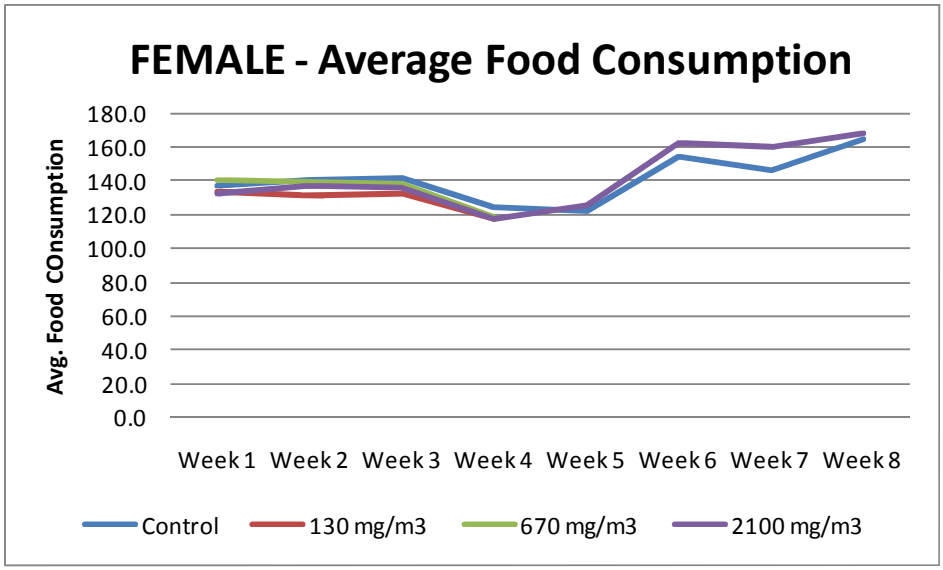
Weeks 6, 7, and 8 showed significant differences between the dosed and the control group. The control group in week 5 had a higher food consumption average, but only by 6.3 grams.

Male Food Consumption: Week 5-8

	Dose	N	Mean	Std. Deviation
Week 5	Control	6	159.7	11.8
	2100 mg/m3	6	153.3	9.7
Week 6	Control	6	242.8	10.3
	2100 mg/m3	6	217.8	20.3
Week 7	Control	6	236.2	13.4
	2100 mg/m3	6	209.3	21.1
Week 8	Control	6	238.2	15.6
	2100 mg/m3	6	210.3	19.5

V.II Female Food Consumption

All four dose groups in the dosing period had similar average food consumption. The 130 mg/m^3 averages appeared slightly lower than the other three doses. In the recovery period, the 2100 mg/m^3 rats appeared to consume more food than the control group, but the two followed the same trend.



V.II.I Female Food Consumption: Week 1-4

The range of mean food consumption for week 1 was 7.7, week 2 was 8.5, week 3 was 8.9 and week 4 was 6.3. **In the future, you could add this to the chart rather than listing it out in the discussion.** With relatively similar standard deviations and similar means within each week, this data suggested that the dosing didn't have too much effect on the food consumption of a female rat.

Female Food Consumption: Week 1-4

		N	Mean	Std. Deviation
Week 1	Control	12	137.2	11.8
	130 mg/m3	6	133.3	8.3
	670 mg/m3	6	140.7	12.8
	2100 mg/m3	12	132.9	10.9
Week 2	Control	12	140.1	14.7
	130 mg/m3	6	131.5	9.5
	670 mg/m3	6	139.0	9.0
	2100 mg/m3	12	136.9	10.6
Week 3	Control	12	141.4	14.4
	130 mg/m3	6	132.5	12.2
	670 mg/m3	6	138.5	11.7
	2100 mg/m3	12	135.6	14.0
Week 4	Control	12	123.8	10.9
	130 mg/m3	6	117.5	10.5
	670 mg/m3	6	118.8	6.5
	2100 mg/m3	12	117.6	7.3

V.II.II Female Food Consumption: Week 5-8

Food consumption was relatively equal for all four recovery weeks between the two groups. However, after week 5, the means of both groups increases by 30 to 40 grams.

Female Food Consumption: Week 5-8

	Dose	N	Mean	Std. Deviation
Week 5	Control	6	121.8	12.1
	2100 mg/m3	6	125.2	5.1
Week 6	Control	6	154.2	16.0
	2100 mg/m3	6	162.3	9.0
Week 7	Control	6	146.3	16.1
	2100 mg/m3	6	159.8	20.3
Week 8	Control	6	164.3	19.4
	2100 mg/m3	5	167.6	13.2

I. INTRODUCTION

A four week inhalation study of Black Smoke was performed with 36 male and 36 female rats. For both males and females, inhalation doses of 130 mg/m^3, 670 mg/m^3, and 2100 mg/m^3 were administered, as well as a control group (0 mg/m^3). The rats were dosed every day for four weeks, excluding non-work days. At the end of the four weeks, 24 rats from each gender (6 rats per dose group) were euthanized and their organ weights, as well as their body weights, were documented. The remaining 12 rats per gender (6 - control, 6 - 2100 mg/m^3) were followed for another four weeks; however they were not exposed to additional Black Smoke. After the additional month had passed, these remaining rats, called the 'recovery group', were euthanized and their organ and body weights were collected.

II. ABSOLUTE ORGAN WEIGHT

II.I DATASET

This dataset consisted of the animal id, gender, dose group, body weight, and multiple organ weights for each animal. The organs of interest were brain, heart, kidney, epididymides, liver, lungs, spleen, testes and thymus. For the female dataset, epididymides and testes were replaced by ovaries and uterus.

II.II STATISTICAL METHODS

The dataset was separated into males in females since the differences in size of rats was so apparent. This was evident from the rats bodyweights and the magnitude of their organ weights. For each gender, the same statistical methods were conducted to analyze the data. A MANCOVA (multivariate analysis of covariance) model was run to test to see which organs' weights were significantly affected by the dose of Black Smoke that the animal received. As a result, dose group is the predictor variable and the 10 organ weights were the dependent variables in the MANCOVA model. Additionally, the body weight of the rat was used as a covariate in the MANCOVA model to adjust the organ weights for any differences in body weights among the dose groups, because heavier animals would tend to have heavier organs. To run the MANCOVA model, we checked to equality of variances between the four dose groups and that the overall distribution of the data was normally distributed for each organ. The interaction of dose*bodyweight was calculated to make sure it was not significant. If it were significant, the MANCOVA model cannot be used. If the MANCOVA model was significant for a specific organ (p-value less than .05), the marginal means were compared to see which two dose groups were statistically different at the 95% confidence level. Marginal means were used instead of the true means so that the covariate was accounted for in determining the mean differences.

II.III OVERALL SIGNIFICANCE RESULTS

Males had significantly different (p-value < .05) organ weights for adrenals, epididymides, lungs, kidneys, and the thymus. The lungs had differences at 670 mg/m^3 and 2100 mg/m^3 compared to the control; however the rest of the organs only had differences at 2100 mg/m^3 compared to the control group. Females only showed statistically different organ weights for kidneys, lungs, and the heart. Lungs were statistically different for both genders, and for both groups (recovery, non-recovery).

It's conclusive that the lungs followed by kidneys were most affected by rats inhaling a high dose of Black Smoke. There is also evidence that the high dose group in the recovery rats started to recover and their organ weights appear to be starting to return to the normal (equal to control group) weights. This is because more differences were found in non-recovery data and p-values (that were found significant) for the recovery groups are closer to the .05 mark.

ABSOLUTE ORGAN WEIGHTSIGNIFICANT DIFFERENCES (alpha = .05)					
Gender	**Recovery Group**	**Organ**	**Doses with Sig. Diff.**	**Mean**	**P-value**
Male	No	Adrenals	Control	0.068	0.029
			2100 mg/m^3	0.089	
Male	No	Epididymides	Control	1.111	0.044
			2100 mg/m^3	0.966	
Male	No	Lungs	Control	1.666	0.00
			670 mg/m^3	2.562	
Male	No	Lungs	Control	1.666	0.00
			2100 mg/m^3	2.598	
Male	Yes	Kidneys	Control	3.238	0.006
			2100 mg/m^3	3.585	
Male	Yes	Lungs	Control	2.118	0.004
			2100 mg/m^3	2.479	
Male	Yes	Thymus	Control	0.415	0.048
			2100 mg/m^3	0.622	
Female	No	Kidneys	Control	1.678	0.05
			130 mg/m^3	1.886	
Female	No	Lungs	Control	1.386	0.00
			670 mg/m^3	2.009	
Female	No	Lungs	Control	1.386	0.00
			2100 mg/m^3	2.162	
Female	Yes	Lungs	Control	1.339	0.033
			2100 mg/m^3	1.664	
Female	Yes	Heart	Control	0.921	0.048
			2100 mg/m^3	1.00	

II.IV MARGINAL MEANS

The marginal means reported below are adjusted for the body weight of each animal. Significantly different dose groups within each organ are in bold. The first table is Male organ weights separated by recovery and non-recovery groups, and the second table is the female marginal means.

MALE ORGAN WEIGHTS

Week 4 Weights			Week 8 Weights (RECOVERY)		
Organ	**Dose**	**Marginal Mean***	**Organ**	**Dose**	**Marginal Mean***
Adrenals	**Control**	0.068	Adrenals	Control	0.073
	130 mg/m^3	0.070			
	670 mg/m^3	0.074		2100 mg/m^3	0.071
	2100 mg/m^3	0.090			
Brain	Control	2.073	Brain	Control	2.055
	130 mg/m^3	1.998			
	670 mg/m^3	2.020		2100 mg/m^3	2.083
	2100 mg/m^3	2.017			
Heart	Control	1.343	Heart	Control	1.541
	130 mg/m^3	1.334			
	670 mg/m^3	1.381		2100 mg/m^3	1.537
	2100 mg/m^3	1.448			
Kidneys	Control	2.967	Kidneys	**Control**	3.238
	130 mg/m^3	2.905			
	670 mg/m^3	2.861		**2100 mg/m^3**	3.585
	2100 mg/m^3	2.856			
Epididymides	**Control**	1.111	Epididymides	Control	1.406
	130 mg/m^3	1.057			
	670 mg/m^3	1.005		2100 mg/m^3	1.383
	2100 mg/m^3	0.966			
Liver	Control	12.073	Liver	Control	15.885
	130 mg/m^3	11.914			
	670 mg/m^3	12.045		2100 mg/m^3	15.704
	2100 mg/m^3	11.720			
Lungs	**Control**	1.666	Lungs	**Control**	2.118
	130 mg/m^3	1.755			
	670 mg/m^3	2.562		**2100 mg/m^3**	2.479
	2100 mg/m^3	2.598			
Spleen	Control	0.644	Spleen	Control	0.798
	130 mg/m^3	0.637			
	670 mg/m^3	0.639		2100 mg/m^3	0.89
	2100 mg/m^3	0.567			
Testes	Control	3.361	Testes	Control	3.484
	130 mg/m^3	3.252			
	670 mg/m^3	3.208		2100 mg/m^3	3.361
	2100 mg/m^3	3.235			
Thymus	Control	0.471	Thymus	**Control**	0.415
	130 mg/m^3	0.464			
	670 mg/m^3	0.476		**2100 mg/m^3**	0.622
	2100 mg/m^3	0.455			

*Adjusted for Bodyweight as a covariate

FEMALE ORGAN WEIGHTS					
Week 4 Weights			Week 8 Weights (RECOVERY)		
Organ	**Dose**	**Marginal Mean***	**Organ**	**Dose**	**Marginal Mean***
Adrenals	Control	0.082	Adrenals	Control	0.062
	130 mg/m^3	0.086			
	670 mg/m^3	0.090		2100 mg/m^3	0.059
	2100 mg/m^3	0.093			
Brain	Control	1.862	Brain	Control	1.912
	130 mg/m^3	1.808			
	670 mg/m^3	1.830		2100 mg/m^3	1.878
	2100 mg/m^3	1.868			
Heart	Control	0.870	Heart	**Control**	0.921
	130 mg/m^3	0.917			
	670 mg/m^3	0.903		**2100 mg/m^3**	1.000
	2100 mg/m^3	0.900			
Kidneys	**Control**	1.678	Kidneys	Control	1.891
	130 mg/m^3	1.886			
	670 mg/m^3	1.776		2100 mg/m^3	1.924
	2100 mg/m^3	1.795			
Ovaries	Control	0.170	Ovaries	Control	0.151
	130 mg/m^3	0.165			
	670 mg/m^3	0.161		2100 mg/m^3	0.140
	2100 mg/m^3	0.159			
Liver	Control	7.163	Liver	Control	8.110
	130 mg/m^3	7.001			
	670 mg/m^3	7.422		2100 mg/m^3	8.438
	2100 mg/m^3	7.246			
Lungs	**Control**	1.386	Lungs	**Control**	1.545
	130 mg/m^3	1.694			
	670 mg/m^3	2.009		**2100 mg/m^3**	1.870
	2100 mg/m^3	2.162			
Spleen	Control	0.523	Spleen	Control	0.553
	130 mg/m^3	0.527			
	670 mg/m^3	0.494		2100 mg/m^3	0.565
	2100 mg/m^3	0.462			
Uterus	Control	0.444	Uterus	Control	0.551
	130 mg/m^3	0.491			
	670 mg/m^3	0.448		2100 mg/m^3	0.594
	2100 mg/m^3	0.537			
Thymus	Control	0.323	Thymus	Control	0.411
	130 mg/m^3	0.426			
	670 mg/m^3	0.446		2100 mg/m^3	0.406
	2100 mg/m^3	0.336			

*Adjusted for Bodyweight as a covariate

III. ORGAN TO BODY/BRAIN RATIO

III.I DATASET

There are two separate datasets: organ to body ratio and organ to brain ratio. Both datasets had each rat's id number, gender, and ratio of organ weight divided by either body weight or brain weight. Each of the ratios had a recovery and non-recovery dataset. The non-recovery dataset (measured at week 4) contained all four dose groups, while the recovery dataset only included the control and highest-dose groups.

III.II STATISTICAL METHODS

The methods for analyzing the organ to body ratio and organ to brain ratio were identical for the non-recovery dataset as well as the recovery dataset.

- For the non-recovery dataset (week 4 measurements), an ANOVA model was used to see if the dose group had any significant effect on the organ to body/brain ratio. If the p-value was less than .05, we concluded that one of the doses had a significantly different mean ratio than did one of the other doses. Homogeneity of variances and normality of data were checked before the ANOVA model was run. If the ANOVA showed significance (p-value less than .05), a Dunnett C test was run to compare the three dosed groups to the control group to find where the significant difference was.
- A T-test was used to compare the control to the 2100 mg/m^3 dose in the recovery dataset. Again, if the p-value was less than .05, we concluded that the two dose groups' means were significantly different and that Black Smoke did have an effect on the weight of the organ. Homogeneity of variances and normality of data was checked before the T-test results were reported.

III.III OVERALL SIGNIFICANCE RESULTS

Overall, there are eighteen ratios that are significant; twelve of them are associated with the lungs. From these results and supporting evidence from the absolute organ weight results above, lungs were affected the most by the Black Smoke inhalation. Adrenals, kidneys and epididymides (in males) seem to also be affected by inhalation but not to the extent that lungs were. The difference between the control and doses doesn't appear to be statistically significant until the high dose (2100 mg/m^3) is compared, having only three significant results between the means of the control and an intermediate dose. A few highlights of the table are reported below.

- Lungs were significantly different for both genders for the recovery and non-recovery rats. This was the case for both organ to body and organ to brain ratio.
- Kidneys, adrenals, brain, epididymides all had at least one measurement where a dose group was different from the control.
- Heart, liver, spleen, testes, thymus, ovaries and uterus never had a significant difference in weight when comparing to dose groups.
- Males had cumulatively more significant differences than did females.
- As was the case with the absolute organ weights, the non-recovery rats had slightly more weigh-ins with significant differences between two dose groups.

ORGAN TO BODY/BRAIN RATIO SIGNIFICANCE RESULTS						
Measurement	**Organ**	**Gender**	**Measurement period**	**Dose Group**	**Mean**	**p-value**
Organ - Body Ratio	Adrenals	Male	Week4	Control	0.00018	0.001*
				2100 mg/m^3	0.00028	
Organ - Body Ratio	Lungs	Male	Week4	Control	0.00497	0.00*
				2100 mg/m^3	0.00772	
Organ - Body Ratio	Lungs	Male	Week4	Control	0.00497	0.00*
				2100 mg/m^3	0.00775	
Organ - Body Ratio	Kidneys	Male	Week8	Control	0.00700	0.017
				2100 mg/m^3	0.00753	
Organ - Body Ratio	Lungs	Male	Week8	Control	0.00443	0.000
				2100 mg/m^3	0.00547	
Organ - Body Ratio	Brain	Male	Week8	Control	0.00428	0.017
				2100 mg/m^3	0.00465	
Organ - Body Ratio	Lungs	Female	Week4	Control	0.00660	0.00*
				670 mg/m^3	0.00807	
Organ - Body Ratio	Lungs	Female	Week4	Control	0.00660	0.00*
				2100 mg/m^3	0.01037	
Organ - Body Ratio	Lungs	Female	Week8	Control	0.00602	0.048
				2100 mg/m^3	0.00740	
Organ - Brain Ratio	Adrenals	Male	Week4	Control	0.03258	.009*
				2100 mg/m^3	0.04442	
Organ - Brain Ratio	Lungs	Male	Week4	Control	0.80628	.000*
				670 mg/m^3	1.26553	
Organ - Brain Ratio	Lungs	Male	Week4	Control	0.80628	0.00*
				2100 mg/m^3	1.28822	
Organ - Brain Ratio	Liver	Male	Week8	Control	7.99522	0.037
				2100 mg/m^3	7.07578	
Organ - Brain Ratio	Epididymides	Male	Week8	Control	0.69674	0.052
				2100 mg/m^3	0.65745	
Organ - Brain Ratio	Lungs	Male	Week8	Control	1.03732	0.011
				2100 mg/m^3	1.17657	
Organ - Brain Ratio	Lungs	Female	Week4	Control	0.75438	.002*
				670 mg/m^3	1.10325	
Organ - Brain Ratio	Lungs	Female	Week4	Control	0.75438	.001*
				2100 mg/m^3	1.13898	
Organ - Brain Ratio	Lungs	Female	Week8	Control	0.80697	0.015
				2100 mg/m^3	0.99717	

* Dunnett-C post hoc test

STATISTICAL ANALYSIS ADDENDUM

During the final data review for this study, several discrepancies were discovered between the datasets initially sent to the statistician and the values on the necropsy sheets. The study director had discussed the values in question with the recorder and made the appropriate changes to the necropsy sheets after the initial datasets were sent to the statistician for evaluation. These changes affected the adrenal weights and weight ratios for the main study male rats and the female recovery rats as well as the testes weights and weight ratios for the main study male rats. The appropriate changes were documented on the original necropsy sheets and are reflected in the tables in Appendix N. Only the corrected datasets were sent to the statistician for reevaluation using the methods originally described. These datasets and statistical results are presented in the following pages since the changes were not made to the original statistics report. Values that were changed are highlighted.

ARTHUR J. O'NEILL
STUDY DIRECTOR

25 AUG 2014
DATE

ABSOLUTE ORGAN WEIGHTS (RECOVERY)

Sex	Dose Group	Animal ID	Adrenals
Female	Control	11-0077	0.053
Female	Control	11-0078	0.052
Female	Control	11-0079	0.061
Female	Control	11-0084	0.058
Female	Control	11-0085	ND
Female	Control	11-0087	0.085
Female	2100 mg/m^3	11-0074	0.062
Female	2100 mg/m^3	11-0081	0.050
Female	2100 mg/m^3	11-0088	0.064
Female	2100 mg/m^3	11-0089	0.061
Female	2100 mg/m^3	11-0090	ND
Female	2100 mg/m^3	11-0093	0.067

The adrenal weight average between the control and 2100 mg/m3 dose group was not statistically significant (p=.885)

Independent Samples Test

		t-test for Equality of Means						
							95% Confidence Interval of the Difference	
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Adrenals	Equal variances assumed	.150	8	.885	.00100	.00668	-.01441	.01641

Group Statistics

	Dose Group	N	Mean	Std. Deviation	Std. Error Mean
Adrenals	Control	5	.0618	.01348	.00603
	2100 mg/m3	5	.0608	.00646	.00289

ABSOLUTE ORGAN WEIGHTS

Sex	Dose Group	Animal ID	Adrenals	Testes
Male	Control	11-0014	0.072	3.176
Male	Control	11-0016	0.069	3.322
Male	Control	11-0018	0.074	3.280
Male	Control	11-0023	0.070	3.598
Male	Control	11-0026	0.083	3.239
Male	Control	11-0037	ND	3.550
Male	130 mg/m^3	11-0017	0.068	3.249
Male	130 mg/m^3	11-0019	0.060	3.664
Male	130 mg/m^3	11-0034	0.072	3.598
Male	130 mg/m^3	11-0038	0.090	2.752
Male	130 mg/m^3	11-0048	0.056	3.547
Male	130 mg/m^3	11-0049	0.075	3.202
Male	670 mg/m^3	11-0013	0.068	3.166
Male	670 mg/m^3	11-0021	0.056	3.218
Male	670 mg/m^3	11-0022	0.074	3.137
Male	670 mg/m^3	11-0030	0.079	3.415
Male	670 mg/m^3	11-0033	0.091	2.996
Male	670 mg/m^3	11-0036	0.071	3.321
Male	2100 mg/m^3	11-0015	0.096	2.978
Male	2100 mg/m^3	11-0020	0.083	3.245
Male	2100 mg/m^3	11-0024	0.101	3.497
Male	2100 mg/m^3	11-0025	0.077	3.457
Male	2100 mg/m^3	11-0027	0.098	2.934
Male	2100 mg/m^3	11-0029	0.081	3.300

Results from the ANOVA reveal that at least two of the dose groups have significantly differen adrenal weight averages (p=.021). Tukey Post-hoc test shows that the significant difference occurse between the 130 mg/m3 and 2100 mg/m3 dose groups.

Tests of Between-Subjects Effects

Dependent Variable: adrenals

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.001[a]	3	.000	4.129	.021
Intercept	.134	1	.134	1241.820	.000
dose	.001	3	.000	4.129	.021
Error	.002	19	.000		
Total	.139	23			
Corrected Total	.003	22			

a. R Squared = .395 (Adjusted R Squared = .299)

Multiple Comparisons

Dependent Variable: adrenals

Tukey HSD

(I) dose	(J) dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
130 mg/m	2100 mg/	-.0191667*	.00599737	.023	-.0360303	-.0023030
	670 mg/m	-.0030000	.00599737	.958	-.0198637	.0138637
	Control	-.0034333	.00629009	.947	-.0211201	.0142534
2100 mg/	130 mg/m	.0191667*	.00599737	.023	.0023030	.0360303
	670 mg/m	.0161667	.00599737	.063	-.0006970	.0330303
	Control	.0157333	.00629009	.092	-.0019534	.0334201
670 mg/m	130 mg/m	.0030000	.00599737	.958	-.0138637	.0198637
	2100 mg/	-.0161667	.00599737	.063	-.0330303	.0006970
	Control	-.0004333	.00629009	1.000	-.0181201	.0172534
Control	130 mg/m	.0034333	.00629009	.947	-.0142534	.0211201
	2100 mg/	-.0157333	.00629009	.092	-.0334201	.0019534
	670 mg/m	.0004333	.00629009	1.000	-.0172534	.0181201

Based on observed means.
The error term is Mean Square(Error) = .000.

*. The mean difference is significant at the .05 level.

Descriptive Statistics

Dependent Variable: adrenals

dose	Mean	Std. Deviation	N
130 mg/m	.0701667	.01207339	6
2100 mg/	.0893333	.01017186	6
670 mg/m	.0731667	.01165190	6
Control	.0736000	.00559464	5
Total	.0766957	.01240760	23

Results from the ANOVA revealed that there were no significant differences among the dose groups when comparing testes weights (p=.629).

Tests of Between-Subjects Effects

Dependent Variable: testes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.099[a]	3	.033	.590	.629
Intercept	258.996	1	258.996	4613.502	.000
dose	.099	3	.033	.590	.629
Error	1.123	20	.056		
Total	260.218	24			
Corrected Total	1.222	23			

a. R Squared = .081 (Adjusted R Squared = -.056)

Descriptive Statistics

Dependent Variable: testes

dose	Mean	Std. Deviation	N
130 mg/m	3.3353333	.34273235	6
2100 mg/	3.2351667	.23617910	6
670 mg/m	3.2088333	.14660343	6
Control	3.3608333	.17267358	6
Total	3.2850417	.23051803	24

ORGAN TO BODY WEIGHT RATIOS

Sex	Dose Group	Animal ID	Adrenals	Testes
Male	Control	11-0014	0.0002	0.0098
Male	Control	11-0016	0.0002	0.0108
Male	Control	11-0018	0.0002	0.0097
Male	Control	11-0023	0.0002	0.0099
Male	Control	11-0026	0.0002	0.0093
Male	Control	11-0037	ND	0.0105
Male	130 mg/m^3	11-0017	0.0002	0.0114
Male	130 mg/m^3	11-0019	0.0002	0.0092
Male	130 mg/m^3	11-0034	0.0002	0.0096
Male	130 mg/m^3	11-0038	0.0003	0.0084
Male	130 mg/m^3	11-0048	0.0002	0.0111
Male	130 mg/m^3	11-0049	0.0002	0.0090
Male	670 mg/m^3	11-0013	0.0002	0.0097
Male	670 mg/m^3	11-0021	0.0002	0.0094
Male	670 mg/m^3	11-0022	0.0002	0.0101
Male	670 mg/m^3	11-0030	0.0003	0.0109
Male	670 mg/m^3	11-0033	0.0002	0.0082
Male	670 mg/m^3	11-0036	0.0002	0.0104
Male	2100 mg/m^3	11-0015	0.0003	0.0086
Male	2100 mg/m^3	11-0020	0.0003	0.0101
Male	2100 mg/m^3	11-0024	0.0003	0.0098
Male	2100 mg/m^3	11-0025	0.0002	0.0109
Male	2100 mg/m^3	11-0027	0.0003	0.0085
Male	2100 mg/m^3	11-0029	0.0003	0.0105

Results from the ANOVA revealed that at least two of the dose groups had significantly different adrenal to body weight ratios (p=.005). Tukeys Post-hoc test revealed that the 2100 mg/m3 dose group had a significantly greater adrenal to body weight ratio compared to the other three dose groups.

Tests of Between-Subjects Effects

Dependent Variable: adrenals

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.370E-008[a]	3	7.899E-009	6.003	.005
Intercept	1.200E-006	1	1.200E-006	912.302	.000
dose	2.370E-008	3	7.899E-009	6.003	.005
Error	2.500E-008	19	1.316E-009		
Total	1.270E-006	23			
Corrected Total	4.870E-008	22			

a. R Squared = .487 (Adjusted R Squared = .406)

Multiple Comparisons

Dependent Variable: adrenals

Tukey HSD

(I) dose	(J) dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
130 mg/m	2100 mg/	-.0000667*	.00002094	.023	-.0001256	-.0000078
	670 mg/m	.0000000	.00002094	1.000	-.0000589	.0000589
	Control	.0000167	.00002196	.872	-.0000451	.0000784
2100 mg/	130 mg/m	.0000667*	.00002094	.023	.0000078	.0001256
	670 mg/m	.0000667*	.00002094	.023	.0000078	.0001256
	Control	.0000833*	.00002196	.006	.0000216	.0001451
670 mg/m	130 mg/m	.0000000	.00002094	1.000	-.0000589	.0000589
	2100 mg/	-.0000667*	.00002094	.023	-.0001256	-.0000078
	Control	.0000167	.00002196	.872	-.0000451	.0000784
Control	130 mg/m	-.0000167	.00002196	.872	-.0000784	.0000451
	2100 mg/	-.0000833*	.00002196	.006	-.0001451	-.0000216
	670 mg/m	-.0000167	.00002196	.872	-.0000784	.0000451

Based on observed means.
The error term is Mean Square(Error) = 1.316E-009.

*. The mean difference is significant at the .05 level.

Descriptive Statistics

Dependent Variable: adrenals

dose	Mean	Std. Deviation	N
130 mg/m	.0002167	.00004082	6
2100 mg/	.0002833	.00004082	6
670 mg/m	.0002167	.00004082	6
Control	.0002000	.00000000	5
Total	.0002304	.00004705	23

Results from the ANOVA revealed that there were no significant differences between the dose groups when analyzing testes to body weight ratio (p=.962)

Tests of Between-Subjects Effects

Dependent Variable: testes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.550E-007[a]	3	8.500E-008	.094	.962
Intercept	.002	1	.002	2567.019	.000
dose	2.550E-007	3	8.500E-008	.094	.962
Error	1.805E-005	20	9.025E-007		
Total	.002	24			
Corrected Total	1.831E-005	23			

a. R Squared = .014 (Adjusted R Squared = -.134)

Descriptive Statistics

Dependent Variable: testes

dose	Mean	Std. Deviation	N
130 mg/m	.0097833	.00120402	6
2100 mg/	.0097333	.00098928	6
670 mg/m	.0097833	.00093684	6
Control	.0100000	.00055136	6
Total	.0098250	.00089212	24

Appendix Q

Benchmark Dose Determinations

Appendix Q – Derivation of the BMD using the dose-response relationships of male and female incidence of moderate to severe alveolar histiocytosis.

Data Used

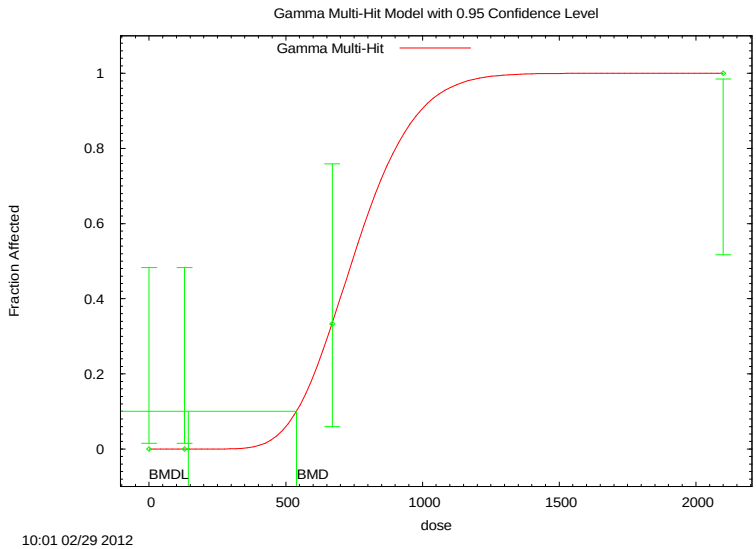
Dose mg/m^3	Male	Female	n
0	0	0	6
130	0	0	6
670	2	5	6
2100	6	6	6

Male Results

Model Name	AIC	P-value	BMD	BMDL	Scaled Residual of Interest
Gamma	9.63817	1	539.558	144.084	0
Logistic	11.6382	1	625.824	253.886	0
LogLogistic	11.6382	1	612.878	196.625	0
LogProbit	11.6382	1	571.08	189.193	0
Multistage	1.30E+54	0	computation failed.		0
Multistage-Cancer	10.0088	0.978	323.967	105.143	-0.32
Probit	11.6382	1	582.44	230.105	0
Weibull	11.6382	1	560.745	135.359	0
Quantal-Linear	13.341	0.5334	110.412	59.233	-0.89

The Gamma Model gave best fit of data

Male
BMD – 539.6
BMDL10 – 144.0



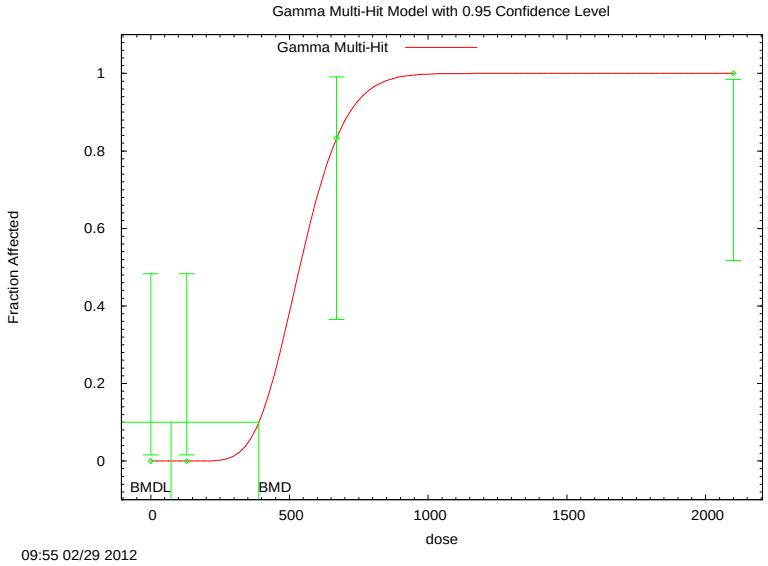
Q-1

Female Results

Model Name	AIC	P-value	BMD	BMDL	Scaled Residual of Interest
Gamma	7.40674	1	389.68	73.4875	-0.002
Logistic	9.40673	1	570.932	145.557	0
LogLogistic	9.40673	1	525.723	89.5967	0
LogProbit	9.40673	1	391.586	90.0952	0
Multistage	1.30E+54	0	computation failed.		0
Multistage-Cancer	8.17704	0.9375	170.601	48.6282	-0.615
Probit	9.40673	1	490.831	133.842	0
Weibull	9.40677	1	473.871	70.6662	0
Quantal-Linear	11.0141	0.5397	53.9047	28.1134	0

The Gamma Model gave best fit of data

Female
BMD – 390.6
BMDL10 – 81.8



Appendix R

Study Protocol With Modifications

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2010/11/16	2. PROTOCOL NUMBER: 0CKC-35-10-08-01	3. MODIFICATION#: 1

4. PROTOCOL TITLE: Acute and Four-Week Repeated-Dose Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Arthur J. O'Neill	410-436-5080	MCHB-IP-TTE

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 0					1b. N/A ☒				
2. ORIGINAL PROTOCOL TOTAL: 94					3. PROTOCOL TOTAL AFTER MODIFICATION: 94				
2a. USDA pain cat:	B: 0	C: 6	D: 78	E: 10	3a. USDA pain cat:	B: 0	C: 6	D: 78	E: 10

4. Yes	No	
☐	☒	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☒	☐	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL: Page, paragraph, section	**SECTION III. MODIFICATION/JUSTIFICATION** *Explain the modification indicated above in the area below. Indicate any changes to the 3Rs (Refinement, Reduction, Replacement) resulting from changes in number or animals*
Section V.1.3., Page 5, Experimental Design; Section V.1.11., Page 9, Clinical Pathology; Section V.1.12., Page 9, Necropsy; Section V.4.1.2.1, Page 12, Anesthesia/ Analgesia; and Section V.4.4.2, Page 14, Biosamples	1. MODIFICATION: Addition of CO2 anesthesia: Anesthesia for the terminal cardiac blood sampling will now be performed using either CO2 or isoflurane gas. The original protocol stated that terminal blood sampling would be performed strictly under isoflurane gas. 1a. JUSTIFICATION/REASON: It has been determined through the conduct of past studies that it can be very difficult to obtain a large enough volume blood from the heart of a rat under isoflurane gas. Having the choice to use either CO2 or isoflurane will ensure that valid samples are obtained from all animals on study. Recent studies allowing the use of CO2 for anesthesia have proven that blood flow into the syringe is greatly increased compared to animals anesthetized using isoflurane gas. In addition, clinical laboratory staff have indicated that the quality of the samples has been better when using CO2 anesthesia with fewer incidents of clotting in the hematology samples and much less hemolysis in the serum.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
	2. MODIFICATION:
	2a. JUSTIFICATION/REASON:
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Arthur J. O'Neill	Signature	DATE: (yyyy/mm/dd) 20101116 17 ENTRY ERROR CORRECTED AO 11/17/10
2. PROGRAM MANAGER:: (Printed Name) Glenn Leach	Signature	DATE: (yyyy/mm/dd) 20101117
3. ATTENDING VETERINARIAN: (Printed Name) Anne M. MacLarty, MAJ, VC	Signature Anne M. MacLarty, DVM	DATE: (yyyy/mm/dd) 20101117
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE) Roy A. Valiant	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) Kristin T. Newkirk	APPROVED / REVIEWED YES ☒ NO ☐ Signature	DATE: (yyyy/mm/dd) 2010/11/17

ANIMAL USE PROTOCOL
TOXICOLOGY DIRECTORATE
U.S. ARMY PUBLIC HEALTH COMMAND (PROVISIONAL)
ABERDEEN PROVING GROUND, MD 21010-5403

PROTOCOL TITLE: Acute and Four-Week Repeated-Dose Inhalation Toxicity Study in Rats Exposed to Pyrotechnically Disseminated Black Smoke

PROTOCOL NUMBER: ØCKC-35-1Ø-Ø8-Ø1

PRINCIPAL INVESTIGATOR/STUDY DIRECTOR:

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SPONSOR: Erik Hangeland
RDECOM/EASLP
Aberdeen Proving Ground, MD 21010

POC: Dr. Mark S. Johnson
Ordnance Environmental Program
USACHPPM/HERP
Aberdeen Proving Ground, MD 21010

I. NON-TECHNICAL SYNOPSIS: The inhalation toxicity of a pyrotechnically disseminated black smoke formulation used by the military will be determined by the inhalation exposure of rats to both an acute exposure (single exposure) and repeated doses (a total of 20 exposures) of the test atmosphere. All rats will be monitored for body weights and clinical signs. The acute study rats will have necropsies performed with limited histopathology (respiratory tract). The 4-week study rats will be monitored for food consumption, receive ophthalmic exams, have blood samples collected, and receive full necropsy/histopathology. Although toxicity testing of military pyrotechnics requires the use of unique generation systems and exposure guidelines, appropriate endpoints will be used based on guidance from a combination of the applicable U.S. Environmental Protection Agency (USEPA) and/or Organisation for Economic Co-Operation and Development (OECD) testing guidelines. Rats are the recommended species by the testing guidelines and have historically been used for inhalation toxicity studies at USAPHC (Prov) and therefore have an extensive historical database.

The data from this study may be used to generate occupational exposure guidelines.

II. BACKGROUND

II.1. Background: The US Military uses colored smokes in a variety of ways, including identification of potential targets and friendly troops, simulation of battlefield events, and as a means of communication. Previously used smoke formulations, including black smoke, were developed strictly based on their ability to produce the desired color for a specified period of time. Recent changes made to the smoke formulations and dissemination systems have focused on soldier safety during training and deployed scenarios as well as preservation of the land on which they train and fight. Disperse Red 9 and Solvent Green 3 are the primary dye components contained in the current formulation being tested.

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched: Medline, TOXFILE, FEDRIP, BIOSIS, EMBASE, CA SEARCH, DTIC, and BRD

II.2.2. Date of Search: 22 July 2010

II.2.3. Period of Search: 1926-2010

II.2.4. Key Words of Search: pyrotechnic, smoke, dye, inhalation, aerosol, toxic, heart stick, cardiac stick, rats, mice

II.2.5. Results of Search: The literature search resulted in 18 hits. A review of the references from the search revealed acute and/or repeated-dose inhalation toxicity studies have been conducted with various pyrotechnically disseminated smokes (e.g., terephthalic acid, titanium dioxide). However, since no inhalation studies were found to be conducted with the current black smoke formulation outlined in this protocol, this project is not considered to be a duplicate effort.

III. OBJECTIVE/HYPOTHESIS:

The objective of this study is to evaluate the acute and repeated dose toxicity of pyrotechnically disseminated black smoke when administered by inhalation to male and female rats.

IV. MILITARY RELEVANCE:

Pyrotechnic black smoke systems are used by the military to simulate battlefield effects for force-on-force and force-on-target training simulations. Although it is imperative that soldiers are trained in a similar manner in which they fight, these training exercises often result in soldiers and training instructors being repeatedly

exposed to materials used to simulate battlefield scenarios. Toxicity data in a mammalian system needs to be generated to assess repeated-dose inhalation toxicity health hazards associated with the material to assure its safety before use by military personnel and to determine an occupational exposure level for training instructors potentially exposed on a daily basis.

V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures: The inhalation toxicity of a pyrotechnically disseminated black smoke formulation used by the military will be determined by the inhalation exposure (nose-only) of rats to a single dose (acute study; one exposure) or repeated doses (4-week study; 20 exposures) of the test atmosphere. The overall experimental design of this study will include three sub-studies: (1) an acute study, (2) a pilot study, and (3) a 4-week repeated-dose study (which includes a control group). For the acute study, rats will be exposed to a single, 30-minute duration of the test atmosphere followed by a 14-day recovery period. Rats will be monitored throughout the study for mortality, body weights, and clinical signs. Following the recovery period, rats will receive a gross necropsy with limited histopathology (respiratory tract only). Prior to conducting the 4-week study, a pilot study will be conducted with a small sample of rats that will be exposed to several 30-minute exposures of the test atmosphere in an attempt to ensure that the design concentration for the high-level exposure chamber in the 4-week study will not produce unwanted and/or unexpected cumulative toxicity effects (i.e., mortality). The 4-week study rats will be exposed 30 minutes per day for 20 days (weekend and holidays not included). Rats will be monitored throughout the study for mortality, body weights, clinical signs, and food consumption. In addition, ophthalmic examinations will be performed. Following the recovery period, rats will have blood samples collected and receive a full necropsy. The study endpoint for all sub-studies is euthanasia when moribund or survival to scheduled time points following exposure and observation periods. Estimated initiation date for acute study is September 2010. Estimated completion date for the 4-week study is November 2010.

The design of the acute study was based primarily on the EPA Health Effects Test Guidelines for an Acute Inhalation Study (reference 1). The design of the 4-week study was based primarily on the EPA Health Effects Test Guidelines for a 90-Day Inhalation Study (reference 2). Changes to the 90-day study design guidelines were made to appropriately reflect a 4-week exposure study design. The OECD Guidelines for a 28-Day Inhalation Study (reference 3) were used as a secondary guide for the 4-week study. Additional changes to the acute and repeated-dose guidelines were made to reflect the unique issues associated with conducting an inhalation study with a pyrotechnically disseminated smoke material and to address the unique military exposure regime.

V.1.1. <u>Experimental Design of Acute Study</u>: In an attempt to determine the acute toxicity associated with single, high-concentration exposures to black

smoke, 5 male and 5 female rats will be exposed for 30 minutes to atmospheric concentrations of the test material targeted to 2000 mg/m^3. Following the exposure, the rats will be held for a 14-day observation period and monitored for morbidity/mortality, weight loss, and/or clinical signs of toxicity. Following the recovery period, rats will be euthanized by CO_2 (euthanasia will be ensured by pneumothorax) and then necropsied. All rats will receive a gross necropsy and limited histopathology (respiratory tract). In an attempt to minimize potential issues related to the shipment of rats that are not within the desired age or weight ranges, one additional rat per sex will be ordered to ensure that the acute study is initiated with 5 male and 5 female rats within the proper age and weight ranges. A total of 12 rats will be ordered for the acute study. Animals not used for the acute exposure will either be transferred to another protocol or humanely euthanized per protocol guidance.

V.1.2. Experimental Design of Pilot Study: Prior to conducting the 4-week study, 3 male and 3 female rats will be exposed approximately 30 minutes/day to the black smoke at the highest design concentration for the 4-week study (e.g., 2000 mg/m^3). Rats will be exposed for multiple days (e.g., 3-4 days) in an attempt to ensure that 2000 mg/m^3 is an appropriate design concentration for the high-level exposure chamber. Although data will be available from the acute toxicity study, this pilot study will attempt to ensure that there will not be unwanted/unexpected cumulative toxicity effects (i.e., mortality) resulting from repeated exposure to the test material. Evaluations will be based on mortality, weight loss, and/or adverse clinical signs of toxicity observed in the exposed rats. Rats will be weighed and observed daily during the exposure and recovery periods. The recovery period will be no more than 5 days following the exposure period. All surviving animals will be euthanized by CO_2. Euthanasia will be ensured by pneumothorax. A total of 6 rats will be used for the pilot study.

V.1.3. Experimental Design of 4-Week Repeated-Dose Study: Four groups of 12 rats each (6 rats/sex/group) will be exposed to atmospheric concentrations of black smoke targeted to 0, 100, 700, and 2000 mg/m^3. Rats will be exposed nose-only for 30 minutes per day, 2-5 days/week, for a total of 20 exposure days (weekends and holidays excluded). In addition, the control and high dose (i.e., 2000 mg/m^3) levels will each have an additional recovery group utilizing 6 rats/sex/group. Both recovery groups will be exposed to the target concentrations for a total of 20 exposure days, but will be held for a one-month period following the exposures. The results of both the acute and pilot studies could result in adjustments to these target concentrations. During the exposure and recovery periods, all rats will be weighed at least once per week and will be observed for clinical signs daily (5 days/week). Food consumption amounts will be monitored at least weekly during the exposure and recovery periods. Prior to initial exposure to the test substance, all test animals will have an ophthalmic examination per TOX SOP 096 (reference 4). Surviving rats in the high dose and control groups will also have an ophthalmic examination at the termination of both the exposure and recovery periods. If changes in the eyes are detected in

the high concentration or control groups, all animals in the other dose groups will be examined as well. On the day following the exposure period, 6 rats/sex/group will be anesthetized with isoflurane gas and a terminal blood sample (approx 1-3 ml) will be collected via a cardiac puncture. Following blood collection, rats will be euthanized by CO_2 and pneumothorax, and then necropsied. After a one-month recovery period, all surviving rats (e.g., 6 rats/sex/group) will receive the same procedures. In an attempt to minimize potential issues related to the shipment of rats that are not within the desired age or weight ranges, two additional rats per sex will be ordered to ensure that the 4-week study is initiated with at least 6 male and 6 female rats per dose group within the proper age and weight ranges. A total of 76 rats will be used for the 4-week study. Animals not used for the exposures will either be transferred to another protocol or humanely euthanized per protocol guidance.

Treatment Group	Test Animals		
ACUTE STUDY	Males	Females	
	5	5	
Additional Rats Ordered For Weight Matching Purposes	1	1	
PILOT STUDY	Males	Females	
	3	3	
4-WEEK STUDY:	Males	Females	
Control Level (0 mg/m^3)	6 + 6 recovery	6 + 6 recovery	
Low Level (100 mg/m^3)	6	6	
Intermediate Level (700 mg/m^3)	6	6	
High Level (2000 mg/m^3)	6 + 6 recovery	6 + 6 recovery	
Additional Rats Ordered For Weight Matching Purposes	2	2	
Total	**47**	**47**	**94**

V.1.4. <u>Test Substance</u>: The black smoke mixture contains a proprietary blend of dyes and accelerants/propellants. A list of the approximate percentages of each ingredient in the black smoke mixture will be documented in the study records.

V.1.5. <u>Concentration Level Selection (4-Week Study)</u>: Results from the acute inhalation study and pilot study will be used to finalize design concentrations for the 4-week study. The approximate target concentrations provided above might be adjusted based on the results of the acute and pilot evaluations. Typically, acute inhalation mortality/toxicity data is helpful in setting the high concentration level for a repeated-dose inhalation study. Since no mortality or significantly adverse clinical signs of toxicity are expected based on historical testing of

military smokes/obscurants, the targeted concentration for the high chamber in the current study has been selected based on an attempt to minimize the potential effects of pulmonary overload that can result from excessive concentrations of an insoluble dust material. With inhalation studies employing aerosols/dusts, the potential exists for overwhelming the lung particle clearance mechanisms. Since pulmonary overload effects can prevent the accurate interpretation of study results from repeated-dose inhalation toxicity studies, the National Toxicology Program (NTP) recommended that maximal aerosol concentrations used on long-term (chronic) studies should not exceed 100 mg/m^3 to avoid adversely affecting lung clearance (reference 5). Keeping in mind that the current study is a 4-week inhalation toxicity study with a much shorter daily exposure duration (30 minutes) than typical repeated-dose inhalation toxicity studies, 2000 mg/m^3 has been selected as the high concentration because it is considered to be a high enough level to produce toxic effects but a low enough level so as not to cause pulmonary overload effects. The low concentration (100 mg/m^3) is 20-fold lower than the high concentration and is expected to be without adverse toxicological effects. The intermediate concentration (700 mg/m^3) is expected to produce some degree of toxicity.

V.1.6. Administration of Test Substance and Exposure Mode: Rats will be exposed nose-only to atmospheres of the test substance. The nose-only (head only) exposure mode is typically used for test atmospheres that contain particulates/aerosols in an attempt to minimize deposition of the test substance onto the fur of the animals, and therefore, minimizing inadvertent dermal and oral exposure of the test substance to the animals. For nose-only exposures, rats will be individually restrained during exposure in perforated, stainless steel cylinders with conical nosepieces. These type cylinders are typically used for nose-only inhalation exposures and are widely accepted equipment for inhalation toxicity test systems (references 6 and 7). Rats will be positioned in the exposure cylinders such that their noses will be at the conical end of the cylinder. In order to secure the rat in this position, a plastic disc with a hole in the center will be inserted over the tail of each rat and positioned within the cylinder close to the base of the rat's tail to prevent the rat from backing out of the rear of the cylinder. Care will be taken to properly insert each rat into its exposure cylinder, such that there is a balance between allowing the rat room to move its body while ensuring that it is positioned properly for adequate exposure. Each exposure cylinder will be inserted into one of the holes in the faceplate of the exposure chamber such that only the nose/head of each rat extends into the exposure chamber. Exposure cylinders will be appropriately cleaned after each use.

V.1.7. Exposure Duration: Rats for the acute study will be exposed to a single, 30-minute exposure of black smoke atmosphere. Rats for the pilot study will be exposed to atmospheres of black smoke for 30 minutes per day for a total of 3-4 days. Rats for the 4-week study will be exposed to atmospheres of black smoke for 30 minutes per day, 2-5 days/week, for a total of 20 exposure days (weekends and holidays excluded). The initiation/completion of the exposure

period for the 4-week study will be staggered over 2 days in order to accommodate practical limitations of the necropsy scheduling process, however, each rat will receive a total of 20 exposures. The starting time of each exposure will be defined as the time when the chamber has reached the desired concentration and the rats have been placed into the faceplate. The ending time of each exposure will be defined as the time when the rats are removed from the faceplate. At the end of each exposure, the rats will be removed from the exposure cylinders and returned to their home cages in the animal room.

V.1.8. Atmosphere Generation: Chamber atmospheres will be generated by the pyrotechnic dissemination of the test material inside an empty inhalation chamber with solid rubber stoppers placed in the faceplate to contain the smoke atmosphere. This inhalation experiment is somewhat unique in that the pyrotechnic dissemination of the test material in the inhalation chamber will serve as the generation system rather than metering a test substance at a constant rate into the chamber. Test atmospheres exiting the exposure chamber will be directed through a high-capacity HEPA filter prior to discharge into the exhaust system. The concentration of black smoke will be monitored gravimetrically after cartridge initiation to determine when a target concentration has been achieved and the rats can placed in the faceplate. This monitoring and exposure scenario will be repeated daily for each target concentration using the atmosphere generated from one black smoke unit /day.

Measurements will be taken during the method development phase of the study and the generation system altered, if needed, to strive for uniform distribution of the test substance within the exposure chamber. The methods for performing the chamber distribution analysis are described in TOX SOP# 152 (reference 8).

A total of two exposure chambers will be used for this study (one chamber for all smoke exposures and one for all control exposures). All exposure chambers are constructed of stainless steel and glass with a nominal internal volume of approximately 1 m^3. The chambers are modeled after the New York University (NYU) style inhalation exposure chambers. The NYU design refers to an inhalation exposure chamber with a cubical mid-section, square-pyramidal inlets and outlets, and a tangential feed at the top of the chamber (inlet) to promote uniform distribution of the test atmosphere (reference 9). The tangential feed feature will not be used for this study since the black smoke unit must be contained inside of the chamber. Several pieces of glass in the chambers will be replaced with faceplates to allow for a nose-only exposure scenario.

V.1.9. Analyses of the Test Atmosphere: The atmospheric concentration of black smoke particulate will be determined at least twice during each exposure for each exposure concentration. The air control chamber will be sampled by gravimetric analysis at least once during each exposure. Known volumes of chamber atmosphere will be drawn from each exposure chamber through a filter cassette that contains a pre-weighed glass fiber filter. Filters will be weighed

again after the sampling period is completed. All filters will be weighed on a Cahn microbalance. The atmospheric concentration of black smoke particulate will be calculated from the difference in the pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled.

Samples to determine particle size distribution (mass median aerodynamic diameter and percent particles less than 1, 3, and 10 µm diameter) of the black smoke particulate will be collected at least once for the acute study and at least 2 times from each of the test atmosphere concentrations for the 4-week study (e.g., during the first week of the exposure period and during the last week of the exposure period). Additional samples may be collected if necessary. Particle size samples will be collected with a Sierra® Series 210 Eight-Stage Cascade Impactor fitted with a cyclone preseparator and an Anderson Series 110 Constant Flow Air Sampler (reference 10).

Combustion gases (e.g., CO_2, CO, NO_x) will be analyzed at least once during the acute study and at least twice during the 4-week study. The appropriate method for analysis will be determined by Aberdeen Test Center personnel. The method and results for the analysis will be appropriately documented in the study records. In addition, analysis of the particulate contained on the filters may also be performed on selected filters collected during the acute and 4-week studies. The appropriate method for analysis will be determined by USAPHC (Prov) Directorate of Laboratory Science (DLS) personnel. The method and results for the DLS analysis will be appropriately documented in the study records.

Since the exposure chamber will essentially be a static system, chamber airflow will not be measured during this study. However, oxygen content will be measured during each exposure to ensure that the rats have an adequate supply of oxygen (e.g., >19%). Oxygen readings will be collected at least twice during each exposure. The chamber temperature and relative humidity will be targeted to be 22 ± 2°C and 50 ± 10%, respectively. The temperature and humidity for each exposure will be monitored continuously and recorded at least 2 times during each exposure.

V.1.10. <u>Body Weights and Observation</u>: All rats will be weighed at least once per week. Weights will be collected during the acclimation period and on specified exposure days during the exposure phase of the study. A thorough physical examination will be performed at least once each day (weekends and holidays excluded) for the acute study animals and prior to each exposure for the 4-week exposure animals. Daily observations will also be performed on the 4-week exposure animals during the recovery period (weekends and holidays excluded). In addition, group observations will be made while the rats are in the exposure chambers and individual observation will be performed after each exposure. The body weight and observation data will be recorded in the laboratory notebook. Observations will include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects,

autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self mutilation, walking backwards). Appropriate actions will be taken to minimize loss of animals to the study. A brief summary related to the collection of body weights and observations will also be recorded in the animal room logbook on days this data is collected.

V.1.11. Clinical Pathology Evaluation (4-Week Study Animals Only): Blood will be collected and evaluated per TOX SOP# 053 (reference 11). Briefly, 1-3 ml of blood will be collected via cardiac puncture following anesthesia (isoflurane gas). A portion of the blood will be transferred to a 1.3 ml EDTA microtube and evaluated for total red blood cell and white blood cell counts, packed cell volume, hemoglobin, and five-part differential. The remainder will be transferred to a 1.3 ml serum-gel microtube and evaluated for the following chemistries: BUN, CREA, GLU, TP, ALB, CHOL, LDH, TBIL, CA, PHOS, and electrolytes. Clinical chemistry and hematology parameters are outlined in TOX SOP# 034 and TOX SOP# 001, respectively (references 12 and 13).

V.1.12. Necropsy and Histopathology Evaluation (Acute and 4-Week Study Animals Only): For the acute study, following the recovery period, all rats will be euthanized and submitted for necropsy. All gross pathology changes will be recorded on CHPPM form 333. If a necropsy cannot be performed immediately after a deceased animal is discovered, death will be ensured by performing a pneumothorax and the animal will be refrigerated at temperatures low enough to minimize autolysis. The organs and tissues representative of the respiratory tract (lungs, trachea, pharynx, larynx, and nose) will be preserved in a suitable medium for future histopathological examination. In addition, the lungs will be weighed. Prior to being weighed, organs will be carefully dissected and trimmed to remove fat and other tissue in a uniform manner. Full histopathological examinations will be performed on all of the respiratory tract tissues collected. For the 4-week study, on the day following the exposure period, 6 rats/sex/group will be anesthetized via isoflurane gas, bled, euthanized, and submitted for a full necropsy. Following a one-month recovery period, the remaining rats (e.g., 6 rats/sex/group) from the high concentration group and the control group will be anesthetized via isoflurane gas, bled, euthanized, and submitted for a full necropsy. All gross pathology changes will be recorded on CHPPM form 333. If a necropsy cannot be performed immediately after a deceased animal is discovered, death will be ensured by performing a pneumothorax and the animal will be refrigerated at temperatures low enough to minimize autolysis. The following organs and tissues, or representative samples, will be preserved in a suitable medium for future histopathological examination: all gross lesions; brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex); pituitary; thyroid/parathyroid; thymus; lungs; trachea; pharynx; larynx; nose; heart; bone marrow (either femur, sternum, or rib at the costochondral junction); salivary glands; liver; spleen; kidney; adrenals; pancreas; testes; epididymides;

seminal vesicles; prostate; ovaries; uterus; aorta; esophagus; stomach; duodenum; jejunum; ileum; caecum; colon; rectum; urinary bladder; representative lymph node; peripheral nerve; sternum with bone marrow; mammary gland; thigh musculature; eyes; femur (including articular surface); spinal cord at three levels (cervical, midthoracic, and lumbar); exorbital lachrymal glands; skin. In addition, the following organs will be weighed: lungs, heart, liver, kidneys, adrenals, spleen, thymus, brain, testes, epididymides, ovaries, and uterus. Prior to being weighed, organs will be carefully dissected and trimmed to remove fat and other tissue in a uniform manner. Full histopathological examinations will be performed on selected organs and tissues of all animals in the control and high dosage groups. Further histopathology from other dosage groups will be performed on organs which show lesions similar to those observed in the high dosage group or for which clinical observations indicate such a need.

V.1.13. Study Conduct: The study described will be conducted in a manner consistent with the principles of the Good Laboratory Practice (GLP) regulations in the Toxic Substances Control Act (TSCA): 40 CFR (Code of Federal Regulations) 792, plus amendments (reference 14). All study records will be made available to oversight organizations such as the Environmental Protection Agency or the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) as needed. The investigators and technicians will adhere to The Guide for Care and Use of Laboratory Animals, 1996 (reference 15).

V.2. Data Analysis: With the exception of clinical chemistry, hematology, and necropsy data, all data will be recorded in the laboratory notebook. The data from the 4-week study will be analyzed with a one-way repeated measures ANOVA used to compare the values from the vehicle control group to the values from the treatment groups across observation times. Daily overt toxicological observations may also be tested using an appropriate categorical test (e.g., Chi square, Fisher's exact) or quantified and evaluated with a one-way ANOVA. Data not normally distributed will be evaluated across treatments with a Kruskal-Wallis One-way ANOVA on ranks (SigmaStat® statistical software, SPSS Science, Chicago, Ill.). Multiple pairwise comparisons will be completed using the Dunn's Method. For all tests $\alpha = 0.05$ is the level of significance. Applicable regulatory guidelines for a repeated-dose inhalation study (references 2 and 3) specify using at least 10 rats (5 male and 5 female) per exposure concentration. In addition, these same guidelines make some general recommendations concerning the possible inclusion of a satellite group in the study design to assess reversibility, persistence, or delayed occurrence of toxic effects during a post-exposure period. Since this study will generally follow the recommendations of the applicable regulatory guidelines for the number of animals per concentration level for this type study, no statistical sample size justification needs to be performed.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: The objective of this study is to determine the adverse health effects resulting from acute and repeated inhalation exposure to black smoke in the rat. To date, there are no appropriate animal substitutes (e.g., computer models, tissue/cell cultures) for the data that will be produced by this study. No non-animal alternative would provide the necessary toxicological information required to make an accurate adverse health effect evaluation of black smoke. Therefore, it is necessary to perform this study in an animal model.

V.3.2. Animal Model and Species Justification: The USEPA Health Effects Test Guidelines (OPPTS 870.3465 90-Day Inhalation Toxicity) and OECD Guidelines for Testing of Chemicals (412 Repeated Dose Inhalation Toxicity: 28-Day or 14-Day Study) state that the rat is the preferred species. Sprague-Dawley rats are the strain of rat that have historically been used for inhalation toxicity studies conducted by the USAPHC (Prov) Directorate of Toxicology (DTOX) and is therefore the recommended strain of rat due to the extensive historical database.

V.3.3. Laboratory Animals

V.3.3.1. Genus and Species: *Rattus norvegicus*

V.3.3.2. Strain/Stock: Sprague-Dawley

V.3.3.3. Source/Vendor: Charles River Laboratories, Wilmington, MA (USDA 14-R-0144) or other USAPHC (Prov) approved vendor

V.3.3.4. Age: Acute & Pilot Studies: approx 7 weeks at delivery
Acute & Pilot Studies: approx 8 weeks at initiation of exposure
4-Week Study: approx 6 weeks at delivery
4-Week Study: approx 7 weeks at initiation of exposures

V.3.3.5. Weight: Age appropriate

V.3.3.6. Sex: 47 male and 47 female (nulliparious and non-pregnant)

V.3.3.7. Special Considerations: Animals will be tested for and certified free from *Bordatella*.

V.3.4. Number of Animals Required (By Species): 94 rats

V.3.5. Refinement, Reduction, Replacement

V.3.5.1. Refinement: For the 4-week study, an approximate 30-minute period

of acclimation for the rats to the modules will be performed at least one day prior to their initial exposure. No additional refinements will be employed other than the enrichment strategy discussed in V.5.3.1.

V.3.5.2. Reduction: The same set of control animals will be used for all parts of the 4-week study.

V.3.5.3. Replacement: None

V.4. Technical Methods

V.4.1. Pain/Distress Assessment:

V.4.1.1. APHIS Form 7023 Information

V.4.1.1.1. Number of animals

V.4.1.1.1.1. Column C: 6

V.4.1.1.1.2. Column D: 78

V.4.1.1.1.3. Column E: 10

V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Anesthesia will be administered prior to cardiac blood collection and euthanasia for the 4-week study animals. Anesthesia will be administered with isoflurane gas. Prior to administering the anesthesia, the oxygen tank and isoflurane levels will be checked to ensure they are sufficiently full and scavenger canisters will be connected to both exhaust lines. At this point in the process, the stopcock to the anesthesia chamber should be in the ON position and the stopcock to the nose cone should be in the OFF position. The oxygen tank can then be turned ON, the oxygen flowmeter set to approximately 1 L/min, the rat placed in the anesthesia chamber, and the chamber lid closed and secured by locking the latches on the chamber. The vaporizer on the anesthesia machine will be adjusted to approximately 3%. Once the rat is sufficiently anesthetized, the stopcock to the nose cone will be turned to the ON position and the stopcock to the anesthesia chamber will be turned to the OFF position. At this point, the rat will be taken out of the anesthesia chamber and placed on the work surface where the bleeding process is to take place. The nose cone will be positioned over the nose of the rat to ensure continual isoflurane anesthesia during the bleeding procedure. Prior to collecting the terminal blood sample, deep anesthesia will be ensured.

V.4.1.2.2. Pre- and Post-procedural Provisions: Prior to their blood collection

and necropsy, rats will be fasted from food overnight. The fasting period will not exceed 16 hours in length. Water will remain available to the rats during the food fasting period. Post-procedural observations, monitoring for exposure complications, and illness are described in section V.1.10 above.

V.4.1.2.3. Paralytics: None

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures

V.4.1.3.1. Sources Searched: Medline, TOXFILE, AGRICOLA, BIOSIS, EMBASE, and CA SEARCH

V.4.1.3.2. Date of Search: 22 July 2010

V.4.1.3.3. Period of Search: 1926-2010

V.4.1.3.4. Key Words of Search: pyrotechnic, smoke, dye, inhalation, aerosol, toxic, heart stick, cardiac stick, alternative, welfare, method, model, in vitro, pain, distress, simulate, video, computer, replacement, refinement, reduction

V.4.1.3.5. Results of Search: The literature search resulted in four hits. However, a review of the references in the search revealed that no validated in vitro tests for acute or 4-week inhalation toxicity studies are currently available, and therefore, it is necessary to conduct this study and expose rats to atmospheres of the test material.

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification: N/A

V.4.2. Prolonged Restraint: Rats will be contained in nose-only exposure cylinders during the 30-minute exposure period. Rats will be contained in the exposure cylinders during both the time it takes to insert the rats into the cylinder prior to the exposure (approx 30 minutes) and the time it takes to remove the rats from the cylinders following the exposure (approx 30 minutes). The total time that the rats will be in the exposure cylinders is estimated to be approximately 90 minutes. This type of exposure cylinder and restraint regimen is a commonly accepted method of restraint for rats exposed nose-only during an inhalation exposure (references 6 and 7). A 30-minute period of acclimation for the rats to the restrainers will be performed at least one day prior to their initial exposure for the 4-week study.

V.4.3. Surgery: None

V.4.3.1. Pre-surgical Provisions: N/A

V.4.3.2. Procedure: N/A

V.4.3.3. Post-surgical Provisions: N/A

V.4.3.4. Location: N/A

V.4.3.5. Surgeon: N/A

V.4.3.6. Multiple Major Survival Operative Procedures: None

V.4.3.6.1. Procedures: N/A

V.4.3.6.2. Scientific Justification: N/A

V.4.4. Animal Manipulations:

V.4.4.1. Injections: None

V.4.4.2. Biosamples: No biosamples will be collected from the acute or pilot study rats. For the 4-week study, approximately 1-3 ml of blood will be collected from each rat just prior to euthanasia. All blood sampling will occur under isoflurane gas anesthesia via a terminal cardiac puncture using an 18-21 gauge, 1-1.5 inch needle, as outlined in TOX SOP #053 (reference 11). Biosampling will be promptly followed by euthanasia via CO_2.

V.4.4.3. Adjuvants: None

V.4.4.4. Monoclonal Antibody (MAbs) Production: None

V.4.4.5. Animal Identification: Rats will be identified by cage cards according to TOX SOP #003 (reference 16). In addition, the last 3 digits of the animal number will be marked on the tail of each rat with a water-insoluble marker in order to ensure proper identification of rats when they are removed from their exposure modules at the end of each exposure.

V.4.4.6. Behavioral Studies: None

V.4.4.7. Other Procedures: All technical methods concerning smoke exposures, observations of animals, and necropsy of animals are described in sections V.1.5 – V.1.12 of this protocol.

V.4.4.8. Tissue Sharing: Tissue sharing may be available upon request provided the request does not interfere with the tissues required for meaningful interpretation of this study.

V.4.5. Study Endpoint: The study endpoint is euthanasia when moribund or survival to scheduled timepoints following exposure and observation periods. The duration of the observational period for the acute study will not exceed 18

days postexposure. The duration of the observational period for the pilot study will not exceed 5 days postexposure. The duration of the observational period for the 4-week study will not exceed 5 days postexposure for the 48 rats scheduled to be necropsied immediately following the exposure period and will not exceed 40 days postexposure for the 24 rats scheduled to be necropsied following a one month recovery. One or more of the following clinical signs will be indicative of a moribund animal: impaired ambulation which prevents animals from reaching food/water; excessive weight loss or extreme emaciation (≥ 20% body weight); lack of physical or mental alertness; prolonged labored breathing; unabated seizure activity; inability to urinate or defecate for greater than 24 hours; or a prolonged inability to remain upright. The Attending Veterinarian may be consulted to evaluate moribund animals if any of these signs are observed and the Attending Veterinarian and/or PI/SD will determine if euthanasia is indicated. At the end of the exposure and/or recovery periods, all appropriate animals from the 4-week study will be anesthetized for cardiac blood sampling, euthanized by CO_2 (euthanasia will be ensured by pneumothorax), and necropsied (acute and 4-week studies only). Animals not used for the acute and repeated-dose exposures will either be transferred to another protocol or humanely euthanized per protocol guidance.

V.4.6. Euthanasia: Euthanasia will be in accordance with TOX SOP #066 (reference 17). Rats will be euthanized by CO_2 and ensured by pneumothorax immediately followed by necropsy, when applicable. Study personnel and/or Veterinary Medicine staff will perform euthanasia.

V.5. Veterinary Care

V.5.1. Husbandry Considerations: Water will be available *ad libitum* throughout the acclimation and study periods except during the exposures. Food will be available *ad libitum* throughout the acclimation and study periods except during the exposures and the pre-necropsy fasting cycle for the acute and 4-week studies. All rats will be maintained in a temperature-, relative humidity-, and light-controlled room. The conditions of the animal room will be targeted to a temperature range of 64-79°F, 30 to 70% relative humidity, and a 12:12 hour light/dark cycle. Rats will be housed individually (except possibly during acclimation) in suspended polycarbonate boxes with bedding.

V.5.1.1. Study Room: Studies will be conducted at the USAPHC (Prov) Toxicology Directorate facilities, Bldg E-2100 or Bldg E-2101 (study room as assigned) or in approved transition facilities.

V.5.1.2. Special Husbandry Provisions: Animals may be pair-housed (sexes separate) during the quarantine/acclimatization period for the acute, pilot, and 4-week studies, however, animals will be singly housed during study conduct due to the unknown toxicity of the test substance. Animals will be fasted overnight (no more than 16 hours) prior to blood collection and/or necropsy for the acute

and 4-week studies, otherwise, they will be given certified rodent feed *ad libitum*.

V.5.1.3. Exceptions: Acclimation to exposure cylinders, as well as body weight measurements, will be conducted during the animals' acclimation period, but not during the first 24 hours following placement into their homecage upon arrival in this facility. These procedures will be performed by the study staff.

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: All animals will be observed at least twice daily by assigned Veterinary Medicine personnel (once daily on weekends and holidays) for husbandry conditions, humane care, and general health. During the exposure periods, animals will be observed by study personnel prior to loading them into the exposure chambers and then again after they are removed from the exposure chambers. In addition during the recovery periods, study animals will be observed at least once daily (weekends and holidays excluded) by study personnel. Observations by study personnel will be documented in the study records. If there is a need for increased frequency of observations, the duty veterinarian will consult with the PI/SD.

V.5.2.2. Emergency Veterinary Medical Care: All emergency animal health care will be provided by the Veterinary staff in consultation with the PI whenever possible.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy: Standard rat enrichment will be implemented in accordance with TOX SOP #122 (reference 18). Animals will be handled on a frequent basis and provided a form of enrichment (e.g., nylabones) throughout the study, except during the 30-minute exposure period.

V.5.3.2. Enrichment Restriction: None

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Staff Member	Procedure	Training	Experience	Qualifications
Lee Crouse	Inhalation exposure, observations/ handling, bleed, euthanasia (CO_2), necropsy	Humane Care and Use of Lab Animals (May 2000); Rodent handling techniques, WRAIR (Nov 1996)	15+ Yrs Animal Research	M.S. Environmental Science

John Houpt	Observations/ handling, euthanasia (CO_2), necropsy	Rodent Handling Workshop, USAMRICD (Nov 2003); Humane Care and Use of Lab Animals (May 2000, April 2003)	22+ Yrs Animal research	B.S. Biology
Wilfred McCain	Observations/ handling, euthanasia (CO_2), necropsy	Animal Care & Use Training (March 1995); Humane Care and Use of Lab Animals (May 2000)	30+ Yrs Animal research	PhD Toxicology
Mark Way	Observations/ handling, euthanasia (CO_2), necropsy Inhalation exposure	Rodent & Small Animal Handling workshops (May 2007); Necropsy procedure training (May 2007); Rat handling training (July 2007) TBS by A. O'Neill	14+ Yrs Animal research	B.S. Biology; LAT (1990)
Terry Hanna	Observations/ handling, euthanasia (CO_2), necropsy	Rodent Handling & Techniques (3/1992); Rodent & Small Animal Handling Workshop (MRICD 2004, 2005, 2006); Rat handling and gavage training (CHPPM 2007); Rat euthanasia via CO_2 with thoractomy (3/2009); Rat isoflurane anesthesia, cardiac blood draw, & CO_2 euthanasia training (5/2009); Necropsy training (2/2009, 1/2010)	15+ Yrs Animal research	LAT
Art O'Neill	Inhalation exposure, observations/ handling, euthanasia (CO_2), necropsy	Inhalation testing experience (Memo from DuPont dated Oct 2008)	30+ yrs Animal research	B.S. Biology; LATG (1990)

TBS = To be scheduled

VII. BIOHAZARD/SAFETY: In accordance with CHPPM Regs. 385-1 and 385-5 and TOX SOP #083, standard laboratory protection (e.g., glasses, gloves) will be used when working with the test material. All required PPE will be used when working with animals (live or deceased). A scavenging system will be used with the isoflurane gas anesthesia system. Test substances shall be stored in sealed containers when not in use. Although the precise toxicity of the test substance may not be known, information regarding its chemical family is provided by the sponsor such that a reasonable assessment of its safety can be made (references 19, 20, and 21).

VIII. EXTRAMURAL COLLABORATION: The sponsor for this study is the U.S. Army Research Development and Engineering Command (RDECOM) Environmental Acquisition and Logistics Sustainment Program (EALSP). The Point of Contact (POC) is Dr. Mark Johnson, Ordnance Environmental Program, USACHPPM/HERP, Aberdeen Proving Ground, MD 21010. The sponsor will provide funding and the test material black smoke cartridges required to conduct this study.

IX. ENCLOSURES:

A. Appe ndix A – References

X. ASSURANCES:

X.1. As the Study Director/Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

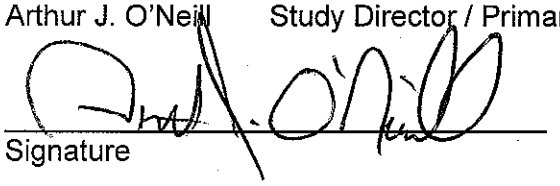
E. Training: I verify that the personnel performing the animal procedures/manipulations/observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R," namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL and WILL NOT be relieved with the use of anesthetics, analgesics, and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Arthur J. O'Neill Study Director / Primary Investigator



Signature

20100825

Date (YYYYMMDD)

X.2. As the Primary Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

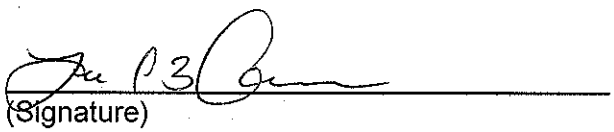
B. Authority: I understand that, as the Primary Co-Investigator, I am authorized and responsible for performing all procedures and manipulations as assigned to the SD/PI in the SD/PI's absence. This includes euthanasia of distressed animals.

C. Training: I verify that I am technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

D. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

E. Painful Procedures: I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL and WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Lee C.B. Crouse Primary Co-Investigator



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(Date) (YYYYMMDD)

APPENDIX A

References

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