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90-Day Inhalation Toxicity Study of FT Fuel

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14. ABSTRACT
FT or S-8 jet fuel is a synthetic organic mixture produced using the Fischer-Tropsch (FT) process that converts natural gas to liquid hydrocarbons. This study was designed to assess the potential inhalation toxicity of FT jet fuel aerosol and vapor via whole-body inhalation exposure to male and female Fischer-344 rats for 6 hours per day, 5 days per week over approximately 90 days, at concentrations of 0, 200, 700, and 2000 mg/m^3. In the motor activity test, males exposed to the highest concentration of FT jet fuel showed a reduction in total activity; the females in the same group displayed a reduction in the initial exploratory activity. Neurobehavioral function was assessed using the Functional Observation Battery (FOB). A reduction in rearing behavior was observed in females exposed at the highest concentration. Olfactory epithelial degeneration and respiratory epithelial hyperplasia was observed in the nasal airways of the high concentration male and female rats. In the lung, minimal to mild multifocal areas of inflammatory cell infiltration were observed in the high concentration groups in male and female rats. Animals exposed to the high concentration of jet fuel showed somewhat lower body weights. Differences in the average organ weights were seen between the high exposure concentration and controls in the liver, thymus, epididymides, spleen, and heart of male rats, and lungs, kidneys, and brain of females. No significant differences were observed for the andrology endpoints or the vaginal cytology results. Toxicity results were comparable to current jet fuel, JP-8.

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FT, S-8, Fischer-Tropsch, jet fuel, subchronic, 13 week, 90-day, inhalation, vapor/aerosol mix, toxicity

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PREFACE

Funding for this project was provided through the Air Force Research Laboratory, Propulsion Directorate, Fuels Branch (Dr Tim Edwards, AFRL/RZPF) and the Alternative Fuels Certification Office (AFMC 77 AESW/LF, now ASC/WNN). This research was conducted under contracts FA8601-07-P-0448 and FA8601-07-P-0473. The program manager for the contracts was LT Dean Wagner, PhD, USN Naval Health Research Center/Environmental Health Effects Laboratory (NHRC/EHEL). The technical manager for the program under which this project was conducted, Fischer Tropsch (F-T) Jet Fuel Toxicity Assessment, was Dr David Mattie of 711 HPW/RHPB (now RHDJ). The authors acknowledge the following individuals who also served on a review panel for this program and this project: John Hinz (USAFSAM/OEHTH, Brooks City Base, TX); Gunda Reddy, PhD (USACHPPM, Aberdeen Proving Ground, MD); David Steup, PhD (Shell Oil Company, Houston, TX; Chairman, American Petroleum Institute-Toxicology Task Force); and Errol Zeiger, Ph.D., J.D. (Errol Zeiger Consulting, Chapel Hill, NC).

This study was conducted in compliance with the United States Environmental Protection Agency (U.S. EPA) Good Laboratory Practice (GLP) Standards (40 CFR Part 792), with few noted exceptions.

The study protocols were designed to be in general compliance with the U.S. EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guidelines 870.3465 90-Day Inhalation Toxicity (U.S. EPA, 1998a) and OPPTS 870.6200 Neurotoxicity Screening Battery (U.S. EPA, 1998b).

This animal study was approved by the Air Force Surgeon General's Human & Animal Research Panel (FWR-2008-0005A) and The Hamner Institutes for Health Sciences Animal Care and Use Committee (protocol number 08013). The study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

The authors would like to acknowledge the contributions of several scientists who performed the procedures in this study. The clinical pathology evaluation was performed by Doug Neptun, Laboratory Director and Statistician, Antech Diagnostics, Morrisville, NC. Analysis of kidney samples for alpha-2μ-globulin binding was performed by Kevin Leiner, PhD, Integrated Laboratory Systems, Inc. (ILS), Durham, NC. Evaluation of sperm motility, count and morphology was conducted by Ms. Carol Sloan, RTI International, RTP, NC. Neurotoxicological effects (motor activity and FOB) were assessed by Ms. Melanie Struve, BS, DABT, Research Associate, College of Veterinary Medicine, North Carolina State University, Raleigh, NC. The fingerprint analysis was performed by Dr. Mark Sochaski of the Hamner Institutes.

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1.0 SUMMARY

Designated as Synthetic Paraffinic Kerosene (SPK) by the Air Force, FT jet fuel is a synthetic organic mixture produced in the Fischer-Tropsch (FT) process using carbon monoxide and hydrogen from natural gas, coal, or biological (bio) feedstock. The candidate SPK fuel tested in this study was produced by Syntroleum (Tulsa, OK) from natural gas and is called S-8. FT jet fuel is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the U.S. armed forces. This study was designed to assess the potential inhalation toxicity of FT jet fuel when administered as an aerosol and vapor via whole-body inhalation exposure to rats for 6 hours per day, 5 days per week over approximately 90 days, at concentrations of 0, 200, 700 and 2000 mg/m^3. Ten male and ten female Fischer-344 rats were in each exposure group, and each exposure group had two replicates of five males and five females each. Assessments included clinical observations, gross pathology, histopathology of target organs, functional observational battery (FOB) and motor activity tests, vaginal cytology and sperm morphology.

After the 12th week of exposure, animals were assessed for motor activity. In animals exposed to the highest concentration of FT jet fuel, the males showed a reduction in total activity, while the females reduced their initial exploratory activity. After the 13th week, neurobehavioral function was assessed using the FOB. A reduction in rearing behavior was observed in females exposed at the highest concentration, but no other evidence of neurotoxicity was seen.

No adverse effects were seen in any target tissues other than the nose and lungs and in male rats, in the kidneys. Olfactory epithelial degeneration and respiratory epithelial hyperplasia was observed in the nasal airways of the high concentration (2000 mg/m^3) male and female rats. In the lung, minimal to mild multifocal areas of inflammatory cell infiltration were observed in the high concentration groups in male and female rats.

Animals exposed to the high concentration of jet fuel showed slightly lower body weights. Differences in the average organ weights were seen between the high exposure concentration and controls, notably in the liver, thymus, epididymides, spleen, and heart of male rats, and lungs, kidneys, and brain of females. These observed differences in organs between the high concentration groups and the controls were correlated to the decreased body weight and were not necessarily a response to the jet fuel.

The α2μ-globulin results showed little or no change in measured levels relative to exposure concentration, consistent with the minimal increase in hyaline droplets in the male rat kidney from the high concentration group compared with the controls. No significant differences were observed for the andrology endpoints of interest. The vaginal cytology results did not show a significant alteration of the estrus cycle.

A chemical analysis of the aerosol and vapor phase of the delivered test chemical mixture was performed which determined the hydrocarbon fingerprint of the FT jet fuel in the chambers by GC/MS. A number of peaks, such as n-octane, n-nonane and n-tetradecane (i.e., n-octane through n-pentadecane) were qualitatively identified in the FT fuel mixture.

Overall, the results of this study indicate that FT jet fuel is similar to or less toxic than JP-8.

2.0 INTRODUCTION

Designated as Synthetic Paraffinic Kerosene (SPK) by the Air Force, FT jet fuel is a synthetic organic mixture produced in the Fischer-Tropsch (FT) process using carbon monoxide and hydrogen from natural gas, coal, or biological (bio) feedstock. The candidate SPK fuel tested in this study was produced by Syntroleum (Tulsa, OK) from natural gas and is called S-8. FT jet fuel is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the U.S. armed forces. FT jet fuel is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the U.S. armed forces. JP-8 fuel contains a mixture of aliphatic and aromatic hydrocarbons. The FT process for S-8 creates a mixture of aliphatic compounds similar to those found in JP-8, but does not form aromatic compounds such as benzene and naphthalene. This difference of composition between FT jet fuel and JP-8 points to a potential difference in the toxicity of the two fuels.

During refueling operations, personnel may be exposed to vapors and aerosols of jet fuel primarily by dermal or inhalation exposure. A review of JP-8 jet fuel toxicology concluded that exposure to JP-8 near the permissible exposure limit (PEL) of 350 mg/m^3 was potentially toxic to the immune, respiratory and nervous systems (NRC, 2003). Physiological effects on pulmonary function and cellular effects have been observed (Herrin *et al.*, 2006), as well as neurobehavioral effects in humans exposed to jet fuels including JP-8 (NRC, 2003; Baldwin *et al.*, 2007). Consequently, the National Research Council (NRC) proposed a reduction in the JP-8 Occupational Exposure Limit (OEL) to 200 mg/m^3, a value formally adopted by the U.S. Air Force in AFOSH 48-8. The American Conference of Industrial Hygienists (ACGIH, 2003) has also set its threshold limit value (TLV) for kerosene and jet fuel at 200 mg/m^3.

Very limited toxicity testing of FT jet fuel has been performed. Since inhalation is a major route of exposure for JP-8 jet fuel, the assessment of toxicity of FT by inhalation is needed to assess the risk of replacing or augmenting JP-8. Data from this study will be used to develop an occupational exposure limit for FT jet fuel.

3.0 METHODS

This study was designed to assess the potential inhalation toxicity of FT jet fuel when administered as an aerosol and vapor via inhalation exposure to rats for 6 hours per day, 5 days per week over approximately 90 days, at concentrations of 0, 200, 700 and 2000 mg/m^3. Ten male and ten female Fischer-344 rats were in each exposure group, and each exposure group had two replicates of five males and five females each (Table 1). The replicates were staggered by one day in the exposure schedule to accommodate the necropsy at the end of exposures. Due to the stagger in exposures, accommodation of holidays and the neurobehavioral test schedule, the study spanned a total of 96 days from first exposure to last necropsy (for a nominal 90-day study), with each replicate receiving 65 exposures. After the completion of exposures, animals were euthanized and necropsied. Assessments included clinical observations, gross pathology, histopathology of target organs, functional observational battery and motor activity tests, vaginal cytology and sperm morphology.

Table 1. Study Design

Group	Exposure Level	Number of Animals	
	mg/m^3	Males	Females
Control			
Replicate 1	0	5	5
Replicate 2	0	5	5
Low Group			
Replicate 1	200	5	5
Replicate 2	200	5	5
Intermediate Group			
Replicate 1	700	5	5
Replicate 2	700	5	5
High Group			
Replicate 1	2000	5	5
Replicate 2	2000	5	5
Total		40	40

3.1 Test Substance

The FT Jet Fuel (S-8 Synthetic Jet Fuel, CAS No. 437986-20-4) was obtained from the manufacturer (Syntroleum Corporation, Tulsa, OK). An additive package was added to the FT jet fuel by the Air Force Research Laboratory Fuels Branch at Wright Patterson Air Force Base. The additive package consists of proprietary icing, static and corrosion inhibitors normally added to JP-8 jet fuel at low concentrations (0.1 percent volume/volume, 2 mg/L and 15 mg/L, respectively). The combination of FT jet fuel with additives was designated as POSF 5109. Information regarding the purity and composition and stability of the FT jet fuel is maintained by the U.S. Air Force. The jet fuel was administered as supplied and was stored under room temperature ambient conditions. The terms FT jet fuel, S-8 jet fuel and jet fuel have been used interchangeably in this report and appended documents.

Three drums of FT jet fuel were used over the course of the study. The contents of the first drum were used in the two previous jet fuel studies (Mattie *et al.*, 2011a). Two samples were taken from the first jet fuel drum after the two-week study. Two more drums were used for the remainder of this study. Samples of Drum 3 were taken when opened and after the end of the study. These samples were analyzed at Wright-Patterson Air Force Base (AFB) using gas chromatography to determine stability. A sample was also taken of the stock at Wright-Patterson AFB from which the jet fuel sent to The Hamner Institutes was drawn.

3.2 Animals and Animal Husbandry

A total of 44 male and 44 female rats (Fischer (CDF®) [F344/DuCrl]), approximately 6 weeks old, were obtained from Charles River Laboratories (Kingston, NY). Animals were assigned temporary identification numbers and cage locations upon receipt. All animals were examined by the animal care staff and 40 male and 40 females rats considered suitable on the basis of physical examination, body weight and pre-study ophthalmologic exam were selected for the study. Animals were acclimated to the facility for approximately two weeks. During the acclimation period, animals were individually housed in stainless steel wire-mesh cages (R-24 cage units, Lab Products, Inc., Seaford, DE) in an animal room. Each R-24 cage unit has a capacity of 24 rats in individual compartments, so a single cage unit held an entire group of 20 rats (10 females and 10 males). Four cage units were used to house the study animals.

Room conditions were maintained at a target of 22°C, 50 percent humidity, with a 12 hour light/dark cycle. Animals were fed a certified rodent diet, NIH-07 (pellets, Zeigler Brothers, Gardners, PA) and reverse osmosis purified municipal tap water, *ad libitum*, except during exposure, when food was withheld.

Prior to exposure, animals were weighed and assigned randomized animal numbers using a Provantis NT-2000 protocol (Provantis, Conshohocken, PA) and identified with stainless steel ear tags. Five days prior to start of exposures, animals in R-24 wire mesh cage units were transferred into 1 m^3 chambers (H1000, Lab Products, Seaford, DE) (one cage unit per chamber) for housing acclimation. For exposures, the cage racks containing animals were transferred into the 1 m^3 exposure chambers and were exposed for 6 hours per day, 5 days per week, for 90 days to the aerosol-vapor mixture of jet fuel. After each day's exposures, animals were transferred back to the housing 1 m^3 chambers during non-exposure periods, except during weekends as noted below. Control animals remained in one 1-m^3 exposure chamber during exposure and non-exposure periods, except during the weekends.

Food consumption was measured during the weekends between exposure weeks for the first eleven weeks. Following the exposure on Friday, animals in the exposure groups were transferred to individual polycarbonate caging. Food pellets were added to the stainless steel wire cage lid hopper and weighed. On Monday morning, prior to start of exposures, animals were transferred back to the exposure chambers. The stainless steel wire cage lids containing food pellets were weighed to determine the amount of food consumed.

3.3 Exposure System

Air for the exposure chambers was pulled by fans through a 95 percent high efficiency purified air (HEPA) filter and a charcoal filter, the temperature and humidity was adjusted as required, and the air was distributed to the exposure chambers. Air flow was measured by monitoring the pressure drop across an orifice plate at the inlet to each chamber. Air flow was calibrated using an in-line mass flow meter (Sierra Instruments, Inc., Monterey, CA). The temperature and relative humidity in the chambers was measured by using a humidity temperature transmitter (Hygromer 200 Series, Rotronic AG, Huntington, NY) located near the center top of the

chamber. The temperature transmitter was calibrated by comparison with a certified thermometer, and humidity was calibrated by comparison with saturated salt solutions.

3.4 Generation System

The jet fuel was generated as a mixture of aerosol and vapor by pumping the liquid jet fuel into an air atomizing nozzle (Model SUJ1A with fluid cap 1650 and air cap 64, Spraying Systems Co., Wheaton, IL). A liquid metering pump (Fluid Metering, Inc. (FMI), Syosset, NY) pumped liquid jet fuel from a glass bottle reservoir to the nozzle. Compressed instrument air at approximately 50 psi was supplied to the nozzle. The nozzle assembly was housed in a stainless steel sanitary tee fitting. The spray was directed into a custom-made glass mixing volume. The total flow of the chamber passed through the glass tube, carrying the generated jet fuel mixture into the exposure chamber (Figure 1).

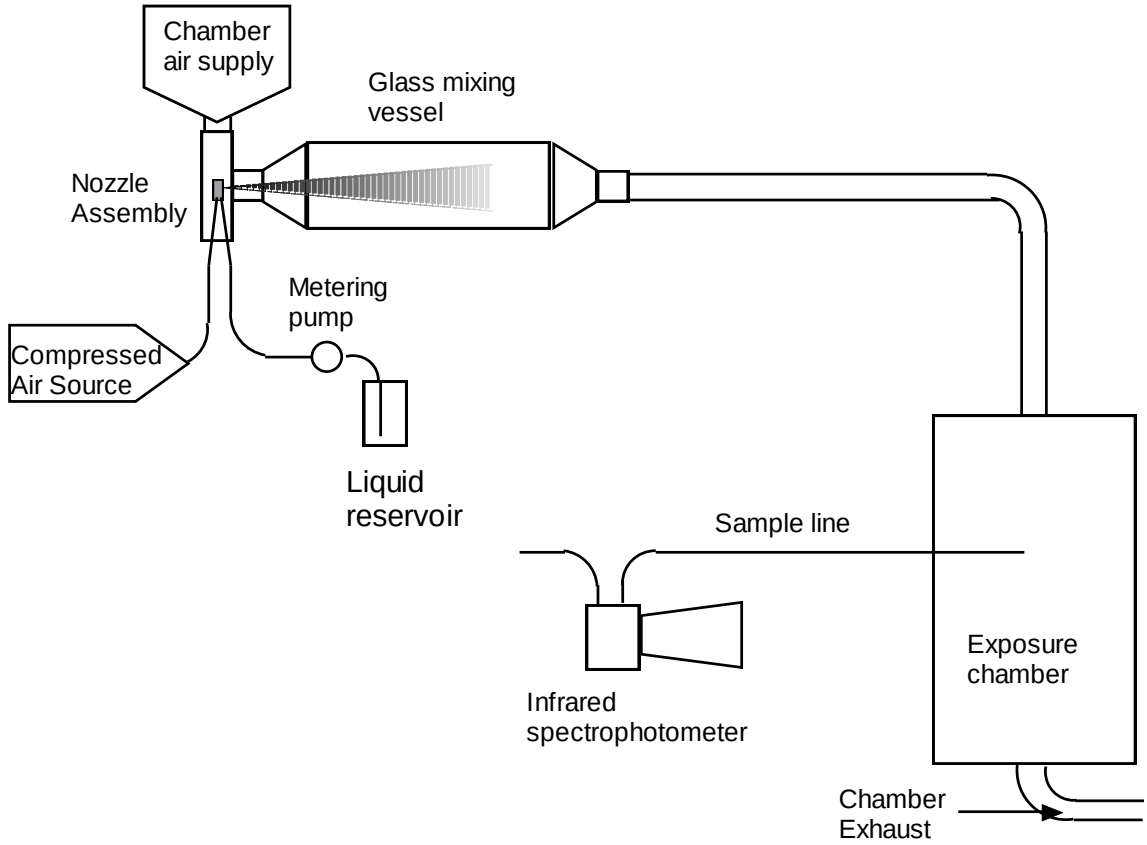

Figure 1. FT Jet Fuel Generation and Exposure System Schematic

3.5 Infrared Spectrometer Concentration Measurement

An infrared spectrophotometer (MIRAN 1A, Foxboro Co., South Norwalk, CT) was used to monitor the concentration of jet fuel in the chamber. The sensing cell of the infrared (IR)

spectrophotometer was warmed to approximately 50°C by a heat tape. A sample of the chamber atmosphere was pulled through the IR spectrophotometer. When the sample was pulled through the heated cell, the aerosol droplets evaporated. A chart recorder was used to continuously record the electrical output of the IR spectrophotometer.

The infrared spectrophotometer was calibrated using a closed loop method. Jet fuel was injected in a series of volumes to produce a set of increasing concentrations of jet fuel. A calibration curve of spectrophotometer response as a function of jet fuel concentration was produced. Nominal concentration was calculated from the air flow rate through the chamber and the FMI pump rate.

3.6 Chamber Distribution

The uniformity of distribution within the exposure chamber was checked by measuring the concentration at different locations within the chamber and from the home port (primary sampling port). As animals were placed in a single cage rack in the middle level of the $1m^3$ exposure chamber, four corner locations in the cage rack were sampled for uniformity. Chamber distribution measurements were conducted using the infrared spectrophotometer and it was determined that the variability in chamber concentration was less than four percent, indicating that the distribution of the test compound within the chamber was uniform.

Aerosol concentration was determined by taking a gravimetric filter sample from the chamber. A sample of the atmosphere was pulled through the filter at a known flow rate and time. The aerosol concentration was calculated from the mass of the jet fuel collected on the filter and the volume of atmosphere pulled through the filter.

Particle size distribution measurement was conducted using an aerodynamic particle sizer (Model 3321, TSI, Inc., St. Paul, MN). The instrument was connected to a sample port on the chamber. Dilution air was added in order to keep the aerosol concentration from overloading the conditions required for the particle sizer.

3.7 Necropsy

Following the last exposure, animals were weighed and euthanized by an overdose of sodium pentobarbital. Blood was collected via cardiac puncture for hematology and clinical chemistry. The animals were exsanguinated by transection of the abdominal aorta; and then necropsied. The necropsy included examination of the external surface and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. The pathology observations were conducted by Dr. Gabrielle A. Willson, B.V.M.S., MRCVS, F.R.C. Path, from Experimental Pathology Laboratories, Inc. (EPL). Wet weights of the lungs, liver, kidneys, adrenals, testes, epididymides, ovaries, uterus, thymus, spleen, brain, and heart were obtained after dissection from the exposure and control animals.

3.8 Clinical Chemistry and Hematology

During necropsy, blood was taken via cardiac puncture into three different vials for clinical chemistry and hematology. On the first necropsy day, all three vials could not always be filled by the lower blood volumes from smaller animals, and there was insufficient sample for some measurements. On the second necropsy day, the volume of blood taken for one of the vials was reduced, which enabled all three vials to be filled, even with lower blood volumes from smaller animals. Samples were transported to Antech Diagnostics, Morrisville, NC, where they were analyzed for clinical chemistry and hematology under the supervision of Doug Neptun, Laboratory Director and statistician.

3.9 Histopathology

The respiratory tract tissues and other tissues from all study animals were fixed and stained for microscopic examination using appropriate methods. Histological slides were prepared at EPL, where microscopic examinations were performed by Dr. Willson. These tissues included the trachea, larynx, lungs (2 sections), liver (2 sections), kidney (right and left), spleen, adrenals (right and left), heart, and nasal cavity (4 sections). Tissues from the high concentration and control groups were initially examined histologically. If treatment-related observations were detected, additional tissues from the low and intermediate concentration groups were examined.

3.10 $\alpha_{2\mu}$-Globulin

A sample of the right and/or left kidney of all male rats and from female rats in the control and high concentration groups were taken and frozen in liquid nitrogen.

During the necropsy, kidney samples were placed in vials and immersed in liquid nitrogen. On the first necropsy day, some identification labels fell off of the vials after placement in liquid nitrogen. Therefore, many of the first necropsy day kidney samples could not be identified. The labels were more securely attached for the second necropsy day. A total of 44 kidney samples were available for analysis.

Kidney samples were taken for $\alpha_{2\mu}$-globulin analysis using a sandwich enzyme-linked immunosorbent assay (ELISA) assay. Sample analysis was carried out by Integrated Laboratory Systems (ILS, Durham, NC) under the supervision of Kevin Leiner, Ph.D.

3.11 Sperm Motility and Concentration

At necropsy of each male rat, the right cauda epididymis was removed and weighed. Seminal fluid from the cauda was assessed manually for sperm motility and slides were made for sperm morphology determination. The remainder of the cauda and the right testis from each male was frozen at approximately -70°C for further analysis. Evaluation of sperm motility, count and morphology was conducted under the guidance of Ms. Carol Sloan, RTI International, RTP, NC.

Sperm motility was determined for all groups. Caudal sperm number, as well as enumeration of testicular homogenization-resistant spermatid heads, and calculation of daily sperm production (DSP) and efficiency of DSP in each testis was evaluated using an Integrated Visual Optic System (IVOS) Automated Sperm Analysis System (Version 12.1c, Hamilton-Thorne Research , Beverly, MA) in samples from adult males in the high dose and control groups.

3.12 Vaginal Cytology

Vaginal cytology was conducted on female rats in all groups during the 12^{th} week of exposure. Vaginal lavage was performed on each female rat daily, prior to exposure, for a five day period. The lavage fluid was placed on a glass slide and dried. The vaginal smears were stained with methylene blue and vaginal cell types were identified under a light microscope.

3.13 Motor Activity

Motor activity was assessed after approximately 12 weeks of exposure, on a non-exposure day following 5 consecutive days of exposure. Animals were transferred into individual polycarbonate cages and spontaneous motor activity was determined using an automated photobeam data collection system. Both fine movements and ambulations were measured and the data were collected by a computer system.

3.14 Functional Observational Battery (FOB)

Neurobehavioral evaluations were performed using an observation battery designed to detect functional deficits. Rats (n = 10 rats/sex/exposure group) were evaluated on non-exposure days after approximately 13 weeks of inhalation exposure to air or FT jet fuel. Two FOB sessions evaluating five rats/sex/exposure group/session were conducted. Conduct of the FOB evaluation was balanced across sex and exposure concentration in each test session. Observations were made: 1) while the rat wais in an observation cage, 2) during removal of the rat from the observation cage, 3) while the rat was being held and examined for clinical observations, 4) as the animal moved freely about the open field, and 5) during manipulative tests. The FOB consisted of non-invasive procedures designed to evaluate and document the absence or presence (or severity, if appropriate) of a predetermined set of behavioral and clinical signs. The animals were observed for:

- Posture
- Signs of involuntary muscular movements (tremors, spasms, and convulsions)
- Palpebral closure
- Handling reactivity
- Muscle tone
- Fur condition (piloerection, fur appearance, facial crust, skin temperature and color)
- Breathing pattern
- Salivation and lacrimation
- Arousal

- Ataxia
- Gait
- Body position
- Excessive vocalization
- Stereotypy and unusual behaviors
- Defecation, diarrhea, urination, and rears
- Approach response
- Startle response
- Tail pinch responses
- Visual placing
- Grip strength
- Surface righting reflexes
- Hind leg splay
- Pupillary reflex
- Body weight
- Any additional observations were recorded

Neurotoxicological effects (motor activity and FOB) were assessed by Ms. Melanie L. Foster, BS, DABT, Research Associate, College of Veterinary Medicine, North Carolina State University, Raleigh, NC. The motor activity and FOB assessments were conducted at the Hamner testing facility.

3.15 Fingerprint Analysis

The FT jet fuel with additives was characterized as part of a separate project (not conducted under GLP) by the Analytical Chemistry Services Group at The Hamner Institutes. Aerosol and vapor phase FT jet fuel samples were collected from the exposure chambers during high (2000 mg/m^3), middle (700 mg/m^3) and low (200 mg/m^3) FT fuel exposures for the purpose of qualitatively comparing the various samples using gas chromatography/mass spectroscopy (GC/MS) analysis. Additionally, samples of the exposure atmosphere were taken and separated into the aerosol and vapor fraction using an electrostatic precipitator. Both fractions were analyzed on a gas chromatograph mass spectrometer to identify major components of the jet fuel. The fingerprint analysis was performed by Dr. Mark Sochaski of the Hamner Institutes.

4.0 RESULTS

4.1 Test Substance Characterization

The results of the gas chromatographic analysis conducted at Wright-Patterson AFB of the jet fuel samples showed no differences between the sample of the drum taken after the completion of the study, and the sample from the original stock material at Wright-Patterson AFB (Mattie *et*

al., 2011a). At the level of resolution between the chromatograms, no change between jet fuel samples was observed which indicated that the jet fuel was stable.

4.2 Exposure Period

For this study, the exposure period started when the compressed air and the jet fuel flow were applied to the nozzle. The concentration in the chamber began to increase immediately, as observed on the infrared spectrophotometer chart recording. At the end of the exposure period, the compressed air and fuel flow to the nozzle were shut off. The aerosol concentration was observed to drop as expected. The overall vapor concentration, however, was observed to take significantly longer to clear the chamber. In order to avoid exposing the animals to this long tail of hydrocarbons, animals were moved from the exposure chamber to the respective housing chamber by moving the entire wire mesh rack from one chamber to the other. Control animals were exposed and housed within the same chamber. However, the rack was pulled out and pushed back into the chamber to simulate the rack movements.

4.3 Exposure Conditions

Over the course of the exposures, concentration, temperature, humidity, air flow, and static pressure readings were recorded (Table 2). The average temperature, humidity, and air flow remained at target level, and did not deviate outside of prescribed ranges. The study average total chamber concentrations were 0.02 ± 0.10, 200.1 ± 5.0, 698.6 ± 16.7, and 1988.4 ± 48.1 mg/m^3 for the 0, 200, 700, and 2000 mg/m^3 chambers, respectively. Nominal concentrations for each chamber, based on the liquid pump flow rate and the chamber air flow, were 173.2 ± 8.2, 598.9 ± 44.6 and 2046.8 ± 76.8 mg/m^3, giving analytical to nominal concentration ratios of 1.16, 1.17, and 0.97, respectively.

The aerosol mass concentration was measured using gravimetric filters. Filter samples were taken during exposures approximately two to three times per week over the course of the study. The average aerosol concentrations were 0.11 ± 0.12, 1.28 ± 0.54, 81.8 ± 14.2, and 656.4 ± 67.7 mg/m^3 (Table 3). The aerosol composed 0.6 percent of the total concentration for the low concentration chamber, and increased to 11.7 and 33.0 percent of the total jet fuel concentration in the intermediate and high concentration chambers, respectively. Thus, as the total FT jet fuel concentration increased, the fraction of the total that existed as aerosol droplets increased as well.

An aerodynamic particle sizer was used to measure the particle size distribution. Measurements were made by sampling from each chamber twice during a week, for a total of 16 measurements over the course of the exposure. The average mass median aerodynamic diameter and geometric standard deviation (MMAD (GSD)) of the aerosols were calculated as 1.6 (1.5), 1.6 (1.5), 2.6 (1.7) and 2.6 (1.6) µm for the control, low, intermediate, and high concentration chambers, respectively (Table 3). Aerosols with particle size distributions between 1 and 4 µm are generally considered as respirable by rodents.

Table 2. Exposure Conditions

	Target Concentration	0 (mg/m^3)	200 (mg/m^3)	700 (mg/m^3)	2000 (mg/m^3)
1-m^3 Temperature (°F)	Mean of daily means	**72.8**	**72.4**	**68.9**	**70.3**
	Std Dev	0.5	0.4	04	0.4
	Maximum daily mean	73.6	73.6	69.8	71.4
	Minimum daily mean	70.7	70.4	67.2	68.8
	No. of Days	67	67	67	67
1 m^3 Relative Humidity (%)	Mean of daily means	**52**	**43**	**45**	**54**
	Std Dev	1	1	1	1
	Maximum daily mean	56	45	47	56
	Minimum daily mean	49	41	43	51
	No. of Days	67	67	67	67
1 m^3 Air Flow (L/min)	Mean of daily means	**225**	**224**	**225**	**226**
	Std Dev	1	1	2	2
	Maximum daily mean	234	226	230	230
	Minimum daily mean	223	219	216	216
	No. of Days	67	67	67	67
Actual Chamber Static Pressure (in H_20)	Mean of daily means	**0.260**	**-0.127**	**-0.247**	**-0.225**
	Std Dev	0.082	0.034	0.071	0.070
	Maximum daily mean	0.388	0.129	-0.113	-0.130
	Minimum daily mean	-0.001	-0.160	-0.475	-0.455
	No. of Days	67	67	67	67
Actual Chamber Concentration (mg/m^3)	Mean of daily means	**0.02**	**200.1**	**698.6**	**1988.4**
	Std Dev	0.10	5.0	16.7	48.1
	Maximum daily mean	0.62	217.4	750.3	2092.0
	Minimum daily mean	0	183.4	664.4	1866.6
	No. of Days	67	67	67	66
Nominal Chamber Concentration (mg/m^3)	Mean	**N/A**	**173.2**	**598.9**	**2046.8**
	Std Dev	N/A	8.2	44.6	76.8
	Maximum daily mean	N/A	151.8	537.1	1892.8
	Minimum daily mean	N/A	192.9	706.2	2219.9
	No. of Days	N/A	67	67	67
	Analytical to nominal ratio		1.16	1.17	0.97
Gravimetric Concentration (mg/m^3)	Mean	**0.11**	**1.28**	**81.8**	**656.4**
	St. Dev	0.12	0.54	14.2	67.7
	N	42	42	42	42
	Proportion of total Concentration	**NA**	**0.006**	**0.117**	**0.330**
Particle Size	MMAD (μm)	**1.60**	**1.58**	**2.62**	**2.59**
	GSD	1.54	1.49	1.67	1.63
	N	16	16	16	16

Table 3. Aerosol Mass Concentration and Particle Size Distribution

Target Exposure Level	Mass Concentration (mg/m^3)	Aerosol Fraction	Particle Size Distribution MMAD (GSD) (µm)
0	0.11 ± 0.12	NA	1.60 (1.54)
200	1.28 ± 0.54	0.006	1.58 (1.49)
700	81.8 ± 14.2	0.117	2.62 (1.67)
2000	656.4 ± 67.7	0.330	2.59 (1.63)

MMAD = mass median aerodynamic diameter
GSD = geometric standard deviation

4.4 Statistics

The study was programmed into a data management system (Provantis™ NT2000 and Provantis™ 8, Instem LSS, Ltd., Staffordshire, UK) with four exposure groups (200, 700, and 2000 mg/m^3 FT jet fuel and a control with clean air). Each exposure group had two replicates, both with five males and five females, for a total of ten males and ten females at each exposure concentration. The replicates were staggered by one day in the exposure schedule. In-life data were recorded in the Provantis data management system based on replicates. The two replicates could not be combined into one group for statistical analysis by the Provantis™ System, so the combined replicates data were put into a spreadsheet for further data analysis. To calculate statistical parameters based on exposure group (and not on replicate groups), a statistical analysis program (JMP, v. 7.0.1, SAS Institute, Cary, NC) was used for the in-life data such as body weight, body weight gain, and food consumption. Levene's test ($p<0.01$) was performed to check for equal variance, followed by a one-way analysis of variance (ANOVA) ($p<0.05$). If means were unequal, then a Dunnett's test was used to compare treatment means to control ($p>0.05$). Provantis was used to calculate statistics for organ weights.

Some individual data points for body weight and food consumption appeared to be outliers. Four male animals appeared to lose weight in week three while the rest of their growth curves were comparable with other animals. Three of the four were control rats, while the remaining animal was in the high dose group. All questionable weights occurred on the same day. An undetected problem with the weighing process is suspected. Although not statistical outliers based on the Outlier Box Plot and Grubb's Test (NIST, 2010), these four body weights in week three were excluded from group analysis. The remaining data were then subject to the same statistical analysis as above.

4.5 Animal Body Weights

Except for the issue mentioned above, all other animals gained weight over the course of the study (Figures 2 and 3). For both the females and males, the average group weight increased through study day 1, when the animals were weighed prior to the start of exposures. Males ranged in weight from 151.1 to 189.1 g, and females from 99.1 to 136.0 g. The average body weight of the males in the control, low (200 mg/m^3), and intermediate (700 mg/m^3) exposure groups continually increased, though the rate of weight gain appeared to slow after the start of exposures. For male rats, there were significant differences in body weight between control and jet fuel treated animals in the high (2000 mg/m^3) concentration group from week six through the end of the exposure and at necropsy. Female average body weight followed a similar pattern, with the high (2000 mg/m^3) concentration group lower in average body weight, but differing significantly from the control group only for weeks 12 and 13. Tabular bodyweight data are found in Appendix A.

The body weight trends discussed above are also illustrated by the body weight gain (Figures 4 and 5). The male rats exposed to the highest concentration of FT jet fuel (2000 mg/m^3) had a lower weight gain than controls at weeks 2 and 3 (statistically significant for week 3), and also for weeks 6 and 12. For the other weeks, the body weight gain for the male rats exposed at the highest concentration tended to be lower than the other groups, though the difference was not statistically significant. There was a statistically significant higher body weight gain for the low concentration group (200 mg/m^3) at week 3, and the intermediate concentration group (700 mg/m^3) at week 8. The females exposed at the high concentration also showed a decreased body weight gain at weeks 2, 3 and 4 (statistically significant). The body weight gain of the high concentration females tended to be on the low side for other time points, though not statistically significant. Tabular weight gain data are in Appendix A.

This body weight gain data for the males and females combined with the group average body weight observations would seem to indicate that the body weight gain was depressed most during the first two or three weeks for animals exposed at the high concentration. After the first few weeks, there was less of an effect on body weight gain, though the high concentration animals continued to lag behind the other groups in body weight.

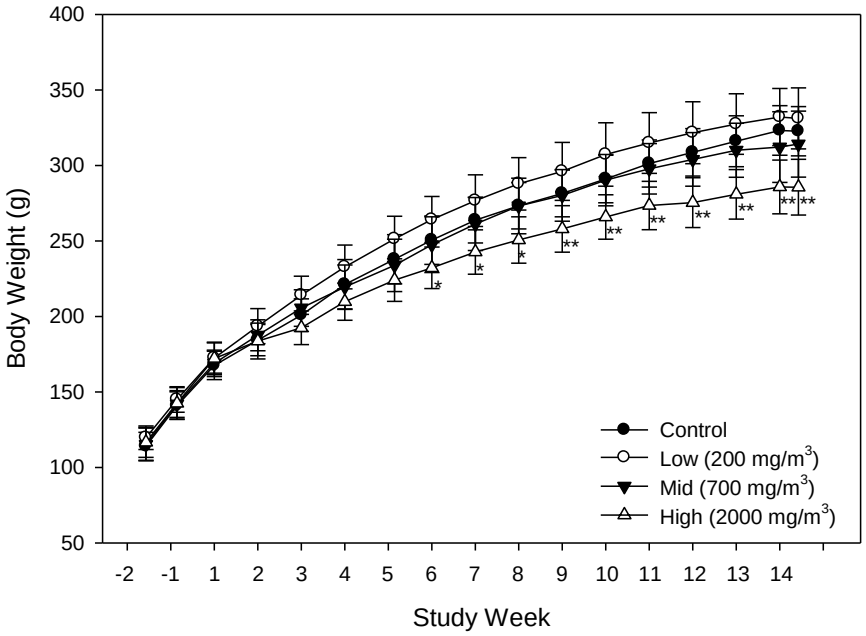

Figure 2. Male Body Weights
Note: *Significant at p<0.05; **
Significant at p<0.01

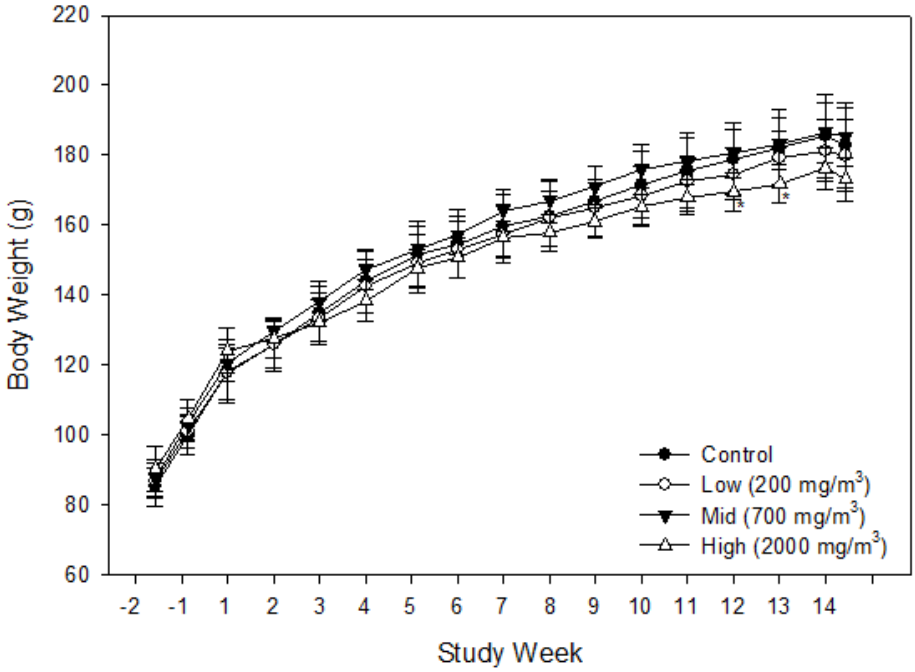

Figure 3. Female Body Weights
Note: *Significant at p<0.05

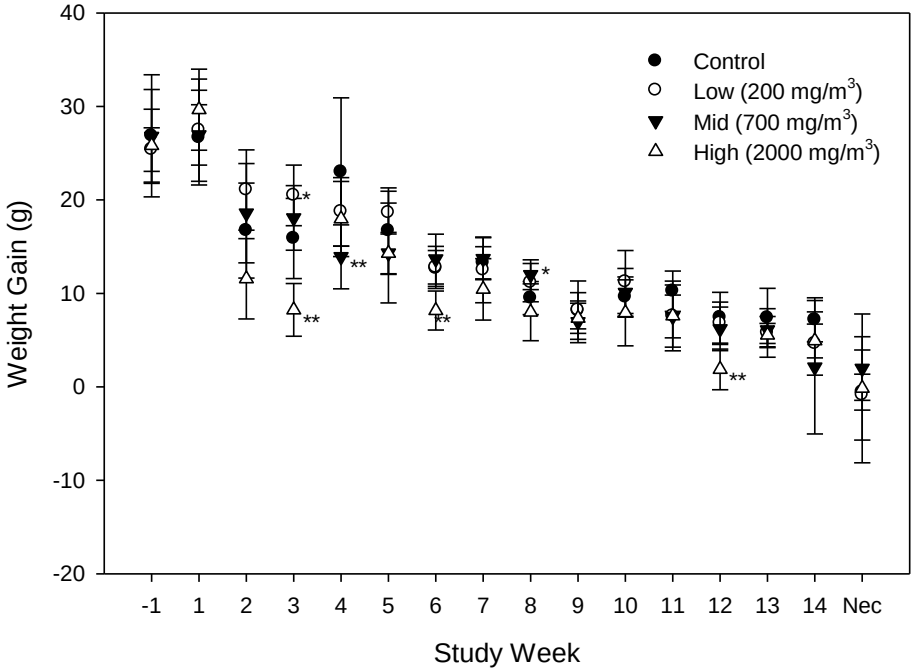

Figure 4. Male Body Weight Gain
Note: *Significant at p<0.05; **Significant at p<0.01

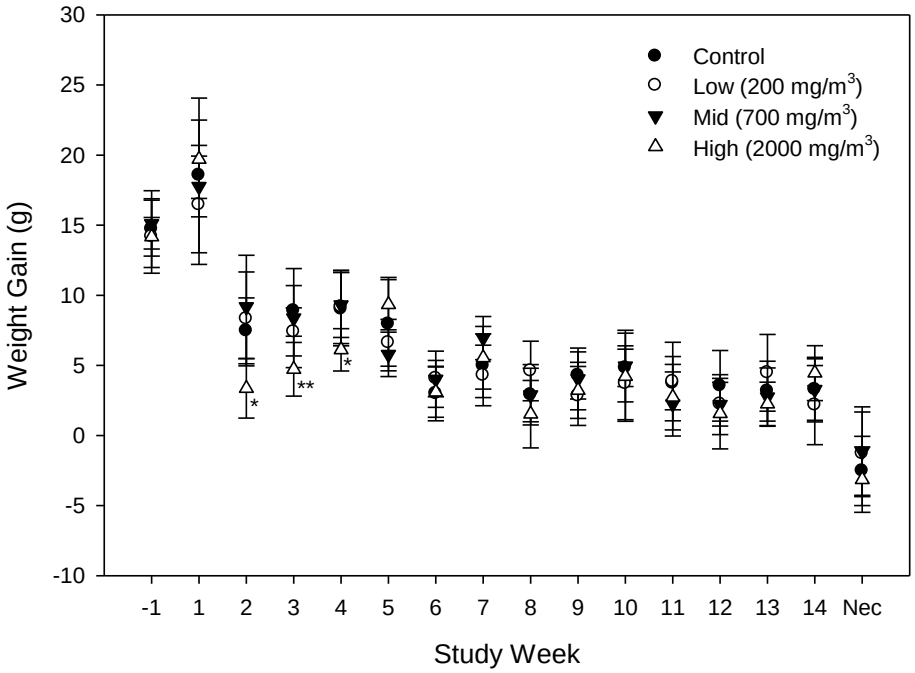

Figure 5. Female Body Weight Gain
Note: *Significant at p<0.05; **Significant at p<0.01

4.6 Food Consumption

There were 11 periods of food consumption measurement during the weekends between exposures. The food consumption for females when compared with controls was generally consistent across the exposure groups, except at week three where consumption was significantly lower for the high concentration group (Figure 7). For males, the data were more scattered, but males in the high concentration group had significantly lower consumption of food at weeks 1, 3, 5, 6 and 11 (Figure 6). Food consumption was significantly lower for the intermediate concentration group at weeks 3, 4, and 6 and higher for the low concentration group (200 mg/m^3) at week 9. Tabular food consumption data are found in Appendix A.

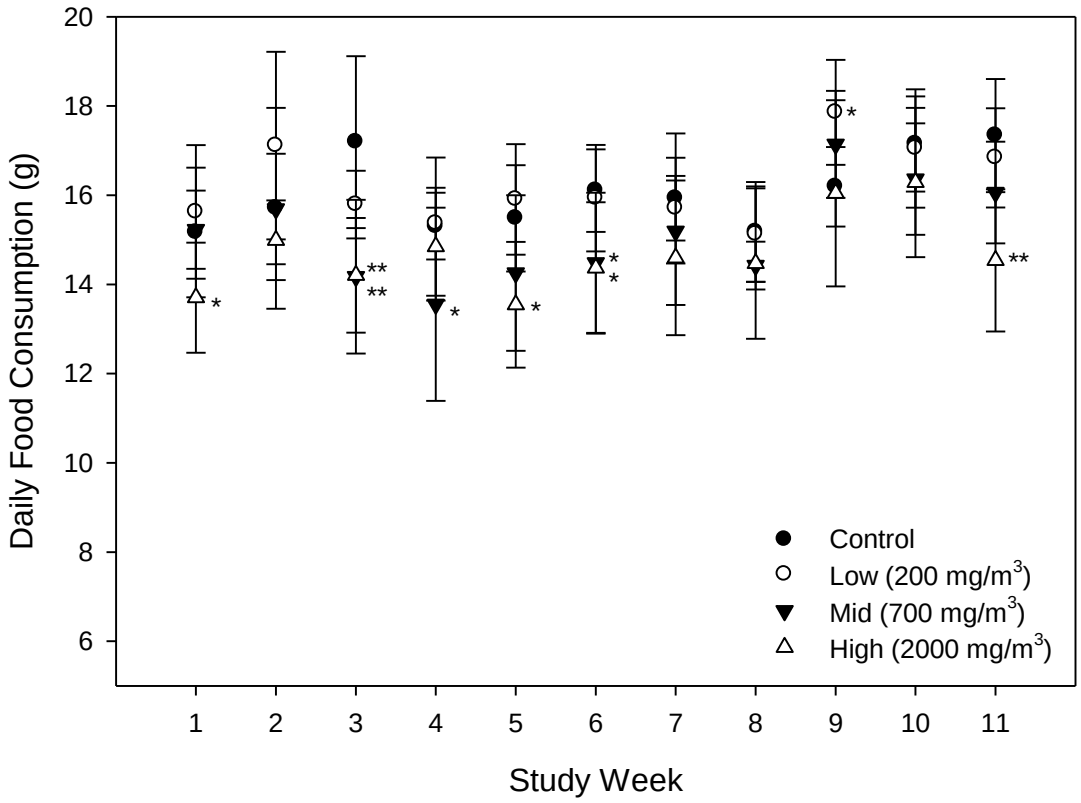

Figure 6. Average Daily Food Consumption in Males
Note: *Significant at p<0.05; **Significant at p<0.01

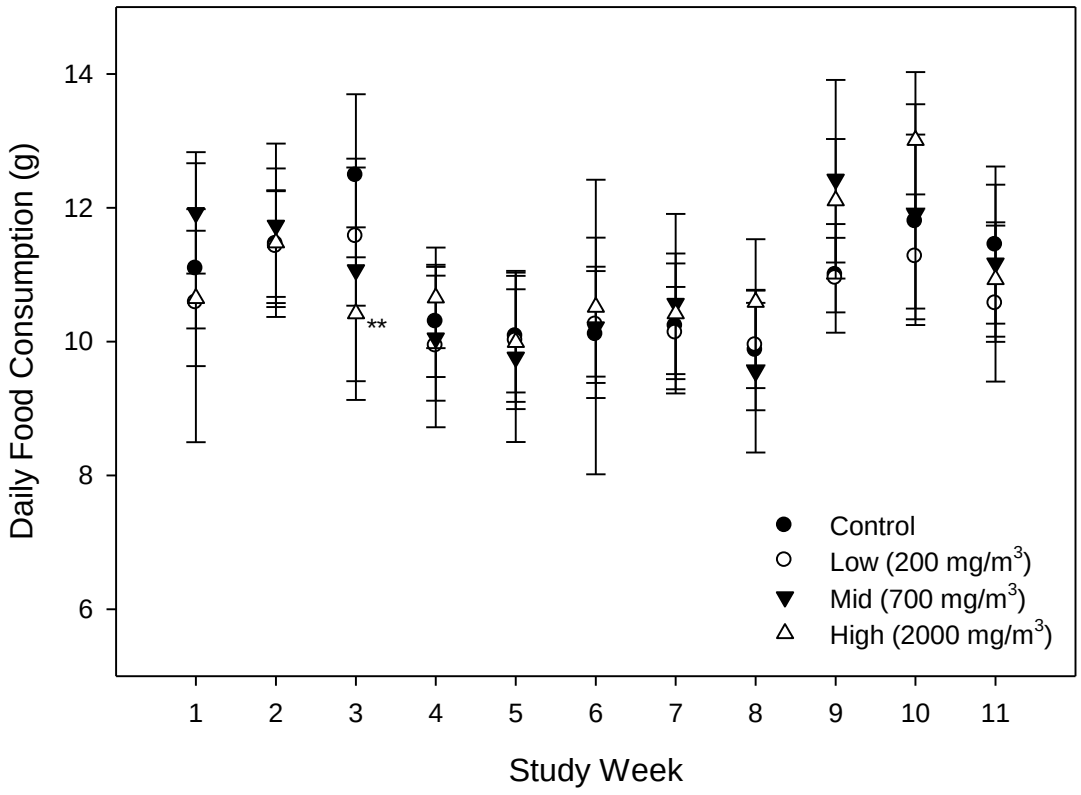

Figure 7. Average Daily Food Consumption in Females
Note: **Significant at p<0.01

4.7 Clinical Observations

Over the course of the study, no unscheduled deaths occurred, nor were any animals found in a moribund condition. Clinical observations were conducted when animals were weighed. The observations that could be related to the exposure regimen included pelage alopecia and nasal discharge in 10 of 10 female rats after 4 weeks of exposure at the high concentration. These observations in female rats typically continued through the end of the study. In the male rats, the clinical observations noted were pelage alopecia in 9 of 10 rats after 4 weeks of exposure, and 1 rat after 5 weeks, at the high dose. Nasal discharge was observed in 1 of 10 male rats after 4 weeks of exposure, and in the remainder after 5 weeks of exposure at the high concentration. These observations in male rats typically continued through the end of the study.

In the observations during the FOB procedure (Section 4.16), alopecia was noted in some males (2/10) around the forelimbs in the control group, and alopecia was observed around the nose (6/10) and flaky or scaly skin (6/10) in the high exposure group. Some alopecia was noted in females in the control or lower concentrations (1/10), but more extensively in the high concentration group (9/10). Flaky or scaly akin was also noted on most of the females in the high exposure group (8/10).

4.8 Ophthalmologic Exam Results

Ophthalmologic examinations were performed prior to the start of exposures and at the end of week 14 of the exposure regimen. For both examinations, no ophthalmologic effects other than those related to normal aging of the rat were seen. No ophthalmological effects attributable to the exposure were found.

4.9 Gross Pathology

In males exposed at the low concentration, one animal had an epididymal mass determined to be a sperm granuloma. One male in the control group had a cyst on the right kidney and 6 control males had thymus foci. Thymus foci were also seen in 4 males in the low concentration group, 5 males in the intermediate concentration group and 3 males that received the high concentration. These findings were not considered to be related to the administration of the jet fuel. The thymus foci were possibly related to the method of euthanasia (Stefanski *et al.*, 1990). No visible lesions were observed in female rats from any group.

The adrenals, brain, heart, liver, spleen, thymus, kidney (right and left) and lungs were weighed in all jet fuel exposure animals at the terminal necropsy. The testes (right and left) and epididymides (right and left) from the male rats and the ovaries and uterus from female rats were also weighed. The mean organ weights are shown for males (Table 4) and females (Table 5). The absolute weights of male adrenal glands were decreased in rats in the intermediate group compared with controls. The mean absolute weight of liver, thymus, epididymides, spleen, and heart were statistically less than that of controls for male rats in the high concentration group. The lower weight of some organs was likely due to the lower average body weight of the high exposure group males.

In female rats, absolute weights of adrenal glands were significantly elevated over controls in the low and high concentration groups, but not in the intermediate concentration group. The mean absolute weight of lung and kidneys in female rats in the high concentration group were elevated over controls. Absolute brain weights were also significantly increased in female rats of the intermediate and high groups. The reason is unknown for the elevated weight of some organs in females exposed at the high concentration with lower average body weights.

Table 4. Group Mean Organ Weights in Males

Males		Control	Low	Intermediate	High
Tissue Weight		Group 1	Group 2	Group 3	Group 4
Terminal Body Weight	Means (g)	322.623	331.154	314.116	285.599
	SD	16.288	20.244	21.855	18.483
	Ratio to Brain	171.117	172.225	168.061	154.572
Adrenal Gland (bilateral)	Means (g)	0.0489	0.0470	0.0426*	0.0453
	SD	0.0082	0.0054	0.0245	0.0050
	% BW	0.02	0.01	0.01*	0.02
	Ratio to Brain	0.0258	0.0245	0.0228	0.0245
Kidney, Right	Means (g)	1.1132	1.0547	1.0061	0.9852
	SD	0.2538	0.0577	0.0806	0.0631
	% BW	0.35	0.32	0.32	0.35
	Ratio to Brain	0.5922	0.5491	0.5383	0.5334
Kidney, Left	Means (g)	1.0255	1.0684	1.0162	0.9941
	SD	0.0454	0.0596	0.0792	0.0613
	% BW	0.32	0.32	0.32	0.35
	Ratio to Brain	0.5443	0.5559	0.5435	0.5385
Liver	Means (g)	11.246	11.834	11.168	10.400*
	SD	0.6524	0.9874	0.6927	0.7774
	% BW	3.49	3.57	3.56	3.64*
	Ratio to Brain	5.9624	6.1549	5.9749	5.6298
Lung	Means (g)	1.4570	1.4048	1.3879	1.4839 [1]
	SD	0.1245	0.1395	0.1130	0.1040
	% BW	0.45	0.42	0.44	0.80
	Ratio to Brain	0.7714	0.7310	0.7432	0.8040
Thymus	Means (g)	0.288	0.278	0.261	0.244*
	SD	0.0514	0.0270	0.0277	0.0369
	% BW	0.09	0.08	0.08	0.09*
	Ratio to Brain	0.1520	0.1450	0.1399	0.1321
Spleen	Means (g)	0.700	0.726	0.701	0.626*
	SD	0.0471	0.0654	0.0791	0.0550
	% BW	0.22	0.22	0.23	0.22*
	Ratio to Brain	0.3719	0.3777	0.3755	0.3386
Brain	Means (g)	1.887	1.923	1.869	1.851
	SD	0.0850	0.0759	0.0586	0.0775
	% BW	0.59	0.58	0.60	0.65
	Ratio to Brain	1.0000	1.0000	1.0000	1.0000
Heart	Means (g)	0.927	0.941	0.909	0.863*
	SD	0.0838	0.0465	0.0484	0.0365
	% BW	0.29	0.28	0.29	0.30*
	Ratio to Brain	0.4921	0.4895	0.4865	0.4672

[1] N = 9, one animal value not included in calculations
SD – Standard Deviation
BW – Body Weight
*p<0.05
**p<0.01

Table 4. Group Mean Organ Weights in Males (continued)

Males		Control	Low	Intermediate	High
Tissue Weight		Group 1	Group 2	Group 3	Group 4
Testis, Right	Means (g)	1.5574	1.5843	1.5167	1.5072
	SD	0.0443	0.0652	0.0779	0.1456
	% BW	0.48	0.48	0.48	0.53
	Ratio to Brain	0.8268	0.8245	0.8115	0.8129
Testis, Left	Means (g)	1.5619	1.5016	1.5630	1.5362
	SD	0.0640	0.2312	0.0847	0.1162
	% BW	0.49	0.45	0.50	0.54
	Ratio to Brain	0.8296	0.7811	0.8364	0.8292
Epididymis, Right	Means (g)	0.5096	0.5099	0.5097	0.4666**
	SD	0.0300	0.0363	0.0295	0.0333
	% BW	0.16	0.15	0.16	0.16*
	Ratio to Brain	0.2706	0.2652	0.2729	0.2522
Epididymis, Left	Means (g)	0.5412	0.4969	0.5364	0.4939*
	SD	0.0651	0.1010	0.0383	0.0334
	% BW	0.17	0.15	0.17	0.17*
	Ratio to Brain	0.2868	0.0502	0.2872	0.2671

SD – Standard Deviation
BW – Body Weight
*$p<0.05$
**$p<0.01$

Table 5. Group Mean Organ Weights in Females

Females		Control	Low	Intermediate	High
Tissue Weight		Group 1	Group 2	Group 3	Group 4
Terminal Body Weight	Means (g)	182.820	179.940	185.310	173.110
	SD	12.0668	10.1998	8.2074	6.2139
	Ratio to Brain	106.4450	102.4252	105.0469	98.0042
Adrenal Gland (bilateral)	Means (g)	0.0489	0.0591**	0.0530	0.0581**
	SD	0.0056	0.0077	0.0038	0.0087
	% BW	0.03	0.03**	0.03	0.03**
	Ratio to Brain	0.0285	0.0336	0.0300	0.0329
Kidney, Right	Means (g)	0.6334	0.6315	0.6452	0.6720*
	SD	0.0513	0.0327	0.0160	0.0537
	% BW	0.35	0.35	0.35	0.39*
	Ratio to Brain	0.3690	0.3600	0.3658	0.3804
Kidney, Left	Means (g)	0.6343	0.6389	0.6585	0.6688*
	SD	0.0349	0.0432	0.0225	0.0415
	% BW	0.35	0.36	0.36	0.39*
	Ratio to Brain	0.3695	0.3638	0.3734	0.3788
Liver	Means (g)	6.249	6.355	6.262	6.219
	SD	0.4331	0.5856	0.3618	0.4686
	% BW	3.42	3.53	3.38	3.59
	Ratio to Brain	3.6387	3.6231	3.5499	3.5184
Lung	Means (g)	0.9704	1.0166	1.2645	1.0777*
	SD	0.0798	0.1083	0.6839	0.0965
	% BW	0.53	0.56	0.68	0.62*
	Ratio to Brain	0.5653	0.5786	0.7146	0.6097
Thymus	Means (g)	0.252	0.248	0.222	0.230
	SD	0.0379	0.0382	0.0308	0.0283
	% BW	0.14	0.14	0.12	0.13
	Ratio to Brain	0.1467	0.1410	0.1258	0.1302
Spleen	Means (g)	0.488	0.495	0.516	0.495
	SD	0.0294	0.0280	0.0337	0.0366
	% BW	0.27	0.28	0.28	0.29
	Ratio to Brain	0.2843	0.2820	0.2926	0.2803
Brain	Means (g)	1.718	1.761	1.764*	1.767*
	SD	0.0581	0.1068	0.0403	0.0395
	% BW	0.94	0.98	0.95*	1.02*
	Ratio to Brain	1.0000	1.0000	1.0000	1.0000
Heart	Means (g)	0.632	0.606	0.625	0.636
	SD	0.0494	0.0420	0.0259	0.0375
	% BW	0.35	0.34	0.34	0.37
	Ratio to Brain	0.3682	0.3454	0.3542	0.3600

SD – Standard Deviation
BW – Body Weight
*$p<0.05$
**$p<0.01$

Table 5. Group Mean Organ Weights in Females (continued)

Females		Control	Low	Intermediate	High
Tissue Weight		Group 1	Group 2	Group 3	Group 4
Ovaries (bilateral)	Means (g)	0.1019	0.1149	0.1073	0.1088
	SD	0.0148	0.0225	0.0128	0.0219
	% BW	0.06	0.06	0.06	0.06
	Ratio to Brain	0.0592	0.0652	0.0608	0.0614
Uterus	Means (g)	0.6020	0.607	0.745	0.568
	SD	0.1560	0.1744	0.2766	0.1149
	% BW	0.33	0.34	0.40	0.33
	Ratio to Brain	0.3510	0.3474	0.4228	0.3210

SD – Standard Deviation
BW – Body Weight
*p<0.05
**p<0.01

4.10 Histopathology

No adverse effects were observed in histological sections of target tissues other than the nose, lungs in both males and females and in the kidneys of male rats at the intermediate and high concentrations. Some lesions observed (e.g. cardiomyopathy, inflammation in the liver, hyaline droplet accumulation in respiratory epithelium) were considered to be spontaneous background occurrences unrelated to treatment, while foci in the thymus were considered to be related to the method of euthanasia (Stefanski *et al.*, 1990).

In the kidneys, males in the high exposure groups showed an accumulation of hyaline droplets in the proximal convoluted tubules. The observation was considered to be minimal as the hyaline droplets were slightly more prominent in the males exposed to 2000 mg/m^3 compared with controls.

Histological evaluations were conducted on nasal tissues of male and female rats in the control, 200, 700, and 2000 mg/m^3 exposure groups. Evaluations were made at four standard levels (I through IV) of the nasal cavity. Treatment-related findings in the nasal cavity were minimal to mild, olfactory epithelial degeneration and respiratory epithelial hyperplasia. Respiratory epithelial hyperplasia was found in the anterior of the nose (Level I) and was characterized by an increase in number and height of the epithelium. The respiratory epithelial hyperplasia was seen at minimal levels in males and females exposed at 700 mg/m^3, and at mild levels in males and females exposed at 2000 mg/m^3. Olfactory epithelial degeneration was generally minimal in the intermediate dose group and increased to minimal to mild in extent in the high dose group of both sexes, and tended to occur near the junctions of respiratory and olfactory epithelium. When olfactory epithelial degeneration was more pronounced, multifocal patchy areas of the septum and turbinates in Levels III and IV were affected. Nasopharyngeal duct goblet cell hypertrophy or hyperplasia was minimal in Level IV at 700 mg/m^3 and mild at 2000 mg/m^3 in both male and female rats.

In the lungs, at the highest concentration in both male and female rats, there were minimal to mild multifocal areas of inflammatory cell infiltration, typically neutrophils and alveolar histiocytes with occasional lymphocytes, throughout the lung parenchyma. No interstitial fibrosis or granuloma formation was evident. Animals exposed at the intermediate concentration showed a lesser effect. The presence of neutrophils and alveolar histiocytes, but the lack of interstitial fibrosis or granuloma formation in the lungs suggests that the infiltrate was a "cleanup response", as opposed to signs of more permanent damage to the lung. Details of the histopathology results and specific incidences of the observed effects are provided in the histopathology report, Appendix B.

4.11 Clinical Chemistry and Hematology

There were no biologically significant or toxicologically significant changes in hematology or clinical chemistry values from exposure to FT Jet Fuel. Details of the clinical chemistry and hematology results are provided in Appendix C.

For male rats, there were no hematologic differences between control and treated animals in any dose group. The results for prothrombin time (PT) and activated partial thromboplastin time (APTT) did not show any effect in clotting factors. From blood clinical chemistry results, alkaline phosphatase (ALP) was decreased in the 700 and 2000 mg/m^3 groups of male rats. Alanine aminotransferase (ALT), cholesterol, total protein and albumin were decreased in the 2000 mg/m^3 groups of male rats. Elevated ALP or ALT could be indicative of damage to the liver or blockage of the biliary ducts. However, decreased levels may be related to deficiency of various vitamins, minerals or other inorganic compounds possibly linked to malnutrition. The decreased ALP and ALT levels may to be related to possible nutritional effects as demonstrated by the decreased weight gain in the 700 and 2000 mg/m^3 groups of male rats. These results were not considered to be toxicologically significant.

For female rats, mean corpuscular hemoglobin (MCH) was decreased in the 2000 mg/m^3 female rats with a slight but not statistically significant decrease in erythrocytes, hemoglobin and hematocrit. Decreased hemoglobin, hematocrit and MCH could be indicative of a problem with anemia but because the decreases were barely distinguishable from controls, the decreases are not considered to be biologically significant. Although there were only three to five results per group for PT and APTT, there did not appear to be any effect in clotting factors. From the clinical chemistry results, chloride was increased and albumin decreased in the 2000 mg/m^3 groups of female rats. The relative differences from controls were small and isolated to two analytes, so the changes were not considered to be biologically significant.

4.12 $\alpha_{2\mu}$-Globulin Quantitation

An ELISA assay was used to measure the concentration of α2μ-globulin in kidney samples of male and female rats. Levels of α2μ-globulin were present in the kidney samples of all male rats, including control animals (except one male rat in the high concentration group) at quantities >100-fold higher than found in the kidney samples of all female rats assayed. There was no

apparent trend relative to increasing exposure concentration. In fact, the concentration observed in the high exposure concentration rats was lower than in the other exposure concentration or control groups, though this difference was not statistically significant. Based on the histopathology observed, FT jet fuel did not induce significant α2μ-globulin nephropathy in F344 male rats. The biochemical measurements showed no increase in α2μ-globulin in any of the exposed males compared with controls, also indicating that exposure to FT jet fuel with additives did not induce significant α2μ-globulin nephropathy. The complete report can be found in Appendix D.

4.13 Sperm Motility and Concentration

Caudal sperm motility, determined for males in all exposures groups, was 91.0, 86.2, 85.5, and 85.0 percent for the control, low, mid, and high exposure concentrations, respectively. The statistical analysis showed no significant difference of the exposed groups from the control group.

Right caudal sperm concentration, determined for the control (790.35 million/mL) and high concentration group (778.48 million/mL) using automated sperm analysis, showed no significant difference between the high concentration and control group. As there was no difference between the high concentration and controls, the samples from the intermediate and low concentration animals were not evaluated.

Other calculated andrology endpoints, including spermatid head count, daily sperm production and efficiency of daily sperm production, also did not show any significant differences between the high concentration and control group. The complete andrology report is located in Appendix E.

4.14 Vaginal Cytology

Vaginal lavage was conducted on female rats in all exposure groups once a day over a five day period. The samples were stained to identify the predominant cell type present each day a sample was taken. The cell types identified represented the proestrus (early and late), estrus, metestrus and diestrus stages. The presence of cells representing at least three of the four stages in all females (but one control female) indicated that these animals were all going through the estrus cycle, regardless of exposure to the FT jet fuel at any concentration. Vaginal cytology was not carried out for a long enough period to determine whether FT jet fuel inhalation altered the length of the estrus cycle. The complete vaginal cytology report can be found in Appendix F.

4.15 Motor Activity

Spontaneous motor activity was determined in male and female F344 rats (10 rats/sex/exposure group) during a one-hour test session in air-exposed (control) and jet fuel-exposed rats. Motor activity was determined on a non-exposure day following approximately 12 weeks of inhalation

exposure. The activity of the control rats approached an asymptote during the last 10-15 minutes of the 60-minute testing session, indicating that the 60 minute time period was sufficient to meet the criteria detailed by the U.S. Environmental Protection Agency (U.S. EPA) testing guideline. There was a significant dose effect seen in male rats exposed to FT jet fuel for both ambulation and total movement seen during the one-hour test session. An ANOVA performed on these parameters revealed a significant decrease in ambulation and total movements in male rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed control males). There was also a decrease in total motor activity during the initial (6 minute) exploratory phase in male rats exposed to 200 or 2000 mg/m^3 (versus air-exposed control males). An overall treatment effect on motor activity was not seen for female rats exposed to FT jet fuel (multivariate analysis of variance (MANOVA)). As with the male rats, a decrease in total motor activity was seen during the initial (6 minute) exploratory phase in female rats exposed to 2000 mg/m^3 (versus air-exposed control females). These results indicate that the highest exposure concentration of FT jet fuel caused an overall reduction in motor activity in the males, and reduced the initial exploratory activity of female rats. The motor activity report is included as Appendix G.

4.16 Functional Observational Battery

Neurobehavioral evaluations using an observation battery designed to detect functional deficits (FOB) were also performed. Rats (10 rats/sex/exposure group) were evaluated on non-exposure days after approximately 13 weeks of inhalation exposure to air or FT jet fuel. Two FOB sessions evaluating five rats/sex/exposure group/session were conducted. Conduct of the FOB sessions was balanced across sex and exposure concentration in each test session. Exposure to FT jet fuel was associated with significant changes. Body weight was decreased in male and female rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed controls). When compared to air-exposed controls, female rats exposed to FT jet fuel at 2000 mg/m^3 had a reduced number of rears during a two-minute observation period in an open field. Rats exposed to the highest concentration of FT jet fuel also had changes in external appearance. Male rats exposed to FT jet fuel at 2000 mg/m^3 had a higher incidence of alopecia or flakey or scaly skin on the nose or feet (8/10) versus air-exposed controls (2/10) or male rats exposed to FT jet fuel at either 200 mg/m^3 (0/10) or 700 mg/m^3 (1/10). Female rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed controls) also had a higher incidence of alopecia or flakey skin on the nose or feet (9/10) versus air-exposed rats (1/10) or female rats exposed to FT jet fuel at either 200 mg/m^3 (2/10) or 700 mg/m^3 (1/10). With the exception of the decrease in rearing behavior seen during the FOB, there was no other evidence of neurotoxicity seen during the FOB evaluation of FT jet fuel exposed rats (Appendix G).

4.17 Fingerprint Analysis

The results of the assay by gas chromatography and mass spectrometry to identify major components of the jet fuel confirmed that the FT Jet Fuel was a complex mixture of C8 to C16 aliphatic compounds. Aerosol/vapor fraction analysis demonstrated an increased presence of high molecular weight compounds in the aerosol phase compared to an increased presence of low molecular weight compounds in the vapor phase. When comparing the aerosol phases of the

different concentration groups, there was no appreciable difference in the distribution of compounds among them. In the vapor phase, there appeared to be more total compounds present in the high concentration exposure samples compared to the low concentration exposure samples. A majority of the compounds (accounting for >90 percent of the total peak area in the sample) found in the low concentration vapor samples were those found between n-undecane and n-tetradecane. The high concentration vapor samples, meanwhile, appeared to contain a much larger range of molecular weight compounds (i.e., n-octane through n-pentadecane). Results are further described in Appendix H.

5.0 DISCUSSION

A study was conducted with male and female Fischer 344 rats exposed to synthetic FT jet fuel by inhalation. The jet fuel was generated as a mixture of aerosol and vapor at three target concentrations, 200, 700, and 2000 mg/m^3, and a control with clean air. A total of 10 male and 10 female rats were exposed at each concentration, in two replicates of 5 male and 5 female each. The replicates were staggered by one day in the exposure schedule. Exposures were conducted for 6 hours/day, 5 days per week for a total of 65 exposure days (nominally a 90-day study).

5.1 Exposures

FT jet was stable for the length of the entire study. There was good agreement between projected chamber concentrations, actual concentrations and nominal concentrations as evidenced by the nominal concentration ratios. There were no issues associated with any of the exposure conditions. Although this is an inhalation study, additional exposure could occur from aerosol deposition on the fur and skin of the animals resulting in dermal absorption and/or ingestion through grooming by the animals. Due to the higher concentrations of FT that were required to achieve significant aerosol concentrations, these alternate routes of exposure were probably minor compared to the inhalation route.

5.2 Animal Body Weights and Food Consumption

Animals exposed to the high concentration of FT jet fuel showed lower body weights. In the high concentration (2000 mg/m^3) group, the average male body weight was decreased by approximately 12 percent, while the average female body weight decreased 5 percent by the end of the exposures relative to the control average. One possible reason for the depression of body weight gain could be a toxicological effect of the FT jet fuel. Based on overall results, this does not appear to be the best explanation. It is possible that there was diminished appetite due an aftertaste following exposure sessions due to a higher level of residual jet fuel in the respiratory tract as a result of the higher aerosol concentrations. The decreased food consumption for the male high concentration group appears to correlate with the observed differences in average body weight and body weight gains at weeks 3 and 6, and for the females at week 3. Whether the body weight decrease is a result of decreased food consumption, or decreased food

consumption is due to smaller animals eating less, and how these relate to the inhalation of jet fuel cannot be determined from these data.

5.3 Observations and Organ Weights

There were very few adverse clinical observations. Gross pathological changes seen were minimal and not related to exposure. Overall changes were not dose response related or were observed only in the highest exposure group. Higher incidences of alopecia and flaky or scaly skin were noted in males and females exposed to the higher concentrations.

Differences in the average organ weights were seen between the high exposure concentration and controls, notably in the liver, thymus, epididymides, spleen, and heart of male rats, and lungs, kidneys, and brain of females. The lower weight of some organs was likely due to the lower average body weight of the high exposure group males. In female rats the reason is unknown for the elevated weight of some organs in females exposed at the high concentration with lower average body weights.

5.4 Motor Activity and FOB

After the 12^{th} week of exposure, animals were assessed for motor activity, and after the 13^{th} week for neurobehavioral function using the FOB. In animals exposed to the highest concentration of FT jet fuel, the males showed a reduction in total activity, and the females a reduction in initial exploratory activity. From the FOB assessment, a reduction in rearing behavior was observed in females exposed at the highest concentration, but no other evidence of neurotoxicity was seen.

5.5 Histopathology

Target tissues were examined histopathologically. No adverse effects were seen in any target tissues other than the nose and lungs and in male rats in the kidneys. Olfactory epithelial degeneration and respiratory epithelial hyperplasia was observed in the nasal airways of the high concentration (2000 mg/m^3) male and female rat. In the lung, minimal to mild multifocal areas of inflammatory cell infiltration were observed in the high concentration groups in male and female rats. A lesser effect was also seen in the lungs of the intermediate exposure groups.

5.6 Analytical Endpoints

There were statistically significant differences in the clinical chemistry and hematology that were not considered to be biologically significant. These observed differences in organ weights and in clinical chemistry between the high concentration groups and the controls were correlated to the decreased body weight, were not consistent with a dose response or necessarily the result of exposure to the jet fuel. The $\alpha 2\mu$-globulin results showed little or no change in measured levels

relative to exposure concentration. This response was consistent with the histopathology which noted only a minimal increase in hyaline droplets in the male rat kidney from the high concentration group compared with the controls. No significant differences were observed for the andrology endpoints of interest. The vaginal cytology results did not show a significant alteration of the estrus cycle.

5.7 Comparison with JP-8

JP-8 was tested in a similar inhalation study by Mattie *et al.* (1991). Ten week old Fischer 344 rats and C57BL/6 mice of both sexes were exposed to 0, 500 or 1000 mg/m^3 JP-8 vapor, continuously, for 90 consecutive days. The animals were then retained until 24 months of age. Male rats in both exposure groups failed to gain weight as well as control rats; the lower weights persisted until study termination. Female rat weight gains were not affected.

Male rats developed alpha-2μ-globulin nephropathy that persisted throughout recovery. Male kidney weights (absolute and relative) were increased in both exposure groups, as compared to controls, following the 90-day exposure and again at the age of 24 months. Exposed rats showed a dose-dependent incidence and severity of epithelial cells in the urine, which resolved two weeks following the exposure. Female rat kidneys were unaffected by exposure.

Exposed mice showed no clinical signs of treatment. Male, and to some extent, female mice had increased incidence of necrotizing dermatitis due to fighting as they were housed in community cages. Male mice losses to the dermatitis were dose-dependent.

JP-8 and FT jet fuels toxicity were found to be comparable. The major difference is the lack of male rat kidney formation of alpha-2μ-globulin with the FT jet fuel, which is associated with the aromatic hydrocarbons present in JP-8. Failure to induce alpha-2μ-globulin may be associated with the fact that aromatic hydrocarbons are not present in FT jet fuel. JP-8 may also contain other hydrocarbons that can induce alpha-2μ-globulin that are not present in FT jet fuel or that fail to bind to this protein when exposed to the FT jet fuel mixture (Leavens and Borghoff, 2009).

5.8 Fingerprint Analysis

The FT jet fuel contains no aromatic compounds. The range of C8 to C16 aliphatic compounds is similar to the C6 to C16 range of JP-8. There is no published comparison of JP-8 to FT fuel although a study was conducted for the Air Force Office of Scientific Research (personal communication, J. L. Campbell and J. W. Fisher, 2006, Department of Environmental Health Science, University of Georgia, Athens, GA).

5.9 Other FT studies

This study is a continuation of the toxicity assessment of FT fuel. Three assays, the reverse mutation assay, chromosome aberration test and micronucleus assay (Mattie *et al.*, 2011a, 2011b), demonstrated that FT fuel was not mutagenic or genotoxic. The acute dermal irritation study showed that FT is similar to JP-8, in that it is slightly to moderately irritating (Hurley *et al.*, 2011). Data from all FT toxicity studies will be used to develop a health hazard assessment for FT jet fuel as well as an occupational exposure limit.

6.0 CONCLUSIONS

FT jet fuel is similar to JP-8 but contains no aromatic compounds. The primary target organs for FT jet fuel appear to be the respiratory system and the central nervous system (CNS). In the nasal cavities and lungs, the greatest effects were seen at the 2000 mg/m^3 dose with effects just beginning at the 700 mg/m^3 level. For the CNS, effects were only seen at the highest dose. Overall, toxicity results were comparable to the current jet fuel, JP-8.

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APPENDIX A. MEAN TABULAR RESULTS

Table 1. Group Mean Body Weights

Males Study Week	Control (g)[1]	Low (g)[1]	Intermediate (g)[1]	High (g)[1]
-2	114.03 (9.269)	119.62 (7.811)	115.18 (10.891)	116.64 (9.948)
-1	140.89 (9.151)	145.00 (8.484)	141.98 (8.876)	142.45 (10.445)
1	167.55 (9.367)	172.46 (9.984)	168.93 (8.754)	172.11 (10.796)
2	184.27 (10.362)	193.52 (11.582)	187.51 (10.249)	183.67 (11.857)
3	200.83 [2] (10.827)	214.01* (12.651)	205.58 (12.080)	192.48 [3] (11.164)
4	220.16 (15.941)	232.74 (14.471)	219.49 (14.528)	209.76 (12.284)
5	237.85 (16.263)	251.38 (14.930)	233.81 (17.388)	224.04 (14.079)
6	250.52 (16.061)	264.16 (15.260)	247.48 (18.438)	232.20 * (13.740)
7	263.79 (15.113)	276.67 (17.076)	261.19 (17.561)	242.63 * (14.687)
8	273.28 (15.363)	287.82 (17.358)	273.19 (18.407)	250.61 * (15.374)
9	281.42 (15.500)	296.03 (19.195)	280.14 (17.095)	257.94 ** (15.375)
10	291.02 (15.754)	307.25 (20.989)	290.24 (17.010)	265.86 ** (14.738)
11	301.25 (15.603)	314.82 (20.095)	297.77 (16.7.4)	273.45 ** (16.022)
12	308.65 (15.740)	321.63 (20.494)	303.97 (17.738)	275.32 ** (16.509)
13	316.02 (16.832)	327.40 (20.032)	310.05 (17.929)	280.85 ** (16.379)
14	323.19 (16.393)	332.03 (18.886)	312.15 (23.353)	285.76 ** (17.771)
Terminal Weight	322.623 (16.288)	331.154 (20.244)	314.116 (21.855)	285.599 ** (18.483)

[1] MEAN (SD) N = 10 except where noted
[2] N = 7. Three animals not included in means and SD
[3] N = 9. One animal not included in mean and SD
*p<0.05
**p<0.01

Table 1. Group Mean Body Weights (continued)

Females Study Week	Control (g)[1]	Low (g)[1]	Intermediate (g)[1]	High (g)[1]
-2	85.12 (5.468)	86.95 (5.080)	87.71 (5.126)	90.3 (6.327)
-1	99.84 (5.576)	101.13 (4.781)	102.80 (4.767)	104.47 (5.700)
1	118.39 (98.982)	117.58 (7.311)	120.56 (5.364)	124.17 (6.281)
2	125.85 (7.709)	125.89 (6.727)	129.73 (3.007)	127.53 (5.678)
3	134.74 (7.872)	133.29 (7.241)	138.10 (5.725)	132.25 (6.320)
4	143.75 (8.821)	142.45 (7.685)	147.31 (5.611)	138.36 (5.918)
5	151.69 (9.638)	149.06 (8.373)	153.19 (6.625)	147.69 (5.279)
6	154.68 (9.776)	153.12 (7.966)	157.20 (5.544)	150.78 (5.661)
7	159.65 (9.056)	157.40 (8.319)	164.15 (6.055)	156.32 (5.042)
8	162.55 (10.172)	162.00 (7.855)	167.04 (6.196)	157.84 (5.147)
9	166.83 (9.809)	164.82 (8.095)	171.07 (5.976)	161.06 (4.651)
10	171.65 (9.720)	168.52 (8.539)	175.98 (7.163)	165.27 (5.480)
11	175.38 (11.168)	172.37 (7.262)	178.26 (6.720)	168.00 (4.987)
12	178.91 (10.242)	174.60 (7.308)	180.46 (7.105)	169.56 * (5.358)
13	182.08 (10.895)	179.07 (7.583)	183.2 (7.332)	171.81 * (5.423)
14	185.35 (11.834)	181.24 (8.901)	186.47 (8.657)	176.26 (5.889)
Terminal Weight	182.820 (12.0668)	179.940 (10.1998)	185.310 (8.2074)	173.110 (6.2139)

[1] MEAN (SD), N = 10
*$p<0.05$

Table 2. Group Mean Body Weight Gains

Males		Control	Low	Intermediate	High
Study Weeks		(g)	(g)	(g)	(g)
From	To				
-2	-1	26.86 (6.53)	25.38 (2.33)	26.79 (5.03)	25.81 (3.90)
-1	1	26.66 (5.07)	27.46 (5.47)	26.95 (3.23)	29.66 (4.33)
1	2	16.72 (5.08)	21.06 (4.29)	18.58 (5.31)	11.56 (4.29)
2	3	15.87[2] (4.28)	20.49 * (3.24)	18.07 (3.45)	8.23[3] ** (2.82)
3	4	22.99[2] (7.94)	18.73 (3.66)	13.91 ** (3.42)	17.96[3] (4.02)
4	5	16.69 (4.59)	18.64 (2.30)	14.32 (5.34)	14.28 (2.24)
5	6	12.67 (1.92)	12.78 (2.25)	13.67 (2.67)	8.16 ** (2.08)
6	7	13.27 (1.72)	12.51 (3.52)	13.71 (2.25)	10.43 (3.28)
7	8	9.49 (1.76)	11.15 (2.05)	12.00 * (1.60)	7.98 (3.04)
8	9	8.14 (1.93)	8.21 (3.12)	6.95 (2.23)	7.33 (1.61)
9	10	9.60 (2.15)	11.22 (3.36)	10.10 (2.56)	7.92 (3.53)
10	11	10.23 (2.16)	7.57 (3.1)	7.53 (2.28)	7.59 (3.73)
11	12	7.40 (2.72)	6.81 (2.26)	6.20 (2.33)	1.87 ** (2.18)
12	13	7.37 (3.17)	5.77 (2.59)	6.08 (1.44)	5.53 (1.25)
13	14	7.17 (2.36)	4.63 (3.39)	2.10 (7.14)	4.91 (1.81)
	Gain at Necropsy	-0.567 1.93	-0.876 4.81	1.966 3.40	-0.161 7.97

[1] MEAN (SD) N = 10 except where noted
[2] N = 7. Three animals not included in means and SD
[3] N = 9. One animal not included in mean and SD
*p<0.05
**p<0.01

Table 2. Group Mean Body Weight Gains (continued)

Females		Control	Low	Intermediate	High
Study Weeks		(g)	(g)	(g)	(g)
From	To				
-2	-1	14.72 (2.74)	14.18 (2.60)	15.09 (1.79)	14.17 (1.38)
-1	1	18.55 (5.51)	16.45 (4.25)	17.76 (2.17)	19.70 (2.79)
1	2	7.46 (2.35)	8.31 (3.35)	9.17 (3.68)	3.36 * (2.12)
2	3	8.89 (1.80)	7.39 (1.71)	8.37 (3.53)	4.72 ** (1.92)
3	4	9.01 (2.62)	9.17 (2.62)	9.31 (2.32)	6.11 * (1.50)
4	5	7.94 (3.33)	6.61 (1.67)	5.78 (1.58)	9.33 (1.79)
5	6	2.99 (1.94)	4.06 (1.30)	4.01 (2.01)	3.09 (1.79)
6	7	4.97 (2.26)	4.28 (2.16)	6.95 (1.54)	5.54 (2.23)
7	8	2.90 (2.16)	4.60 (2.13)	2.89 (1.91)	1.52 (2.40)
8	9	4.28 (1.69)	2.82 (2.10)	4.03 (2.20)	3.22 (2.00)
9	10	4.82 (1.33)	3.70 (2.69)	4.95 (2.56)	4.21 (3.09)
10	11	3.73 (1.90)	3.85 (2.80)	2.25 (2.29)	2.73 (2.34)
11	12	3.54 (2.53)	2.23 (1.56)	2.20 (2.14)	1.56 (2.52)
12	13	3.16 (2.14)	4.47 (2.74)	2.74 (2.08)	2.25 (1.55)
13	14	3.27 (2.31)	2.17 (2.82)	3.27 (2.19)	4.45 (1.96)
	Gain at Necropsy	-2.53 2.47	-1.30 2.97	-1.16 3.20	-3.15 2.33

*p<0.05
**p<0.01

Table 3. Group Average Food Consumption (g)

Males		Study Week										
Group		1	2	3	4	5	6	7	8	9	10	11
1	Mean	15.163	15.706	17.190	15.297	15.480	16.103	15.927	15.176	16.188	17.149	17.337
	SD	1.454	2.252	1.927	1.548	1.191	0.926	1.457	1.118	0.890	1.068	1.270
	N	10	10	10	10	10	10	10	10	8	10	10
2	Mean	15.623	17.1	15.790	15.365	15.905	15.932	15.707	15.127	17.857*	17.048	16.829
	SD	1.498	2.1	0.758	0.801	1.240	1.193	0.726	1.068	1.178	1.325	1.125
	N	10	9	9	10	8	10	10	10	10	10	10
3	Mean	15.226	15.690	14.173**	13.555*	14.255	14.484*	15.188	14.423	17.139	16.363	16.060
	SD	0.876	1.237	1.721	2.168	1.743	1.570	1.649	0.536	1.199	1.250	1.139
	N	10	10	10	10	10	10	10	9	10	10	10
4	Mean	13.704*	14.990	14.204**	14.850	13.544*	14.368*	14.595	14.468	16.044	16.287	14.537**
	SD	1.234	0.892	1.285	1.207	1.411	1.471	1.733	1.685	2.087	1.673	1.593
	N	10	10	10	10	10	10	10	10	10	10	10

Females		Study Week										
Group		1	2	3	4	5	6	7	8	9	10	11
1	Mean	11.088	11.457	12.478	10.294	10.077	10.104	10.227	9.867	10.993	11.795	11.441
	SD	0.891	0.789	1.219	0.823	0.980	0.949	0.941	0.894	0.557	1.297	1.175
	N	8	10	9	10	10	10	10	10	10	10	10
2	Mean	11.234	11.418	11.569	9.935	10.010	10.249	10.127	9.941	10.944	11.268	10.568
	SD	1.035	0.842	1.033	1.211	0.770	0.869	0.689	0.638	0.812	0.934	1.166
	N	7	10	10	10	10	10	10	10	9	10	10
3	Mean	11.923	11.737	11.070	10.056	9.764	10.217	10.567	9.556	12.425	11.899	11.170
	SD	0.908	1.223	1.663	0.935	1.265	2.202	1.342	1.216	1.484	1.649	1.173
	N	10	10	9	10	10	10	10	10	10	10	10
4	Mean	10.644	11.477	10.417**	10.656	9.987	10.514	10.415	10.587	12.104	13.008	10.927
	SD	1.012	1.110	1.289	0.750	0.996	1.038	0.902	0.942	0.922	1.020	0.854
	N	10	10	10	10	10	10	10	10	10	10	10

*$p<0.05$
**$p<0.01$

APPENDIX B. FINAL PATHOLOGY REPORT

Study Name: A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing

Protocol: The Hamner Institutes for Health Sciences Protocol 08013

EPL Project Number: 304-434

Submitted to: The Hamner Institutes for Health Sciences
6 Davis Drive
Research Triangle Park, NC 27709

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

October 28, 2009

INTRODUCTION

The Fischer-Tropsch (FT) process converts carbon monoxide and hydrogen to liquid hydrocarbons. S-8 jet fuel is made using the FT process. S-8 jet fuel is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the US Armed Forces. JP-8 fuel contains a mixture of aliphatic and aromatic hydrocarbons. The FT process for S-8 creates a mixture of aliphatic compounds similar to those found in JP-8, but does not form aromatic benzene and naphthalene compounds. This difference of composition between S-8 and JP-8 fuel points to a potential difference in the toxicity of the two fuels.

During refueling operations, personnel may be exposed to vapors and aerosols of jet fuel primarily by dermal or inhalation exposure. A review of JP-8 jet fuel toxicology concluded that exposure to JP-8 near the permissible exposure limit (PEL) of 350 mg/m^3 was potentially toxic to the immune, respiratory and nervous systems (NRC, 2003). In the respiratory system, physiological effects on pulmonary function and cellular effects have been observed (Herrin *et al.*, 2006) from exposure to JP-8. Neurobehavioral effects have been observed in humans exposed to jet fuels, including JP-8 (NRC, 2003; Baldwin *et al.*, 2007). Studies of animals exposed to jet fuel also indicated some neurobehavioral effects (NRC, 2003). Studies that exposed rats to vapor and aerosol of JP-8 utilized the Functional Observational Battery to demonstrate neurobehavioral effects (Baldwin *et al.*, 2007).

METHODS

This study is designed to assess the potential inhalation toxicity of a test substance when administered via inhalation exposure to Fischer 344 rats on a repeated basis for 90 days (5 days per week).

This Hamner Institutes for Health Sciences Study 08013 was conducted in male and female F344 rats. The experimental design is summarized in the Table 1 below:

Table 1. Experimental Design

Group	Exposure Level mg/m^3	Number of Animals	
		Males	Females
Control	0	10	10
Low	200	10	10
Intermediate	700	10	10
High	2000	10	10

Table 2. Animal Identification

Group	Animal Identification Number 08013-	
	Males	Females
Control Replicate 1	101-105	201-205
Control Replicate 2	106-110	206-210
Low Replicate 1	111-115	211-215
Low Replicate 2	116-120	216-220
Intermediate Replicate 1	121-125	221-225
Intermediate Replicate 2	126-130	226-230
High Replicate 1	131-135	231-235
High Replicate 2	136-140	236-240

Inhalation Exposures

The animals were exposed by inhalation via whole-body exposure. The test substance was administered for approximately 6 hours per exposure day, 5 days per week for at least 90 days.

The in-life phase of the study and the necropsies were performed at the Hamner Institute. Dr. Gabrielle Willson of EPL, NC conducted post mortem examinations at the terminal necropsy. Abnormal observations were recorded.

Necropsy Procedures

Animals to be euthanized were deeply anesthetized with sodium pentobarbital (intraperitoneal injection, approximately 30 mg/kg) and exsanguinated by transection of the abdominal aorta.

A complete macroscopic examination was performed on all animals. The necropsy included examination of the external surface and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

Histopathology Procedures

Histological sections of the tissues listed below were stained with hematoxylin and eosin (H&E) and evaluated via light microscopy.

- Digestive System
    - Salivary glands
    - Esophagus
    - Stomach
    - Duodenum
    - Jejunum

    - Ileum
    - Cecum
    - Colon
    - Rectum
    - Liver
    - Pancreas
- Nervous System
    - Brain (including sections of medulla/pons, cerebellum, and cerebrum)
    - Pituitary
    - Peripheral sciatic nerve
    - Spinal cord
    - Eyes (optic nerve)
- Glandular System
    - Adrenals
    - Parathyroids
    - Thyroids
- Respiratory System
    - Trachea
    - Lung
    - Pharynx
    - Larynx
    - Nose: 4 sections (Young, 1981)
- Cardiovascular/hematopoietic system
    - Aorta
    - Heart
    - Bone marrow (femur)
    - Lymph nodes (mesenteric and trancheobronchial)
    - Spleen
    - Thymus
- Urogenital system
    - Kidneys
    - Urinary bladder
    - Prostate
    - Testes
    - Epididymides
    - Seminal Vesicles
    - Uterus
    - Ovaries
- Other
    - Mammary gland (females)
    - Lacrimal gland
    - Skin
    - All gross lesions and masses

Histological evaluation of the high concentration and control groups of animals was done initially. When a treatment-related effect was observed, tissues from the intermediate groups were examined.

During the light microscopic examination, histopathologic diagnoses for tissues of each animal were recorded. Microscopic findings were graded using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high). Non-gradable changes are indicated as Present (P). After individual animal histopathology findings were reviewed, incidence tables that summarized histopathology findings by treatment groups were prepared.

RESULTS AND DISCUSSION

All study animals survived until the scheduled necropsy. There were a few gross findings found which were not considered to be treatment related. A variety of spontaneous background lesions were observed in this study (e.g., cardiomyopathy; inflammation in the liver, hyaline droplet accumulation in respiratory epithelium, etc.). These findings vary both in incidence and severity from study to study and are not related to treatment (Boorman, 1990; Greaves, 2007). Treatment-related histological findings were found in nose, lungs and kidneys. The latter tissue was only affected in male rats. These tissues were examined from the intermediate and low-dose exposure groups.

Nasal Sections

Treatment-related findings in the nasal cavity were olfactory epithelial degeneration and respiratory epithelial hyperplasia. Respiratory epithelial hyperplasia was evident in the anterior of the nose (Level I) and was characterized by an increase in number and height of the epithelium.

Olfactory epithelial degeneration, when minimal, was characterized by disorganization of the layers of olfactory epithelium. This was evident at the junction of respiratory and olfactory epithelium and multifocally in more rostral sections. It was minimal to mild in extent in the high dose animals of both sexes. More severe olfactory epithelial degeneration was characterized by thinning, disorganization with loss of cells and vacuoles within the epithelium. There was a predilection of olfactory epithelial degeneration near the junctions of respiratory and olfactory epithelium. However, when more pronounced, multifocal patchy areas of the septum and turbinates in Levels III and IV were affected.

Nasopharyngeal goblet cell hypertrophy hyperplasia of the nasopharyngeal duct was characterized by an increase in the number/height of the cells in this area.

Table 3. Incidence and [average severity] of selected findings in the noses of male rats (ten per group)

Group mg/m3	Olfactory Epithelial Degeneration (LIII)	Hypertrophy Hyperplasia Goblet Cells Nasopharyngeal Duct (LIV)
Control (0)	0 [0]	0 [0]
Low (200)	0 [0]	0 [0]
Intermediate (700)	9 [0.9]	10 [1]
High (2,000)	10 [2]	10 [2]

Table 4. Incidence and [average severity] of selected findings in the noses of female rats (ten per group)

Group mg/m3	Olfactory Epithelial Degeneration (LIII)	Hypertrophy Hyperplasia Goblet Cells Nasopharyngeal Duct (LIV)
Control (0)	0 [0]	0 [0]
Low (200)	0 [0]	0 [0]
Intermediate (700)	8 [0.8]	9 [.9]
High (2,000)	10 [1.8]	10 [1.9]

Lungs

At the highest-exposure level, there were minimal to mild multifocal areas of inflammatory cell infiltration throughout the lung parenchyma in both male and female rats. These cell aggregates were composed of alveolar histiocytes and neutrophils with occasional lymphocytes. There was no interstitial fibrosis or granuloma formation evident. A lesser effect was seen in intermediate exposure groups with only one male and three females having a minimal focal alveolar inflammatory cell infiltration.

Table 5. Incidence and [average severity] of inflammatory cell infiltration in the lungs of male rats (ten per group)

Group mg/m3	Multifocal Inflammatory Cell Infiltration	Focal Inflammatory Cell Infiltration
Control (0)	0 [0]	0 [0]
Low (200)	0 [0]	1 [0.1]
Intermediate (700)	0 [0]	1 [0.1]
High (2,000)	10 [1.9]	0 [0]

Table 6 Incidence and [average severity] of inflammatory cell infiltration in the lungs of female rats (ten per group)

Group mg/m3	Multifocal Inflammatory Cell Infiltration	Focal Inflammatory Cell Infiltration
Control (0)	0 [0]	0 [0]
Low (200)	0 [0]	0 [0]
Intermediate (700)	0 [0]	3 [0.3]
High (2,000)	10 [1.3]	0 [0]

Kidneys

In the high-dose male kidneys, there was a slight increase of hyaline droplets in the proximal convoluted tubules in comparison to controls. This was minimal. The hyaline droplets were slightly more prominent in the high-dose animals when compared to the background level in controls.

Table 7 Incidence and [average severity] of selected findings in the kidneys of male rats (ten per group)

Group mg/m3	Hyaline Droplet Tubular Epithelium
Control (0)	0 [0]
Low (200)	0 [0]
Intermediate (700)	0 [0]
High (2,000)	10 [1.7]

CONCLUSIONS:

Treatment-related findings were evident in kidneys (high-dose males only) and in noses and lung of both male and female rats in the high- and intermediate-dose groups.

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

28 Oct '09
Date

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COMPLIANCE STATEMENT

Client Name	The Hamner Institutes for Health Sciences	EPL Project Coordinator/ Principal Investigator	Dr. Gabrielle Willson
Client Study	Protocol No. 08013	EPL Pathologist	Dr. Gabrielle Willson
Species	F344 Rats	EPL Project Number	304-434
Study Title	A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing		
Test Article	S-8 Jet Fuel with JP-8 additives		

The histopathology procedures for this study were completed in accordance with applicable standard operating procedures, the protocol, all amendments, and the protocol-specified EPA Toxic Substances Control Act (TSCA) Good Laboratory Practices (GLP) regulations as noted in 40 CFR 792.

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

28 October 2009
Date

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing

Client Study: Protocol No. 08013 EPL Principal Investigator: Dr. Gabrielle Willson

EPL Project Number: 304-434 EPL Pathologist: Dr. Gabrielle Willson

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

	Dates	
Area Inspected	Inspection	Reporting
EPL Project Sheets	May 15, 2008; Aug. 6, 2008; Oct. 13, 2008; Oct. 21, 2008; Nov. 4, 2008	May 15, 2008; Aug. 6, 2008; Oct. 13, 2008; Oct. 21, 2008; Nov. 4, 2008
Project Setup	Aug. 8, 2008; Aug. 11, 2008; Oct. 15, 2008	Aug. 8, 2008; Aug. 11, 2008; Oct. 15, 2008
Data Review	Aug. 25, 2008; Sept. 19, 2008; Sept. 22, 2008; Oct. 31, 2008	Aug. 25, 2008; Sept. 19, 2008; Sept. 22, 2008; Oct. 31, 2008
Draft Pathology Report	Nov. 21 & 24, 2008; Aug. 4, 2009	Nov. 25, 2008; Aug. 4, 2009
Final Pathology Report	October 29, 2009	October 29, 2009

Date reported to Study Director/Management: October 29, 2009

Date of last quarterly facility inspection: July 2009

Jane I Hollingsworth
EPL Quality Assurance Unit

October 29, 2009
Date

Male Rat Summary Incidence Table

08013
90-Day Sacrifice
Male Rat

	GROUP 0	GROUP 200	GROUP 700	GROUP 2000		
ADRENAL (NO. EXAMINED)	(10)			(10)		
AORTA (NO. EXAMINED)	(10)			(10)		
BRAIN (NO. EXAMINED)	(10)			(10)		
EPIDIDYMIS (NO. EXAMINED)	(10)	(1)		(10)		
Sperm Granuloma		1				
ESOPHAGUS (NO. EXAMINED)	(10)			(10)		
EXORBITAL LACRIMAL GLAND (NO. EXAMINED)	(10)			(10)		
EYE (NO. EXAMINED)	(10)			(10)		
FEMUR (NO. EXAMINED)	(10)			(9)		
HEART (NO. EXAMINED)	(10)			(10)		
Cardiomyopathy	10			8		
INTESTINE-LARGE, CECUM (NO. EXAMINED)	(10)			(10)		
INTESTINE-LARGE, COLON (NO. EXAMINED)	(10)			(10)		
INTESTINE-LARGE, RECTUM (NO. EXAMINED)	(10)			(10)		
INTESTINE-SMALL, DUODENUM (NO. EXAMINED)	(10)			(10)		
INTESTINE-SMALL, ILEUM (NO. EXAMINED)	(10)			(10)		
INTESTINE-SMALL, JEJUNUM (NO. EXAMINED)	(10)			(10)		
KIDNEY (NO. EXAMINED)	(10)	(10)	(10)	(10)		
Cyst	1					
Hyaline Droplet, Tubular Epithelium				10		
Infiltrate Mononuclear Cells		1	1	1		

Male Rat Summary Incidence Table (continued)

08013
90-Day Sacrifice
Male Rat

	GROUP 0	GROUP 200	GROUP 700	GROUP 2000		
KIDNEY (CONTINUED)						
Regeneration, Tubular						
Epithelium	10	7	10	10		
Renal Tubule, Cast			2			
LARYNX W/PHARYNX (NO. EXAMINED)	(10)			(10)		
LIVER (NO. EXAMINED)	(10)			(10)		
Inflammation, Chronic, Focal	6			5		
LUNG (NO. EXAMINED)	(10)	(10)	(10)	(10)		
Alveolus, Inflammatory Cell						
Infiltration, Focal		1	1			
Alveolus, Inflammatory Cell						
Infiltration, Multifocal				10		
Subpleural, Lymphocytes		5	3			
LYMPH NODE, MESENTERIC (NO.						
EXAMINED)	(10)			(10)		
Sinus Dilation				1		
LYMPH NODE, TRACHEOBRONCHIAL						
(NO. EXAMINED)	(10)			(10)		
Hyperplasia				2		
MANDIBULAR SALIVARY GLAND (NO.						
EXAMINED)	(10)			(10)		
MARROW (NO. EXAMINED)	(10)			(9)		
NASAL CAVITY, LEVEL I (NO.						
EXAMINED)	(10)	(10)	(10)	(10)		
Respiratory Epithelium,						
Hyaline Droplet Accumulation	2	2	3	2		
Respiratory Epithelium,						
Hyperplasia			8	7		
Respiratory Epithelium,						
Inflammation						
NASAL CAVITY, LEVEL II (NO.						
EXAMINED)	(10)	(10)	(10)	(10)		
Olfactory Epithelial						
Degeneration				2		

Male Rat Summary Incidence Table (continued)

08013
90-Day Sacrifice
Male Rat

	GROUP 0	GROUP 200	GROUP 700	GROUP 2000		
NASAL CAVITY, LEVEL III (NO.						
EXAMINED)	(10)	(10)	(10)	(10)		
Olfactory Epithelial						
Degeneration			9	10		
Respiratory Epithelium,						
Hyaline Degeneration				8		
Respiratory Epithelium,						
Hyaline Droplet Accumulation						
NASAL CAVITY, LEVEL IV (NO.						
EXAMINED)	(10)	(10)	(10)	(10)		
Nasopharyngeal Duct, Goblet						
Cell Hyperplasia/Hypertrophy			10	10		
Olfactory Epithelial						
Degeneration				10		
OPTIC NERVE (NO. EXAMINED)	(9)			(7)		
PANCREAS (NO. EXAMINED)	(10)			(10)		
PARATHYROID (NO. EXAMINED)	(9)			(10)		
PITUITARY (NO. EXAMINED)	(10)			(10)		
PROSTATE (NO. EXAMINED)	(10)			(10)		
SCIATIC NERVE (NO. EXAMINED)	(10)			(10)		
SEMINAL VESICLE (NO. EXAMINED)	(10)			(10)		
SKIN (NO. EXAMINED)	(10)			(10)		
SPINAL CORD (NO. EXAMINED)	(10)			(10)		
SPLEEN (NO. EXAMINED)	(10)			(10)		
STOMACH (NO. EXAMINED)	(10)			(10)		
TESTES (NO. EXAMINED)	(10)			(10)		
Tubule Degeneration				1		
THYMUS (NO. EXAMINED)	(10)	(4)	(5)	(10)		
Hemorrhage	6	4	5	3		

Male Rat Summary Incidence Table (continued)

08013
90-Day Sacrifice
Male Rat

	GROUP 0	GROUP 200	GROUP 700	GROUP 2000		
THYROID (NO. EXAMINED)	(10)			(10)		
TRACHEA (NO. EXAMINED)	(10)			(10)		
URINARY BLADDER (NO. EXAMINED)	(10)			(10)		

Female Rat Summary Incidence Table

08013
90-Day Sacrifice
Female Rat

	GROUP 0	GROUP 200	GROUP 700	GROUP 2000		
ADRENAL (NO. EXAMINED)	(10)			(10)		
AORTA (NO. EXAMINED)	(10)			(10)		
BRAIN (NO. EXAMINED)	(10)			(10)		
ESOPHAGUS (NO. EXAMINED)	(10)			(10)		
EXORBITAL LACRIMAL GLAND (NO. EXAMINED)	(10)			(10)		
EYE (NO. EXAMINED)	(10)			(10)		
FEMUR (NO. EXAMINED)	(10)			(10)		
HEART (NO. EXAMINED)	(10)			(10)		
Cardiomyopathy	6			6		
INTESTINE-LARGE, CECUM (NO. EXAMINED)	(10)			(10)		
INTESTINE-LARGE, COLON (NO. EXAMINED)	(10)			(10)		
INTESTINE-LARGE, RECTUM (NO. EXAMINED)	(10)			(10)		
INTESTINE-SMALL, DUODENUM (NO. EXAMINED)	(10)			(10)		
INTESTINE-SMALL, ILEUM (NO. EXAMINED)	(10)			(10)		
INTESTINE-SMALL, JEJUNUM (NO. EXAMINED)	(10)			(10)		
KIDNEY (NO. EXAMINED)	(10)	(10)	(10)	(10)		
Cyst						
Hyaline Droplet, Tubular Epithelium						
Infiltrate Mononuclear Cells		1	1			
Regeneration, Tubular Epithelium	1		2			
Renal Tubule, Cast	1		1			

Female Rat Summary Incidence Table (continued)

08013
90-Day Sacrifice
Female Rat

	GROUP 0	GROUP 200	GROUP 700	GROUP 2000		
LARYNX W/PHARYNX (NO. EXAMINED)	(10)			(10)		
LIVER (NO. EXAMINED)	(10)			(10)		
Inflammation, Chronic, Focal	3			8		
LUNG (NO. EXAMINED)	(10)	(10)	(10)	(10)		
Alveolus, Inflammatory Cell						
Infiltration, Focal			3			
Alveolus, Inflammatory Cell						
Infiltration, Multifocal				10		
Subpleural, Lymphocytes	2	2	3			
LYMPH NODE, MESENTERIC (NO.						
EXAMINED)	(10)			(10)		
Sinus Dilation						
LYMPH NODE, TRACHEOBRONCHIAL						
(NO. EXAMINED)	(10)			(10)		
Hyperplasia						
MAMMARY (NO. EXAMINED)	(9)			(10)		
MANDIBULAR SALIVARY GLAND (NO.						
EXAMINED)	(10)			(10)		
MARROW (NO. EXAMINED)	(10)			(10)		
NASAL CAVITY, LEVEL I (NO.						
EXAMINED)	(10)	(10)	(10)	(10)		
Respiratory Epithelium,						
Hyaline Droplet Accumulation			1			
Respiratory Epithelium,						
Hyperplasia			9	7		
Respiratory Epithelium,						
Inflammation	1	4	7			
NASAL CAVITY, LEVEL II (NO.						
EXAMINED)	(10)	(10)	(10)	(10)		
Olfactory Epithelial						
Degeneration				2		

Female Rat Summary Incidence Table (continued)

08013
90-Day Sacrifice
Female Rat

	GROUP 0	GROUP 200	GROUP 700	GROUP 2000		
NASAL CAVITY, LEVEL III (NO.						
EXAMINED)	(10)	(10)	(10)	(10)		
Olfactory Epithelial						
Degeneration			8	10		
Respiratory Epithelium,						
Hyaline Degeneration						
Respiratory Epithelium,						
Hyaline Droplet Accumulation				9		
NASAL CAVITY, LEVEL IV (NO.						
EXAMINED)	(10)	(10)	(10)	(10)		
Nasopharyngeal Duct, Goblet						
Cell Hyperplasia/Hypertrophy			9	10		
Olfactory Epithelial						
Degeneration			3	10		
OPTIC NERVE (NO. EXAMINED)	(8)			(8)		
OVARY (NO. EXAMINED)	(10)			(10)		
PANCREAS (NO. EXAMINED)	(10)			(10)		
PARATHYROID (NO. EXAMINED)	(10)			(9)		
PITUITARY (NO. EXAMINED)	(10)			(10)		
SCIATIC NERVE (NO. EXAMINED)	(10)			(10)		
SKIN (NO. EXAMINED)	(10)			(10)		
SPINAL CORD (NO. EXAMINED)	(10)			(10)		
SPLEEN (NO. EXAMINED)	(10)			(10)		
STOMACH (NO. EXAMINED)	(10)			(10)		
THYMUS (NO. EXAMINED)	(10)			(10)		
Hemorrhage						
THYROID (NO. EXAMINED)	(10)			(10)		
TRACHEA (NO. EXAMINED)	(10)			(10)		
URINARY BLADDER (NO. EXAMINED)	(10)			(10)		

Female Rat Summary Incidence Table (continued)

08013
90-Day Sacrifice
Female Rat

	GROUP 0	GROUP 200	GROUP 700	GROUP 2000		
UTERUS (NO. EXAMINED)	(10)			(10)		

APPENDIX C. REPORT OF CLINICAL PATHOLOGY RESULTS

Study Name: A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing

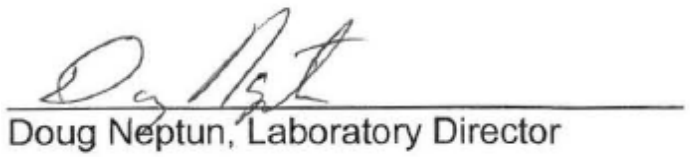
Protocol: The Hamner Institutes for Health Sciences Protocol 08013

Testing Facility: Antech Diagnostics
507 Airport Blvd., Suite 113
Morrisville, NC 27560

Sponsor: The Hamner Institutes for Health Sciences
6 Davis Drive
Research Triangle Park, NC 27709

Key Personnel: Doug Neptun - Laboratory Director, Statistician

Written and approved by:



Doug Neptun, Laboratory Director

8/18/05

Date

GOOD LABORATORY PRACTICES STATEMENT

A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing

Study Number: 08013

Timeperiod	Collection Date	Date Samples were received (at Antech Diagnostics)	Date Samples were analyzed
Term-Necropsy	7/31/08	7/31/08	8/1/08
Term-Necropsy	8/1/08	8/1/08	8/1/08

Study Activities at Antech Diagnostics:
Start Date: 7/31/08 **Completion Date of Analysis: 8/1/08**

I confirm that the clinical pathology portion of this study performed at Antech Diagnostics was in compliance with the United States Environmental Protection Agency (EPA), Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards, 40 CFR Part 792.

Exception: The statistical program, SigmaStat software by SYSTAT Software, Inc. (San Jose, CA) was not fully validated.

Doug Neptun, Laboratory Director — Date: 8/18/09

QUALITY ASSURANCE STATEMENT (QAS)

To	The Hamner Institutes for Health Sciences
Study Director	Brian Wong, PhD (and Study Director Management)
From Quality Assurance Auditor	Katie Powell
Protocol referenced	A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing (08013)
Regulations followed	U.S. EPA, Toxic Substances Control Act (TSCA), GLP Standards, 40 CFR Part 792
Timeperiod(s), material audited, inspection date(s)	Term Necropsy: 811108, Phase Audit of Sample Receipt, Accessioning and Worksheets, August 1,2008 Term – Necropsy: 7/31108, Study Data, August 18, 2008 Term – Necropsy: 811108, Study Data, August 18, 2008 Interpretive Report: Draft, Text and Summary Tables, October 1, 2008 Interpretive Report: Draft, Statistical Significance, October 1, 2008 Interpretive Report: Final, August 18, 2009
Date the Phase Report was issued	August 1, 2008
Date the Audit Report was issued:	August 18, 2009
Study Director and Study Director Management notified (Dates sent):	Phase Audit Report, August 1,2008 Audit Report, August 18, 2009 QAS, August 18, 2009

Printed Name: Katie Powell

Signature: Katie Powell Date: 8/18/09

Title: Senior QA Auditor

INTRODUCTION

Male and female rats, Fischer (CDF^{TM}) [F344/DuCrl], were exposed to 0 (Room air), 200,700 or 2000 mg/m^3 of total hydrocarbons as FT Jet Fuel with JP-8 additives in an aerosol/vapor combination. All surviving animals were evaluated for clinical pathology toxicologic effects at the terminal euthanasia.

STUDY DESIGN AND METHODS

Male and female rats were divided into four groups for clinical pathology analyses. Groups of female and male rats are described as 1-F to 4-F and 1-M to 5-M, respectively.

Blood was collected from all rats at terminal euthanasia. Blood samples were transported to Antech Diagnostics on the day of collection. All samples were evaluated for Complete Blood Count, coagulation testing and clinical chemistry analysis. When insufficient blood was available, tests not run were labeled as QNS (quantity not sufficient for analysis). Results were entered into the ClinAxys v2.2 computer system.

Complete blood count (CBC) consisted of a total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), leukocyte differential: (neutrophils [NEU], lymphocytes [LYM], monocytes [MON], eosinophils [EOS], basophils [BAS] and large unstained cells [LUC]) with RBC morphology were analyzed by the Siemens Advia 120 automated hematology system (Norwood, MA).

Prothrombin time (PT) and activated partial thromboplastin time (APTT) were evaluated using the Trinity CSI90 system (Berkeley Heights, NJ).

Clinical chemistry testing was performed using Olympus reagents and the Olympus 640e clinical chemistry analyzer (Center Valley, PA). Tests performed by the Olympus included: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), calcium (CA), cholesterol (CHOL), chloride (CL), creatine kinase (CPK), creatinine (CREA), glucose (GLU), potassium (K), sodium (NA), phosphorus (PHOS) and total protein (TPRO).

Statistical evaluation of the data was performed using SigmaStat software. The data were analyzed for normality followed by an ANOVA ($p<0.05$) and, if significant, a comparison of groups by Holm-Sidak. If the test for normality failed, the ANOVA was based on Kruskal-Wallis ANOVA on Ranks ($p<0.05$) and, if significant, Dunn's comparison of groups. Values are reported as means, standard deviation (SD) and number of sample (N).

RESULTS

Discussion of results refers to a comparison of the group mean to the control group mean. Unless otherwise stated, the difference is a statistically significant difference.

Termination, Male Rats

There were no hematologic differences between control and treated animals in any dose group. ALP was decreased in the 700 and 2000 mg/m^3 groups of male rats. ALT, cholesterol, total protein and albumin were decreased in the 2000 mg/m^3 groups of male rats. These changes are not biologically significant individually, but appear to be related to possible nutritional effects as demonstrated in the general weight gain differences and not toxicologically significant (based on animal body weight data supplied by The Hamner Institutes from study 08013, and listed in the final report).

Termination, Female Rats

The MCH was decreased in the 2000 mg/m^3 female rats with a slight but not statistically significant decrease in erythrocytes, hemoglobin and hematocrit. These changes were not believed to be biologically significant. Although there were only 3to 5 results per group for PT and APTT, there did not appear to be any effect in clotting factors. Chloride was increased and ALT and albumin were decreased in the 2000 mg/m^3 groups of female rats. These changes were not believed to be biologically significant.

CONCLUSIONS

There were no biologically significant or toxicologically significant changes in hematology or clinical chemistry values from exposure to FT Jet Fuel with JP-8 additives in an aerosol/vapor combination. Slight changes in clinical chemistry values in males exposed to 2000 mg/m^3 of FT appear to be related to possible nutritional effects as demonstrated in the general weight gain differences and not toxicologically significant.

SUMMARY TABLES

Table 1. Hematology

Study: 08013 Species: RAT

Time point: TERM

Group		WBC 10^3/uL	RBC 10^6/uL	HB g/dL	HCT %
1M CONTROL	Mean	3.24	8.88	14.7	46.9
	SD	0.509	0.196	0.30	1.14
	n	10	10	10	10
2M LOW	Mean	3.66	8.68	14.4	45.7
	SD	1.249	0.278	0.40	1.93
	n	10	10	10	10
3M MID	Mean	3.36	8.76	14.4	46.2
	SD	0.950	0.358	0.55	1.60
	n	10	10	10	10
4M HIGH	Mean	3.26	8.75	14.6	46.4
	SD	0.780	0.186	0.25	0.98
	n	10	10	10	10

Group		MCV fL	MCH pg	MCHC g/dL	PLT 10^3/uL
1M CONTROL	Mean	52.9	16.5	31.3	791
	SD	0.57	0.18	0.36	44.4
	n	10	10	10	10
2M LOW	Mean	52.6	16.6	31.5	797
	SD	0.79	0.26	0.64	182.8
	n	10	10	10	10
3M MID	Mean	52.7	16.4	31.1	808
	SD	0.66	0.14	0.35	162.8
	n	10	10	10	10
4M HIGH	Mean	53.0	16.7	31.4	748
	SD	0.38	0.19	0.23	49.4
	n	10	10	10	10

Table 1. Hematology (continued)

Study: 08013 Species: RAT

Time point: TERM

Group		NEU% %	NEU 10^3/uL	LYM% %	LYM 10^3/uL
1M CONTROL	Mean	19.9	0.64	76.5	2.48
	SD	3.67	0.129	4.10	0.443
	n	10	10	10	10
2M LOW	Mean	22.9	0.90	73.3	2.62
	SD	6.94	0.672	7.25	0.602
	n	10	10	10	10
3M MID	Mean	22.6	0.82	73.6	2.41
	SD	8.81	0.663	8.75	0.396
	n	10	10	10	10
4M HIGH	Mean	19.3	0.63	76.6	2.50
	SD	2.65	0.179	2.86	0.600
	n	10	10	10	10

Group		MON% %	MON 10^3/uL	EOS% %	EOS 10^3/uL
1M CONTROL	Mean	1.5	0.05	1.2	0.04
	SD	0.57	0.022	0.51	0.020
	n	10	10	10	10
2M LOW	Mean	1.7	0.06	1.2	0.04
	SD	0.31	0.035	0.39	0.022
	n	10	10	10	10
3M MID	Mean	1.6	0.05	1.2	0.04
	SD	0.31	0.013	0.35	0.020
	n	10	10	10	10
4M HIGH	Mean	1.7	0.06	1.4	0.04
	SD	0.42	0.016	0.34	0.014
	n	10	10	10	10

Table 1. Hematology (continued)

Study: 08013 Species: RAT

Time point: TERM

Group		BAS% %	BAS 10^3/uL	LUC% %	LUC 10^3/uL
1M CONTROL	Mean	0.4	0.01	0.5	0.02
	SD	0.18	0.005	0.11	0.005
	n	10	10	10	10
2M LOW	Mean	0.4	0.01	0.6	0.02
	SD	0.15	0.004	0.25	0.012
	n	10	10	10	10
3M MID	Mean	0.4	0.01	0.6	0.02
	SD	0.14	0.004	0.22	0.009
	n	10	10	10	10
4M HIGH	Mean	0.3	0.01	0.7	0.02
	SD	0.13	0.003	0.15	0.005
	n	10	10	10	10

Group		PT sec	APTT sec
1M CONTROL	Mean	16.7	21.2
	SD	0.64	4.96
	n	10	10
2M LOW	Mean	16.4	19.1
	SD	0.48	2.08
	n	10	10
3M MID	Mean	16.8	21.6
	SD	0.95	5.46
	n	9	9
4M HIGH	Mean	16.8	20.3
	SD	0.84	3.63
	n	9	9

Table 1. Hematology (continued)

Study: 08013 Species: RAT

Time point: TERM

Group		WBC 10^3/uL	RBC 10^6/uL	HB g/dL	HCT %
1F CONTROL	Mean	3.23	8.61	15.1	46.9
	SD	0.567	0.148	0.32	0.98
	n	10	10	10	10
2F LOW	Mean	3.49	8.48	14.7	46.2
	SD	0.351	0.137	0.21	1.11
	n	10	10	10	10
3F MID	Mean	3.38	8.54	15.0	46.3
	SD	0.401	0.251	0.56	1.78
	n	10	10	10	10
4F HIGH	Mean	3.48	8.46	14.7	45.9
	SD	0.609	0.140	0.26	0.97
	n	10	10	10	10

Group		MCV fL	MCH pg	MCHC g/dL	PLT 10^3/uL
1F CONTROL	Mean	54.4	17.5	32.2	773
	SD	0.50	0.13	0.36	98.4
	n	10	10	10	10
2F LOW	Mean	54.4	17.4	31.9	783
	SD	0.74	0.13	0.48	71.5
	n	10	10	10	10
3F MID	Mean	54.2	17.5	32.3	749
	SD	0.72	0.21	0.44	83.7
	n	10	10	10	10
4F HIGH	Mean	54.3	17.3 *	31.9	781
	SD	0.82	0.14	0.44	41.5
	n	10	10	10	10

* Statistically different from control p <0.05

Table 1. Hematology (continued)

Study: 08013 Species: RAT

Time point: TERM

Group		NEU% %	NEU 10^3/uL	LYM% %	LYM 10^3/uL
1F CONTROL	Mean	14.7	0.46	80.3	2.61
	SD	4.20	0.120	5.18	0.610
	n	10	10	10	10
2F LOW	Mean	13.8	0.48	82.0	2.86
	SD	3.28	0.132	3.46	0.321
	n	10	10	10	10
3F MID	Mean	15.1	0.51	79.6	2.70
	SD	3.05	0.112	3.64	0.381
	n	10	10	10	10
4F HIGH	Mean	14.1	0.49	80.4	2.81
	SD	1.32	0.073	2.09	0.540
	n	10	10	10	10

Group		MON% %	MON 10^3/uL	EOS% %	EOS 10^3/uL
1F CONTROL	Mean	2.2	0.07	1.7	0.05
	SD	1.28	0.037	0.98	0.022
	n	10	10	10	10
2F LOW	Mean	2.0	0.07	1.0	0.04
	SD	0.72	0.026	0.31	0.012
	n	10	10	10	10
3F MID	Mean	2.6	0.09	1.5	0.05
	SD	0.72	0.023	0.81	0.021
	n	10	10	10	10
4F HIGH	Mean	2.3	0.08	2.1	0.07
	SD	0.70	0.027	0.42	0.010
	n	10	10	10	10

Table 1. Hematology (continued)

Study: 08013 Species: RAT

Time point: TERM

Group		BAS% %	BAS 10^3/uL	LUC% %	LUC 10^3/uL
1F CONTROL	Mean	0.4	0.01	0.7	0.02
	SD	0.10	0.006	0.50	0.015
	n	10	10	10	10
2F LOW	Mean	0.4	0.01	0.8	0.03
	SD	0.11	0.005	0.27	0.009
	n	10	10	10	10
3F MID	Mean	0.5	0.02	0.7	0.03
	SD	0.33	0.008	0.13	0.005
	n	10	10	10	10
4F HIGH	Mean	0.3	0.01	0.9	0.03
	SD	0.15	0.006	0.48	0.013
	n	10	10	10	10

Group		PT sec	APTT sec
1F CONTROL	Mean	10.6	10.7
	SD	1.77	3.32
	n	3	3
2F LOW	Mean	11.0	11.5
	SD	0.74	3.08
	n	5	5
3F MID	Mean	11.7	13.0
	SD	1.05	3.27
	n	5	5
4F HIGH	Mean	11.5	12.7
	SD	1.00	3.00
	n	4	4

Table 2. Clinical Chemistry

Study: 08013 Species: RAT

Time point: TERM

Group		BUN mg/dl	CREA mg/dl	GLU mg/dl	NA mmol/L
1M CONTROL	Mean	23	0.5	201	145
	SD	2.0	0.07	21.9	1.4
	n	10	10	10	10
2M LOW	Mean	22	0.4	196	145
	SD	1.2	0.05	20.8	0.9
	n	10	10	10	10
3M MID	Mean	21	0.5	201	145
	SD	1.7	0.05	25.5	1.0
	n	10	10	10	10
4M HIGH	Mean	22	0.5	197	145
	SD	1.5	0.05	16.9	0.8
	n	10	10	10	10

Group		K mmol/L	CL mmol/L	ALP U/L	ALT U/L
1M CONTROL	Mean	5.0	99	287	66
	SD	0.41	0.9	55.3	12.9
	n	10	10	10	10
2M LOW	Mean	5.2	99	243	74
	SD	0.38	0.7	38.1	27.6
	n	10	10	10	10
3M MID	Mean	5.2	99	244 *	62
	SD	0.31	0.7	52.1	11.6
	n	10	10	10	10
4M HIGH	Mean	5.1	99	220 *	61 *
	SD	0.42	0.9	13.5	50.6
	n	10	10	10	10

* Statistically different from control p <0.05

Table 2. Clinical Chemistry (continued)

Study: 08013 Species: RAT

Time point: TERM

Group		AST U/L	CPK U/L	TPRO g/dl	ALB g/dl
1M CONTROL	Mean	127	681	5.9	3.4
	SD	31.9	239.9	0.11	0.07
	n	10	10	10	10
2M LOW	Mean	134	778	5.9	3.4
	SD	40.2	486.6	0.15	0.12
	n	10	10	10	10
3M MID	Mean	116	604	5.9	3.4
	SD	30.8	244.0	0.19	0.09
	n	10	10	10	10
4M HIGH	Mean	116	698	5.7 *	3.3 *
	SD	69.6	250.5	0.15	0.08
	n	10	10	10	10

Group		CA mg/dl	PHOS mg/dl	CHOL mg/dl
1M CONTROL	Mean	10.5	7.6	66
	SD	0.11	0.45	3.4
	n	10	10	10
2M LOW	Mean	10.5	8.0	72
	SD	0.19	0.62	14.5
	n	10	10	10
3M MID	Mean	10.5	8.0	69
	SD	0.17	0.86	16.1
	n	10	10	10
4M HIGH	Mean	10.5	8.3	61 *
	SD	0.16	0.70	3.3
	n	10	10	10

* Statistically different from control p <0.05

Table 2. Clinical Chemistry (continued)

Study: 08013 Species: RAT

Time point: TERM

Group		BUN mg/dl	CREA mg/dl	GLU mg/dl	NA mmol/L
1F CONTROL	Mean	19	0.4	209	144
	SD	1.3	0.04	29.9	1.2
	n	9	9	9	9
2F LOW	Mean	19	0.4	205	144
	SD	1.7	0.00	28.6	1.1
	n	10	10	10	10
3F MID	Mean	19	0.4	211	144
	SD	1.7	0.05	30.1	1.1
	n	10	10	10	10
4F HIGH	Mean	18	0.4	198	144
	SD	1.6	0.05	27.7	1.1
	n	10	10	10	10

Group		K mmol/L	CL mmol/L	ALP U/L	ALT U/L
1F CONTROL	Mean	5.0	100	199	67
	SD	0.85	0.6	35.3	18.2
	n	9	9	9	9
2F LOW	Mean	4.9	100	190	61
	SD	0.59	0.6	43.4	12.6
	n	10	10	10	10
3F MID	Mean	5.1	101	175	60
	SD	0.85	1.3	33.4	21.2
	n	10	10	10	10
4F HIGH	Mean	5.3	101 *	167	46 *
	SD	1.06	0.8	25.6	8.4
	n	10	10	10	10

* Statistically different from control p <0.05

Table 2. Clinical Chemistry (continued)

Study: 08013 Species: RAT

Time point: TERM

Group		AST U/L	CPK U/L	TPRO g/dl	ALB g/dl
1F CONTROL	Mean	122	558	5.8	3.4
	SD	27.2	185.0	0.19	0.09
	n	9	9	9	9
2F LOW	Mean	109	470	5.7	3.4
	SD	21.0	231.3	0.19	0.08
	n	10	10	10	10
3F MID	Mean	111	488	5.7	3.4
	SD	33.1	140.7	0.26	0.14
	n	10	10	10	10
4F HIGH	Mean	92	497	5.6	3.2 *
	SD	20.3	238.5	0.23	0.08
	n	10	10	10	10

Group		CA mg/dl	PHOS mg/dl	CHOL mg/dl
1F CONTROL	Mean	10.5	8.5	90
	SD	0.30	0.73	5.6
	n	9	9	9
2F LOW	Mean	10.5	8.3	87
	SD	0.25	0.89	4.8
	n	10	10	10
3F MID	Mean	10.5	8.2	90
	SD	0.28	0.83	6.9
	n	10	10	10
4F HIGH	Mean	10.4	8.7	86
	SD	0.22	0.72	5.2
	n	10	10	10

* Statistically different from control p <0.05

APPENDIX D. α2μ-GLOBULIN IN KIDNEY SAMPLES FROM RATS EXPOSED BY INHALATION TO SYNTHETIC JET FUEL

Study Title: A 90-Day Inhalation Toxicity Study of A Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing.

Study Protocol: 08013

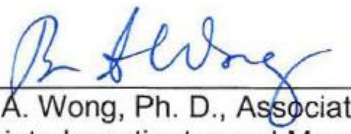
Author: Brian A. Wong
Associate Investigator and Manager, Inhalation Exposure and Aerosol Science Facility

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

Test Site: ILS, Inc.
601 Keystone Park Drive
Suite 100
Durham, NC 27713

Study Sponsor: Naval Health Research Center
Environmental Health Effects Laboratory
2729 R St., Bldg 837, Area B
Wright-Patterson AFB, OH 45433-5707

REPORT PREPARATION

Report prepared by:  Date 9/17/2010

Brian A. Wong, Ph. D., Associate Investigator and Associate Investigator and Manager, Inhalation Exposure and Aerosol Science Facility

KEY PERSONNEL

Performing Laboratory: The Hamner Institutes for Health Sciences
6 Davis Drive
Research Triangle Park, NC 27709
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Study Director: Brian A. Wong, Ph.D.
Associate Investigator

Contract Laboratory: ILS, Inc.
601 Keystone Park Drive
Suite 100
Durham, NC 27713

Principal Investigator: Kevin Leiner, Ph.D.

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was not conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 792. The study was conducted in accordance with the Hamner Institutes Research Quality Standard Procedures. For the portion of this study conducted at ILS, applicable ILS standard operating procedures were followed.

The study dates listed in Section 5.0 of the Final Report refer to dates of ILS work only.

Study Director: B A C Wong Date 9/17/2010
Brian A. Wong, Ph.D.

INTRODUCTION

α2μ-Globulin is a low molecular weight protein that is synthesized in the male rat liver. Synthesis of α2μ-globulin (α2μ) is initiated in the male rat with the onset of sexual maturation, increases, plateaus around 20 weeks of age, and then decreases with aging. The protein is secreted into the plasma and is filtered through the kidney. About half of the α2μ is excreted in the urine, and half is reabsorbed into the proximal tubule of the kidney nephron, where it undergoes hydrolytic digestion. The female rat liver does not readily produce α2μ, and levels of the protein in the urine are approximately two orders of magnitude lower than in the rat (Borghoff *et al.*, 1992).

Exposure to certain chemicals (especially branched hydrocarbons) is associated with the accumulation of α2μ in renal tubular epithelial cells. These chemicals have been hypothesized to form a complex with α2μ that resists lysosomal degradation, leading to accumulation in protein (hyaline) droplets in the cells. The accumulation of α2μ leads to cytotoxicity where injured cells are released into the tubule lumen, and leaving casts in the loop of Henle, or being excreted into the urine. The cytotoxicity also causes regeneration of cells in the area (Borghoff *et al.*, 2001). Histopathologically, α2μ nephropathy is characterized by an excessive accumulation of protein droplet in lysosomes of the proximal tubules, the presence of cellular casts from injured cells in the junction of the proximal tubule and the loop of Henle, and regenerative tubules (Borghoff *et al.*, 2009). Various chemicals have been shown to cause α2μ nephropathy, including some jet fuel formulations (Borghoff *et al.*, 1992).

The objective of this study was to determine whether α2μ nephropathy was associated with exposure to FT Jet Fuel. F344 rats were exposed by inhalation to an aerosol and vapor mixture of FT Jet Fuel with additives. Whole body inhalation exposures were conducted 6 hours/day, 5 days/week over a 90-day period, at a concentration of 0 (control), 200, 700, or 2000 mg/m^3. Groups of 10 males and 10 females were exposed at each exposure concentration for a total of 40 males and 40 females. At necropsy, samples of rat kidneys were taken for α2μ-globulin measurements.

METHODS

The α2μ-Globulin analysis was conducted as a non-GLP (Good Laboratory Practices) study for research purposes. Samples of rat kidneys from all males and from the control and high concentration females were taken, placed in a vial, and frozen in liquid nitrogen. The samples were transferred to a freezer and held at approximately -80°C. The kidney samples were analyzed by an outside laboratory, Integrated Laboratory Systems, Inc. (ILS), Durham, NC. Kevin Leiner, PhD, was the principal investigator.

Kidney Sample Preparation

Forty-four frozen rat kidney samples were provided to ILS by the Hamner Institutes for Health Sciences. The kidney samples were thawed, weighed, and homogenized in three volumes (3

mL/g wet weight) of pre-chilled phosphate buffered saline. The homogenates were centrifuged at 3,000 x g for 30 minutes at 2 to 8°C. Aliquots of the resulting supernatants were stored at -65 to -85°C until assay. No aliquot was thawed more than once prior to assay.

Analysis of Kidney Supernatant

Analysis of Total Protein

The total protein concentration of each kidney homogenate sample was determined with the Pierce Micro BCA™ Protein Assay Kit using bovine serum albumin as the standard.

Analysis of $\alpha_{2\mu}$-Globulin

The $\alpha_{2\mu}$-globulin content of each kidney homogenate sample was determined using a "sandwich" ELISA with a monoclonal anti-rat $\alpha_{2\mu}$-globulin capture antibody (immobilized) and a polyclonal goat anti-rat $\alpha_{2\mu}$-globulin "detection" antibody (in solution). A secondary antibody, donkey anti-goat IgG conjugated to horseradish peroxidase, was used to detect the unlabeled polyclonal goat anti-rat $\alpha_{2\mu}$-globulin "detection" antibody. 3,3',5 ,5'-Tetramethylbenzidine was used as the substrate for horseradish peroxidase, and after acidification of the blue-colored reaction product, the absorbance was measured at a wavelength of 450 nm. This assay was calibrated against $\alpha_{2\mu}$-globulin protein purified from rat urine. The concentration of $\alpha_{2\mu}$-globulin in the standard curve was determined with BCA reagent using purified bovine serum albumin as the standard. This assay has previously been used and reported in a recent study (Borghoff *et al.*, 2009).

Data Analysis

The concentration of $\alpha_{2\mu}$-globulin in each kidney sample is expressed as μg of $\alpha_{2\mu}$-globulin/mg of total protein. The data are presented in a tabular format by sample number (Table 1).

Record Retention

All original data, including the original signed protocol and all amendments, if any, kidney homogenates, and the original final report, will be maintained at ILS for the remainder of the contract. Upon acceptance of the Final Report by the Sponsor, all documents and samples can be picked up by the Sponsor.

RESULTS

Measurements of α2μ-globulin protein in rat kidney samples were reported in the ILS study report (Table 1 is a compilation of the data with additional labeling for clarification). An ELISA

assay (Borghoff *et al.*, 2009) was used to measure the concentration of α2μ in kidney samples of male and female rats. Levels of α2μ were present in the kidney samples of all male rats, including control animals (except one male rat in the high concentration group, which was excluded from the group statistics) at quantities >100-fold higher than found in the kidney samples of all female rats assayed.

Average and standard deviations for the different groups were calculated (Table 2). There was no apparent trend relative to increasing exposure concentration. In fact, the concentration observed in the high exposure concentration rats was lower than in the other exposure concentration or control groups. The difference was not statistically significant (JMP, version 7.0.1, SAS Institute, Cary, NC). Levene's test for equal variance, at $p<0.01$, indicated that variances were equal. A one-way ANOVA, $F<0.05$ then indicated that the means were not significantly different.

DISCUSSION

α2μ-Globulin Measurements

Histopathology results in the kidney showed a slight increase in hyaline droplets in the proximal convoluted tubules in high concentration males compared to controls. In the biochemical analysis, there was an apparent decrease of α2μ in kidney samples from the high concentration-exposed animals, though the decrease was not statistically significant. For the exposed male groups, then, there was no change in α2μ concentration across exposure concentrations. The apparent discrepancy between the histopathology observations and the α2μ biochemical measurements could be caused by problems with the measurement of the α2μ concentration or because of hyaline droplet size is not representative of α2μ content. Other biochemical measurement issues include age-related changes in α2μ production or inadequate sample number.

The reported average concentration of α2μ in the control group was 49.4 μg α2μ-globulin/mg total protein. In previous studies, animals were exposed to gasoline by gavage for 10 days (Borghoff *et al.*, 1992), methyl-tert-butyl ether (MTBE) by inhalation for 10 days (Prescott-Mathews *et al.*, 1996), and tert-butyl alcohol by inhalation for 10 days (Borghoff *et al.*, 2001). In those studies, α2μ was measured using an ELISA method (Borghoff *et al.*, 1992) which gave control values between 140 and 150 μg α2μ-globulin/mg total protein. These control values were higher than the average value measured in this FT jet fuel study.

Using a different ELISA method (the same as used for this study), a recent study reported a control value of α2μ-globulin at approximately 65 ng/μg total protein (Borghoff *et al.*, 2009), approximately equivalent to the control value found in this study. Another study that used the ILS ELISA method measured the α2μ-globulin during and at the end of a 13 week administration of MTBE by drinking water (Bermudez, 2007). The control value measured at the end of the 13 week study was 26.9 μg/mg total protein, which was lower than the control value for this study. However, that MTBE study used a different strain of rat (Wistar instead of

F344), so the difference in control value could be due to rat strain differences. Finally, control values of α2μ-globulin from four different 13- week studies ranged from 60 to 204 ng/μg of total protein (Doi *et al.*, 2007). Thus, levels of α2μ-globulin reported in various studies varied widely from study to study. The values reported for this study were within range of those studies, so there does not appear to be a problem with α2μ-globulin measurement methodology.

The decreased value for the high concentration compared with control may be related to another factor, the effects of aging. In the male rat, α2μ-globulin production begins at puberty and then declines after maturity. Hepatic production of α2μ -globulin increases from puberty (approximately day 50), plateaus until approximately 100 days, and then decreases (Roy *et al.*, 1983). Urinary output of α2μ -globulin decreases sometime after 150 days (5 months) (Motwani *et al.*, 1984; Hard *et al.*, 1993). Animals on a 90-day study may be at or beyond 5 months of age at the end of this study, so they have reached the approximate age when α2μ-globulin begins to decrease. The decrease in production of α2μ-globulin could offset the accumulation in hyaline droplets in kidneys. In the MTBE 90-day drinking water study (Bermudez, 2007), α2μ-globulin measured in the high dose rats at the end of 13 weeks (37.3 μg/g total protein) was lower than at an intermediate time point (4 weeks, 73.7 μg/g total protein), and lower than the 13 week value of the middle dose group (40.1 μg/g total protein). However the value was higher than the control at 13 weeks (26.7 μg/g total protein). A 13-week inhalation exposure to decalin (Dill *et al.*, 2003) provided another example in which the 13-week highest exposure concentration α2μ-globulin value (10 mg/g kidney) was lower than at the 6 week time point (15 mg/g) and lower than the 13 week intermediate exposure concentration value (14 mg/g). These two studies provide evidence that the α2μ-globulin concentration can decline over time at the end of a 13 week study. However, other longer term studies demonstrated the expected pattern in which the animals exposed to the highest dose had the highest α2μ-globulin concentrations at the end of the study (Doi *et al.*, 2007). In order to conclusively demonstrate that α2μ-globulin production was affected by age during a long term (13-week) study, additional measurements at intermediate time points would be required.

Finally, some of the kidney samples could not be used due to loss of labels (see Protocol Deviations). The number of samples that could be used each group ranged from six to ten. The number of samples for most of the studies discussed above was five, so group average values for this study were not compromised by too few samples. The apparent discrepancy between histological observations and the biochemical measurements is not due to problems with methodology.

Histopathology Observations

From the histopathology results, the kidneys showed a slight increase in hyaline droplets in the proximal convoluted tubules in high concentration males compared to controls. This was considered minimal. The hyaline droplets were slightly more prominent in the high-dose animals when compared to the background level in controls. The kidney histopathology slides were not immunohistochemically stained for α2μ-globulin, so there was no visual observation of

α2μ-globulin stain intensity or density that could be compared across exposure groups to correlate α2μ-globulin amount with the hyaline droplet size.

Tubular epithelial regeneration was observed in the kidneys of all males at a minimal to mild level. Observations considered mild were seen at a rate of 6/10 in controls, 4/10 in low, 7/10 in mid, and 8/10 in the high exposure groups, showing a very slight increase in severity with increasing exposure concentration. In female kidneys, minimal tubular epithelial regeneration was seen in one control animal and two intermediate exposure concentration animals.

Renal tubule casts were seen in kidney samples in two males at the intermediate exposure concentration, only. In the female kidney, one control and one intermediate exposure concentration animal had a renal tubule cast.

Characteristics of α2μ-globulin nephropathy include accumulation of hyaline droplets in the proximal tubule, presence of cellular casts in the junction of the proximal tubule and loop of Henle, and tubular epithelial regeneration. The accumulation of hyaline droplets in severe nephropathy may lead to cell injury and death, and subsequent increased or compensatory cell division. Cell proliferation in the kidney was not assessed in this study, so no judgment can be made regarding any change in kidney cell proliferation.

In this study, minimal (or mild) increases in hyaline droplets were observed only in the high exposure concentration males when compared with controls. Cellular casts were seen only in two intermediate concentration males. Tubular epithelial regeneration was observed to increase very slightly from controls to the high exposure concentration males. Based on the histopathology observed, FT jet fuel did not induce significant α2μ-globulin nephropathy in F344 male rats. The biochemical measurements showed no increase in α2μ-globulin in any of the exposed males compared with controls, also indicating that exposure to FT jet fuel with additives did not induce significant α2μ-globulin nephropathy.

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Table 1. α2μ-Globulin Measurements in Kidneys

Gender	Exposure Group	Sample No.	α_{2u}-globulin μg/ml	Total protein mg/ml	α_{2u}-globulin μg/mg total protein
Male	Control	101	1900	39.5	48.10
		102	1970	42.4	46.46
		103	1990	38.1	52.23
		104	2910	40.5	71.85
		105	2060	40.1	51.37
		106	1420	41.1	34.55
		107	1390	66.4	20.93
		108	1540	65.2	23.62
		109	2320	42.2	54.98
		110	3230	35.8	90.22
	Low	111	1910	40.3	47.39
		112	1650	40.0	41.25
		113	1200	32.2	37.27
		114	1580	43.8	36.07
		115	2170	40.3	53.85
		116	2730	26.2	104.20
		117	1580	29.4	53.74
		118	1780	38.5	46.23
		119	1610	34.5	46.67
		120	1220	39.9	30.58
	Mid	126	1340	36.5	36.71
		127	2090	32.7	63.91
		128	1750	42.0	41.67
		129	1760	28.7	61.32
		130	1090	24.6	44.31
	High	133	1260	38.1	33.07
		135	5.125[1]	38.9	0.13[1]
		136	1150	39.3	29.26
		137	1110	41.8	26.56
		138	927	26.0	35.65
		139	903	23.2	38.92
		140	1100	21.8	50.46
Female	Control	205	4.227	42.1	0.10
		206	4.734	34.7	0.14
		207	7.228	41.9	0.17
		208	6.003	25.0	0.24
		209	6.15	34.7	0.18
		210	3.61	33.7	0.11
	High	235	4.212	29.8	0.14
		236	5.932	37.3	0.16
		237	6.984	45.3	0.15
		238	6.094	37.9	0.16
		239	4.88	35.7	0.14
		240	5.491	38.6	0.14

[1]This sample (Animal No. 135) was not included in group mean or standard deviation calculations. The value was distinctly in line with female samples, and completely out of line with male samples. The sample analysis was checked and re-performed by the contract laboratory with similar results. It was likely that an incorrectly labeled vial was used when the samples were collected.

Table 2. α2μ-Globulin Group Averages

Males	Average α_{2u} μg/mg total protein	SD	N
Controls	49.43	20.87	10
Low concentration (200 mg/m^3)	49.72	20.57	10
Intermediate concentration (700 mg/m^3)	50.90	13.72	5
High concentration (2000 mg/m^3)	35.65	8.49	6
Females			
Controls	0.16	0.05	6
High Concentration (2000 mg/m^3)	0.15	0.01	6

APPENDIX E. ANALYSIS OF ANDROLOGY PARAMETERS

Study Title: A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing

Date of Issue of RTI Report: August 23 , 2010

Task Leader: Carol D. Sloan, M.S.

Testing Facility: Laboratory of Reproductive and Endocrine Toxicology
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RTI Analytical Project Plan No.: RTI-1056-AN

Sponsor: The Hamner Institutes for Health Sciences
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Sponsor Protocol No.: 08013

Carol D. Sloan 8-23-10

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Biomarkers and Systems Biology Research
RTI International

PRINCIPAL ANALYTICAL INVESTIGATOR STATEMENT

This portion of the study was conducted in compliance with U.S. Environmental Protection Agency (U.S. EPA) Good Laboratory Practice Standards, 40 CFR 792.

Carol D. Sloan

8-23-10

Carol D. Sloan, M.S.
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RTI International

Date

RTI Study Plan Title: Determination of Andrology Parameters for the Hamner Institutes of Health Sciences

Study 08013: A 90·Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing

Sponsor Protocol Title: A 90 - Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing

Sponsor: The Hamner Institutes for Health Sciences

RTI Protocol Number: RTI-1056-AN

Sponsor Protocol Number: 08013

This study was audited by the Regulatory and Quality Assurance - Quality Assurance Unit and the results of the inspections and audits were reported to the Principal Investigator and management as well as Study Director and management as identified below.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Principal Investigator and Management	Date Inspection/Audit Report Sent to Study Director and Management
Protocol Audit	July 23 & 29, 2008	July 29, 2008	December 9, 2009
Sperm Analysis Inspection	October 3, 2008	October 3, 2008	December 9, 2009
Data & Report Audit	June 22-23, 2009	June 23, 2009	December 9, 2009
Updated Report	August 13, 2010	August 13, 2010	August 16, 2010

Prepared by:

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INTRODUCTION

Sperm motility, epididymal cauda sperm concentration and spermatid head count were determined for The Hamner Institutes of Health Sciences study 08013 entitled: A 90-day inhalation toxicity study of a synthetic jet fuel in Fischer 344 rats with neurobehavioral testing. Male and female rats were exposed by inhalation to an aerosol and vapor of FT jet fuel with JP-8 additives at 0, 200, 700, and 2000 mg/m^3. Animals were exposed 6 hours/day, 5 days/week, for a minimum of 65 exposure days.

METHODS

At the time of adult male necropsies at The Hamner Institutes for Health Sciences, July 31 and August 1, 2008, the right epididymis was immediately removed, the cauda dissected away from the remainder of the epididymis, and weighed, and fluid from the cauda assessed for sperm number and motility by RTI personnel (Carol Sloan and Susan Pearce). Sperm motility was assessed manually immediately after necropsy in all control and treated adult males and slides were prepared for possible future sperm morphology determination by RTI personnel while at The Hamner Institutes. Sperm motility was determined manually using a hemacytometer and a light microscope.

The remaining right epididymis (cauda, from which the above samples were taken) and the right testis from each male were frozen and transported to RTI International and stored at -70°C until concentrations were determined.

Caudal sperm number was evaluated initially in high-dose and control adult males from a properly retained cauda. Testicular spermatid head counts, daily sperm production (DSP), and efficiency of DSP (LeBlond and Clermont, 1952; Robb *et al.*, 1978) were evaluated on 10-2-08 from the frozen right testes, one testis/male, from each high-dose and control male. As treatment-related effects were not observed, testes from low and middle concentration exposures were not examined, nor was sperm morphology evaluated manually. Sperm motility was determined manually using a hemacytometer. Cauda sperm concentrations, and testicular homogenization resistant spermatid head counts were assessed using an Integrated Visual Optic System (IVOS) Computer Automated Sperm Analysis (CASA) System (Version 12.1 c, Hamilton Thorne, Inc., Beverly, MA) by Susan Pearce.

STATISTICAL ANALYSES

The unit of comparison was the male. The percent motile sperm was compared amount the three treatment groups and one vehicle control group and the rest of the andrology endpoints were compared between the high dose group and one vehicle control group using either parametric ANOVA under the standard assumptions robust regression method (Huber, 1967; Royall, 1986; Zeger and Liang , 1986), which do not assume homogeneity of variance or normality. The homogeneity of variance assumption will be examined via Levene's Test (Levene, 1960). If Levene's Test indicated lack of homogeneity of variance ($p<0.05$), robust regression methods

were used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They will be used to test for overall treatment group differences (via Wald Chi-Square Test), followed by individual t-tests for exposed versus control group comparisons when the overall treatment effect is significant available in the REGRESS procedure of SUDAAN® Release 9.0 (RTI , 2004), a package that is currently in use on GLP studies. If Levene's Test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques will be applied for comparing the treatment groups. The General Linear Model procedure in SAS® Version 8 (SAS Institute Inc., 2004, 2005, 2006a,b) will be used to evaluate the overall effect of treatment and, when a significant treatment effect is present, to compare each exposed group value to control group value via Dunnett's Test (Dunnett, 1955, 1964)

RESULTS

The results for all of the andrology parameters determined are shown in Table 1. The caudal sperm motility was determined for all groups, whereas the right caudal sperm concentration was determined for the control and high dose group. The average for each group is shown for the parameters measured. A statistical analysis was performed and the summary of these results is shown in Table 2. No significant differences were found for the andrology endpoints of interest.

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ARCHIVING

All specimens, raw data, and documentation generated at RTI International will be transferred to The Hamner Institutes for Health Sciences for archiving after the RTI report is finalized. The RTI facility data will be retained in the RTI archive.

Table 1. Individual Andrology Parameters and Means

GROUP

0 mg/m3			Cauda Sperm Concentration		Spermatid Head Count	
Animal #	Sperm Motility (%)	Cauda Weight (gm)	[1]Millions/mL*	[2]Millions/mL	Right Testis Weight (gm)	Millions/mL
101	100.00	0.2032	9	149.4	1.509	224.8
102	70.45	0.2300	22	149.9	1.595	188.4
103	81.25	0.2249	30	125.1	1.568	259.1
104	85.10	0.2015	27	131.0	1.600	171.2
105	97.85	0.2305	21	180.7	1.576	217.4
106	91.80	0.2179	18	164.8	1.485	186.1
107	100.00	0.2078	25	138.5	1.578	189.9
108	92.85	0.2304	20	132.5	1.613	227.1
109	94.25	0.2105	17.5	172.7	1.549	207.0
110	94.45	0.2026	36	133.5	1.503	146.7
Mean	**90.80**	**0.2159**	**23**	**147.8**	**1.5574**	**201.8**
SD	**9.35**	**0.0122**	**7**	**19.2**	**0.044**	**32.1**
200mg/m3						
111	77.2	0.2083	24		1.522	
112	80.5	0.2215	20.5		1.581	
113	92.4	0.2102	13.5		1.588	
114	85.25	0.2554	23		1.746	
115	90.6	0.2305	15.5		1.576	
116	88.2	0.2240	26		1.551	
117	86.7	0.2220	15		1.612	
118	85.65	0.2053	17.5		1.508	
119	88.85	0.2105	18		1.566	
120	87.1	0.2243	15.5		1.593	
Mean	**86.2**	**0.2212**	**18.9**		**1.584**	
SD	**4.5**	**0.0146**	**4.3**		**0.065**	

Table 1. Individual Andrology Parameters and Means (Con't)

GROUP			Cauda Concentration		Spermatid Head Count	
700 mg/m3						
Animal Number	Sperm Motility (%)	Cauda Weight (gm)	[1]Millions/mL*	[2]Millions/mL	Right Testis Weight (gm)	Millions/mL
121	70.2	0.2190	23.5		1.575	
122	88	0.2218	33.5		1.583	
123	87.23	0.2005	23.5		1.542	
124	84.1	0.2025	21		1.628	
125	88.8	0.2262	22		1.492	
126	87.5	0.2186	20		1.398	
127	84.8	0.2171	19.5		1.519	
128	88.2	0.2156	21		1.521	
129	87.4	0.2219	20		1.530	
130	88.4	0.1914	17.5		1.379	
Mean	**85.5**	**0.2135**	**22.15**		**1.517**	
SD	**5.6**	**0.0113**	**4.38**		**0.078**	
2000 mg/m3						
131	69	0.2332	23.5	135	1.633	250.9
132	80	0.1730	17.5	107.7	1.533	185.4
133	84.45	0.2012	16.5	118.5	1.568	236.0
134	86.7	0.2025	15.0	167.3	1.600	165.3
135	91.45	0.1760	23.5	114.2	1.510	186.1
136	86.2	0.1374	14.5	91.8	1.121	194.3
137	91.6	0.2194	18.5	177.2	1.613	255.4
138	85.8	0.2038	17.5	153.9	1.492	222.6
139	88.85	0.1947	23.0	129.1	1.474	205.5
140	86	0.2068	18.0	136.5	1.528	245.7
Mean	**85.01**	**0.1948**	**18.8**	**133.1**	**1.507**	**214.7**
SD	**6.56**	**0.0269**	**3.4**	**26.9**	**0.146**	**31.7**

* [1]Concentration from initial sampling of caudal for motility determinations (at The Hamner).

[2] Concentration from epididymal sample (automated analysis at RTI).

Table 2. Summary and Statistical Analysis of the Andrology Assessments

	Group			
	Control	Low	Mid	High
No. Males at Scheduled Sacrifice	10	10	10	10
Percent Motile Sperm[a]				
	91.0	86.2	85.5	85.0
	± 3.0	± 1.4	± 1.8	± 2.1
	N=10	N=10	N=10	N=10
Cauda Epididymal Sperm Concentration (10^6/g)[a,b,c]				
	790.35			778.48
	± 24.30			± 25.27
	N=10			N=10
Spermatid Head Concentration (10^6/g)[a,b,d]				
	129.50			143.27
	± 6.35			± 6.79
	N=10			N=10
Daily Sperm Production per Testis (10^6/testis/day)[a,b,e]				
	43.77			46.58
	± 2.20			± 2.18
	N=10			N=10
Efficiency of Daily Sperm Production (10^6/g. testis/day)[a,b,f]				
	28.09			31.08
	± 1.38			± 1.47
	N=10			N=10

[a] Reported as the mean ± S.E.M.

[b] Only males in the control group and high group were evaluated for this endpoint.

[c] Cauda Epididymal sperm concentration = Cauda concentration (initial) plus cauda concentration (RTI) / cauda weight.

[d] Spermatid Head Concentration = Spermatid head count in testis divided by testis weight.

[e] Daily Sperm Production per Testis = Spermatid Head Count divided by divided by 4.61 (constant for rats, the duration in days of the homogenization-resistent stages, VI-VIII, Steps 18 and 19-LeBlond and Clermond, 1952)).

[f] Efficiency = Spermatid Head Concentration divided by 4.61 (constant for rats).

APPENDIX F. VAGINAL CYTOLOGY TO IDENTIFY ESTROUS CYCLICITY IN FEMALE RATS EXPOSED TO FT JET FUEL

Study Title: A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing.

Study Protocol: 08013

Author: Brian A. Wong
Associate Investigator and Manager, Inhalation Exposure and Aerosol Science Facility

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REVIEW AND APPROVAL

Report prepared by:  Date 9/17/2010

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GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This vaginal cytology portion of Protocol 08013 was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 792, except that the data, in some cases, did not follow 792.130(e).

PRINCIPAL INVESTIGATOR: Brian A. Wong, Ph.D. Date 9/17/2010

STUDY DIRECTOR Brian A. Wong, Ph.D. Date 9/17/2010

INTRODUCTION

Exposure to some exogenous chemicals may cause disruption of the estrous cycle in female rats (Barlow and Sullivan, 1982). A synthetic jet fuel produced using the Fischer- Tropsch (FT) process is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the U.S. armed forces. During fueling operations, personnel may be exposed to vapors and aerosols of jet fuel by inhalation. A study was performed to assess the potential toxicity of FT Jet fuel by inhalation. F344 Rats were exposed by inhalation to an aerosol and vapor mixture of FT Jet Fuel with additives. Whole body inhalation exposures were conducted 6 hours/day, 5 days/week over a 90-day period, at concentrations of 0 (Control), 200, 700, or 2000 mg/m^3. Groups of 10 males and 10 females were exposed at each exposure concentration for a total of 40 males and 40 females. In order to determine if exposure to jet fuel had any effect on the estrous cycle of the female rat, vaginal cytology was performed on each female rat over five day period.

METHODS

Vaginal cytology was conducted on all female rats. During the 12th week of exposure, July 14 to July 18, 2008, a vaginal lavage was performed on each female rat daily, prior to exposure, over the five day period. Using a fire polished glass dropper, 50 to 100 µL physiological saline was gently flushed into the vaginal opening and aspirated back into a glass dropper. The aspirate was placed on individual pre-labeled, gridded glass slides for each test subject and allowed to air dry in a slide folder. The slides were prepared and read after all lavages were completed (October 24 and 25, 2008).

A stain/rehydrating solution was made using 1 mL of new methylene blue in 100 mL of sterile 0.9 percent saline. A drop of this solution was placed in each gridded space. The slides were

read on a BX41 Olympus microscope at 10 x power. The predominant vaginal cell types observed were used to identify a particular estrus stage for the day.

RESULTS AND DISCUSSION

The vaginal lavage and cytology identified the predominant cell type present each day over a five day span (Table 1). The cell types identified represented the proestrus (early and late), estrus, metestrus and diestrus stages of the estrous cycle. The normal rat estrous cycle lasts from 4 to 5 days, with metestrus lasting 6 to 8 hours, diestrus 55 to 57 hours, proestrus 12 to 14 hours, and estrus 25to 27 hours (Becker *et al.*, 2005). A female rat with a normal estrous cycle should proceed through all stages over the five day lavage period. In order to confirm a disruption of the estrous cycle, it would be preferable to continue vaginal cytology for additional days. However, for this study, other endpoints (motor activity) were scheduled, which precluded a continuing of vaginal cytology.

The vaginal cytology for each female rat was determined over the five day period, and one might expect to see cytology representing all four stages. However, because some of the stages are shorter than 24 hours, each stage may not be seen in the cytology (Becker *et al.*, 2005). The number of stages observed over the five day period for each female rat was tabulated. All of the females, except for control rat number 208, showed the presence of cells representing at least three of the four stages. As vaginal cytology over additional days was not conducted, the potential effect on estrous cycle timing could not be addressed. However, all of the exposed female rats appeared to be going through the estrus cycle, regardless of exposure to the FT jet fuel at any concentration.

REFERENCES

Barlow, S. M. and Sullivan, F. M. Reproductive Hazards of Industrial Chemicals. Academic Press, New York, 1982, p. 15.

Becker, J. B., Arnold, A. P., Berkley, K. J., Blaustein, J. D., Eckel, L. A., Hampson, E., Herman, J. P., Marts, S., Sadee, W., Steiner, M., Taylor, J., and Young, E. (2005). Strategies and Methods for Research on Sex Differences in Brain and Behavior. Endocrinology, 146:1650-1673.

ARCHIVES

Raw data and the final report will be archived at The Testing Facility. Items will be maintained in the archives for a period of one year after submission of the signed final report. The Sponsor will be contacted in order to determine the final disposition of these materials.

Table 1. Vaginal Cytology. Estrous Cycle Stage Identification from slides of vaginal lavage.

Exposure Group	Animal No.	Day 1	Day 2	Day 3	Day 4	Day 5	Number of stages seen
Control	201	P	E	M	D	D	4
	202	P	E	M	D	D	4
	203	D	P	E	M	D	4
	204	D	P	P	E	M	4
	205	P	P	E	M	M	3
	206	P	P	M	D	E	4
	207	P	E	M	P	E	3
	208	E	E	P	P	E	2
	209	P	P	E	D	D	3
	210	P	E	E	D	P	3
200 mg/m^3	211	M	D	D	P	E	4
	212	E	M	D	P	P	4
	213	E	M	D	P	E	4
	214	P	E	M	M	D	4
	215	P	E	E	M	M	3
	216	P	P	E	M	P	3
	217	P	E	E	M	P	3
	218	E	M	D	P	P	4
	219	D	P	P	E	M	4
	220	P	E	E	E	M	3
700 mg/m^3	221	D	P	E	E	M	4
	222	P	E	E	M	D	4
	223	D	P	P	E	M	4
	224	P	E	E	M	M	3
	225	P	E	M	D	E	4
	226	E	E	M	D	P	4
	227	P	E	M	D	D	4
	228	P	P	E	M	D	4
	229	E	E	M	M	D	3
	230	P	P	E	M	D	4
2000 mg/m^3	231	E	M	D	P	P	4
	232	E	M	D	P	E	4
	233	P	P	E	E	M	3
	234	E	M	P	M	D	4
	235	P	P	E	M	D	4
	236	P	E	E	M	D	4
	237	P	E	E	M	D	4
	238	P	E	M	P	E	3
	239	D	P	E	E	M	4
	240	D	P	E	E	M	4

M = Metestrus
D = Diestrus
P = Proestrus
E = Estrus

APPENDIX G. NEUROTOXICITY (MOTOR ACTIVITY AND FOB) STUDIES IN RATS EXPOSED TO JET FUEL

Forward: The main report and protocol title, "A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing." used the term "neurobehavioral testing". This appendix contains the report using the term "neurotoxicity". The neurobehavioral testing refers to procedures in the U.S. EPA Health Effects Test Guidelines, OPPTS 870.6200 Neurotoxicity Screening Battery. The OPPTS 870.6200 guideline, with "Neurotoxicity" in the title, describes the purpose of the functional observational battery "…to better quantify behavioral or neurological effects". The neurobehavioral testing in the protocol title is included in the neurotoxicity testing described in the guidelines and the Scientist's Report, "Neurotoxicity (Motor Activity and FOB) Studies in Rats Exposed to Jet Fuel."

Study Title: A 90-Day Inhalation Toxicity Study of a Synthetic Jet Fuel in Fischer 344 Rats with Neurobehavioral Testing

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Report Prepared By:

Melanie L. Foster, B.S., DABT — 9/16/10
Consultant, Principal Investigator — Date

Report Reviewed By:

Brian A. Wong, Ph.D. — 9/17/2010
Study Director — Date

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described by this report was conducted in compliance with the U. S. Environmental Protection Agency (U.S. EPA) Good Laboratory Practice Standards, 40 CFR 792, except that computer validation was not completed on some equipment (e.g. neurobehavioral test equipment).

Melanie L. Foster, B.S., DABT — 9/16/10
Consultant, Principal Investigator — Date

SUMMARY

The purpose of this study was to assess the potential inhalation toxicity of a synthetic jet fuel (FT) with additives when administered via inhalation exposure at either 0, 200, 700, or 2000 mg/m^3 to adult male and female Fischer 344 rats on a repeated basis over 90 days (6 hours per day, 5 days per week). Neurotoxicological effects in exposed rats were assessed using functional observational battery (FOB) and motor activity tests as defined in the United States Environmental Protection Agency (U.S. EPA) neurotoxicology guidelines. This report describes the methods and results of the neurobehavioral testing.

Spontaneous motor activity was determined in male and female F344 rats (n = 10 rats/sex/exposure group) during a one-hour test session in air-exposed (control) and jet fuel exposed rats. Motor activity was determined on a non-exposure day following approximately 12 weeks of inhalation exposure. The activity of the control rats approached an asymptote during the last 10-15 minutes of the 60-minute testing session indicating that the 60 minute time period

was sufficient to meet the criteria detailed by the U.S. EPA testing guideline. Using a repeated measure multivariate analysis of variance (MANOVA), there was a significant dose effect seen in male rats exposed to FT jet fuel for both ambulation and total movement seen during the one-hour test session. An ANOVA performed on these parameters revealed a significant decrease in ambulation and total movements in male rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed control males). There was also a decrease in total motor activity during the initial (6 minute) exploratory phase in male rats exposed to 200 or 2000 mg/m^3 (versus air-exposed control males). An overall treatment effect on motor activity was not seen for female rats exposed to FT jet fuel (MANOVA). As with the male rats, a decrease in total motor activity was seen during the initial (6 minute) exploratory phase in female rats exposed to 2000 mg/m^3 (versus air exposed control females). These results indicate that the highest exposure concentration caused an overall reduction in motor activity in the males, but only reduced the initial exploratory activity of jet fuel exposed female rats.

Neurobehavioral evaluations using an observation battery designed to detect functional deficits (FOB) were also performed. Rats (n = 10 rats/sex/exposure group) were evaluated on non-exposure days after approximately 13 weeks of inhalation exposure to air or FT jet fuel. Two FOB sessions evaluating 5 rats/sex/exposure group/session were conducted. Conduct of the FOB sessions was balanced across sex and exposure concentration in each test session. Exposure to FT jet fuel was associated with significant changes. Body weight was decreased in male and female rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed controls). When compared to air-exposed controls, female rats exposed to FT jet fuel at 2000 mg/m^3 had a reduced number of rears during a 2-minute observation period in an open field. Rats exposed to the highest concentration of FT jet fuel also had changes in external appearance. Male rats exposed to FT jet fuel at 2000 mg/m^3 had a higher incidence of alopecia or flaky or scaly skin on the nose or feet (8/10) versus air-exposed controls (2/10) or male rats exposed to FT jet fuel at either 200 mg/m^3 (0/10) or 700 mg/m^3 (1/10). Female rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed controls) also had a higher incidence of alopecia or flaky skin on the nose or feet (9/10) versus air-exposed rats (1/10) or female rats exposed to FT jet fuel at either 200 mg/m^3 (2/10) or 700 mg/m^3 (1/10).

INTRODUCTION

Civilian and military personnel are occupationally exposed to gasoline, jet fuel, diesel fuel, kerosene, and other hydrocarbon fuels. These exposures may occur acutely or chronically to raw fuel, vapor, aerosol, or fuel combustion exhaust by dermal, respiratory inhalation, or ingestion routes (Ritchie *et al.*, 2001). Hydrocarbon fuels represent a complex mixture and can include a variety of aliphatic and aromatic hydrocarbon neurotoxicants. A number of published studies have documented neurotoxicity arising from acute, subchronic, or chronic exposure of humans or animals to hydrocarbon fuels (Ritchie *et al.*, 2001). Exposure to JP-8 commonly occurs within the military. Previous studies have connected JP-8 exposure to numerous adverse health effects including pulmonary, developmental, hepatic, immunological, neurological, and dermal effects (Anand *et al.*, 2007; Chao *et al.*, 2006; Cooper and Mattie, 1996; Dossing *et al.*, 1985; Harris *et al.*, 1997; Keil *et al.*, 2003, 2004; McDougal and Rogers, 2004; Robledo *et al.*, 2000; Smith *et al.*, 1997; Ullrich and Lyons, 2000). Alternative fuels including a Fischer-Tropsch (FT) process

synthetic jet fuel (S-8) containing fewer aromatic hydrocarbons (versus JP-8) have been developed. The toxicological profile of FT jet fuel is incomplete and is the subject of the current investigation. This study focused on inhalation exposure, a route of exposure relevant for civilian and military personnel handling FT jet fuel.

Purpose

The purpose of this study was to assess the potential inhalation neurotoxicity of FT jet fuel with additives when administered via inhalation exposure at 0, 200, 700, or 2000 mg/m^3 to adult male and female Fischer 344 rats on a repeated basis over 90 days (6 hours per day, 5 days per week). Neurotoxicological effects in exposed rats were assessed using functional observational battery (FOB) and motor activity tests as defined in the United States Environmental Protection Agency (U.S. EPA) neurotoxicology guidelines. This report describes the methods and results of the neurobehavioral testing.

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MATERIALS AND METHODS

Test Guidelines/Regulations

The study described by this report was conducted in compliance with the following guidelines:

- U.S. EPA, 40 CFR Part 792. Good Laboratory Practice Standards
- U.S. EPA, Health Effects Test Guidelines OPPTS 870.6200 Neurotoxicity Screening Battery

with the following protocol-specified exceptions: (a) specialized neuropathology assessments using in situ perfusion of the nervous system were not conducted; and (b) FOB and motor activity assessments were performed once.

Animals

F344 rats were used for this study. The rat was selected since the neurotoxicology guidelines specifically state that rats or mice should be used and they are a common representative species for toxicity studies. These animals (10/sex/exposure concentration) were also evaluated for inhalation toxicity and reproductive toxicity as described in the main body of the report. Details concerning the animals used on this study and their husbandry may be found in the main body of

the report. The animals were exposed by whole body inhalation to 0, 200, 700, or 2000 mg/m^3 FT jet fuel for 6 hours/day, 5 days/ week for a minimum of 65 exposure days. Details concerning the test compound, FT jet fuel with additives, and the animal exposure conditions are described in the final report. The terms jet fuel, S-8, and S-8 jet fuel and FT jet fuel are used synonymously in this report to describe the test substance.

Motor Activity

Motor activity was measured once after approximately 12 weeks of exposure. It was assessed on a non-exposure day following five consecutive days of exposure. Motor activity was measured during 10 six-minute intervals for a total of 60 minutes using an automated cage rack photobeam activity system (San Diego Instruments, San Diego, CA). Activity was recorded as ambulations (when the rat broke two adjacent photobeams consecutively), and total movements (fine movements consisting of breaking the same photobeam consecutively, plus ambulations). Cumulative totals for the session were also recorded. Four sessions of 20 rats each were balanced for exposure groups and gender. Illumination and white noise levels, as well as temperature and humidity in the motor activity laboratory were documented during the day of testing. White noise was generated using a Coulbourn Instruments (Allentown, PA) white noise generator. Advance notice of the testing was sent to all staff and signs with barriers were placed around the laboratory in order to reduce other environmental distractions.

Functional Observational Battery

The FOB including quantitative assessment of grip strength and foot splay was performed after approximately 13 weeks of exposure. Sessions were conducted on two nonexposure days at the end of an exposure week. Observations were made: 1) while the rat was in the observation cage, 2) during removal of the rat from the observation cage, 3) while the rat was being held and examined for clinical observations, 4) as the animal moved freely about the open field, and 5) during manipulative tests. The observers were blinded to the treatment groups by the use of a randomized order and temporary identification numbers for the rats. One trained technician performed the FOB on all animals on the study. Two additional technicians assisted with the conduct and recording of the FOB data.

Illumination and ambient noise levels, as well as temperature and humidity in the motor activity laboratory were documented during the week of testing. Advance notice of the testing was sent to all staff and signs with barriers were placed around the laboratory in order to reduce other environmental distractions.

The animals were observed for:

- Posture
- Signs of involuntary muscular movements (tremors, spasms, and convulsions)
- Palpebral closure
- Handling reactivity
- Muscle tone

- Fur condition (piloerection, fur appearance, facial crust, skin temperature and color)
- Breathing pattern
- Salivation and lacrimation
- Arousal
- Ataxia
- Gait
- Body position
- Excessive vocalization
- Stereotypy and unusual behaviors
- Defecation, diarrhea, urination, and rears
- Approach response
- Startle response
- Tail pinch responses
- Visual placing
- Grip strength
- Surface righting reflexes
- Hind leg splay
- Pupillary reflex
- Body weight
- Any additional observations were recorded

Behavior Testing Schedule

Motor Activity		FOB			
# Male rats	# Female rats	# Male rats	# Female rats	Date	Session ID[a]
40	40			7/19/08	MA Sessions 1 and 2
		20	20	7/25/08	FOB Session 1
		20	20	7/26/08	FOB Session 2

a: MA: Motor activity; FOB: functional observational battery

Statistical Procedures

Motor activity

Mean and standard deviation values were calculated for total motor activity and ambulations at each six minute interval during the measurement period. All motor activity data were evaluated using the following computer-generated statistical tests of significance (JMP 7.0, Cary, NC): a nested analysis of motor activity data was performed using a repeated-measures analysis with

exposure as a grouping factor and test period and test session time as within subject factors (multivariate analysis of variance (MANOVA)) ($p<0.05$). Levene's test for homogeneity ($p<0.01$) followed by one-way analysis of variance (ANOVA) ($p<0.05$) and Dunnett's t-test ($p<0.05$) were performed for homogeneous data. A one-way analysis of variance (ANOVA) ($p<0.05$) and Dunnett's t-test ($p<0.05$) were performed on the transformed data. In the event that the Levene's test on the transformed data indicated non-homogenous data, a Kruskal-Wallis H test ($p<0.05$) and Wilcoxon 2-sample Rank-Sum test ($p<0.05$) were used.

FOB

Levene's test for homogeneity ($p<0.01$) followed by one-way analysis of variance (ANOVA) ($p<0.05$) and Dunnett's t-test ($p<0.05$) were performed for continuous data. Categorical data were converted to ordinal scores as noted on Table 8 and analyzed using a contingency analysis. Treatments indicating significant changes were further analyzed using a log-likelihood model ($p<0.05$) and Pearson's Chi Square.

Unless otherwise indicated, data presented in all figures represents mean values ± standard error of the mean (SEM).

Project Participants

Study Director: Brian A. Wong, Ph.D.
FOB Technician and Report Author: Melanie L. Struve, B.S., DABT (consultant)
Behavior Technician: Carol M. Bobbitt, A.A., RVT
Behavior Technician: Lisa Hubbard, B.S.

Protocol and SOP Deviations

There were no protocol or SOP deviations for this study.

Data Storage

The data sheets and all nonperishable raw data were stored in the archives of the Hamner Institutes for Health Sciences.

RESULTS

Motor Activity

Motor activity was determined in air-exposed (control) and FT-exposed rats using a one-hour test session. Motor activity data are presented as group summary data in Tables 1-2, and as individual data in Tables 3-6.

The activity of the male and female control rats approached an asymptote during the last 10-15 minutes of the 60-minute testing session (Figures 1-2), thereby meeting a criteria for the test guidelines.

When compared to air-exposed controls, male rats exposed to FT jet fuel at 2000 mg/m^3 had decreased total motor activity as assessed using movement or ambulation as the experimental parameter (Figure 3). An ANOVA performed on these parameters revealed a significant decrease in ambulation and total movements in male rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed control males). There was also a decrease in motor activity during the initial (6 minute) exploratory phase in male rats exposed to 200 or 2000 mg/m^3 (versus air-exposed control males, Figure 2).

An overall treatment effect on total movement or ambulation was not seen for female rats exposed to FT jet fuel (MANOVA, Figures 1-2). As with the male rats, a decrease in motor activity was seen during the initial (6 minute) exploratory phase in female rats exposed to 2000 mg/m^3 (versus air-exposed control females) (Figures 1-2). These results indicate that the highest exposure concentration caused an overall reduction in motor activity in the males, but only reduced the initial exploratory activity of jet fuel exposed female rats.

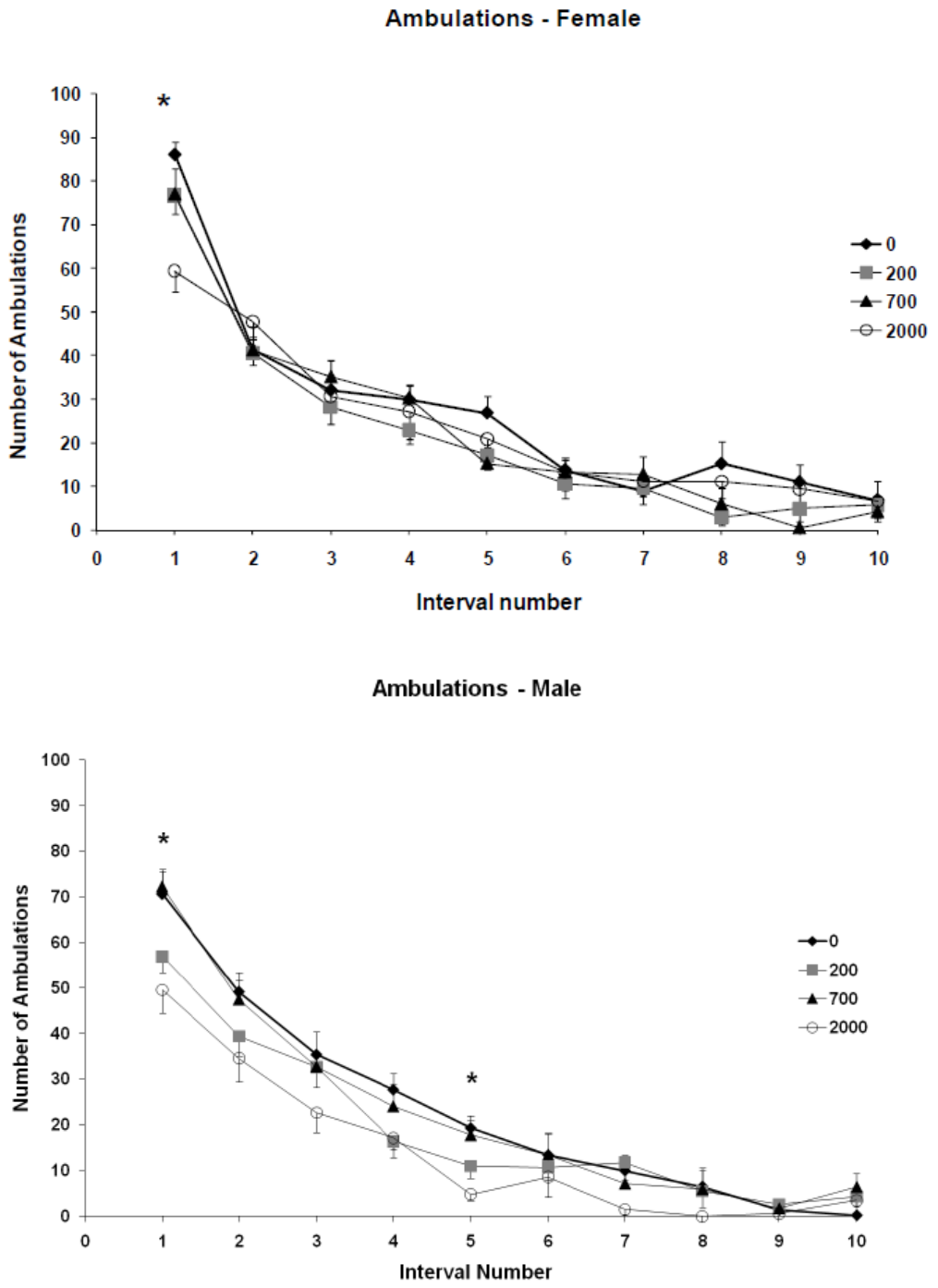

Figure 1. Ambulation recorded during the one-hour motor activity test session in male and female rats exposed to FT jet fuel

*Indicates decreased motor activity in jet fuel-exposed rats versus air-exposed controls ($p<0.05$; individual interval).

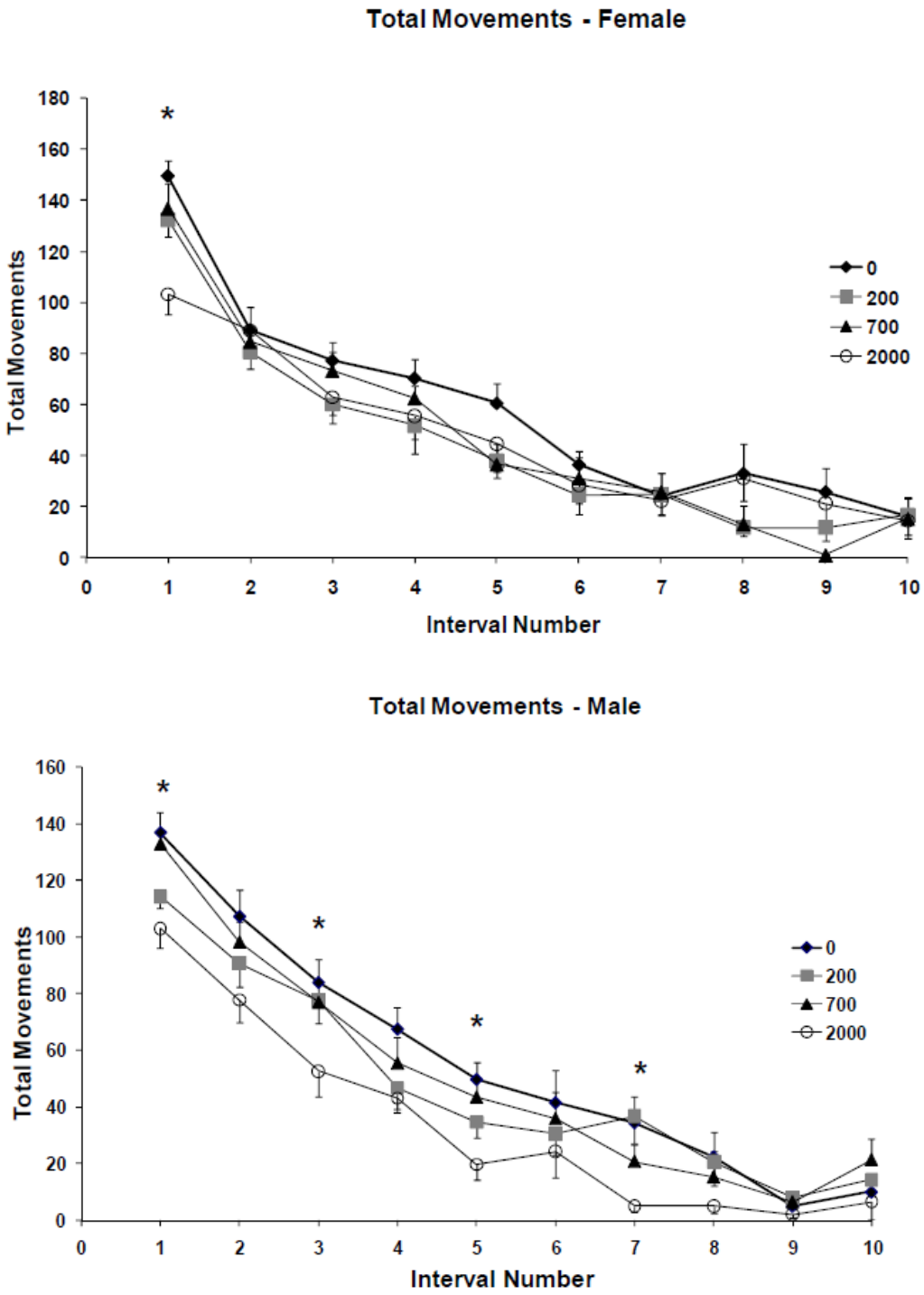

Figure 2. Total movements recorded during the one-hour motor activity test session in male and female rats exposed to FT jet fuel

*Indicates decreased motor activity in jet fuel-exposed rats versus air-exposed controls ($p<0.05$; individual interval).

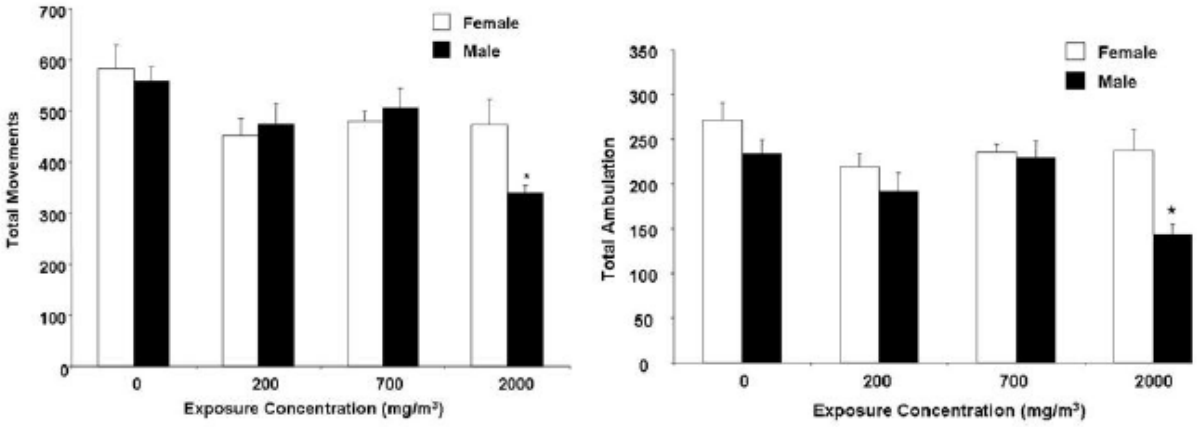

Figure 3. Cumulative total movement (left) and ambulation (right) counts recorded during the one-hour motor activity test session in male and female rats exposed to FT jet fuel
* Indicates decreased motor activity in male rats (versus air-exposed controls; $p<0.05$).

Functional Observation Battery

Neurobehavioral evaluations using an FOB designed to detect functional deficits were also performed. Rats (10 rats/sex/exposure group) were evaluated on non-exposure days after approximately 13 weeks of inhalation exposure to air or FT jet fuel. Two FOB sessions evaluating five rats/sex/exposure group/session were conducted. Conduct of the FOB sessions was balanced across sex and exposure concentration in each test session.

FOB group summary data for the quantitative measures are presented in Table 7, a scoring key for categorical data is presented in Table 8, and individual data are presented in Tables 9 through 20. Exposure to FT jet fuel was associated with several statistically significant changes. Body weight was decreased in male and female rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed controls) (Figure 4, Tables 7, 17, 18). Male rats exposed to FT jet fuel at 2000 mg/m^3 had an approximate 12 percent decrease in body weight (versus air-exposed controls). Female rats exposed to FT jet fuel at 2000 mg/m^3 had an approximate 6 percent decrease in body weight (versus air-exposed controls).

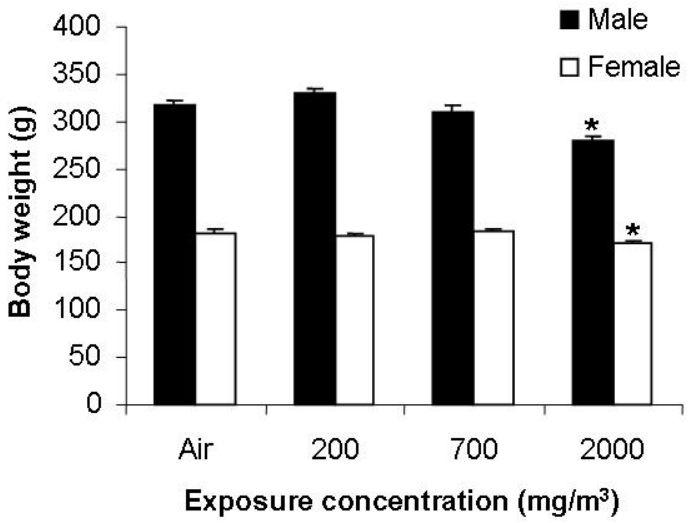

Figure 4. Body weight (g) in male and female rats exposed to FT jet fuel
* Indicates decreased body weight (versus air-exposed controls; $p<0.05$).

When compared to air-exposed controls, female rats exposed to FT jet fuel at 2000 mg/m^3 had a reduced number of rears during a two-minute observation period in an open field (Figure 5; Tables 7, 14-15).

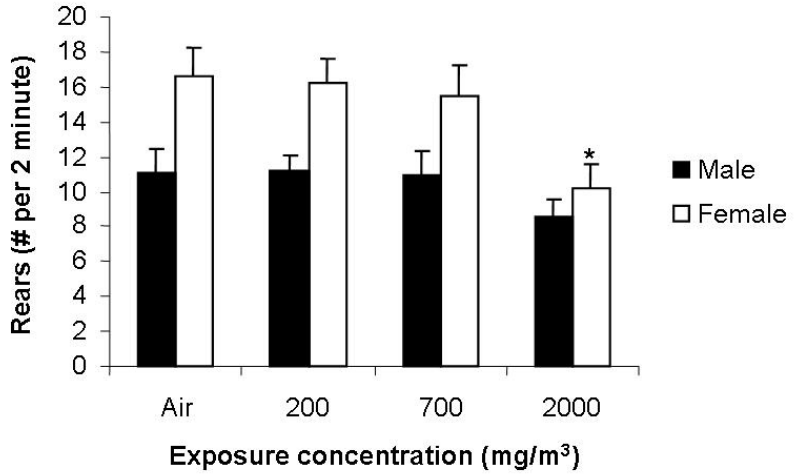

Figure 5. Number of rears in male and female rats exposed to FT jet fuel
*Indicates decreased rearing behavior (versus air-exposed controls; $p<0.05$).

Rats exposed to the highest concentration of FT jet fuel also had changes in external appearance (Tables 11, 12, 19, 20). Male rats exposed to FT jet fuel at 2000 mg/m^3 had a higher incidence

of alopecia or flaky or scaly skin on the nose or feet (8/10) versus air exposed controls (2/10) or male rats exposed to FT jet fuel at either 200 mg/m^3 (0/10) or 700 mg/m^3 (1/10). Female rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed controls) had a higher incidence of alopecia or flaky skin on the nose or feet (9/10) versus air-exposed rats (1/10) or female rats exposed to FT jet fuel at either 200 mg/m^3 (2/10) or 700 mg/m^3 (1/10). No other changes were seen in behaviors or observations that could be linked to the jet fuel exposure during the FOB.

DISCUSSION AND INTERPRETATION

The information from human and laboratory animal studies indicates that neurotoxicity may occur by all routes of exposure and that all jet fuels may be neurotoxic (ATSDR, 1998). As is common with many hydrocarbons, the primary acute neurotoxic effect of jet fuels is central nervous depression (ATSDR, 1998). Several petroleum distillates have been evaluated using motor activity and FOB as part of a subchronic inhalation study in rats. Lapin *et al.*(2001) conducted a 15-week, whole-body inhalation study of the vapors of a distillate of light catalytic cracked naphtha (LCCN-D, CAS no. 64741-55-5) in Sprague-Dawley rats. Target exposure concentrations were 0, 750, 2500 and 7500 ppm for 6 hours/day, 5 days/week (≥ 65 exposures). They reported no evidence of neurotoxicity at any exposure level. Schreiner and coworkers (2000) conducted a 13-week whole-body inhalation study in Sprague-Dawley CD rats exposed to LCRN-D at target concentrations of 0, 750, 2500 and 7500 ppm for 6 hours/day, 5 days/week. The only effect of LCRN-D on neurobehavioral parameters reported by Schreiner *et al.*(2000) was significantly higher motor activity counts among high-dose (7500 ppm) males after a four-week recovery period which was suggestive of a possible delayed effect of LCRN-D. However, there was no evidence of hyperactivity or abnormal behavior from their FOB evaluations, and there were no microscopic changes in neural tissue to support this observation. Rossi *et al.*, (2001) reported that Sprague-Dawley rats exposed to JP-8 (1000 mg/m^3 for 6 hours/day, 5 days/week) or JP-5 (1200 mg/m^3 for 6 hours/day, 5 days/week) jet fuel vapor did not develop changes in total locomotor activity (versus air exposed controls).

The purpose of this study was to assess the potential inhalation neurotoxicity of a synthetic jet fuel (FT) when administered via inhalation exposure at 0, 200, 700, or 2000 mg/m^3 to adult male and female Fischer 344 rats on a repeated basis over 90 days (6 hours per day, 5 days per week). Neurotoxicological effects in exposed rats were assessed using a screening test consisting of an FOB and test of motor activity as defined in the U.S. EPA neurotoxicology guidelines.

High dose exposure to FT jet fuel was associated with decreased body weight in male and female rats exposed to FT jet fuel at 2000 mg/m^3 (versus air-exposed controls). Male rats exposed to FT jet fuel at 2000 mg/m^3 had an approximate 12 percent decrease in body weight (versus air-exposed controls). Female rats exposed to FT jet fuel at 2000 mg/m^3 had an approximate 6 percent decrease in body weight (versus air-exposed controls). The highest exposure concentration used in this study (2000 mg/m^3, 6 hours/day, 5 days/week) was not associated with overt morbidity (as assessed using the FOB) or mortality. In male rats it was associated with a 12 percent retardation of body weight gain as compared with control animals which is consistent with the highest exposure concentration being close to or in excess of a maximum tolerated dose (MTD) in male rats.

In addition to changes in body weight, sub-chronic exposure to the highest concentration of FT was associated with an approximate 39 percent decrease in total motor activity in male rats (Figure 3). Subchronic exposure was also associated with a decrease in motor activity in female rats exposed to FT jet fuel at 2000 mg/m^3. This effect in females was confined to the first six minutes of the test session which is part of the initial exploratory behavior of the rat in a novel environment. In female rats, this effect dissipated and was no longer apparent during the later stages of the test session. Male rats exposed to 2000 mg/m^3 had decreased motor activity during the first six-minute interval and additional intervals, as well as a significant overall effect (MANOVA). Male rats exposed to 200 mg/m^3 FT jet fuel also had significantly decreased total movements only during the first six-minute interval. This effect was not seen in the animals exposed to FT jet fuel at 700 mg/m^3; the reason for this lack of dose response is unknown. Female rats exposed to FT jet fuel at 2000 mg/m^3 also had an approximate 39 percent decrease in rearing behavior in an open field. The FOB open field test is assessed during a two minute test interval and involves a novel test environment and represents another form (vertical) of motor activity. Vertical activity is not measured separately in the motor activity test, but the open field activity is reflective of the first six-minute interval in the motor activity session, and may contribute to the decrease in total activity seen in that interval in females. It is unknown whether the effects seen on motor activity in rats exposed to the highest exposure concentration are indicative of direct neurotoxicity or an indirect response stemming from systemic toxicity.

With the exception of the aforementioned decrease in rearing behavior seen during the FOB, there was no other evidence of neurotoxicity seen during the FOB evaluation of FT exposed rats. The toxicological significance of the alopecia and flaky skin seen in rats subchronically exposed to FT jet fuel at 2000 mg/m^3 is unknown.

In conclusion, subchronic exposure of F344 rats to FT vapors resulted in evidence of changes in motor activity. This effect was most pronounced in male rats.

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Table 1. Summary Motor Activity Ambulations – Mean (Standard Deviation)

Males	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
N	10	10	10	10
Time period (min)				
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
6	70.7 (17.4)	57.0 (11.1)	72.3 (10.7)	**49.6 (16.1)****
12	49.2 (13.5)	39.4 (13.6)	47.5 (13.3)	34.6 (16.1)
18	35.4 (15.6)	32.7 (14.0)	32.8 (9.6)	22.7 (13.5)
24	27.7 (11.3)	16.4 (11.2)	24.1 (14.8)	17.2 (8.1)
30	19.3 (8.5)	11.0 (8.2)	17.8 (10.0)	**4.8 (4.3)****
36	13.4 (15.4)	10.7 (8.8)	13.5 (14.6)	8.6 (14.0)
42	10.0 (10.4)	11.8 (12.5)	7.2 (7.4)	1.5 (3.2)
48	6.5 (13.2)	5.5 (11.2)	6.0 (12.5)	0.0 (0.0)
54	1.4 (2.7)	2.6 (5.7)	1.8 (3.3)	0.6 (1.9)
60	0.2 (0.6)	4.3 (6.8)	6.5 (9.3)	3.5 (11.1)
Total	233.8 (50.1)	191.4 (66.4)	229.5 (59.2)	**143.1 (39.0)****

Females	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
N	10	10	10	10
Time period (min)				
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
6	86.1 (9.3)	76.8 (13.4)	77.1 (17.9)	**59.4 (15.4)****
12	41.2 (17.3)	40.6 (8.8)	41.3 (9.6)	47.8 (12.9)
18	32.0 (10.6)	28.1 (12.1)	35.1 (11.9)	30.6 (8.6)
24	29.9 (11.0)	22.9 (10.2)	30.2 (8.8)	27.2 (20.0)
30	26.8 (11.9)	17.1 (10.3)	15.2 (11.9)	20.9 (16.7)
36	13.7 (7.2)	10.6 (10.4)	13.2 (10.9)	13.2 (12.8)
42	8.7 (12.0)	9.5 (11.5)	12.7 (13.1)	11.2 (10.8)
48	15.1 (16.0)	2.9 (5.3)	5.9 (11.8)	11.1 (12.0)
54	10.9 (12.7)	4.9 (9.9)	0.4 (1.3)	9.4 (13.6)
60	6.8 (13.7)	5.7 (13.4)	4.1 (10.5)	6.6 (11.9)
Total	271.2 (61.5)	219.1 (46.3)	235.2 (28.7)	237.4 (75.6)

Bold indicates significant difference from control (0 mg/m^3 exposed group).
*p<0.05, **p<0.01

Table 2. Summary Motor Activity Total Movements – Mean (Standard Deviation)

Males	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
N	10	10	10	10
Time period (min)				
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
6	136.9 (23.3)	**114.3 (13.1)***	132.9 (15.7)	**103.1 (22.6)****
12	107.3 (29.7)	90.8 (26.1)	98.2 (22.5)	77.8 (24.4)
18	84.0 (25.4)	77.5 (24.7)	76.9 (22.8)	**52.7 (28.3)***
24	67.6 (23.7)	46.7 (23.3)	55.4 (29.6)	43.1 (16.3)
30	49.8 (18.3)	34.6 (17.7)	43.4 (19.4)	**19.7 (17.6)****
36	41.6 (36.5)	30.5 (23.6)	35.8 (30.6)	24.3 (28.7)
42	34.5 (29.7)	36.8 (31.0)	20.7 (18.8)	**5.0 (6.9)***
48	22.5 (26.9)	20.6 (26.9)	15.2 (28.7)	5.1 (8.3)
54	5.1 (10.5)	7.9 (12.8)	6.4 (7.9)	2.1 (3.1)
60	10.1 (16.9)	14.3 (17.3)	21.3 (23.1)	6.5 (20.2)
Total	559.4 (87.0)	474.0 (132.9)	506.2 (121.5)	**339.4 (45.3)****
Females	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
N	10	10	10	10
Time period (min)				
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
6	149.7 (19.4)	132.3 (20.5)	136.9 (30.6)	**103.2 (24.5)****
12	89.3 (28.1)	80.4 (19.9)	84.6 (14.9)	89.0 (25.2)
18	77.3 (22.6)	60.0 (23.1)	73.3 (23.1)	62.8 (21.9)
24	70.4 (23.7)	51.8 (17.0)	62.5 (15.2)	55.6 (46.5)
30	60.6 (23.7)	38.0 (21.9)	36.6 (25.1)	44.7 (35.2)
36	36.5 (17.2)	24.6 (23.2)	31.1 (26.0)	28.5 (23.4)
42	24.4 (27.2)	24.8 (24.6)	25.7 (24.4)	22.3 (18.5)
48	33.1 (36.1)	11.7 (10.1)	13.2 (22.7)	31.2 (28.0)
54	25.8 (29.5)	11.7 (16.8)	1.0 (1.6)	21.3 (29.9)
60	16.2 (22.6)	16.7 (25.0)	15.3 (25.9)	14.4 (22.2)
Total	583.3 (142.4)	452.0 (105.0)	480.2 (60.1)	473.0 (158.9)

Bold indicates significant difference from control (0 mg/m^3 exposed group).
*p<0.05, **p<0.01

Table 3. Motor Activity (Ambulation) in Male Rats

0 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
101	M	1	9	0	55	24	24	10	6	15	0	0	0	0	134
102	M	1	20	0	87	66	17	17	14	0	0	0	0	0	201
103	M	1	16	0	61	53	41	14	11	0	10	5	0	0	195
104	M	3	13	0	76	58	66	40	15	12	19	1	0	0	287
105	M	3	3	0	73	61	29	27	28	11	21	1	0	0	251
106	M	2	11	0	69	51	52	36	27	1	8	43	8	0	295
107	M	2	10	0	43	39	29	36	22	6	13	9	2	2	201
108	M	4	1	0	56	34	17	22	26	50	29	6	4	0	244
109	M	4	14	0	99	45	36	41	13	28	0	0	0	0	262
110	M	4	9	0	88	61	43	34	31	11	0	0	0	0	268
			Mean	0.0	70.7	49.2	35.4	27.7	19.3	13.4	10.0	6.5	1.4	0.2	233.8
			SD	0.0	17.4	13.5	15.6	11.3	8.5	15.4	10.4	13.2	2.7	0.6	50.1
			N	10	10	10	10	10	10	10	10	10	10	10	10

200 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
111	M	1	8	0	51	67	44	29	21	23	8	0	0	0	243
112	M	1	2	0	53	32	8	3	1	0	0	0	0	16	113
113	M	3	11	0	56	40	24	5	8	0	4	0	0	0	137
114	M	3	6	0	47	26	32	29	6	12	8	0	0	0	160
115	M	3	14	0	65	39	46	29	14	24	20	5	0	1	243
116	M	2	14	0	82	37	26	8	25	14	16	36	16	12	272
117	M	2	20	0	47	17	29	3	3	9	0	0	0	0	108
118	M	2	3	0	48	40	20	25	8	14	25	4	0	0	184
119	M	4	3	0	66	51	53	20	19	11	37	10	10	14	291
120	M	4	4	0	55	45	45	13	5	0	0	0	0	0	163
			Mean	0.0	57.0	39.4	32.7	16.4	11.0	10.7	11.8	5.5	2.6	4.3	191.4
			SD	0.0	11.1	13.6	14.0	11.2	8.2	8.8	12.5	11.2	5.7	6.8	66.4
			N	10	10	10	10	10	10	10	10	10	10	10	10

Table 3. Motor Activity (Ambulation) in Male Rats (continued)

700 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
121	M	1	13	0	63	64	29	47	10	9	13	0	0	0	235
122	M	1	18	0	63	23	11	11	22	0	2	0	0	0	132
123	M	1	3	0	59	52	39	29	5	43	15	23	0	11	276
124	M	3	18	0	69	43	24	8	16	0	6	0	0	0	166
125	M	3	10	0	72	46	39	10	24	23	21	0	4	0	239
126	M	2	12	0	86	59	40	16	13	1	0	0	0	26	241
127	M	2	15	0	76	51	44	28	38	20	11	35	4	16	323
128	M	2	15	0	75	39	36	12	20	6	0	0	0	0	188
129	M	4	2	0	67	34	33	34	6	4	0	0	10	12	200
130	M	4	20	0	93	64	33	46	24	29	4	2	0	0	295
			Mean	0.0	72.3	47.5	32.8	24.1	17.8	13.5	7.2	6.0	1.8	6.5	229.5
			SD	0.0	10.7	13.3	9.6	14.8	10.0	14.6	7.4	12.5	3.3	9.3	59.2
			N	10	10	10	10	10	10	10	10	10	10	10	10

2000 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
131	M	1	6	0	44	29	7	7	0	2	0	0	0	0	89
132	M	1	11	0	47	74	13	28	0	0	0	0	6	35	203
133	M	3	20	0	30	39	33	26	8	42	10	0	0	0	188
134	M	3	17	0	49	35	29	10	2	0	0	0	0	0	125
135	M	3	1	0	41	32	36	14	12	0	2	0	0	0	137
136	M	2	5	0	32	9	0	24	4	11	3	0	0	0	83
137	M	2	6	0	48	30	22	24	11	0	0	0	0	0	135
138	M	2	16	0	75	33	44	12	6	0	0	0	0	0	170
139	M	4	10	0	51	36	19	7	2	24	0	0	0	0	139
140	M	4	17	0	79	29	24	20	3	7	0	0	0	0	162
			Mean	0.0	49.6	34.6	22.7	17.2	4.8	8.6	1.5	0.0	0.6	3.5	143.1
			SD	0.0	16.1	16.1	13.5	8.1	4.3	14.0	3.2	0.0	1.9	11.1	39.0
			N	10	10	10	10	10	10	10	10	10	10	10	10

Table 4. Motor Activity (Ambulation) in Female Rats

0 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
201	F	1	17	0	76	28	31	42	26	5	0	0	0	0	208
202	F	1	5	0	83	18	19	35	2	4	0	0	4	0	165
203	F	3	2	0	103	25	48	11	20	19	4	14	18	0	262
204	F	3	12	0	93	65	37	35	34	6	6	0	0	0	276
205	F	3	7	0	77	51	25	28	40	8	0	32	23	6	290
206	F	2	18	0	75	30	16	36	44	14	18	5	5	16	259
207	F	2	4	0	90	58	26	38	23	20	2	35	6	0	298
208	F	2	8	0	93	26	37	22	20	22	33	30	39	43	365
209	F	4	7	0	80	54	36	13	32	18	0	0	0	0	233
210	F	4	19	0	91	57	45	39	27	21	24	35	14	3	356
			Mean	0.0	86.1	41.2	32.0	29.9	26.8	13.7	8.7	15.1	10.9	6.8	271.2
			SD	0.0	9.3	17.3	10.6	11.0	11.9	7.2	12.0	16.0	12.7	13.7	61.5
			N	10	10	10	10	10	10	10	10	10	10	10	10

200 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
211	F	1	15	0	83	37	26	24	30	0	0	0	0	0	200
212	F	1	12	0	83	30	17	23	13	0	0	0	3	35	204
213	F	1	14	0	83	48	38	8	34	23	0	0	28	19	281
214	F	3	16	0	62	36	20	37	24	19	20	0	0	0	218
215	F	3	5	0	55	38	8	35	11	15	1	0	0	0	163
216	F	2	17	0	65	53	45	11	14	8	4	4	0	0	204
217	F	2	9	0	71	32	40	27	14	6	25	15	0	0	230
218	F	4	16	0	77	32	34	14	0	0	0	0	0	0	157
219	F	4	8	0	93	48	35	33	9	29	27	10	18	3	305
220	F	4	13	0	96	52	18	17	22	6	18	0	0	0	229
			Mean	0.0	76.8	40.6	28.1	22.9	17.1	10.6	9.5	2.9	4.9	5.7	219.1
			SD	0.0	13.4	8.8	12.1	10.2	10.3	10.4	11.5	5.3	9.9	13.4	46.3
			N	10	10	10	10	10	10	10	10	10	10	10	10

Table 4. Motor Activity (Ambulation) in Female Rats (continued)

700 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
221	F	1	10	0	92	25	34	26	6	30	0	0	0	0	213
222	F	1	1	0	49	59	54	20	8	0	28	31	4	0	253
223	F	3	19	0	75	40	35	42	14	14	20	0	0	0	240
224	F	3	9	0	78	42	37	34	13	20	5	1	0	0	230
225	F	3	8	0	79	41	27	18	8	8	5	25	0	0	211
226	F	2	19	0	89	47	40	28	12	21	29	2	0	0	268
227	F	2	7	0	70	32	36	30	35	27	32	0	0	8	270
228	F	2	1	0	47	43	49	44	36	6	8	0	0	33	266
229	F	4	18	0	101	34	28	24	20	6	0	0	0	0	213
230	F	4	12	0	91	50	11	36	0	0	0	0	0	0	188
			Mean	0.0	77.1	41.3	35.1	30.2	15.2	13.2	12.7	5.9	0.4	4.1	235.2
			SD	0.0	17.9	9.6	11.9	8.8	11.9	10.9	13.1	11.8	1.3	10.5	28.7
			N	10	10	10	10	10	10	10	10	10	10	10	10

2000 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
231	F	1	7	0	62	45	28	8	8	31	13	9	0	0	204
232	F	1	4	0	50	52	29	56	14	0	15	32	6	0	254
233	F	1	19	0	73	56	32	17	6	11	23	23	18	34	293
234	F	3	15	0	75	33	21	11	29	3	0	0	0	0	172
235	F	3	4	0	64	48	22	16	0	0	0	0	0	0	150
236	F	2	2	0	42	66	24	46	32	30	30	26	38	21	355
237	F	2	13	0	74	64	51	56	54	21	17	10	0	0	347
238	F	4	11	0	68	37	35	16	7	0	0	0	0	0	163
239	F	4	6	0	28	26	32	6	26	10	14	0	27	11	180
240	F	4	5	0	58	51	32	40	33	26	0	11	5	0	256
			Mean	0.0	59.4	47.8	30.6	27.2	20.9	13.2	11.2	11.1	9.4	6.6	237.4
			SD	0.0	15.4	12.9	8.6	20.0	16.7	12.8	10.8	12.0	13.6	11.9	75.6
			N	10	10	10	10	10	10	10	10	10	10	10	10

Table 5. Motor Activity (Total Movement) in Male Rats

0 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
101	M	1	9	0	121	74	62	40	14	47	23	7	2	0	390
102	M	1	20	0	148	157	57	42	53	0	0	0	0	51	508
103	M	1	16	0	122	99	101	53	36	1	47	27	0	1	487
104	M	3	13	0	147	108	121	95	44	40	56	14	2	5	632
105	M	3	3	0	145	125	80	56	71	46	81	16	1	4	625
106	M	2	11	0	137	123	116	84	48	12	37	93	34	7	691
107	M	2	10	0	109	84	78	94	51	39	23	27	3	30	538
108	M	4	1	0	102	57	42	40	61	112	74	29	9	0	526
109	M	4	14	0	164	114	90	94	41	92	4	0	0	0	599
110	M	4	9	0	174	132	93	78	79	27	0	12	0	3	598
			Mean	0.0	136.9	107.3	84.0	67.6	49.8	41.6	34.5	22.5	5.1	10.1	559.4
			SD	0.0	23.3	29.7	25.4	23.7	18.3	36.5	29.7	26.9	10.5	16.9	87.0
			N	10	10	10	10	10	10	10	10	10	10	10	10

200 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
111	M	1	8	0	100	138	97	63	54	62	32	3	0	2	551
112	M	1	2	0	113	75	40	32	10	0	0	0	0	31	301
113	M	3	11	0	121	106	94	16	40	2	30	5	0	0	414
114	M	3	6	0	97	61	77	64	36	37	35	31	3	13	454
115	M	3	14	0	121	86	92	48	34	55	66	43	14	4	563
116	M	2	14	0	125	79	46	23	53	57	41	84	35	40	583
117	M	2	20	0	105	57	68	14	14	21	5	2	0	3	289
118	M	2	3	0	111	101	52	69	30	40	67	9	0	0	479
119	M	4	3	0	141	123	109	64	60	28	92	28	26	44	715
120	M	4	4	0	109	82	100	74	15	3	0	1	1	6	391
			Mean	0.0	114.3	90.8	77.5	46.7	34.6	30.5	36.8	20.6	7.9	14.3	474.0
			SD	0.0	13.1	26.1	24.7	23.3	17.7	23.6	31.0	26.9	12.8	17.3	132.9
			N	10	10	10	10	10	10	10	10	10	10	10	10

Table 5. Motor Activity (Total Movement) in Male Rats (continued)

700 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
121	M	1	13	0	103	99	69	93	36	28	35	0	1	0	464
122	M	1	18	0	128	64	32	25	46	3	17	0	0	0	315
123	M	1	3	0	130	112	61	65	11	80	52	59	17	35	622
124	M	3	18	0	117	85	63	20	38	2	13	0	1	10	349
125	M	3	10	0	133	109	96	27	60	77	46	0	11	18	577
126	M	2	12	0	152	100	98	40	36	7	0	0	0	61	494
127	M	2	15	0	144	101	110	52	74	49	25	78	8	43	684
128	M	2	15	0	136	83	84	46	47	27	1	2	0	0	426
129	M	4	2	0	130	82	88	104	21	17	0	0	22	46	510
130	M	4	20	0	156	147	68	82	65	68	18	13	4	0	621
			Mean	0.0	132.9	98.2	76.9	55.4	43.4	35.8	20.7	15.2	6.4	21.3	506.2
			SD	0.0	15.7	22.5	22.8	29.6	19.4	30.6	18.8	28.7	7.9	23.1	121.5
			N	10	10	10	10	10	10	10	10	10	10	10	10

2000 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
131	M	1	6	0	108	75	21	22	2	3	6	0	0	0	237
132	M	1	11	0	97	114	33	47	0	0	0	0	9	64	364
133	M	3	20	0	65	82	71	60	30	73	14	0	0	0	395
134	M	3	17	0	111	124	82	30	6	0	0	0	0	0	353
135	M	3	1	0	87	78	86	44	33	11	2	0	0	0	341
136	M	2	5	0	103	47	0	54	48	63	19	18	3	0	355
137	M	2	6	0	99	63	46	63	38	7	9	0	0	0	325
138	M	2	16	0	138	60	78	17	29	3	0	0	5	1	331
139	M	4	10	0	85	58	52	36	4	55	0	15	0	0	305
140	M	4	17	0	138	77	58	58	7	28	0	18	4	0	388
			Mean	0.0	103.1	77.8	52.7	43.1	19.7	24.3	5.0	5.1	2.1	6.5	339.4
			SD	0.0	22.6	24.4	28.3	16.3	17.6	28.7	6.9	8.3	3.1	20.2	45.3
			N	10	10	10	10	10	10	10	10	10	10	10	10

Table 6. Motor Activity (Total Movement) in Female Rats

0 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
201	F	1	17	0	144	75	53	88	48	15	5	4	3	12	447
202	F	1	5	0	169	42	56	76	11	11	0	0	16	6	387
203	F	3	2	0	167	79	88	30	51	47	28	31	30	0	551
204	F	3	12	0	140	128	120	85	61	16	21	0	0	0	571
205	F	3	7	0	119	115	66	73	95	56	2	50	48	18	642
206	F	2	18	0	134	68	70	96	93	37	38	14	23	30	603
207	F	2	4	0	170	107	72	90	64	51	12	99	14	18	697
208	F	2	8	0	166	67	76	68	68	54	80	53	98	74	804
209	F	4	7	0	126	89	61	28	59	32	0	0	0	0	395
210	F	4	19	0	162	123	111	70	56	46	58	80	26	4	736
			Mean	0.0	149.7	89.3	77.3	70.4	60.6	36.5	24.4	33.1	25.8	16.2	583.3
			SD	0.0	19.4	28.1	22.6	23.7	23.7	17.2	27.2	36.1	29.5	22.6	142.4
			N	10	10	10	10	10	10	10	10	10	10	10	10

200 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
211	F	1	15	0	128	69	45	51	65	7	0	3	8	0	376
212	F	1	12	0	147	56	37	75	22	1	18	4	12	67	439
213	F	1	14	0	134	96	73	24	61	47	0	21	52	32	540
214	F	3	16	0	134	101	44	75	69	53	65	19	8	2	570
215	F	3	5	0	93	91	20	59	42	49	4	1	3	2	364
216	F	2	17	0	108	92	83	33	30	8	18	6	0	0	378
217	F	2	9	0	139	62	77	64	23	12	48	26	0	1	452
218	F	4	16	0	126	47	54	38	1	0	1	0	4	0	271
219	F	4	8	0	150	90	78	49	26	55	47	24	30	10	559
220	F	4	13	0	164	100	89	50	41	14	47	13	0	53	571
			Mean	0.0	132.3	80.4	60.0	51.8	38.0	24.6	24.8	11.7	11.7	16.7	452.0
			SD	0.0	20.5	19.9	23.1	17.0	21.9	23.2	24.6	10.1	16.8	25.0	105.0
			N	10	10	10	10	10	10	10	10	10	10	10	10

Table 6. Motor Activity (Total Movement) in Female Rats (continued)

700 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
221	F	1	10	0	163	68	80	59	21	76	1	3	1	0	472
222	F	1	1	0	92	101	112	50	46	0	44	60	5	0	510
223	F	3	19	0	120	79	61	78	42	41	45	3	0	13	482
224	F	3	9	0	147	77	92	61	17	31	14	2	2	0	443
225	F	3	8	0	131	74	54	34	11	18	12	52	0	2	388
226	F	2	19	0	146	98	84	57	23	42	55	7	1	0	513
227	F	2	7	0	140	101	63	69	69	68	65	4	1	11	591
228	F	2	1	0	89	91	83	86	80	21	20	0	0	68	538
229	F	4	18	0	189	60	76	55	51	14	1	1	0	0	447
230	F	4	12	0	152	97	28	76	6	0	0	0	0	59	418
			Mean	0.0	136.9	84.6	73.3	62.5	36.6	31.1	25.7	13.2	1.0	15.3	480.2
			SD	0.0	30.6	14.9	23.1	15.2	25.1	26.0	24.4	22.7	1.6	25.9	60.1
			N	10	10	10	10	10	10	10	10	10	10	10	10

2000 mg/m^3 jet fuel (inhalation)

				Time Period (min)										
Rat #	Sex	Session	Cage #	0	6	12	18	24	30	36	42	48	54	60	Total
231	F	1	7	0	99	92	57	11	11	56	21	32	0	0	379
232	F	1	4	0	94	102	56	140	51	8	39	71	16	2	579
233	F	1	19	0	131	86	69	34	8	21	37	63	45	58	552
234	F	3	15	0	117	66	53	24	44	12	0	0	1	0	317
235	F	3	4	0	114	79	48	28	0	1	8	37	2	4	321
236	F	2	2	0	79	122	52	86	79	54	49	70	93	45	729
237	F	2	13	0	115	133	123	123	105	59	40	15	0	0	713
238	F	4	11	0	135	73	61	31	17	6	4	1	9	2	339
239	F	4	6	0	54	49	55	12	75	18	25	9	39	33	369
240	F	4	5	0	94	88	54	67	57	50	0	14	8	0	432
			Mean	0.0	103.2	89.0	62.8	55.6	44.7	28.5	22.3	31.2	21.3	14.4	473.0
			SD	0.0	24.5	25.2	21.9	46.5	35.2	23.4	18.5	28.0	29.9	22.2	158.9
			N	10	10	10	10	10	10	10	10	10	10	10	10

Table 7. Summary Quantitative FOB Data

Males – Mean (Standard Deviation)

	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
N	10	10	10	10
Body Weight (g)	317.3 (15.7)	328.7 (19.7)	308.6 (25.5)	**279.2 (16.2) ***
Splay (cm)	7.6 (2.4)	7.5 (1.8)	6.8 (1.2)	7.3 (1.2)
Hindlimb Grip Strength (kg)	0.290 (0.065)	0.344 (0.114)	0.271 (0.075)	0.250 (0.069)
Forelimb Grip Strength (kg)	0.663 (0.160)	0.706 (0.138)	0.817 (0.788)	0.605 (0.119)
# Rears	11.4 (4.1)	11.2 (2.7)	11.0 (4.2)	8.6 (2.9)
# Urine Pools	0.5 (1.1)	0.2 (0.4)	0.1 (0.3)	0.0 (0.0)
# Fecal Boluses	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Females – Mean (Standard Deviation)

	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
N	10	10	10	10
Body Weight (g)	182.3 (11.4)	179.6 (8.5)	184.9 (6.2)	**171.7 (6.1) ***
Splay (cm)	5.5 (1.3)	5.6 (1.2)	6.0 (1.3)	6.6 (1.1)
Hindlimb Grip Strength (kg)	0.222 (0.072)	0.200 (0.057)	0.217 (0.058)	0.191 (0.048)
Forelimb Grip Strength (kg)	0.512 (0.104)	0.512 (0.108)	0.528 (0.065)	0.515 (0.065)
# Rears	16.6 (5.3)	16.2 (4.3)	15.5 (5.4)	**10.2 (4.2) ***
# Urine Pools	0.0 (0.0)	0.0 (0.0)	0.1 (0.3)	0.1 (0.3)
# Fecal Boluses	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

*Significantly different from control (ANOVA and Dunnett's, $p<0.05$)

Table 8. FOB Ordinal Scoring Key[a]

I. Cageside Observations

Posture	1. Normal/awake 2. Normal/asleep 3. On side/prostrate 4. On Stomach/prostrate 5. Other
Tremor	0. None 1. Present 2. Other
Spasm	0. None 1. Present 2. Other
Tremor/Spasm Severity	0. None 1. Present 2. Excessive 3. Other
Tremor/Spasm Induction	0. None 1. Action 2. Resting 3. Action and resting 4. Other
Tremor/Spasm Location	0. None 1. Head 2. Forelimbs 3. Hindlimbs 4. Whole body 5. Other
Seizures	0. None 1. Clonic convulsion 2. Tonic convulsion
Clonic Convulsions	0. None 1. Running fits 2. Writhing 3. Explosive jumps 4. Paddling 5. Other
Seizure Severity	0. None 1. Present 2. Excessive 3. Other
Palpebral Closure	1. Wide open 2. Partially closed 3. Completely shut 4. Other

[a] Multiple findings are scored as "Other" and noted on the Additional FOB Comments and Observations tables.

II. Removal From Cage

Handling Reactivity	1. Normal (slight/moderate) 2. Animal limp 3. High resistance/aggressive 4. Other
Muscle Tone	1. Normal 2. Increased 3. Decreased
Piloerection	0. None 1. Present
Lacrimation	0. None 1. Present 2. Excessive
Salivation	0. None 1. Present 2. Excessive
Fur Appearance	1. Normal 2. Rough 3. Urine stains/wet 4. Other
Facial Crust	0. None 1. One eye 2. Both eyes 3. Nose 4. Mouth 5. Other (multiple locations)
Skin Temperature/Color	1. Normal 2. Pale 3. Cold 4. Warm 5. Flushed 6. Other
Breathing Patterns	1. Normal 2. Mouth breathing/labored 3. Audible 4. Other
Additional Observations	0. None 1. Sneezing 2. Dehydration 3. Emaciation 4. Exopthalmus 5. Tremors 6. Other

Table 8. FOB Ordinal Scoring Key (continued)

III. Open Field Observations

Arousal	1. Alert 2. Not alert 3. Hyperalert
Activity	1. Active 2. Inactive 3. Hyperactive
Ataxia	0. None 1. Present 2. Excessive 3. Other
Gait	1. Normal 2. Hypotonic (present) 3. Hypotonic (excessive) 4. Toe (present) 5. Toe (excessive) 6. Splayed (present) 7. Splayed (excessive) 8. Other
Body Position	1. Normal 2. Hunched 3. On stomach 4. On side 5. Other
Excessive Vocalization	0. None 1. Present 2. Other
Unusual Behaviors	0. None 1. Sneezing 2. Retropulsion 3. Head bobbing/weaving 4. Stereotypy 5. Other
Tremors	0. None 1. Present 2. Other
Spasms	0. None 1. Present 2. Other
Tremor/Spasm Severity	0. None 1. Present 2. Excessive 3. Other
Tremor/ Spasm Induction	0. None 1. Action 2. Resting 3. Action and resting 4. Other

III. Open Field Observations (continued)

Tremor/Spasm Location	0. None 1. Head 2. Forelimbs 3. Hindlimbs 4. Whole body 5. Other
Seizures	0. None 1. Clonic convulsions 2. Tonic convulsions
Clonic Convulsions	0. None 1. Paddling 2. Explosive jumps 3. Writhing 4. Running fits 5. Other
Seizure Severity	0. None 1. Present 2. Excessive 3. Other
Fecal Quality	0. None 1. Normal 2. Soft 3. Diarrhea

IV. Manipulative Procedures

Approach Response	1. Noticeable 2. No reaction 3. Exaggerated
Acoustic Response	1. Noticeable 2. No reaction 3. Exaggerated
Tail Pinch Response	1. Noticeable 2. No reaction 3. Exaggerated
Visual Placing	1. Present 2. None
Surface Righting	1. Normal 2. Abnormal (slow) 3. Abnormal (incomplete) 4. Absent
Pupil Reflex	1. Normal 2. Abnormal 3. Other

Table 9. Cage-Side Observations in Male Rats

Date	Rat #	Posture	Tremors	Spasm	Tremor severity	Tremor induction	Tremor location	Seizures	Clonic Convulsions	Seizure severity	Palpebral closure
0 mg/m^3											
07/25/2008	101	1	0	0	0	0	0	0	0	0	1
07/25/2008	102	1	0	0	0	0	0	0	0	0	1
07/25/2008	103	1	0	0	0	0	0	0	0	0	1
07/25/2008	104	1	0	0	0	0	0	0	0	0	1
07/25/2008	105	1	0	0	0	0	0	0	0	0	1
07/26/2008	106	1	0	0	0	0	0	0	0	0	1
07/26/2008	107	1	0	0	0	0	0	0	0	0	1
07/26/2008	108	1	0	0	0	0	0	0	0	0	1
07/26/2008	109	1	0	0	0	0	0	0	0	0	1
07/26/2008	110	1	0	0	0	0	0	0	0	0	1
200 mg/m^3											
07/25/2008	111	1	0	0	0	0	0	0	0	0	1
07/25/2008	112	1	0	0	0	0	0	0	0	0	1
07/25/2008	113	1	0	0	0	0	0	0	0	0	1
07/25/2008	114	1	0	0	0	0	0	0	0	0	1
07/25/2008	115	1	0	0	0	0	0	0	0	0	1
07/26/2008	116	1	0	0	0	0	0	0	0	0	1
07/26/2008	117	1	0	0	0	0	0	0	0	0	1
07/26/2008	118	1	0	0	0	0	0	0	0	0	1
07/26/2008	119	1	0	0	0	0	0	0	0	0	1
07/26/2008	120	1	0	0	0	0	0	0	0	0	1

Date	Rat #	Posture	Tremors	Spasm	Tremor severity	Tremor induction	Tremor location	Seizures	Clonic Convulsions	Seizure severity	Palpebral closure
700 mg/m^3											
07/25/2008	121	1	0	0	0	0	0	0	0	0	1
07/25/2008	122	1	0	0	0	0	0	0	0	0	1
07/25/2008	123	1	0	0	0	0	0	0	0	0	1
07/25/2008	124	1	0	0	0	0	0	0	0	0	1
07/25/2008	125	1	0	0	0	0	0	0	0	0	1
07/26/2008	126	1	0	0	0	0	0	0	0	0	1
07/26/2008	127	1	0	0	0	0	0	0	0	0	1
07/26/2008	128	1	0	0	0	0	0	0	0	0	1
07/26/2008	129	1	0	0	0	0	0	0	0	0	1
07/26/2008	130	1	0	0	0	0	0	0	0	0	1
2000 mg/m^3											
07/25/2008	131	1	0	0	0	0	0	0	0	0	1
07/25/2008	132	1	0	0	0	0	0	0	0	0	1
07/25/2008	133	1	0	0	0	0	0	0	0	0	1
07/25/2008	134	1	0	0	0	0	0	0	0	0	1
07/25/2008	135	1	0	0	0	0	0	0	0	0	1
07/26/2008	136	1	0	0	0	0	0	0	0	0	1
07/26/2008	137	1	0	0	0	0	0	0	0	0	1
07/26/2008	138	1	0	0	0	0	0	0	0	0	1
07/26/2008	139	1	0	0	0	0	0	0	0	0	1
07/26/2008	140	1	0	0	0	0	0	0	0	0	1

Table 10. Cage-Side Observations in Female Rats

Date	Rat #	Posture	Tremors	Spasm	Tremor severity	Tremor induction	Tremor location	Seizures	Clonic Convulsions	Seizure severity	Palpebral closure
0 mg/m^3											
07/25/2008	201	1	0	0	0	0	0	0	0	0	1
07/25/2008	202	1	0	0	0	0	0	0	0	0	1
07/25/2008	203	1	0	0	0	0	0	0	0	0	1
07/25/2008	204	1	0	0	0	0	0	0	0	0	1
07/25/2008	205	1	0	0	0	0	0	0	0	0	1
07/26/2008	206	1	0	0	0	0	0	0	0	0	1
07/26/2008	207	1	0	0	0	0	0	0	0	0	1
07/26/2008	208	1	0	0	0	0	0	0	0	0	1
07/26/2008	209	1	0	0	0	0	0	0	0	0	1
07/26/2008	210	1	0	0	0	0	0	0	0	0	1
200 mg/m^3											
07/25/2008	211	1	0	0	0	0	0	0	0	0	1
07/25/2008	212	1	0	0	0	0	0	0	0	0	1
07/25/2008	213	1	0	0	0	0	0	0	0	0	1
07/25/2008	214	1	0	0	0	0	0	0	0	0	1
07/25/2008	215	1	0	0	0	0	0	0	0	0	1
07/26/2008	216	1	0	0	0	0	0	0	0	0	1
07/26/2008	217	1	0	0	0	0	0	0	0	0	1
07/26/2008	218	1	0	0	0	0	0	0	0	0	1
07/26/2008	219	1	0	0	0	0	0	0	0	0	1
07/26/2008	220	1	0	0	0	0	0	0	0	0	1

Date	Rat #	Posture	Tremors	Spasm	Tremor severity	Tremor induction	Tremor location	Seizures	Clonic Convulsions	Seizure severity	Palpebral closure
700 mg/m^3											
07/25/2008	221	1	0	0	0	0	0	0	0	0	1
07/25/2008	222	1	0	0	0	0	0	0	0	0	1
07/25/2008	223	1	0	0	0	0	0	0	0	0	1
07/25/2008	224	1	0	0	0	0	0	0	0	0	1
07/25/2008	225	1	0	0	0	0	0	0	0	0	1
07/26/2008	226	1	0	0	0	0	0	0	0	0	1
07/26/2008	227	1	0	0	0	0	0	0	0	0	1
07/26/2008	228	1	0	0	0	0	0	0	0	0	1
07/26/2008	229	1	0	0	0	0	0	0	0	0	1
07/26/2008	230	1	0	0	0	0	0	0	0	0	1
2000 mg/m^3											
07/25/2008	231	1	0	0	0	0	0	0	0	0	1
07/25/2008	232	1	0	0	0	0	0	0	0	0	1
07/25/2008	233	1	0	0	0	0	0	0	0	0	1
07/25/2008	234	1	0	0	0	0	0	0	0	0	1
07/25/2008	235	1	0	0	0	0	0	0	0	0	1
07/26/2008	236	1	0	0	0	0	0	0	0	0	1
07/26/2008	237	1	0	0	0	0	0	0	0	0	1
07/26/2008	238	1	0	0	0	0	0	0	0	0	1
07/26/2008	239	1	0	0	0	0	0	0	0	0	1
07/26/2008	240	1	0	0	0	0	0	0	0	0	1

Table 11. Observations during Removal from Cage in Male Rats

Date	Rat #	Handling Reactivity	Piloerection	Muscle tone	Lacrimation	Salivation	Fur appearance	Facial crust	Skin color	Skin temperature	Breathing pattern
0 mg/m^3											
07/25/2008	101	1	0	1	0	0	4	1	1	3	1
07/25/2008	102	1	0	1	0	0	4	3	1	1	1
07/25/2008	103	1	0	1	0	0	4	3	1	1	1
07/25/2008	104	1	0	1	0	0	1	0	1	1	1
07/25/2008	105	1	0	1	0	0	4	3	5	1	1
07/26/2008	106	1	0	1	0	0	4	0	1	1	1
07/26/2008	107	1	0	1	0	0	4	3	1	3	1
07/26/2008	108	1	0	1	0	0	4	0	1	1	1
07/26/2008	109	2	0	1	0	0	4	3	1	1	1
07/26/2008	110	1	0	1	0	0	4	3	1	1	1
200 mg/m^3											
07/25/2008	111	1	0	1	0	0	4	3	1	3	1
07/25/2008	112	2	0	1	0	0	4	3	1	1	1
07/25/2008	113	2	0	1	0	0	4	3	1	1	1
07/25/2008	114	2	0	1	0	0	4	0	1	1	1
07/25/2008	115	1	0	1	0	0	2	0	1	1	1
07/26/2008	116	2	0	1	0	0	4	3	1	1	1
07/26/2008	117	1	0	1	0	0	4	3	1	3	1
07/26/2008	118	1	0	1	0	0	1	0	1	1	1
07/26/2008	119	2	0	1	0	0	4	0	1	1	1
07/26/2008	120	1	0	1	0	0	4	0	1	1	1

Date	Rat #	Handling Reactivity	Piloerection	Muscle tone	Lacrimation	Salivation	Fur appearance	Facial crust	Skin color	Skin temperature	Breathing pattern
700 mg/m^3											
07/25/2008	121	1	0	1	0	0	4	0	1	3	1
07/25/2008	122	1	0	1	0	0	1	0	1	1	1
07/25/2008	123	1	0	1	0	0	4	0	1	1	1
07/25/2008	124	1	0	1	0	0	4	3	1	1	1
07/25/2008	125	1	0	1	0	0	1	3	1	1	1
07/26/2008	126	2	0	1	0	0	1	0	1	1	1
07/26/2008	127	2	0	1	0	0	4	0	1	1	1
07/26/2008	128	1	0	1	0	0	4	3	1	1	1
07/26/2008	129	1	0	1	0	0	4	0	1	3	1
07/26/2008	130	1	0	1	0	0	4	3	1	1	1
2000 mg/m^3											
07/25/2008	131	1	0	1	0	0	1	0	1	1	1
07/25/2008	132	1	0	1	0	0	1	0	1	1	1
07/25/2008	133	1	0	1	0	0	1	0	1	1	1
07/25/2008	134	1	0	1	0	0	1	0	1	1	1
07/25/2008	135	1	0	1	0	0	2	3	1	1	1
07/26/2008	136	1	0	1	0	0	1	5	1	1	1
07/26/2008	137	1	0	1	0	0	1	0	1	3	1
07/26/2008	138	1	0	1	0	0	1	3	1	1	1
07/26/2008	139	1	0	1	0	0	1	3	1	1	1
07/26/2008	140	2	0	1	0	0	1	3	1	1	1

Table 12. Observations during Removal from Cage in Female Rats

Date	Rat #	Handling Reactivity	Piloerection	Muscle tone	Lacrimation	Salivation	Fur appearance	Facial crust	Skin color	Skin temperature	Breathing pattern
0 mg/m^3											
07/25/2008	201	1	0	1	0	0	3	5	1	1	1
07/25/2008	202	1	0	1	0	0	1	0	1	1	1
07/25/2008	203	1	0	1	0	0	3	0	1	1	1
07/25/2008	204	1	0	1	0	0	3	5	1	1	1
07/25/2008	205	1	0	1	0	0	4	5	1	3	1
07/26/2008	206	1	0	1	0	0	3	5	1	3	1
07/26/2008	207	1	0	1	0	0	1	0	1	1	1
07/26/2008	208	1	0	1	0	0	1	1	1	3	1
07/26/2008	209	1	0	1	0	0	1	0	1	3	1
07/26/2008	210	1	0	1	0	0	1	0	1	3	1
200 mg/m^3											
07/25/2008	211	1	0	1	0	0	3	0	1	1	1
07/25/2008	212	1	0	1	0	0	3	3	1	1	1
07/25/2008	213	1	0	1	0	0	3	5	1	1	1
07/25/2008	214	1	0	1	0	0	2	5	1	3	1
07/25/2008	215	1	0	1	0	0	1	0	1	3	1
07/26/2008	216	1	0	1	0	0	3	1	1	3	1
07/26/2008	217	1	0	1	0	0	3	5	1	1	1
07/26/2008	218	1	0	1	0	0	3	0	1	1	1
07/26/2008	219	1	0	1	0	0	1	0	1	3	1
07/26/2008	220	1	0	1	1	0	3	0	1	1	1

Date	Rat #	Handling Reactivity	Piloerection	Muscle tone	Lacrimation	Salivation	Fur appearance	Facial crust	Skin color	Skin temperature	Breathing pattern
700 mg/m^3											
07/25/2008	221	1	0	1	0	0	1	0	1	3	1
07/25/2008	222	1	0	1	0	0	3	0	1	1	1
07/25/2008	223	1	0	1	0	0	3	0	1	3	1
07/25/2008	224	1	0	1	0	0	3	0	1	1	1
07/25/2008	225	1	0	1	0	0	3	3	1	1	1
07/26/2008	226	1	0	1	0	0	3	0	1	3	1
07/26/2008	227	1	0	1	0	0	3	5	1	3	1
07/26/2008	228	1	0	1	0	0	3	2	1	3	1
07/26/2008	229	1	0	1	0	0	3	3	1	3	1
07/26/2008	230	1	0	1	0	0	3	1	1	1	1
2000 mg/m^3											
07/25/2008	231	1	0	1	0	0	1	5	1	1	1
07/25/2008	232	1	0	1	0	0	3	0	1	1	1
07/25/2008	233	1	0	1	0	0	3	3	1	3	1
07/25/2008	234	1	0	1	0	0	3	0	1	1	1
07/25/2008	235	1	0	1	0	0	3	0	1	1	1
07/26/2008	236	1	0	1	0	0	3	3	1	3	1
07/26/2008	237	1	0	1	0	0	3	0	1	1	1
07/26/2008	238	1	0	1	0	0	3	0	1	3	1
07/26/2008	239	1	0	1	0	0	3	0	1	3	1
07/26/2008	240	1	0	1	0	0	3	0	1	1	1

Table 13. Open Field FOB Data in Male Rats

Date	Rat #	Arousal	Activity	Ataxia	Gait	Body position	Vocal†	Unusual behaviors	Tremors	Spasms	Tremor severity	Tremor induction	Tremor location	Seizures	Clonic convulsions	Seizure severity
0 mg/m³																
07/25/2008	101	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	102	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	103	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	104	1	1	0	1	1	0	4	0	0	0	0	0	0	0	0
07/25/2008	105	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	106	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	107	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	108	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	109	1	1	0	6	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	110	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
200 mg/m³																
07/25/2008	111	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	112	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	113	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	114	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	115	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	116	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	117	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	118	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	119	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	120	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0

† Vocalization

Date	Rat #	Arousal	Activity	Ataxia	Gait	Body position	Vocal†	Unusual behaviors	Tremors	Spasms	Tremor severity	Tremor induction	Tremor location	Seizures	Clonic convulsions	Seizure severity
700 mg/m³																
07/25/2008	121	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	122	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	123	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	124	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	125	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	126	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	127	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	128	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	129	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	130	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
2000 mg/m³																
07/25/2008	131	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	132	1	1	0	1	1	0	4	0	0	0	0	0	0	0	0
07/25/2008	133	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	134	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	135	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	136	1	1	0	1	1	0	4	0	0	0	0	0	0	0	0
07/26/2008	137	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	138	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	139	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	140	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0

† Vocalization

Table 14. Open Field FOB Data in Female Rats

Date	Rat #	Arousal	Activity	Ataxia	Gait	Body position	Vocal†	Unusual behaviors	Tremors	Spasms	Tremor severity	Tremor induction	Tremor location	Seizures	Clonic convulsions	Seizure severity
0 mg/m³																
07/25/2008	201	1	1	0	4	2	0	0	0	0	0	0	0	0	0	0
07/25/2008	202	1	1	0	4	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	203	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	204	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	205	3	3	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	206	1	1	0	4	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	207	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	208	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	209	1	3	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	210	1	1	0	4	1	0	0	0	0	0	0	0	0	0	0
200 mg/m³																
07/25/2008	211	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	212	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	213	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	214	1	3	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	215	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	216	1	1	0	6	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	217	1	3	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	218	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	219	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	220	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0

† **Vocalization**

Date	Rat #	Arousal	Activity	Ataxia	Gait	Body position	Vocal†	Unusual behaviors	Tremors	Spasms	Tremor severity	Tremor induction	Tremor location	Seizures	Clonic convulsions	Seizure severity
700 mg/m³																
07/25/2008	221	3	3	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	222	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	223	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	224	1	3	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	225	1	1	0	1	1	0	4	0	0	0	0	0	0	0	0
07/26/2008	226	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	227	1	3	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	228	1	1	0	4	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	229	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	230	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
2000 mg/m³																
07/25/2008	231	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	232	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	233	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	234	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/25/2008	235	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	236	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	237	1	1	0	6	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	238	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	239	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
07/26/2008	240	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0

† **Vocalization**

Table 15. Manipulative FOB Data in Male Rats

Date	Rat #	Approach response	Acoustic response	Tail pinch response	Visual placing	Surface righting	Pupil Reflex
0 mg/m^3							
07/25/2008	101	1	1	1	1	2	1
07/25/2008	102	1	1	1	1	1	1
07/25/2008	103	1	1	1	1	2	1
07/25/2008	104	1	1	1	1	2	1
07/25/2008	105	1	1	1	1	2	1
07/26/2008	106	1	1	1	1	1	1
07/26/2008	107	1	1	1	1	1	1
07/26/2008	108	1	1	1	1	1	1
07/26/2008	109	1	1	1	1	2	1
07/26/2008	110	1	1	1	1	2	1
200 mg/m^3							
07/25/2008	111	1	1	1	1	2	1
07/25/2008	112	1	1	1	1	2	1
07/25/2008	113	1	1	1	1	1	1
07/25/2008	114	1	1	1	1	3	1
07/25/2008	115	1	1	1	1	2	1
07/26/2008	116	1	1	3	1	1	1
07/26/2008	117	1	1	1	1	2	1
07/26/2008	118	1	1	1	1	1	1
07/26/2008	119	1	1	1	1	1	1
07/26/2008	120	1	1	1	1	2	1

Date	Rat #	Approach response	Acoustic response	Tail pinch response	Visual placing	Surface righting	Pupil Reflex
700 mg/m^3							
07/25/2008	121	1	1	1	1	1	1
07/25/2008	122	1	1	1	1	2	1
07/25/2008	123	1	1	1	1	2	1
07/25/2008	124	1	1	1	1	2	1
07/25/2008	125	1	1	1	1	2	1
07/26/2008	126	1	1	1	1	1	1
07/26/2008	127	1	1	1	1	2	1
07/26/2008	128	1	1	1	1	2	1
07/26/2008	129	1	1	1	1	1	1
07/26/2008	130	1	1	1	1	2	1
2000 mg/m^3							
07/25/2008	131	1	1	1	1	1	1
07/25/2008	132	1	1	1	1	2	1
07/25/2008	133	1	1	1	1	1	1
07/25/2008	134	1	1	1	1	2	1
07/25/2008	135	1	1	1	1	1	1
07/26/2008	136	1	1	1	1	1	1
07/26/2008	137	1	3	1	1	2	1
07/26/2008	138	1	1	1	1	2	1
07/26/2008	139	1	1	1	1	1	1
07/26/2008	140	1	1	1	1	2	1

Table 16. Manipulative FOB Data in Female Rats

Date	Rat #	Approach response	Acoustic response	Tail pinch response	Visual placing	Surface righting	Pupil Reflex
0 mg/m^3							
07/25/2008	201	1	1	1	1	1	1
07/25/2008	202	3	1	1	1	1	1
07/25/2008	203	1	1	1	1	1	1
07/25/2008	204	1	1	1	1	1	1
07/25/2008	205	1	3	3	1	1	1
07/26/2008	206	1	1	1	1	1	1
07/26/2008	207	1	3	1	1	2	1
07/26/2008	208	1	1	1	1	1	1
07/26/2008	209	3	1	1	1	1	1
07/26/2008	210	1	3	3	1	2	1
200 mg/m^3							
07/25/2008	211	1	1	1	1	2	1
07/25/2008	212	1	1	1	1	1	1
07/25/2008	213	1	1	1	1	2	1
07/25/2008	214	1	1	1	1	3	1
07/25/2008	215	1	1	1	1	3	1
07/26/2008	216	3	1	1	1	1	1
07/26/2008	217	1	1	1	1	1	1
07/26/2008	218	1	1	1	1	3	1
07/26/2008	219	1	1	3	1	1	1
07/26/2008	220	1	3	1	1	1	1

Date	Rat #	Approach response	Acoustic response	Tail pinch response	Visual placing	Surface righting	Pupil Reflex
700 mg/m^3							
07/25/2008	221	1	3	1	1	1	1
07/25/2008	222	1	1	1	1	1	1
07/25/2008	223	1	1	1	1	1	1
07/25/2008	224	1	1	1	1	1	1
07/25/2008	225	1	1	1	1	1	1
07/26/2008	226	3	1	1	1	1	1
07/26/2008	227	1	1	1	1	1	1
07/26/2008	228	1	1	1	1	1	1
07/26/2008	229	1	1	1	1	1	1
07/26/2008	230	1	1	1	1	2	1
2000 mg/m^3							
07/25/2008	231	1	1	1	1	1	1
07/25/2008	232	1	1	1	1	1	1
07/25/2008	233	1	1	1	1	2	1
07/25/2008	234	1	1	1	1	1	1
07/25/2008	235	1	3	1	1	1	1
07/26/2008	236	1	1	1	1	2	1
07/26/2008	237	1	1	1	1	3	1
07/26/2008	238	1	1	1	1	2	1
07/26/2008	239	1	1	1	1	1	1
07/26/2008	240	1	1	1	1	2	1

Table 17. Quantitative FOB Data in Male Rats

Date	Rat #	Body weight (g)	Hindlimb Splay (cm)	Hindlimb Splay (cm)	Hindlimb Grip strength (kg)	Hindlimb Grip strength (kg)	Forelimb Grip strength (kg)	Forelimb Grip strength (kg)	# Rears	# Urine pools	# Fecal Boluses
0 mg/m^3											
07/25/2008	101	315.8	7.0	6.3	0.270	0.275	0.930	0.765	11	0	0
07/25/2008	102	312.1	6.6	9.5	0.185	0.245	0.485	0.615	9	2	0
07/25/2008	103	312.0	12.3	9.5	0.205	0.230	0.580	0.058	12	0	0
07/25/2008	104	298.0	5.1	3.5	0.175	0.230	0.610	0.585	15	0	0
07/25/2008	105	340.5	11.0	4.0	0.290	0.300	0.610	0.805	7	0	0
07/26/2008	106	323.1	7.9	3.3	0.335	0.245	0.490	0.775	18	0	0
07/26/2008	107	337.1	12.6	10.8	0.340	0.460	0.540	0.790	4	3	0
07/26/2008	108	326.1	8.5	6.3	0.490	0.195	0.890	0.655	14	0	0
07/26/2008	109	318.4	9.8	8.2	0.440	0.250	0.585	0.740	10	0	0
07/26/2008	110	289.9	2.2	7.7	0.260	0.375	0.945	0.815	14	0	0
	Mean	**317.3**	**7.6**		**0.290**		**0.663**		**11.4**	**0.5**	**0.0**
	SD	15.7	2.4		0.065		0.160		4.1	1.1	0.0
	N	10	10		10		10		10	10	10
200 mg/m^3											
07/25/2008	111	315.7	6.3	5.9	0.390	0.425	0.745	0.785	9	0	0
07/25/2008	112	308.4	4.3	7.2	0.255	0.355	0.805	1.090	13	0	0
07/25/2008	113	342.6	8.0	9.6	0.445	0.419	0.475	0.725	10	1	0
07/25/2008	114	374.6	14.0	8.5	0.460	0.590	1.025	0.750	10	0	0
07/25/2008	115	316.8	7.6	6.7	0.300	0.335	0.645	0.355	10	0	0
07/26/2008	116	311.0	10.5	5.2	0.480	0.445	0.605	0.820	10	0	0
07/26/2008	117	327.6	6.5	7.6	0.155	0.190	0.685	0.685	16	0	0
07/26/2008	118	322.6	5.6	8.2	0.195	0.490	0.720	0.750	7	0	0
07/26/2008	119	327.6	5.4	4.5	0.220	0.330	0.615	0.715	14	1	0
07/26/2008	120	339.9	11.0	7.0	0.230	0.165	0.470	0.660	13	0	0
	Mean	**328.7**	**7.5**		**0.344**		**0.706**		**11.2**	**0.2**	**0.0**
	SD	19.7	1.8		0.114		0.138		2.7	0.4	0.0
	N	10	10		10		10		10	10	10

Date	Rat #	Body weight (g)	Hindlimb Splay (cm)	Hindlimb Splay (cm)	Hindlimb Grip strength (kg)	Hindlimb Grip strength (kg)	Forelimb Grip strength (kg)	Forelimb Grip strength (kg)	# Rears	# Urine pools	# Fecal Boluses
700 mg/m^3											
07/25/2008	121	312.0	8.4	4.0	0.245	0.260	0.760	0.790	15	0	0
07/25/2008	122	311.9	10.5	7.0	0.410	0.315	0.720	5.200	11	0	0
07/25/2008	123	319.8	5.9	6.4	0.130	0.350	0.965	0.815	11	0	0
07/25/2008	124	339.6	5.8	6.0	0.340	0.390	0.087	0.078	18	0	0
07/25/2008	125	300.0	3.2	5.5	0.185	0.250	0.575	0.505	8	0	0
07/26/2008	126	298.6	7.5	6.7	0.295	0.340	0.245	0.770	8	0	0
07/26/2008	127	297.9	5.0	10.5	0.195	0.160	0.745	0.860	3	1	0
07/26/2008	128	330.1	7.8	8.2	0.470	0.220	0.470	0.415	12	0	0
07/26/2008	129	327.9	6.1	8.0	0.275	0.280	0.440	0.500	14	0	0
07/26/2008	130	248.6	7.7	5.8	0.130	0.185	0.775	0.615	10	0	0
	Mean	**308.6**	**6.8**		**0.271**		**0.817**		**11.0**	**0.1**	**0.0**
	SD	25.5	1.2		0.075		0.788		4.2	0.3	0.0
	N	10	10		10		10		10	10	10
2000 mg/m^3											
07/25/2008	131	283.0	7.8	6.0	0.295	0.300	0.545	0.595	9	0	0
07/25/2008	132	255.4	8.6	6.2	0.210	0.180	0.540	0.615	9	0	0
07/25/2008	133	278.6	6.2	7.0	0.145	0.140	0.555	0.655	6	0	0
07/25/2008	134	270.7	6.3	6.0	0.165	0.235	0.780	0.735	12	0	0
07/25/2008	135	283.3	12.8	5.5	0.235	0.245	0.900	0.500	14	0	0
07/26/2008	136	269.3	6.3	9.8	0.235	0.360	0.500	0.720	10	0	0
07/26/2008	137	313.9	8.8	6.9	0.280	0.275	0.480	0.880	5	0	0
07/26/2008	138	281.6	6.9	4.0	0.230	0.270	0.325	0.475	9	0	0
07/26/2008	139	264.2	7.6	6.0	0.285	0.140	0.665	0.785	6	0	0
07/26/2008	140	292.0	10.7	7.0	0.295	0.480	0.410	0.445	6	0	0
	Mean	**279.2***	**7.3**		**0.250**		**0.605**		**8.6**	**0.0**	**0.0**
	SD	16.2	1.2		0.069		0.119		2.9	0.0	0.0
	N	10	10		10		10		10	10	10

* $p \leq 0.05$

Overall Mean and SD of hindlimb splay, grip strength and forelimb grip strength calculated from the average of two values for each rat.

Table 18. Quantitative FOB Data in Female Rats

Date	Rat #	Body weight (g)	Hindlimb Splay (cm)	Hindlimb Splay (cm)	Hindlimb Grip strength (kg)	Hindlimb Grip strength (kg)	Forelimb Grip strength (kg)	Forelimb Grip strength (kg)	# Rears	# Urine pools	# Fecal Boluses
0 mg/m^3											
07/25/2008	201	182.1	9.5	5.3	0.400	0.280	0.520	0.805	17	0	0
07/25/2008	202	169.6	6.4	2.5	0.105	0.125	0.555	0.635	19	0	0
07/25/2008	203	177.4	3.2	4.7	0.130	0.170	0.380	0.435	16	0	0
07/25/2008	204	181.9	7.2	5.6	0.275	0.185	0.365	0.635	15	0	0
07/25/2008	205	195.8	4.5	4.2	0.230	0.245	0.240	0.440	27	0	0
07/26/2008	206	182.4	6.1	8.4	0.425	0.226	0.630	0.535	16	0	0
07/26/2008	207	188.5	6.3	4.8	0.300	0.195	0.590	0.480	11	0	0
07/26/2008	208	201.8	4.8	5.7	0.225	0.135	0.440	0.580	15	0	0
07/26/2008	209	180.0	4.3	3.6	0.170	0.180	0.755	0.440	22	0	0
07/26/2008	210	163.0	7.3	4.6	0.235	0.195	0.380	0.405	8	0	0
	Mean	**182.3**	**5.5**		**0.222**		**0.512**		**16.6**	**0.0**	**0.0**
	SD	11.4	1.3		0.072		0.104		5.3	0.0	0.0
	N	10	10		10		10		10	10	10
200 mg/m^3											
07/25/2008	211	179.1	6.1	4.3	0.100	0.230	0.495	0.610	13	0	0
07/25/2008	212	181.0	4.7	5.0	0.140	0.225	0.685	0.390	17	0	0
07/25/2008	213	185.3	3.0	5.6	0.190	0.070	0.575	0.470	16	0	0
07/25/2008	214	165.9	3.8	6.6	0.175	0.210	0.605	0.425	20	0	0
07/25/2008	215	188.5	4.9	5.5	0.295	0.245	0.500	0.700	22	0	0
07/26/2008	216	193.5	4.6	6.0	0.235	0.185	0.245	0.295	16	0	0
07/26/2008	217	177.6	7.0	3.6	0.250	0.165	0.775	0.355	22	0	0
07/26/2008	218	182.4	6.9	6.0	0.140	0.235	0.655	0.675	12	0	0
07/26/2008	219	173.2	6.8	3.7	0.090	0.185	0.580	0.350	15	0	0
07/26/2008	220	169.2	10.0	7.1	0.345	0.285	0.380	0.465	9	0	0
	Mean	**179.6**	**5.6**		**0.200**		**0.512**		**16.2**	**0.0**	**0.0**
	SD	8.5	1.2		0.057		0.108		4.3	0.0	0.0
	N	10	10		10		10		10	10	10

Date	Rat #	Body weight (g)	Hindlimb Splay (cm)	Hindlimb Splay (cm)	Hindlimb Grip strength (kg)	Hindlimb Grip strength (kg)	Forelimb Grip strength (kg)	Forelimb Grip strength (kg)	# Rears	# Urine pools	# Fecal Boluses
700 mg/m^3											
07/25/2008	221	186.6	4.8	6.9	0.205	0.145	0.415	0.465	27	0	0
07/25/2008	222	190.5	5.2	3.0	0.345	0.195	0.570	0.555	11	0	0
07/25/2008	223	190.0	8.4	4.6	0.065	0.175	0.565	0.610	13	0	0
07/25/2008	224	192.0	6.3	5.1	0.210	0.150	0.265	0.755	22	0	0
07/25/2008	225	176.6	7.8	4.0	0.245	0.200	0.535	0.480	13	0	0
07/26/2008	226	183.2	10.6	5.8	0.285	0.300	0.695	0.485	15	0	0
07/26/2008	227	186.2	7.8	5.3	0.155	0.155	0.625	0.330	18	0	0
07/26/2008	228	188.1	7.1	5.3	0.290	0.200	0.715	0.465	13	0	0
07/26/2008	229	172.9	8.0	6.6	0.175	0.270	0.575	0.600	14	1	0
07/26/2008	230	182.6	2.5	5.8	0.240	0.330	0.300	0.550	9	0	0
	Mean	**184.9**	**6.0**		**0.217**		**0.528**		**15.5**	**0.1**	**0.0**
	SD	6.2	1.3		0.058		0.065		5.4	0.3	0.0
	N	10	10		10		10		10	10	10
2000 mg/m^3											
07/25/2008	231	167.9	4.5	8.0	0.135	0.200	0.420	0.595	12	1	0
07/25/2008	232	173.3	7.6	4.5	0.265	0.235	0.490	0.310	1	0	0
07/25/2008	233	163.0	8.6	5.0	0.170	0.250	0.430	0.720	9	0	0
07/25/2008	234	163.7	7.3	8.6	0.105	0.055	0.500	0.695	9	0	0
07/25/2008	235	173.3	10.0	5.0	0.140	0.340	0.645	0.375	14	0	0
07/26/2008	236	170.9	11.0	6.0	0.170	0.175	0.520	0.465	10	0	0
07/26/2008	237	170.1	7.0	6.0	0.145	0.235	0.695	0.535	6	0	0
07/26/2008	238	172.6	7.6	3.7	0.210	0.245	0.630	0.315	13	0	0
07/26/2008	239	180.7	6.5	6.5	0.255	0.125	0.490	0.535	15	0	0
07/26/2008	240	181.0	5.3	3.8	0.165	0.195	0.365	0.570	13	0	0
	Mean	**171.7***	**6.6**		**0.191**		**0.515**		**10.2***	**0.1**	**0.0**
	SD	6.1	1.1		0.048		0.065		4.2	0.3	0.0
	N	10	10		10		10		10	10	10

* $p \leq 0.05$

Overall Mean and SD of hindlimb splay, grip strength and forelimb grip strength calculated from the average of two values for each rat.

Table 19. Additional FOB Comments and Observations in Male Rats

Date	Rat #	Group	Field	Comment
07/25/2008	101	0	Fur Appearance	Rough fur and Red stains on head and back
07/25/2008	102	0	Fur Appearance	Red stain on back
07/25/2008	103	0	Additional Observations	Alopecia on both forelimbs
07/25/2008	104	0	Open Field Comments	Excessive licking
07/25/2008	105	0	Fur Appearance	Red staining head and back
			Additional Observations	Alopecia on both forelimbs
07/26/2008	106	0	Fur Appearance	Staining on back and forelegs
07/26/2008	107	0	Fur Appearance	Red staining on head and back
			Comments	Rat hung on side of enclosure for extended time. Had to place rat on to bottom of the open field.
07/26/2008	108	0	Fur Appearance	Red stain back and head
07/26/2008	109	0	Fur Appearance	Red stain back and head
07/26/2008	110	0	Fur Appearance	Red stain back and head
07/25/2008	111	200	Fur Appearance	Red stain back and head
07/25/2008	112	200	Fur Appearance	Red staining on head and back
07/25/2008	113	200	Fur Appearance	Red staining on head and back
07/25/2008	114	200	Fur Appearance	Red staining on head and back
07/26/2008	116	200	Fur Appearance	Red staining on head and back, Dirty tail
07/26/2008	117	200	Fur Appearance	Red staining on head and back
07/26/2008	119	200	Fur Appearance	Red staining on head and back
07/26/2008	120	200	Fur Appearance	Red staining on head and back
07/25/2008	121	700	Fur Appearance	Red staining on head and back
07/25/2008	123	700	Fur Appearance	Red staining on head and back
07/25/2008	124	700	Fur Appearance	Red staining on head and back
07/25/2008	125	700	Additional Observations	Alopecia-forelimbs
07/26/2008	127	700	Fur Appearance	Red stain on head and back
			Additional Observations	Mild pododermatitis on right rear foot
07/26/2008	128	700	Fur Appearance	Red stain head and back
07/26/2008	129	700	Fur Appearance	Red staining on head and back
07/26/2008	130	700	Fur Appearance	Red stain on back and head
07/25/2008	131	2000	Additional Observations	All four feet scaly, nose scaly
07/25/2008	132	2000	Additional Observations	Alopecia around nose and flaky skin on feet
			Open Field Comments	Excessive licking
07/25/2008	133	2000	Additional Observations	Alopecia on nose
			Comments	Stereotypy behavior of facial grooming (excessive)
07/25/2008	134	2000	Additional Observations	Alopecia around nose
07/26/2008	136	2000	Facial Crust	Facial crust on nose and mouth
			Additional Observations	Alopecia around nose. Flaky skin on nose and all four feet.
			Open Field Comments	Stereotypy-grooming - excessive
07/26/2008	137	2000	Additional Observations	Alopecia on nose. Flaky skin on all four feet.
07/26/2008	138	2000	Additional Observations	Red nose
07/26/2008	139	2000	Additional Observations	Flaky skin on all four feet. Alopecia on nose.
07/26/2008	140	2000	Additional Observations	Flaky skin on front feet.

Table 20. Additional FOB Comments and Observations in Female Rats

Date	Rat #	Group	Field	Comment
07/25/2008	201	0	Facial Crust	Facial crust one eye and nose
07/25/2008	204	0	Facial Crust	Facial crust one eye and nose
07/25/2008	205	0	Fur Appearance	Urine stain/wet and rough
			Facial Crust	Facial crust one eye and nose
07/25/2008	206	0	Facial Crust	Facial crust nose and mouth
07/26/2008	207	0	Additional Observations	Alopecia both front paws
07/26/2008	208	0	Comments	Tried to climb out of Open Field- had to re-place in Open Field.
07/25/2008	211	200	Additional Observations	Right rear- 4th digit deformity
07/25/2008	212	200	Additional Observations	Skin red and thickened on rear feet near toenails.
07/25/2008	213	200	Facial Crust	Facial crust nose and mouth
07/25/2008	214	200	Facial Crust	Facial crust nose and mouth
07/26/2008	216	200	Additional Observations	Left forefoot alopecia
07/26/2008	217	200	Facial Crust	Facial crust nose and mouth
07/26/2008	218	200	Comments	Had to be re-placed in Open Field Box when tried to climb out.
07/26/2008	220	200	Additional Observations	Alopecia on front feet
			Comments	Tried to climb out of Open Field 2 times- had to re-place in Open
07/25/2008	224	700		Field.
07/25/2008	225	700	Open Field Comments	Frequent licking
07/26/2008	226	700	Additional Observations	Alopecia on forelimbs
07/26/2008	227	700	Facial Crust	Facial crust one eye and mouth
			Comments	Tried to climb out of Open Field 2 times- had to re-place in Open Field.
07/25/2008	231	2000	Facial Crust	Facial crust one eye and nose
07/25/2008	232	2000	Additional Observations	Alopecia around nose. Flaky skin on feet and nose
07/25/2008	233	2000	Additional Observations	Alopecia around nose. Flaky skin on all 4 feet and nose
07/25/2008	234	2000	Additional Observations	Alopecia on nose and flaky skin on all 4 feet
07/25/2008	235	2000	Additional Observations	Mild alopecia on nose
07/26/2008	236	2000	Additional Observations	Alopecia around nose and front feet. Flaky skin on all 4 feet.
07/26/2008	237	2000	Additional Observations	Alopecia around nose
			Additional Observations	Missing end of tail. Alopecia around nose. Flaky skin on nose and
07/26/2008	238	2000		all 4 feet
07/26/2008	239	2000	Additional Observations	Alopecia around nose. Flaky skin on all 4 feet.
07/26/2008	240	2000	Additional Observations	Alopecia around nose. Flaky skin around nose and all 4 feet.

APPENDIX H. FINGERPRINT ANALYSIS OF FT JET FUEL BY GAS CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION

DED-NAV-705

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08/09/2010

SUMMARY

Aerosol and vapor phase 8-8 jet fuel samples were collected from high (2000 mg/m^3 middle (700 mg/m^3), and low (200 mg/m^3) concentration groups for the purpose of qualitatively comparing the various samples using gas chromatography/mass spectrometry (GC/MS) analysis. The samples were collected from inhalation exposure chambers used to conduct toxicity studies in laboratory animals with the FT jet fuel. The following conclusions were made based on the qualitative analysis of the GC/MS analysis of the collected samples:

1. In all cases, there was an apparent shift in the distribution of the chemical species present in the jet fuel samples. This shift demonstrated an increased presence of high molecular weight compounds in the aerosol phase compared to an increased presence of low molecular weight compounds in the vapor phase. This trend was observed at all three exposure concentrations.

2. There did not appear to be an appreciable difference in the distribution of compounds when comparing the different aerosol fractions from each concentration group.

3. In the vapor phase, there appeared to be more total compounds present in the high concentration exposure samples compared to the low concentration exposure samples.

4. A majority of the compounds (accounting for >90 percent of the total peak area in the sample) found in the low concentration vapor samples were those found between n-undecane and n-tetradecane. The high concentration vapor samples, meanwhile, appeared to contain a much larger range of molecular weight compounds (i.e., n-octane through n-pentadecane was observed).

INTRODUCTION AND PURPOSE

Production and use of a synthetic jet fuel (FT) for use in military aircrafts promises to reduce the exposure of military personnel to the potentially toxic aromatic hydrocarbons associated with the currently used JP-8 fuels. Although this new FT fuel has very little, if any, aromatics in it, very little toxicity testing has been conducted in order to prove that it is a healthier alternative to

working with the JP-8 fuels. The purpose of the exposures conducted at The Hamner was to assess the potential inhalation toxicology associated with a typical workplace exposure to FT jet fuel. Exposures were carried out at three concentrations and for two different time periods. In order to better understand any potential adverse biological observations the study may produce, a fingerprint analysis of the aerosol and vapor phase of the delivered test chemical mixture was requested by the sponsor. The goals associated with the fingerprint analysis were as follows:

1. Determination of the hydrocarbon fingerprint of FT jet fuel by gas chromatography/mass spectrometry (GC/MS) for future comparison to aerosolized FT jet fuel samples.

2. Qualitatively identify as many of the peaks present in the FT fuel mixture.

3. Collect and analyze FT jet fuel during an animal exposure with the intent of analyzing the aerosol phase and the gas phase independent of each other.

METHODS

Materials

- GC Column: Petrocol DH 150, Supelco, 150 m x 0.25 mm, 1.00 μm film, Cat #24155
- GC Liners: Siltek 4mm split with glass wool, Restek, Cat # 20782-213.5
- GC syringe: Hamilton gas tight syringe, Restek, Cat # 1701ASRN
- GC septa: 11 mm partial hole septa, Agilent, Part # 5181-3383
- Diesel range organics (DRO) test mix (Tenn/Miss), Restek, Cat # 31214
- Electrostatic precipitator, Aerosol Associates (Chapel Hill, NC)
- Dry Ice
- Ethanol
- Glass cold finger condenser, Prism Research Glass (RTP, NC)
- FT Jet Fuel (POSF 5109)

Instrumental Conditions

- Gas Chromatograph: Agilent 6890 or Shimadzu GC-2010
- Mass Spectrometer: Agilent 5973N or Shimadzu GCMS QP-2010 plus
- Autosampler: Agilent 7683B (manual for Shimadzu)
- Chemstation software: Agilent version D.01.02.16
- Shimadzu software: GCMS solution V.2.50 SU3
- NIST MS Database: NIST 2005 (Shimadzu)
- Total run time: 596 minutes
- Injection Size: 1 μL
- Split Ratio: 100:1
- Injector temperature: 250°C

- Transfer Line Temp: 280°C
- MS Quad Temp: 150°C
- MS Source Temp: 230°C
- MS Ionization Mode: Electron Impact
- Mass Scan Range: 35.0-350.0 amu

Table 1. Temperature program for Petrocol DH 150 column

Time (minutes)	**Initial Temperature** (°C)	**Final Temperature** (°C)	**Temperature Rate** (°C/min)	**Column Flow** (mL/min)	**Total Time** (minutes)
0.0	35	35	0	1	16
16.0	35	70	0.5	1	70
86	70	70	0	1	15
101	70	110	0.5	1	80
181	110	110	0	1	35
216	110	140	0.5	1	60
276	140	140	0	1	35
311	140	170	0.5	1	60
371	170	170	0	1	35
406	170	200	0.5	1	60
466	200	200	0	1	30
496	200	250	0.5	1	100
596	250	250	0	1	NA

Compound Identification in FT Fuel

The compound identification phase of the fingerprint analysis was performed during the two-week inhalation toxicity study with FT[5]. Briefly, FT fuel was transferred directly to a silinated GC auto-sampler vial using a disposable Pyrex glass pipette which had been rinsed twice with FT prior to sample transfer. Samples were capped with Teflon lined crimp top caps and placed in the GC/MS auto-sampler for analysis. The quality control sample was a diesel range organics (DRO) test mixture. The DRO samples were transferred using the identical procedure and analyzed prior to and immediately following FT.

Identification of the peaks was accomplished by matching with our mass spectral database. Matches were considered similar with QUAL values of 75 or better. These scores were calculated through a software algorithm (Agilent Chemstation) comparing the fragmentation ion pattern of the unknown to a known fragmentation pattern (National Institutes of Standards and Technology (NIST) database). The better match is indicated with a higher score (scores of 100 indicate a perfect match).

An effort was made to identify as many of the additional compounds present in the chromatogram as possible. Some of the assigned identifications were made through library

matches that did not have scores above the arbitrarily set QUAL score acceptance criteria of 75, while the remaining identifications were made through manual mass spectral interpretation.

Analysis of Aerosol and Vapor Components

An electrostatic precipitator was generously donated by Dr. David Leith from the University of North Carolina at Chapel Hill, Department of Environmental Sciences. The selection of an electrostatic precipitator (ESP) for the collection of the aerosols was based on the published differences in the loss of sample (mass evaporated) from filter based aerosol collectors versus ESP collection methods[1-4]. At lower vapor concentrations, a significant amount of collected aerosol can be lost if filter sampling is used, as compared to ESP sampling, or that the more volatile components in the collected aerosol will be lost, thus producing a non-representative fingerprint of the actual aerosol components. The use of a cold trap in conjunction with the ESP was to ensure that even the most volatile components of the vapor phase were collected for analysis.

In order to collect the aerosol and vapor phase components of the jet fuel atmospheres, the electrostatic precipitator was hooked up, in series, with a cold finger glass trap using an ethanol/dry ice slurry bath. The precipitator removed any aerosol components present in the sample, while the cold finger trap removed all of the remaining volatile compounds. House vacuum (set to approximately 1.5 L/min)[4] was used to pull sample from the exposure chambers through the electrostatic precipitator/cold trap set-up. For the high concentration exposure (2000 mg/m^3), a collection time of approximately 30 minutes was sufficient to collect an acceptable (10-30 µL) amount of condensed aerosol. For the middle concentration exposure (700 mg/m^3), a collection time of 75 minutes was used. For the low concentration exposure (200 mg/m^3), a collection time of >120 minutes was required. Following collection, samples were either directly injected onto a GC/MS (aerosol samples) or extracted with a minimal volume of carbon tetrachloride (vapor samples, comprised primarily of collected water vapor) prior to analysis. Samples were analyzed on an Agilent 6890 GC with 5973 inert MSD (Agilent, Santa Clara, CA).

Confirmation of chamber fuel concentrations (total hydrocarbons) was conducted through the use of an infrared spectrophotometer (MIRAN 1A, Foxboro Co., South Norwalk, CT). A sample of the chamber atmosphere was continuously pulled through the heated cell of the IR spectrophotometer and the total signal obtained from the instrument was recorded. IR spectrophotometers were calibrated prior to use by analyzing a series of known jet fuel concentrations.

RESULTS

Compound Identification in FT Fuel

The compound identification phase of the fingerprint analysis was performed during the two-week inhalation toxicity study with FT[5]. Identification of the peaks present in the FT fuel blend provided a considerable challenge as most of the highly branched hydrocarbons in the sample were not included in our mass spectral database. Table 2 (reprinted from Mattie *et al.*, 2011[5]) summarizes the compounds with similarity match (or QUAL values) scores of 75 or better. A complete chromatogram for the FT fuel analysis is shown in Figure 1 (pre-exposure analysis, ten-day exposure[5].

Despite the effort made to identify as many of the additional compounds present in the chromatogram as possible, there were still a large number of peaks which we were not able to assign to a specific compound due to the highly branched nature of many of the higher molecular weight hydrocarbons.

Analysis of Aerosol and Vapor Components

Actual chamber concentrations for the starting and ending days of the 90-day exposure are located Figures 2 through 7. These figures compare the aerosol and vapor phases for each test concentration on both days.

Table 2. Identification of Analyte Peaks from FT Fuel

Compound	Retention time (minutes)	Qual Score
2-methyl-heptane	109.90	91
4-methyl-heptane	110.59	86
3-methyl-heptane	113.17	87
3-ethyl-hexane	113.74	78
n-Octane	124.37	91
2,2-dimethyl-heptane	132.83	78
2,4-dimethyl-heptane	134.49	91
2,6-dimethyl-heptane	137.11	78
Ethyl-cyclohexane	138.34	78
2,5-dimethyl-heptane	139.98	91
2,3-dimethyl-heptane	148.58	91
3,4-dimethyl-heptane	149.51	75
4-ethyl-heptane	150.38	91
4-methyl-octane	151.52	95
2-methyl-octane	151.93	94
3-ethyl-heptane	154.24	83
3-methyl-octane	154.74	91
n-Nonane	165.84	94
2-(2-methoxyethoxy)-ethanol	168.71	78
2,4,6-trimethyl-heptane	173.33	75
2,5-dimethyl-octane	176.05	95
3,5-dimethyl-octane	176.50	86
2,6-dimethyl-octane	179.55	90
3,3-dimethyl-octane	180.41	78
2,3-dimethyl-octane	187.57	91
4-ethyl-octane	187.87	93
5-methyl-nonane	189.56	95
4-methyl-nonane	190.33	91
2-methyl-nonane	191.398	96
3-ethyl-octane	192.93	90
3-methyl-nonane	194.27	91
1,3,5-trimethyl-benzene	199.58	76
n-Decane	207.16	97
5-ethyl-2-methyl-octane	214.628	94
2,5-dimethyl-nonane	217.653	94
5-ethyl-2-methyl-octane	233.34	80
2,3-dimethyl-nonane	234.398	94
5-methyl-decane	235.87	93
4-methyl-decane	237.38	95
2-methyl-decane	239.05	95
3-methyl-decane	242.10	95
n-Undecane	254.99	96
4-ethyl-decane	260.080	87
3,5-dimethyl-decane	261.748	78
2,6-dimethyl-decane	262.978	95
3,6-dimethyl-decane	264.916	97
3,7-dimethyl-decane	266.981	94
5-propyl-nonane	267.578	78
5-ethyl-decane	273.61	93

Compound	Retention time (minutes)	Qual Score
4-ethyl-decane	275.69	76
6-methyl-undecane	277.395	90
5-methyl-undecane	277.94	93
4-methyl-undecane	279.498	94
2-methyl-undecane	281.238	91
3-methyl-undecane	284.03	90
n-Dodecane	296.581	96
2,5-dimethyl-undecane	303.677	95
3,8-dimethyl-undecane	310.874	80
2,9-dimethyl-undecane	313.461	83
4-ethyl-undecane	320.013	90
6-methyl-dodecane	321.922	81
5-methyl-dodecane	322.904	86
4-methyl-dodecane	325.085	97
2-methyl-dodecane	327.233	97
3-ethyl-dodecane	328.118	90
3-methyl-dodecane	330.397	90
n-Tridecane	342.914	98
7-methyl-tridecane	362.793	81
6-methyl-tridecane	363.134	90
5-methyl-tridecane	364.184	96
4-methyl-tridecane	366.013	96
2-methyl-tridecane	367.899	94
3-methyl-tridecane	370.440	96
n-Tetradecane	381.026	98
7-methyl-tetradecane	400.692	85
6-methyl-tetradecane	401.378	85
5-methyl-tetradecane	402.731	98
4-methyl-tetradecane	405.160	98
2-methyl-tetradecane	407.462	98
3-methyl-tetradecane	410.671	98
n-Pentadecane	422.591	98
7-methyl-pentadecane	440.787	90
6-methyl-pentadecane	441.511	75
5-methyl-pentadecane	442.830	75
4-methyl-pentadecane	444.899	94
2-methyl-pentadecane	446.766	91
3-methyl-pentadecane	449.379	75
n-Hexadecane	458.856	98

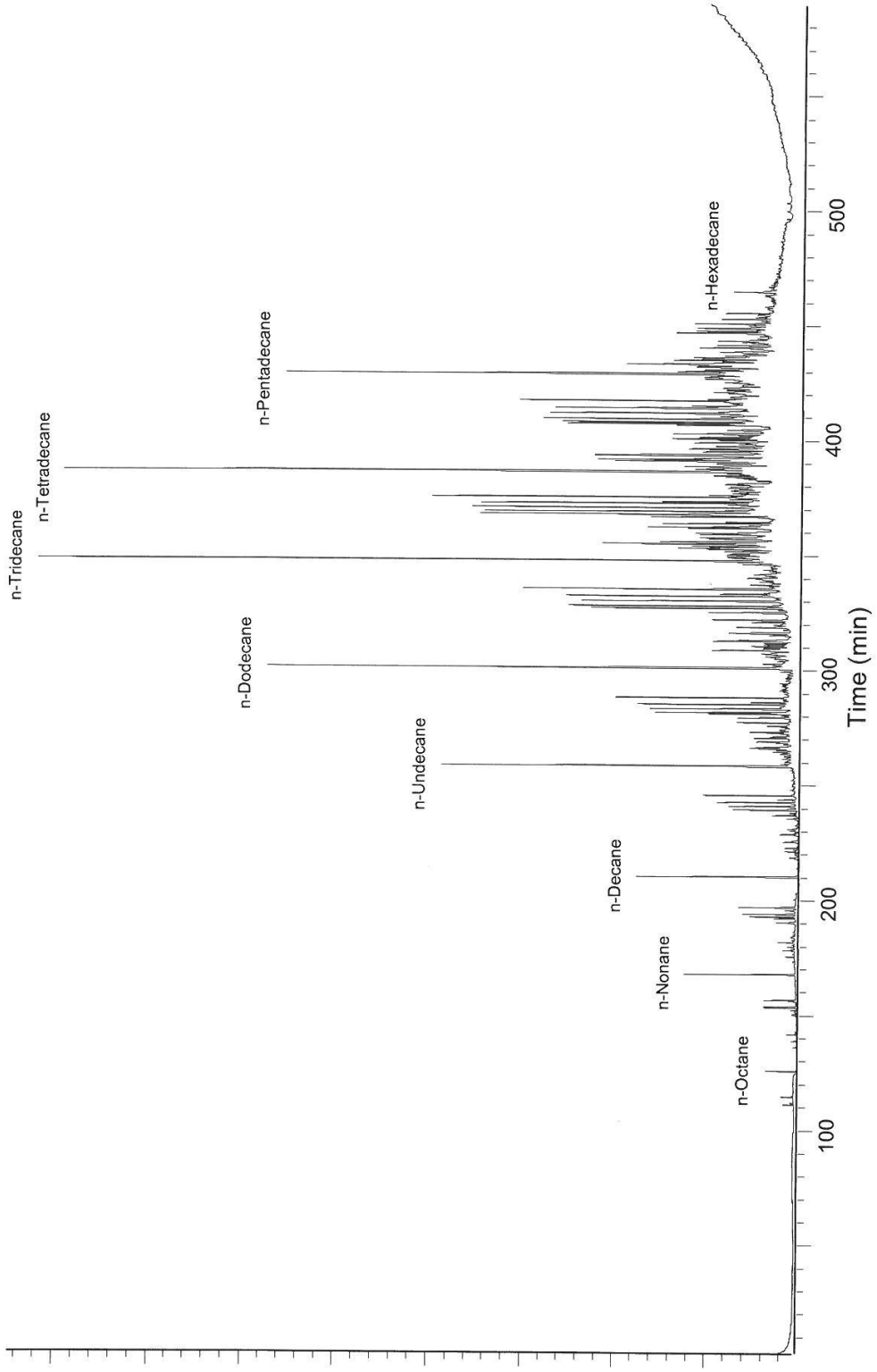

Figure 1. GC/MS Pre-Study Analysis of FT Jet Fuel during the Two-Week Study[5]

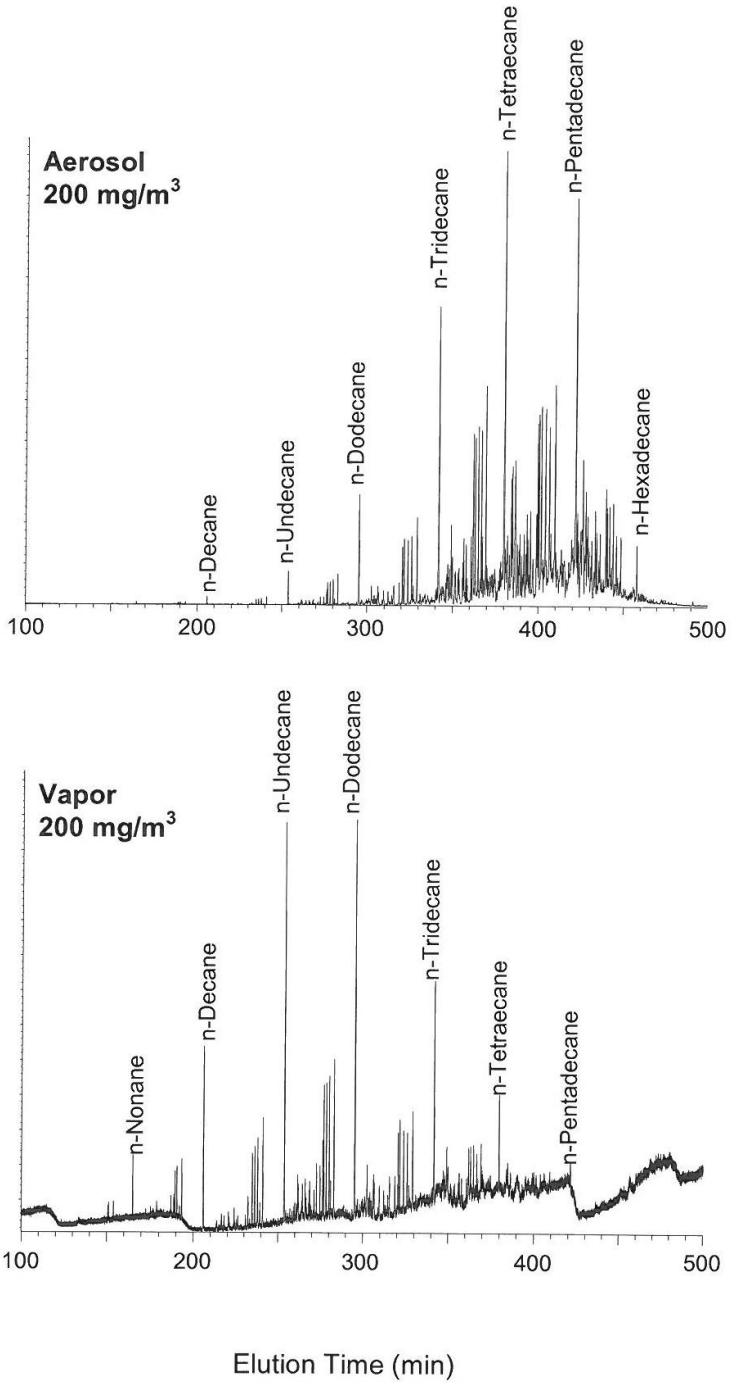

Figure 2. Aerosol/Vapor Fraction, Start, 90-Day Exposure, 200 mg/m^3

Analysis of aerosol and vapor fractions collected during the 05/19/2008 exposures. Aerosol samples were collected using an electrostatic precipitator and vapor fractions were collected using a cold finger trap. Actual chamber concentration (total hydrocarbons) for this day was 201 .7 mg/m^3 Samples were analyzed using the Shimadzu GC/MS.

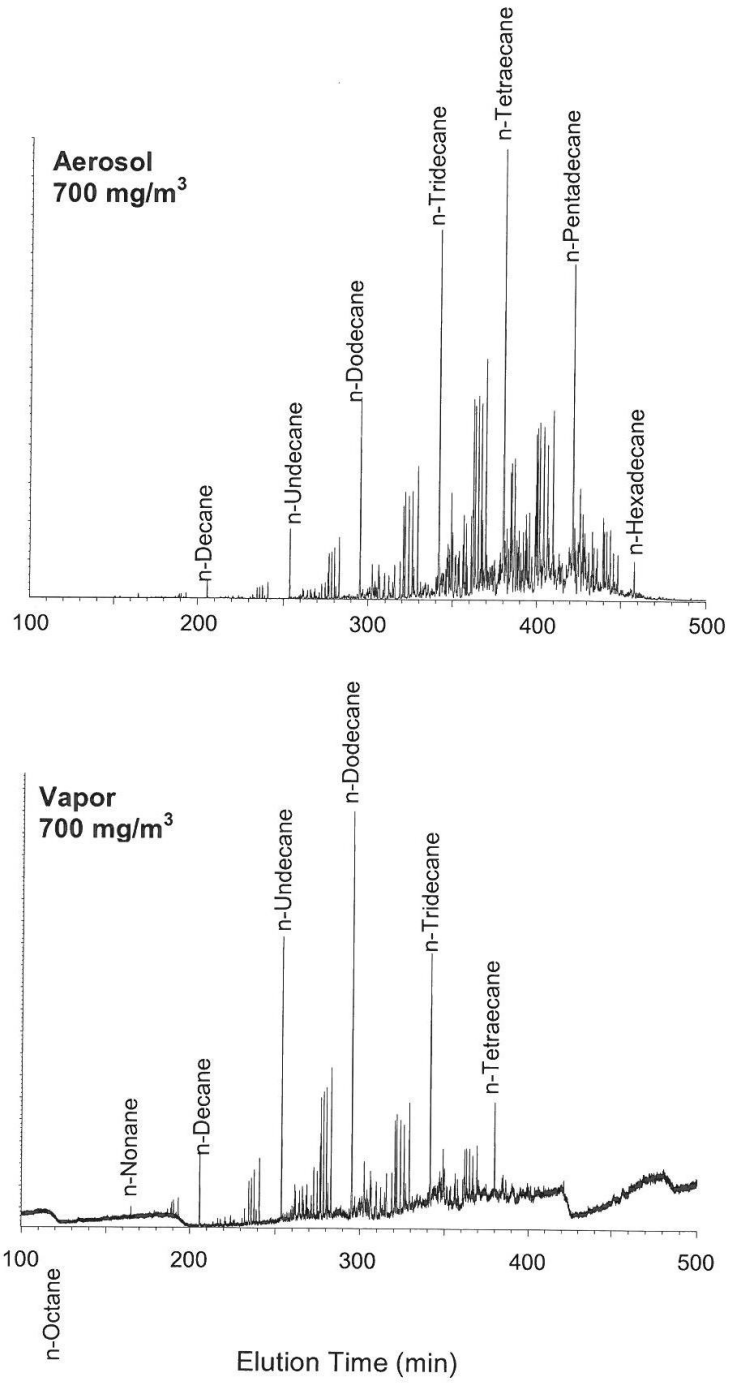

Figure 3. Aerosol/Vapor Fraction, Start, 90-Day Exposure, 700 mg/m^3

Analysis of aerosol and vapor fractions collected during the 05/19/2008 exposures. Aerosol samples were collected using an electrostatic precipitator and vapor fractions were collected using a cold finger trap. Actual chamber concentration (total hydrocarbons) for this day was 676.7 mg/m^3. Samples were analyzed using the Shimadzu GC/MS.

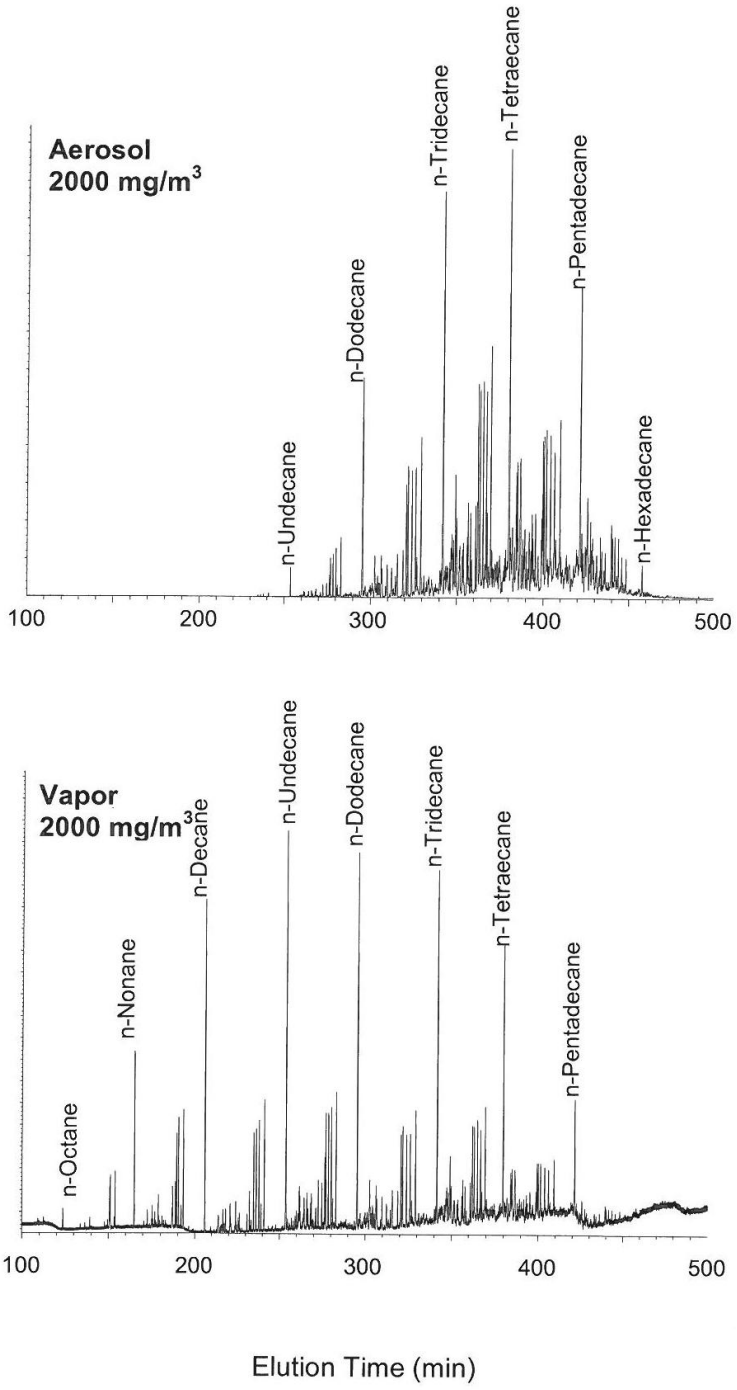

Figure 4. Aerosol/Vapor Fraction, Start, 90-Day Exposure, 2000 mg/m^3

Analysis of aerosol and vapor fractions collected during the 05/19/2008 exposures. Aerosol samples were collected using an electrostatic precipitator and vapor fractions were collected using a cold finger trap. Actual chamber concentration (total hydrocarbons) for this day was 2092.0 mg/m^3. Samples were analyzed using the Shimadzu GC/MS.

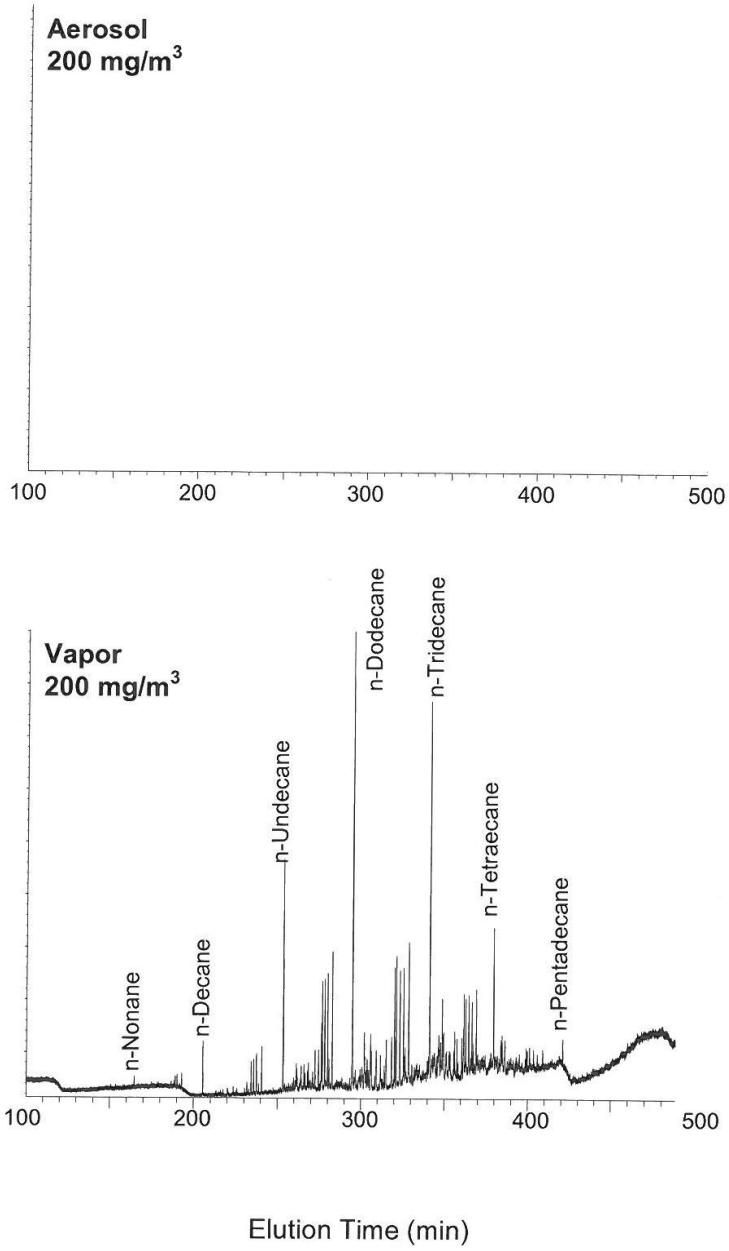

Figure 5. Aerosol/Vapor Fraction, End, 90-Day Exposure, 200 mg/m^3

Analysis of aerosol and vapor fractions collected during the 07/29/2008 exposures. Aerosol samples were collected using an electrostatic precipitator and vapor fractions were collected using a cold finger trap. The aerosol sample was collected for a minimum of 120 minutes with no observable precipitate. An effort was made to recover any aerosol on the inner precipitator liner by washing with two 50 µL aliquots of carbon tetrachloride and analyzing the resulting washes using GC/MS. No jet fuel fingerprint was observed in this sample. Actual chamber concentration (total hydrocarbons) for this day was 200.6 mg/m^3. Samples were analyzed using the Shimadzu GC/MS.

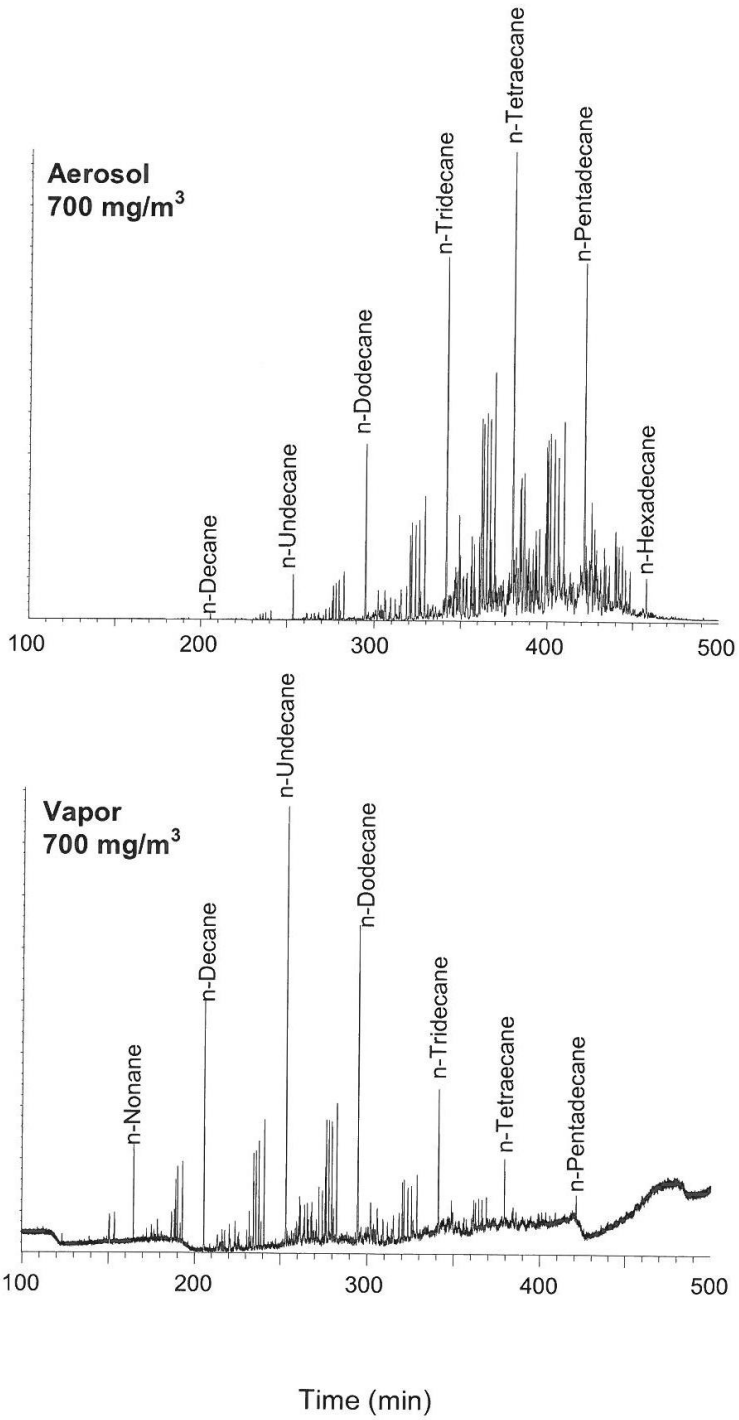

Figure 6. Aerosol/Vapor Fraction, End, 90-Day Exposure, 700 mg/m^3

Analysis of aerosol and vapor fractions collected during the 07/29/2008 exposures. Aerosol samples were collected using an electrostatic precipitator and vapor fractions were collected using a cold finger trap. Actual chamber concentration (total hydrocarbons) for this day was 696.8 mg/m^3. Samples were analyzed using the Shimadzu GC/MS.

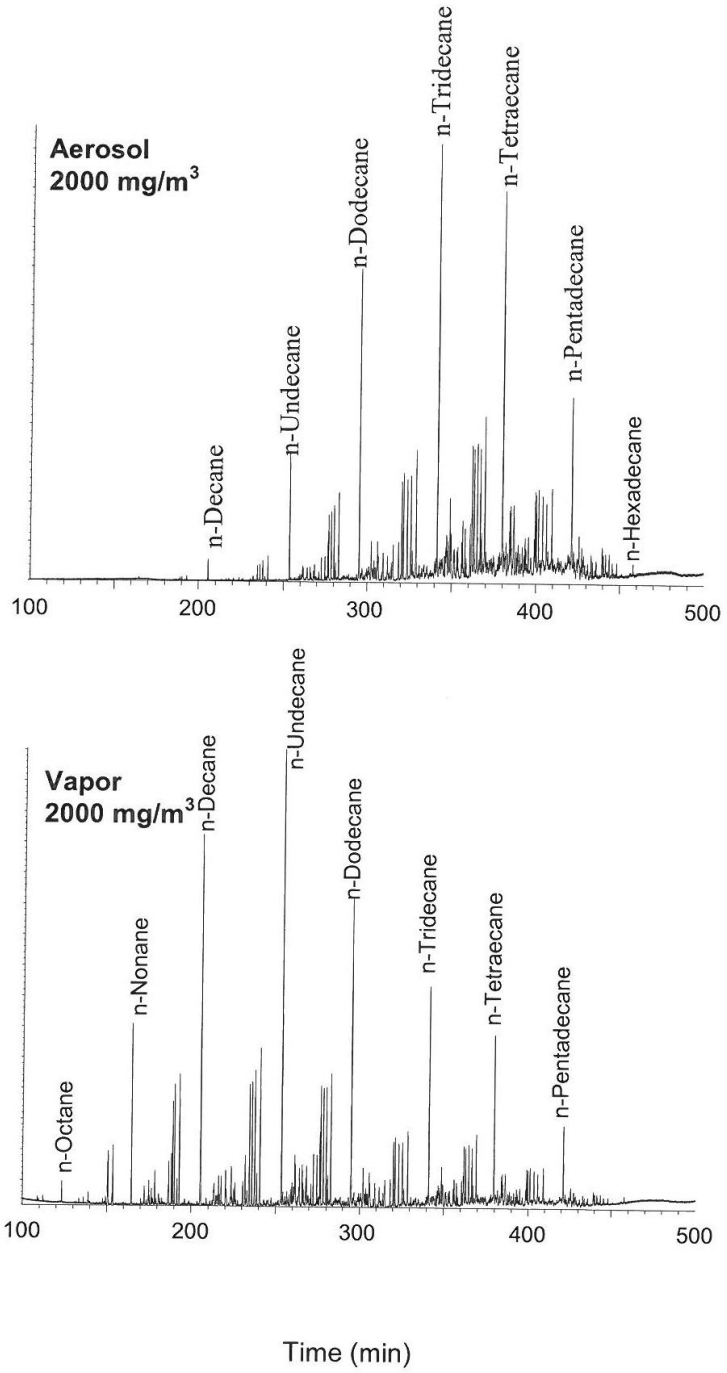

Figure 7. Aerosol/Vapor Fraction, End, 90-Day Exposure, 2000 mg/m^3

Analysis of aerosol and vapor fractions collected during the 07/29/2008 exposures. Aerosol samples were collected using an electrostatic precipitator and vapor fractions were collected using a cold finger trap. Actual chamber concentration (total hydrocarbons) for this day was 2016.5 mg/m^3. Samples were analyzed using the Shimadzu GC/MS.

CONCLUSIONS

The following conclusions were made based on the qualitative analysis of the GC/MS analysis of the collected samples:

1. In all cases, there was an apparent shift in the distribution of the chemical species present in the jet fuel samples. This shift demonstrated an increased presence of high molecular weight compounds in the aerosol phase compared to an increased presence of low molecular weight compounds in the vapor phase. This trend was observed at all three exposure concentrations.

2. There did not appear to be an appreciable difference in the distribution of compounds when comparing the different aerosol fractions from each concentration group.

3. In the vapor phase, there appeared to be more total compounds present in the high concentration exposure samples compared to the low concentration exposure samples.

4. A majority of the compounds (accounting for >90 percent of the total peak area in the sample) found in the low concentration vapor samples were those found between n-undecane and n-tetradecane. The high concentration vapor samples, meanwhile, appeared to contain a much larger range of molecular weight compounds (i.e., n-octane through n-pentadecane was observed).

REFERENCES

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5) Mattie DR, Sterner TR, Wong BA, Dodd DE, Ross PW, Gross EA, Gao P, Wang X-J, Sochaski MA, Willson GA, Wagner DJ. Acute and short term inhalation toxicity study of FT fuel. Air Force Research Laboratory, Applied Biotechnology Branch. AFRL-RH-TR-2011-XXXX. In process.

APPROVAL SIGNATURES

This report is an accurate and complete representation of the data for this study (Protocol 08013).

Signature: _____ 08/09/2010

Mark Sochaski,
The Hamner Institutes for Health Sciences
Analytical Chemistry Services, Manager

Date

LIST OF ACRONYMS

ACGIH	American Conference of Industrial Hygienists
AFB	Air Force Base
ALB	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BAS	basophils
BUN	urea nitrogen
CA	calcium
CASA	Computer Automated Sperm Analysis
CBC	complete blood count
CHOL	cholesterol
CL	chloride
CNS	central nervous system
CPK	creatine kinase
CREA	creatinine
DRO	diesel range organics
DSP	daily sperm production
ELISA	enzyme-linked immunosorbent assay
EOS	eosinophils
EPL	Experimental Pathology Laboratories, Inc.
ESP	electrostatic precipitator
FMI	Fluid Metering, Inc.
FOB	functional observational battery
FT	Fischer-Tropsch
GC/MS	gas chromatography/mass spectrometry
GLP	Good Laboratory Practice
GLU	glucose
H&E	hematoxylin and eosin
HB	hemoglobin
HCT	hematocrit
HEPA	high efficiency particulate air
ILS	Integrated Laboratory Systems, Inc.
IR	infrared
IVOS	Integrated Visual Optic System
K	potassium
LUC	large unstained cells
LYM	lymphocytes
MANOVA	multivariate analysis of variance
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume

MMAD (GSD)	mass median aerodynamic diameter (geometric standard deviation)
MON	monocytes
MTBE	methyl-tert-butyl ether
MTD	maximum tolerated dose
N	number
NA	sodium
NEU	neutrophils
NIST	National Institutes of Standards and Technology
NRC	National Research Council
OEL	occupational exposure limit
PEL	permissible exposure limit
PHOS	phosphorus
PLT	platelet
PT	prothrombin time
QNS	quantity not sufficient for analysis
RBC	red blood cell (erythrocyte count)
SD	standard deviation
SEM	standard error of the mean
SPK	Synthetic Paraffinic Kerosene
TERM	termination
TLV	threshold limit value
TPRO	total protein
U.S. EPA	United States Environmental Protection Agency
WBC	white blood cell (total leukocyte count)
α2μ	$\alpha_{2\mu}$-globulin