mr#
302442

AR226-3779

TRADE SECRET

Study Title

Ammonium Perfluorooctanoate: 28-Day Immunotoxicity Study in Male Rats

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.7800 (1998)

AUTHOR: Denise Hoban, B.A, MLT (ASCP)

STUDY COMPLETED ON: February 1, 2007

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell[SM] Laboratory for Health and Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

Exygen Research
3058 Research Drive
State College, Pennsylvania 16801
U.S.A.

Experimental Pathology Laboratories, Inc.
615 Davis Drive, Suite 500
Durham, North Carolina 27713
U.S.A.

Laboratory for Advanced Electron and Light Optical Methods
College of Veterinary Medicine
North Carolina State University
4700 Hillsborough Street
Raleigh, North Carolina 27606
U.S.A.

LABORATORY PROJECT ID: DuPont-18317

CONTAINS NO CBI

WORK REQUEST NUMBER: 16160

SERVICE CODE NUMBER: 1545

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

PAGE RESERVED

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA FIFRA (40 CFR part 160) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices, except for the item documented below. The item listed does not impact the validity of the study.

A non-GLP characterization was performed prior to the initiation of this study. The accuracy of the composition at the concentrations documented in this report is considered sufficient for the purpose of this study and is based on the process chemistry provided by the sponsor. GLP characterization was performed concurrently during the course of the study.

Applicant / Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: Denise Hoban 01 Feb 2007

Denise Hoban, B.A, MLT (ASCP) — Date
Staff Medical Technologist and Supervisor

Applicant / Sponsor: _____ _____

DuPont Representative — Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 16160
Study Code Number: 1545

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:	October 17, 2005	October 17, 2005	October 17, 2005
Conduct:	November 11, 2005	November 11, 2005	November 11, 2005
	November 14, 2005	November 14, 2005	November 14, 2005
	May 30, 2006*	October 31, 2006*	November 2, 2006*
	June 14, 2006*	October 31, 2006*	November 2, 2006*
	June 27, 2006*	October 31, 2006*	November 2, 2006*
	October 25, 2006*	October 31, 2006*	November 2, 2006*
Report/Records:	February 2, 7, 2006	February 7, 2006	February 8, 2006
	August 10, 11, 14-18, 2006	August 18, 2006	September 11, 2006
	November 13-14, 2006	November 14, 2006	December 12, 2006

* EPL QA Dates

Reported by: Joseph C Hamill 01-Feb-2007
Joseph C. Hamill
Quality Assurance Auditor
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Analytical Evaluation by: _____ 01-Feb-2007
Z. Amanda Shen, Ph.D. Date
Research Chemist

Clinical Pathology Evaluation by: _____ 01-Feb-2007
Nancy E. Everds, D.V.M., Diplomate A.C.V.P. Date
Principal Research Clinical Pathologist and Manager

Anatomic Pathology Evaluation by: _____ 01-Feb-2007
Greg P. Sykes, V.M.D., Diplomate A.C.V.P., Date
A.C.L.A.M., A.B.T.®
Veterinary Pathologist

Anatomic Pathology Evaluation Peer Review by: _____ 01-Feb-2007
Steven R. Frame, D.V.M., Ph.D., Diplomate A.C.V.P. Date
Research Fellow and Manager

Reviewed and Approved by: _____ 01-Feb-2007
Scott E. Loveless, Ph.D. Date
Research Manager and Director

Issued by Study Director: _____ 01Feb2007
Denise Hoban, B.A, MLT (ASCP) Date
Staff Medical Technologist and Supervisor

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Page

STUDY INFORMATION

Substance Tested:
- Ammonium Perfluorooctanoate [AFPO (linear)]
- 3825-26-1 (CAS Number)

Haskell Number: 27308

Composition: Ammonium Perfluorooctanoate Solution 19.5% in water

Purity: 19.5%

Physical Characteristics: White to slightly opaque liquid

Stability: The test substance was stable under the conditions of the study based on analytical results.

Study Initiated/Completed: October 14, 2005 / (see report cover page)

Experimental Start/Termination: October 16, 2005 / February 1, 2007

SUMMARY

The purpose of this study was to evaluate the potential of ammonium perfluorooctanoate [APFO (linear)] to suppress the primary humoral immune response following exposure via oral gavage for up to 28 consecutive days. Groups of 10 male rats each were administered the test substance at daily levels of 0, 0.3, 1, 10, 30, and 30/0 mg/kg. The group designated 30/0 mg/kg was included to assess potential reversibility/recovery and was therefore administered the test substance for 22 consecutive days followed by 6 consecutive days of vehicle (water) administration. Body weights, food consumption measurements, and clinical observations were recorded during the in-life period. Prior to sacrifice, the immune system was stimulated by injecting sheep red blood cells (SRBC) on test day 22 and blood samples were collected from each rat on test day 29. The serum samples were assayed for their concentration of SRBC-specific IgM antibodies to provide a quantitative assessment of humoral immune response. Serum from animals dosed with cyclophosphamide, a positive control immunosuppressive agent, were analyzed concurrently to provide confirmation that the assay performance was acceptable for detection of immunosuppression. Clinical pathology data were collected at day 29 to assess effects on hematology and clinical chemistry. At sacrifice, each animal was examined grossly and selected organs were weighed (brain, spleen, and thymus); selected tissues (as outlined in the methods section) were retained and examined histologically. Thymus and spleen cells were manually counted from single-cell suspensions prepared from the collected tissue.

Samples of the dosing formulations were chemically analyzed and the results indicated that the test substance was at the targeted concentrations, homogeneously mixed, and stable under the conditions of the study.

Test substance-related toxicity was observed during the in-life portion of the study at 1 mg/kg and higher. Adverse reductions in body weights, weight changes, food consumption, and food efficiency occurred at 10 mg/kg and higher; at 30 and 30/0 mg/kg, these reductions were accompanied by low incidences of clinical observations indicative of toxicity. Effects on body weight and food consumption parameters were detected at 1 mg/kg, but these reductions were not considered adverse. There were no test substance-related effects observed at 0.3 mg/kg during the in-life portion of the study.

Rats dosed with ≥0.3 mg/kg had decreased serum total, HDL, and non-HDL cholesterol, and decreased triglycerides. Rats dosed with ≥1 mg/kg had increased microscopic red cell morphologic changes and hemolyzed serum. Rats dosed with ≥10 mg/kg had decreased hemoglobin, hematocrit, mean cell volumes, and mean cell hemoglobin concentrations; increased reticulocyte counts and red cell distribution width, increased total white blood cell, neutrophil, monocyte, and LUC counts; increased serum albumin and decreased serum globulin concentrations; and increased serum corticosterone concentrations. Rats in the 30/0 mg/kg group had more pronounced red cell mass effects and red cell morphologic changes compared to those dosed with 30 mg/kg for 29 days. Parameters with complete recovery in rats dosed with 30/0 mg/kg were serum total, HDL, and non-HDL cholesterol, globulin, and corticosterone concentrations.

There was a test substance-related decrease in final body weights and increase in liver weights. Mean final body weights were decreased at dose levels ≥10 mg/kg of the test substance. Mean liver weight parameters were increased at dose levels ≥0.3 mg/kg. At the terminal sacrifice, test substance-related gross observations were limited to discoloration of the liver in a few rats at doses ≥10 mg/kg. Microscopic examination of the liver demonstrated minimal to mild hepatocellular hypertrophy at 0.3 and 1 mg/kg and moderate hepatocellular hypertrophy at ≥10 mg/kg. Microscopic examination of lymphohematopoietic organs (spleen, thymus, bone marrow, lymph nodes) revealed increased hematopoiesis in the spleen of rats dosed with 30/0 mg/kg.

No test substance-related evidence of immunosuppression was observed in male rats at any concentration tested; the IgM titers were generally comparable across all groups.

No significant changes in total spleen cell or thymocyte number compared to control rats were noted in any animals treated with any dose of APFO.

Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for APFO for systemic toxicity in male rats was less than 0.3 mg/kg, whereas the NOAEL for immunotoxicity was 30 mg/kg.

INTRODUCTION

The primary objective of this study was to evaluate the potential of ammonium perfluorooctanoate [APFO (linear)] to suppress the primary humoral immune response to sheep red blood cells (SRBC) when administered by oral gavage to male rats for at least 28 days. Additional endpoints of toxicity were also evaluated. The oral route of administration was selected because it is a potential route of human exposure.

Ammonium perfluorooctanoate (APFO; FC-143, C_8; $C_7F_{15}COO^-NH_4^+$; CAS Registry number 3825-26-1) is a surfactant used as a processing aid in the production of fluoropolymers. Perfluorooctanoate (PFOA; $C_7F_{15}COO^-$), the dissociation product of APFO, is not metabolized[1] and has been identified in blood samples from exposed workers and the general population.[2,3,4]

PFOA has been reported to inhibit the ability of mice to make antibodies to a T-cell dependent antigen.[5] The reported study employed a single 0.02% PFOA in chow (approximately 30 mg/kg) for 16 days. In order to better characterize the immune response following exposure to this material, APFO was administered by oral gavage using a broad range of doses.

Dosages for this study were selected based on results of a 14-day oral gavage study in male rats and mice.[6]

STUDY DESIGN

A. Design Concentrations

Group	Number/ Group	Daily Dosage (mg/kg)[a]	Dose Solution Concentration (mg/mL)[b]
I	10	0 (Control)	0
III	10	0.3	0.03
V	10	1	0.1
VII	10	10	1
IX	10	30	3
XI	10	30/0[c]	3

a Weight of test substance/kg or animal body weight.
b Solutions were adjusted for purity (19.5%).
c This group (XI) was dosed with 30 mg/kg of test substance through test day 22. Following injection of SRBC on test day 23, group XI was dosed with NANOpure® water, at a volume of 10 mL/kg of body weight, until sacrifice.

B. Study Overview

Study Parameters	Frequency
Body Weight	Day 0, 3, 6, and daily thereafter
Food Consumption	Weekly
Daily Animal Health Observation	Twice daily
General Clinical Observation[a]	Day 0 and weekly thereafter
Detailed Clinical Observation	At each weighing
SRBC Injection	Prior to dosing (test day 23)
Clinical Pathology Evaluation	Test day 29
Serum Collection for Antibody Determination	At sacrifice (test day 29)
Anatomic Pathology Evaluation	Test day 29

a A check for acute signs of toxicity was conducted approximately 2 hours post-dosing.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 870.7800: Immunotoxicity, *Health Effects Test Guidelines* (1998)

B. Test Substance

(Appendix A)

APFO (linear), was supplied by the sponsor as a white to slightly opaque liquid in a 19.5% aqueous solution. The bulk test substance was used within the period of approved use as defined by the expiration date listed on the Certificate of Analysis (COA) that is provided in Appendix A. No evidence of instability, such as a change in color or physical state, was observed.

C. Test System

On October 6, 2005, 66 male Crl:CD(SD) rats, with an assigned birth date of August 22, 2005, were received from Charles River Laboratories, Raleigh, North Carolina.

The Crl:CD(SD) rat was selected based on consistently acceptable health status and on extensive experience with this strain at Haskell Laboratory. By utilizing the Crl:CD(SD) rat, immunotoxicity studies can be conducted in the same strain that is used for other toxicology studies.

D. Animal Husbandry

1. Housing

All animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26°C and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

All rats were provided tap water *ad libitum*. All rats were fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*.

4. Animal Health and Environmental Monitoring Program

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

E. Pretest Period

Upon arrival at Haskell Laboratory, all rats were housed in quarantine. The rats were:

- quarantined for 6 days.
- identified temporarily by cage identification.
- weighed at least 3 times during quarantine.

- observed with respect to weight gain and any gross signs of disease or injury.

The rats were released from quarantine by the laboratory animal veterinarian or designee on the bases of acceptable body weights and clinical signs of all rats.

F. Assignment to Groups

Rats, selected on the bases of adequate body weight gain and freedom from any clinical signs of disease or injury, were distributed by computerized, stratified randomization into study groups as designated in the Study Design, so that there were no statistically significant differences among group body weight means within a sex. The weight variation of selected rats did not exceed ± 20% of the mean weight for each sex.

At grouping, each rat was assigned an animal number/cage identification number. The animal number/cage identification number were tattooed on the tail of each rat and included on the cage label.

At study start (test day 0) the rats were 8 weeks of age.

G. Dose Formulation Preparation and Administration

The dosing solutions were prepared in NANOpure® water. The formulations were adjusted based on the percentage of APFO in the bulk test substance to achieve the desired concentrations. Dosing formulations were prepared on a daily basis.

Animals were dosed daily at approximately the same time (± 2 hours) by intragastric intubation at a dose volume of 10 mL/kg body weight for at least 28 consecutive days; individual dose volumes were calculated based on the most recently collected body weight data. Control rats were similarly dosed with NANOpure® water at a volume of 10 mL/kg of body weight. The 30/0 mg/kg group (XI) was dosed with 30 mg/kg of test substance through test day 22. Following injection of SRBC on test day 23, group XI was dosed with NANOpure® water at a volume of 10 mL/kg of body weight until sacrifice.

One rat from group XI (1109) was not dosed on test days 6 through 8 due to a decrease in body weight gain. Once body weight increases were observed for this rat, dosing resumed.

H. Dose Formulation Sampling and Analysis

1. Recovery Sample Analysis

Concurrent with dosing formulation analyses, recovery of APFO from spiked NANOpure® water was tested at the low level (approximately 0.03 mg/mL), the middle levels (approximately 0.1 and 1 mg/mL), and the high level (approximately 3 mg/mL) to confirm the analytical method. A stock solution of APFO was prepared in NANOpure® water. For all concentration levels, an appropriate aliquot of the stock solution was used to make the spiked solution upon further dilution with NANOpure® water. These spiked recovery samples were then processed and analyzed in the same manner as the dosing samples at similar concentrations.

2. Dosing Solution Treatment

Each dosing sample (1 mL) was initially diluted with NANOpure® water to a nominal concentration of 0.3, 1, 10, and 30 ppm APFO for the 0.03, 0.1, 1, and 3 mg/mL dosing samples, respectively. The samples were further diluted to a final expected concentration of 0.03 ppm with NANOpure® water for analysis. The 0 mg/mL sample followed the 0.03 mg/mL sample dilutions. Before the final dilution, the internal standard (1, 2-di-13C PFOA) was added to each sample to give an equivalent final concentration of the internal standard in all dosing samples; the 0.1, 1, and 3 mg/mL samples were matrix corrected with the initial diluted solution of the control sample.

3. Chromatographic Conditions

LC Parameters

Instrument: Agilent (Hewlett-Packard) 1100 liquid chromatograph
Column: Zorbax® RX-C8, 2.1 x 150 mm, 5 µm
Flow Rate: 0.4 mL/min
Oven Temperature: 35°C
Injection Volume: 20 µL
Mobile Phase: A) 0.15% Acetic acid in NANOpure® water
B) Acetonitrile
Gradient:

Time (min)	% Acetonitrile
0	5
0.9	5
1.0	80
5.0	80
5.1	5
7.0	5

MS Parameters

Instrument: Waters (Micromass) Quattro Micro
Ionization Mode: Electrospray (ESI), negative ion
Capillary Voltage: 2.7 kV
Cone Voltage: 15 V
Source Temperature: 120°C
Desolvation Temperature: 350°C
Scan Function: PFOA: 413 m/z (parent) to 369 m/z (daughter)
1, 2-di-13C PFOA: 415 m/z (parent) to 370 m/z (daughter)

4. Calibration and Quantitation

The analytical reference of APFO (H-22703-376, 100%) was used for quantitation of this study. A stock solution was prepared in NANOpure® water. This stock solution was mixed to ensure that all material was dissolved in solution. Before analysis, appropriate aliquots of the stock solution were diluted with NANOpure® water to make calibration standards that bracketed the target concentration of the diluted dosing samples after matrix correction with the initial diluted solution of the control sample. Before these aliquots were brought to the final volume, an

appropriate amount of 1, 2-di-13C PFOA internal standard was added to give an equivalent final concentration of the internal standard in all standard solutions.

The 369 m/z daughter ion of PFOA dissociated from APFO measured by LC/MS/MS was used against the 370 m/z daughter ion of 1, 2-di-13C PFOA internal standard to determine the concentrations of the dosing samples. Peak area ratios (369 m/z peak versus 370 m/z peak) of these standards were used to construct a calibration curve by least square regression (see Figure 1 for a representative calibration curve). Measured concentrations for dosing solutions were determined by applying the peak area ratios from replicate injections of each sample to the calibration curve.

Concentration verification of APFO in dosing samples was evaluated by the mean result of the duplicate analyses for each respective dosing level.

Uniformity of mixing of APFO in dosing samples was evaluated by calculating the coefficient of variation (C.V. = standard deviation/mean x 100) of the measured concentration in the duplicate analyses of the concentration verification samples. A coefficient of variation of less than or equal to 10% is the standard criterion at Haskell Laboratory for acceptable distribution of the test substance throughout the solution.

Stability of APFO in dosing samples was evaluated by using the mean result of the duplicate concentration verification analyses as the baseline for comparing the corresponding stability results.

I. Body Weights

During the test period, all rats were weighed on test days 0, 3, 6, and daily thereafter.

J. Food Consumption and Food Efficiency

During the test period, the amount of food consumed by each rat over the weighing interval was determined by weighing each feeder at the beginning and end of the interval and subtracting the final weight and the amount of spillage from the feeder during the interval from the initial weight. From these measurements, mean daily food consumption over the interval was determined. From the food consumption and body weight data, the mean daily food efficiency of test substance was calculated for each animal.

K. Clinical Observations

1. Daily Animal Health Observations

Cage-site examinations to detect moribund or dead rats and abnormal behavior and/or appearance among rats were conducted at least twice daily throughout the study. Abnormal behavior/appearance was recorded by exception.

2. General Clinical Observations

An additional cage-site evaluation was conducted approximately 2 hours after dosing to detect acute clinical signs of systemic toxicity.

3. Detailed Clinical Observations

At every weighing, each rat was individually handled and examined for abnormal behavior and appearance. Detailed clinical observations in a standardized arena were also evaluated on all rats. The detailed clinical observations included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling, presence of clonic, tonic, stereotypical, or bizarre behavior. Any abnormal clinical signs noted were recorded.

L. Clinical Pathology Evaluation

A clinical pathology evaluation was conducted on all animals approximately 29 days after initiation of the study. These animals were fasted after 3 p.m. for at least 15 hours. Blood samples for hematology measurements were collected from the orbital sinus of each animal while the animal was under carbon dioxide anesthesia. Blood samples for clinical chemistry and humoral immune system evaluations were collected at sacrifice from the abdominal *vena cava* of each animal while the animal was under carbon dioxide anesthesia. Bone marrow smears were prepared at sacrifice from all surviving animals. Bone marrow smears were stained with Wright-Giemsa stain, but analysis was not necessary to support experimental findings. Blood smears, stained with new methylene blue, were prepared from each animal undergoing a hematology evaluation, but were not needed for examination. All blood samples were evaluated for quality by visual examination. Results were maintained in the study records and reported only if the sample was analyzed.

1. Hematology

Complete blood counts, including reticulocytes, were determined on a Bayer® Advia 120 hematology analyzer or determined from microscopic evaluation of the blood smear. Wright-Giemsa-stained blood smears from all animals were examined microscopically for confirmation of automated results and evaluation of cellular morphology.

The following parameters were determined:

red blood cell count
hemoglobin
hematocrit
mean corpuscular (cell) volume
mean corpuscular (cell) hemoglobin
mean corpuscular (cell) hemoglobin concentration
red cell distribution width
absolute reticulocyte count
platelet count
white blood cell count
differential white blood cell count
microscopic blood smear examination

2. Clinical Chemistry

Routine serum clinical chemistry parameters were determined on an Olympus® AU640 clinical chemistry analyzer. Serum corticosterone was measured using a commercial RIA assay (Diagnostic Products Corporation, Los Angeles, CA; Catalog #TKRC1). Corticosterone concentrations were determined according to the manufacturer's recommended procedure (aspirating aqueous contents of the assay tube rather than decanting). If necessary, the standard curve was extended at the low end of the range by including standards of 5 and 10 ng/mL.

The following parameters were determined:

cholesterol	globulin (calculated)
triglycerides	high-density lipoprotein cholesterol
total protein	non-high-density lipoprotein cholesterol (calculated)
albumin	serum corticosterone

M. Humoral Immune Function

On test day 23, animals were injected intravenously in the lateral tail vein with 0.5 mL of 4×10^8 SRBC/mL (Covance, Denver, Pennsylvania, U.S.A.). On test day 29, serum was collected from each rat and frozen (see L.2. Clinical Chemistry).

Humoral immune function was evaluated by examining sera from individual animals for SRBC-specific IgM levels with an enzyme-linked immunosorbent assay (ELISA).[7] The serum from each animal was assayed as 10 serial, 2-fold dilutions, with 1 replicate per dilution. The optical density (OD) of the contents of the reaction well was measured at the 405 nm wavelength with a MR 5000 Microplate Reader (Dynex Technologies). SRBC-specific serum IgM titer data were analyzed with Revelation Software Version 2.0 (Dynex Technologies). For each serum sample, a semi-log graph of the data was created and the linear portion of the curve was identified by using a log-log curve fit. A slope between -0.600 and -1.200 was obtained. The serum dilution expected to produce an OD of 0.5 was determined by regression analysis. The "titer" of each animal was defined as the reciprocal of the serum dilution that had an OD value of 0.5. If no points had an OD value of greater than or equal to 0.5, the reciprocal of the starting dilution closest to an OD value of 0.5 was used as the titer.

Sera previously collected from rats injected with SRBC and dosed for 6 days with 20 mg/kg of the known immunosuppressive agent, cyclophosphamide monohydrate, or vehicle were run concurrently with the study samples to demonstrate that the assay functioned properly. For any test samples that needed to be rerun due to a poor curve fit or slope, pooled male or female cyclophosphamide monohydrate or vehicle serum samples were concurrently run. The pooled samples consisted of equal aliquots of serum taken from either the male or female rats dosed with cyclophosphamide monohydrate or vehicle.

N. Anatomic Pathology Evaluation

After 29 days on study, all rats from each dose group (0, 0.3, 1, 10, 30, and 30/0 mg/kg body weight) were sacrificed and necropsied for evaluation of subchronic toxicity. The order of

sacrifice for scheduled deaths was stratified across groups. Rats were fasted at least 15 hours before their scheduled sacrifice.

All rats survived the duration of the study and were euthanized by carbon dioxide anesthesia and exsanguination. Gross examinations were performed and final body and organ weights were recorded.

The following tissues were collected from all 60 rats (10/sex/group) on study.

Digestive System	Nervous System
liver[a]	brain[a,c] (3 sections)
Hematopoietic System	**Musculoskeletal System**
spleen[a]	femur/knee joint
thymus[a]	sternum
popliteal lymph node	
mesenteric lymph node	Other
bone marrow[b]	gross observations

a Organs were weighed at necropsy.
b Bone marrow was collected with the femur and sternum.
c Including cerebrum, cerebellum, medulla/pons

Organ weight ratios (% final body weight, % brain weight) and group mean values for weighed organs were calculated.

All tissues were fixed in 10% neutral buffered formalin. Processed tissues were embedded in paraffin, sectioned approximately 5-6 microns thick, stained with hematoxylin and eosin (H&E), and examined microscopically by a veterinary pathologist. Microscopic findings were graded on a 4-point scale based on the severity or extent of the change (grade 1 = minimal; grade 2 = mild; grade 3 = moderate; grade 4 = severe).

All tissues collected from control (0 mg/kg) and high-dose (30 and 30/0 mg/kg) rats were processed to slides and evaluated microscopically. In addition, the following organs were examined from intermediate-dose rats in order to determine a no-observed-effect level for test substance-related microscopic findings: liver and spleen.

Gross observations (recorded at necropsy) were examined microscopically for all animals.

O. Total Cell Counts

The following procedures were used for preparation of spleen and thymus single-cell suspensions for enumeration of total cell counts from each spleen or thymus:

- The weight of the halved spleen or thymus (tissue) was documented; the half was placed in a labeled 15 mL centrifuge tube containing 5 mL Hank's Balanced Salt Solution (HBSS/H) and put on ice.

- The halved tissue/HBSS/H was poured into a small petri dish and cut into small pieces.
- The tissue/HBSS/H was poured into a Stomacher 80 Lab System® bag and placed into the Stomacher 80 Lab System® on "high" setting for 120 seconds (spleen) or 60 seconds (thymus).
- After the Stomacher 80 Lab System® stopped, the cell suspension was pipetted back into the original centrifuge tube, rinsing the bag with 3 mL HBSS/H and adding that to the centrifuge tube.
- The centrifuge tube was inverted 2 or 3 times and left on ice for approximately 10 minutes to allow debris to settle to the bottom of the tube.
- The supernatant was transferred to a new centrifuge tube and the volume of the supernatant was documented.
- Total cell counts were determined from each tissue by hemacytometer.

P. Electron Microscopy Evaluation

A section of liver from 2 control rats (105 and 106) and 2 rats from the 30 mg/kg group (905 and 906) was placed in cassettes, in a container of formalin, and shipped to Experimental Pathology Laboratories, Inc (EPL®) and evaluated by transmission electron microscopy. As a subcontractor to EPL®, the Laboratory for Advanced Electron and Light Optical Methods, College of Veterinary Medicine, North Carolina State University processed the tissues for electron microscopy and prepared electron photomicrographic images under the direction of Dr. Michael Dykstra. The printed electron photomicrographic images were provided to EPL® for evaluation by an ACVP-certified veterinary pathologist who interpreted the images and prepared a final report of the electron microscopic evaluation.

Q. Statistical Analyses

For all statistical analyses, significance was judged at $p < 0.05$. Comparisons were made of the dosed groups to the control group (Group I). Comparisons were also made between Group IX and Group XI.

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight Body Weight Gain Food Consumption Food Efficiency Humoral Immune Function Data[a] Clinical Pathology Organ Weights Total Cell Counts	Levene's test for homogeneity[8] and Shapiro-Wilk test[9] for normality[b]	One-way analysis of variance[10] followed by Dunnett's test[11,12,13]	Kruskal-Wallis test[14] followed by Dunn's test[15]

a SRBC-specific serum IgM antibody titer data were transformed to Log_2 to obtain normality or homogenous variances.

b If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

RESULTS AND DISCUSSION

Analytical Evaluation

A. Chromatography

(Figures 1-2)

PFOA dissociated from APFO and 1, 2-di-^{13}C PFOA eluted together from the HPLC column with a retention time of approximately 4.5 minutes. The mixture was separated into 2 resolved peaks at 369 m/z and 370 m/z, respectively, by MS/MS detection. Representative LC/MS/MS chromatograms are shown in Figures 2(a - e). Test substance was not detected in the 0 mg/mL samples.

B. Recovery Samples

(Table 1)

Detailed analytical results of recovery samples are summarized in Table 1. The variability of the analytical method was demonstrated by the coefficients of variation of the recovery results at each targeted dosing concentration (approximately 0.03, 0.1, 1, and 3 mg/mL) over the course of the study. The range of the measured concentrations of APFO for the 0.03 mg/mL level was 101.7% to 108.3% of nominal (mean percent recovery = 105.0% ± 5%, C.V. = 5%). The range of the measured concentrations of APFO for the 0.1 mg/mL level was 104.0% to 109.6% of nominal (mean percent recovery = 106.8% ± 4%, C.V. = 4%). The range of the measured concentrations of APFO for the 1 mg/mL level was 102.0% to 105.0% of nominal (mean percent recovery = 103.5 ± 2%, C.V. = 2%). The range of the measured concentrations of APFO for the 3 mg/mL level was 101.7% to 107.0% of nominal (mean percent recovery = 104.4 ± 4%, C.V. = 4%). Based on these data, the analytical method performed satisfactorily for the concentration range of the dosing samples in the study.

C. Concentration Verification, Uniformity of Mixing, and 5-Hour Room Temperature Stability Samples

(Table 2)

Analytical results from dosing solutions prepared on October 17, 2005 analyzed for concentration verification, uniformity of mixing, and 5-hour room temperature stability are shown in Table 2.

The following table summarizes the results for concentration verification, uniformity of mixing, and 5-hour room temperature stability analyses.

Preparation Date	Nominal mg/mL	Measured[a] mg/mL	Average % Nominal	C.V. (%)	Stability[b] % Nominal
17-October-2005	0	ND[c]	---	---	---
	0.03	0.0278, 0.0277	92.7	0.3	96.3
	0.1	0.0966, 0.0979	97.3	0.9	99.0
	1	0.979, 1.04, 1.03[d]	102.0	3	96.9
	3	3.16, 3.06	103.7	2	102.0

a Duplicate samples analyzed.
b Stability samples held for 5 hours at room temperature.
c Denotes not detected.
d Data obtained from one of the duplicate initial analyses and 2 repeats from the re-diluted sample.

The data for samples submitted on October 17, 2005 show that the test substance was at the targeted levels (± 7.3% of nominal), uniformly mixed (CV's = 0.3%, 0.9%, 3%, and 2%, respectively), and stable when held for 5 hours at room temperature in the vehicle. Test substance was not detected in the 0 mg/mL sample.

D. Concentration Verification and Uniformity of Mixing Samples

(Table 3)

Analytical results from dosing solutions prepared on November 15, 2005 analyzed for concentration verification and uniformity of mixing are shown in Table 3.

The following table summarizes the results for concentration verification and uniformity of mixing analyses.

Preparation Date	Nominal mg/mL	Measured[a] mg/mL	Average % Nominal	C.V. (%)
15-November-2005	0	ND[b]	---	---
	0.03	0.0276, 0.0272	91.3	1
	0.1	0.0954, 0.0986	97.0	2
	1	1.02, 1.01	102.0	0.7
	3	3.21, 3.23	107.3	0.4

a Duplicate samples analyzed.
b Denotes not detected.

The data for samples submitted on November 15, 2005 show that the test substance was at the targeted levels (± 8.7% of nominal) and uniformly mixed (CV's = 1%, 2%, 0.7%, and 0.4%, respectively). Test substance was not detected in the 0 mg/mL sample.

E. Analytical Conclusions

Data from the analysis of the samples during the study indicate that the test substance was at the targeted concentrations, mixed uniformly, and stable under the conditions of the study. Test substance was not found in the 0 mg/mL samples.

In-Life Measurements

A. Mean Body Weights and Body Weight Gains

(Tables 4-5, Figure 3, Appendix B)

Test substance related adverse reductions in mean body weights and body weight gains were observed at 10, 30 and 30/0 mg/kg. Mean final body weights were 10, 25, and 21% lower than the control group at 10, 30, and 30/0 mg/kg, respectively, as a result of reduced body weight gains; overall body weight gains during test days 0 to 28 were 26, 63 and 50% lower for the same respective doses. The magnitude and onset of the effects on body weight parameters were dose related in that the effects at 30 mg/kg were evident sooner and were more pronounced. There was no appreciable difference in the magnitude of the reduction between the 30 and 30/0 mg/kg, indicating that the shortened dosing period did not have a significant impact on this endpoint.

At 1 mg/kg, overall body weight gain during test days 0 to 28 was 10% lower, resulting in a 4% reduction in mean final body weight. These slight reductions appear to be test substance related; however, they were not statistically significant nor were they considered to be adverse.

Body weight data for animals dosed at 0.3 mg/kg were generally comparable to control group data.

B. Food Consumption and Food Efficiency

(Tables 6-7, Appendix C)

Test substance related adverse reductions in mean daily food consumption and food efficiency were observed at 10, 30 and 30/0 mg/kg; test substance-related effects on these parameters were also observed at 1 mg/kg but these effects at 1 mg/kg were not considered adverse based on the magnitude of the reductions.

Mean daily food consumption was 4, 17 and 16% lower than controls at 10, 30, and 30/0 mg/kg, respectively, during test days 0 to 28.

The combined test substance-related reductions in mean body weight, weight gain, and food consumption resulted in test substance-related reductions in food efficiency. During test days 0 to 28, mean food efficiency was 23, 57 and 42% lower at 10, 30 and 30/0 mg/kg/day.

The effects on food consumption parameters were similar to and consistent with the effects on body weight parameters in that the magnitude and onset of the effects on food consumption and efficiency were dose related terms of onset, severity, and duration of the effects. Additionally, there was no appreciable difference in the magnitude of the reduction of the overall mean daily food consumption between the 30 and 30/0 mg/kg, indicating that the shortened dosing period did not have a significant impact on this endpoint.

At 1 and 10 mg/kg, mean daily food consumption and food efficiency was 3 and 7% lower than controls, respectively. These slight reductions appear to be test substance related; however, they were not statistically significant nor were they considered to be adverse.

Food consumption and food efficiency data for animals dosed at 0.3 mg/kg were generally comparable to control group data.

C. Clinical Observations and Mortality

(Tables 8-9, Appendices D-E)

There was no test substance-related mortality at any level tested; all animals survived to the scheduled sacrifice on test day 29.

Test substance related clinical observations were observed at 30 and 30/0 mg/kg and included wet and/or stained fur, absent or decreased feces, not eating, high carriage, and lethargy. These signs were reported for up to 3 animals in these groups and thus, the incidence was not overwhelming. However, the nature of the signs combined with the effects on body weight and food consumption discussed previously supported that these observations were test substance-related and adverse. Hair loss was reported in up to 3 animals per group; this unremarkable finding was not considered test substance related since the incidence was not dose-related.

Clinical Pathology Evaluation

A. Hematology

(Table 10, Appendix F)

1. Red Blood Cells

Hemolysis was evident in serum of rats dosed with ≥1 mg/kg (see Clinical Chemistry section).

Hemoglobin and hematocrit were mildly decreased in rats dosed with 10 or 30 mg/kg for 29 days (means were 91-92% of the control group mean, respectively; statistically significant), but there were no effects on red blood cell counts. The discordance between red blood cell count and hematocrit was likely due to decreased mean cell volume in rats dosed with 10 or 30 mg/kg for 29 days (hematocrit is the product of mean cell volume and red blood cell count). Means for mean cell volumes were 97 and 95% of the control group mean; (statistically significant at 30 mg/kg). Mean cell hemoglobin, which generally closely parallels mean cell volume, was also decreased in these 2 dose groups (means were 95 and 94% of the control group mean, respectively; statistically significant).

A few rats dosed with 10 or 30 mg/kg for 29 days had increased reticulocytes, although there were no significant changes in mean reticulocyte counts (means were 109 and 112% of the control group mean). Increased red cell distribution width generally correlated with increased reticulocytes in rats from these groups. Mean red cell distribution widths were 111 and 115%, respectively, of the control group mean. Microscopically, some of the rats in these 2 groups had

increased anisocytosis (variation in red cell size; also observed in rats dosed with 1 mg/kg), macrocytosis (increased numbers of larger cells), polychromasia (increased bluish staining of red blood cells), and hypochromasia (pale staining of red blood cells). These changes were consistent with minimally increased reticulocytes in some animals.

Effects on red cell mass parameters were present (red blood cell count) or more pronounced (hemoglobin, hematocrit) in the 30/0 mg/kg group of rats compared to those dosed with 30 mg/kg for 29 days. On test day 29, mean red blood cell count, hemoglobin, and hematocrit ranged from 86-88% of the respective control group means for these 3 parameters (all statistically significant). Decreased red cell mass parameters on test day 29 could be due to one or more of the following processes: increased red cell destruction, red cell loss, or increased plasma volume. The mechanism for decreased red cell mass parameters was not evident from in-life, clinical pathology, or anatomic pathology data. Therefore, the cause of the decreased red cell mass was not determined.

Reticulocytes were moderately increased in rats dosed with 30/0 mg/kg. Mean reticulocyte count was 197% of the control group mean. Consistent with the increase in reticulocyte counts, red cell distribution width was increased (mean was 123% of the control group mean). Microscopically, this group had increased anisocytosis, macrocytosis, polychromasia, hypochromasia, and acanthocytosis (red blood cells with blunt surface projections). All morphologic changes in red cells occurred at greater incidence or at higher severity grades in these rats compared to rats dosed with APFO for 29 days. These red blood cell changes also correlated with histologic evidence of increased extramedullary hematopoiesis, which was observed in 7 of ten 30/0 mg/kg rats, but in none of the 30 mg/kg rats after 29 days of dosing.

2. White Blood Cells

White blood cell counts were minimally increased, primarily due to increases in lymphocytes, in some rats dosed with 10 or 30 mg/kg for 29 days (variable statistical significance). Means were 130 and 137% (total white blood cell count), and 133 and 140% (lymphocyte count) of respective control group means. Individual rats dosed with 10 or 30 mg/kg with higher total white blood cell and lymphocyte counts generally had higher neutrophil, monocyte, and large unstained cell (LUC) counts as well, resulting in mean neutrophil, monocyte and LUC counts that were 114-147% of the control group means. Due to the normal range and variability of total and individual white blood cell counts, these changes did not result in statistically different means. LUCs are cells that cannot be identified as one of the 5 major leukocyte types by the Advia 120 automated hematology analyzer, and normally comprise a small percentage of the total leukocyte population. The LUC count normally includes mostly lymphocytes and monocytes. Consistent with this observation, in this study, the rats with the highest LUC counts usually had the highest lymphocyte and/or monocyte counts. The changes observed in total and individual white blood cell counts are consistent with inflammation. Histologically, there were no findings observed that correlated with these white blood cell changes.

In rats that were dosed with 30/0 mg/kg, total white cell and lymphocyte counts were generally similar to their respective control group means, with the exception of a few rats with higher total white blood cell and lymphocyte counts (rats 1106 and 1107). Monocyte and large unstained cell counts for rats dosed with 30/0 mg/kg were similar to those of rats dosed for 29 days with 10

or 30 mg/kg in that the counts of most rats were similar to controls, but a few rats had higher monocyte and LUC counts. Therefore, there was no recovery.

Mean eosinophil counts were minimally decreased in rats dosed with ≥0.3 mg/kg for 29 days (not statistically significant). These decreases were the result of high eosinophil counts in 3 control rats. There was no dose response in changes in eosinophil counts despite the 100-fold difference in dose administered in either terminal or recovery animals. In rats that were dosed with 30/0 mg/kg, eosinophil counts were similar to groups dosed with ≥0.3 mg/kg for 29 days. Therefore, the apparent decreases in eosinophil counts after 29 days of dosing at ≥0.3 mg/kg and in 30/0 mg/kg rats is of uncertain relationship to treatment.

B. Clinical Chemistry

(Table 11, Appendix F)

Hemolysis was evident in serum of rats dosed with ≥1 mg/kg. Hemolysis is graded as none, trace, small, moderate or large. In this study, all samples had either none, trace, or small hemolysis. The incidence of serum graded trace to small for hemolysis was 1/10, 0/10, 3/10, 9/10, and 7/10 in rats dosed with 0, 0.3, 1, 10, or 30 mg/kg, respectively. In rats that were dosed with 30/0 mg/kg, trace to small hemolysis was observed in 6/10 rats, and the severity was similar to that observed after 29 days of dosing at 30 mg/kg.

Total cholesterol was decreased in rats dosed with 0.3 or 1 mg/kg for 29 days. Means were 64 and 69% of the control group mean, respectively (statistically significant). Cholesterol concentrations of rats dosed with 10 or 30 mg/kg, although higher than those dosed with lower doses, were still lower than controls (means were 81 and 84% of the control group mean, respectively; not statistically significant). The decreases in cholesterol were due to decreases in both HDL and non-HDL cholesterol. HDL cholesterol was decreased by a similar degree in all groups dosed with the test substance for 29 days; means were 75-79% of the control group mean (variable statistical significance). Non-HDL cholesterol, like total cholesterol, was lower in rats dosed with 0.3 or 1 mg/kg (means were 58 and 63% of control group mean; statistically significant) than in rats dosed with 10 or 30 mg/kg (means were 85 and 88% of control group mean; statistically significant).

In rats that were dosed with 30/0 mg/kg, total, HDL, and non-HDL cholesterol concentrations were similar to controls, suggesting recovery for most rats. However, total, HDL, and non-HDL cholesterol concentrations were mildly higher in one recovery rat (1103), and lower in another recovery rat (1107) compared to other animals in the 30/0 mg/kg group.

Triglyceride was decreased in rats dosed with ≥0.3 mg/kg for 29 days. The dose-response was flat across the doses tested; means were 69, 75, 68, and 66% of control group means for rats dosed with 0.3, 1, 10, or 30 mg/kg, respectively (variable statistical significance). Triglycerides were still decreased in rats that were dosed with 30/0 mg/kg (mean was 69% of the control group mean), indicating a lack of recovery for triglyceride concentrations.

Albumin was increased in a few rats dosed with 1 mg/kg, and in most rats dosed with 10 or 30 mg/kg. Means were 106, 112, and 115% of the control group mean, respectively (variable statistical significance). In rats that were dosed with 30/0 mg/kg, albumin was similar to that of

rats dosed with 30 mg/kg for 29 days (with the exception of one male, rat 1109, with lower albumin). Mean concentration for rats dosed with 30/0 mg/kg was 112% of the control group mean, indicating a lack of recovery for albumin concentration.

Globulin was decreased in rats dosed with 10 or 30 mg/kg. Means were both 89% of the control group mean. In rats dosed with 30/0 mg/kg, globulin was similar to that of controls (with the exception of one male, rat 1109, with low globulin), indicating recovery for globulin concentrations.

Serum corticosterone was increased in a few rats dosed with 10 or 30 mg/kg for 29 days. Concentrations greater than 300 ng/mL (approximate upper bound for corticosterone concentration in non-stressed rats) were observed in 0/10, 0/10, 0/10, 2/10, and 4/10 rats dosed with 0, 0.3, 1, 10, or 30 mg/kg, respectively. The higher corticosterone concentrations in some rats dosed with 10 or 30 mg/kg resulted in mean concentrations that were 135 and 196% of controls, respectively. These changes are indicative of physiological stress. In rats that were dosed with 30/0 mg/kg, serum corticosterone concentrations were generally similar to controls, indicating recovery.

C. Clinical Pathology Conclusions

Rats dosed with ≥0.3 mg/kg had decreased serum total, HDL, and non-HDL cholesterol, and decreased triglycerides. Rats dosed with ≥1 mg/kg had increased microscopic red cell morphologic changes and hemolyzed serum. Rats dosed with ≥10 mg/kg had decreased hemoglobin, hematocrit, mean cell volumes, and mean cell hemoglobin concentrations; increased reticulocyte counts and red cell distribution width, increased total white blood cell, neutrophil, monocyte, and LUC counts; increased serum albumin and decreased serum globulin concentrations, and increased serum corticosterone concentrations. Rats in the 30/0 mg/kg group had more pronounced red cell mass effects and red cell morphologic changes compared to those dosed with 30 mg/kg for 29 days. Parameters with complete recovery in rats dosed with 30/0 mg/kg were serum total, HDL, and non-HDL cholesterol, globulin, and corticosterone concentrations.

Immunotoxicity

A. Humoral Immune Function

(Tables 12-13, Appendices G-H)

No test substance-related evidence of immunosuppression was observed in male rats at any concentration tested; the IgM titers were generally comparable across all groups.

For the individual and pooled positive control sera, the primary humoral immune response to SRBC was decreased by 57 and 55%, respectively. Therefore, the SRBC-specific ELISA test system was valid for this study.

Anatomic Pathology Evaluation

A. Cause of Death

There were no test substance-related deaths. All 60 rats on study survived until the scheduled sacrifice on test day 29.

B. Final Body and Organ Weight Data

(Table 14, Appendix I)

Following 28-days of daily gavage administration of the test substance, there was a test substance-related decrease in final body weights and increase in liver weights. Mean final body weights were decreased at dose levels ≥10 mg/kg of the test substance. Mean liver weight parameters were increased at dose levels ≥0.3 mg/kg.

Text Table 1: Mean Absolute and Relative (% body weight) Organ Weights in Male Rats

Group:	I	III	V	VII	IX	XI
Dose (mg/kg):	0	0.3	1	10	30	30/0
Number of Rats/Sex:	10	10	10	10	10	10
Final Body Wt. (g)	423.1	419.7	410.0	377.0*	314.4*	333.8*
Liver	(10)	(10)	(10)	(10)	(10)	(10)
absolute wt. (g)	13.179	14.379	17.227*	21.469*	18.684*	16.206*^
% body wt.	3.113	3.419	4.194	5.680**	5.931**	4.849**
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
absolute wt. (g)	0.844	0.872	0.835	0.808	0.674	0.780
% body wt.	0.199	0.208	0.203	0.215	0.215	0.232
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
absolute wt. (g)	0.568	0.604	0.559	0.581	0.487	0.639^
% body wt.	0.133	0.144	0.136	0.153	0.153	0.191*^

wt. = weight; ( ) = number in parenthesis is the number of organs weighed.
Underlined values were interpreted to be test-substance related effects, as compared to control values.
* = statistically significant (Dunnett/Tamhane-Dunnett parametric pairwise test), compared to control value.
** = statistically significant (Dunn's non-parametric pairwise test), compared to control value.
^ = statistically significant (Dunn's non-parametric pairwise test) change in Group XI value compared to Group IX value.

1. Final Body Weight

Mean final body weights were decreased 11%, 26%, and 21% in the 10, 30, and 30/0 mg/kg dose groups, respectively, as compared to the control value. All decreases were statistically significant. Mean final body weights in the 0.3 and 1 mg/kg dose groups were similar to the control values.

There was a small, statistically insignificant increase in the mean final body weight of the 30/0 mg/kg dose group, as compared to the 30 mg/kg dose group. This increase suggests partial recovery from the test substance-related final body weight decrease in the 6 recovery days following the injection of sheep red blood cells.

2. Liver

Mean absolute liver weights were increased 9%, 31%, 63%, 42%, and 23% in the 0.3, 1, 10, 30, and 30/0 mg/kg dose groups, respectively, as compared to the control value. Mean relative (% body weight) liver weights were similarly increased (10%, 35%, 82%, 91%, and 56%, respectively). All increases were statistically significant, except for those in the 0.3 mg/kg dose group and the mean relative liver weight in the 1 mg/kg dose group.

The increased liver weights, at all dose levels, correlated with the microscopic finding of minimal to moderate hepatocellular hypertrophy. It also correlated with the gross observation of liver discoloration in a few rats at doses ≥10 mg/kg.

3. Other

All other individual and mean organ weight differences were considered to be spurious or secondary to the decrease in final body weights. Mean relative brain weights (% body weight) were increased only at doses (≥10 mg/kg) that produced significantly decreased body weights. Similarly, small, statistically insignificant, decreases in mean absolute, and increases in mean relative (% body weight), spleen and thymus weights were interpreted to be secondary to changes in final body weights. The lack of any gross or microscopic effects in the brain, spleen, and thymus further suggests that these organ weight differences were a function of body weight and not organ-specific effects.

C. Gross Observations

(Table 15, Appendix J)

At the terminal sacrifice, test substance-relate gross observations were limited to discoloration of the liver in a few rats at doses ≥10 mg/kg.

Text Table 2: Incidences of Test Substance-Related Gross Observations in Male Rats

Group:	I	III	V	VII	IX	XI
Dose (mg/kg):	0	0.3	1	10	30	30/0*
Number of Rats/Group:	10	10	10	10	10	10
Liver						
Discoloration	0	0	0	1	2	1

Underlined values were interpreted to be test-substance related increases, as compared to control values.
* Not dosed with test substance following immunology challenge.

The gross liver discoloration observed in the 4 rats given ≥10 mg/kg of the test compound was considered to be a result of the microscopic finding of hepatocellular hypertrophy.

D. Microscopic Findings

(Table 16, Appendix J)

Microscopic examination of the liver demonstrated minimal to mild hepatocellular hypertrophy at 0.3 and 1 mg/kg and moderate hepatocellular hypertrophy at ≥10 mg/kg.

Microscopic examination of lymphohematopoietic organs (spleen, thymus, bone marrow, lymph nodes) revealed increased hematopoiesis in the spleen of high-dose recovery rats (30/0 mg/kg).

The thymus, mesenteric lymph nodes and popliteal lymph nodes had no test substance-related effects.

Text Table 3: Incidences of Test Substance-Related Microscopic Findings in Male Rats

Group:	I	III	V	VII	IX	XI
Dose (mg/kg):	0	0.3	1	10	30	30/0*
Number of Rats/Group:	10	10	10	10	10	10
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hypertrophy, hepatocellular	0	5 [1.0]	10 [1.7]	10 [3.0]	10 [3.0]	10 [3.0]
Necrosis, focal	0	0	0	1 [1.0]	4 [1.0]	1 [1.0]
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
EMH, increased	0	0	1 [1.0]	0	0	7 [1.3]

[ ] = Number in brackets is the average grade (grades 1 – 4) when lesion is present (i.e., sum of grades ÷ # animals with lesion). Grading scale: 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
( ) = number in parenthesis is the number of organs examined; EMH = Extramedullary hematopoiesis. Underlined values were interpreted to be test-substance related increases, as compared to control values.
* Not dosed with test substance following immunology challenge.

1. Liver

a. Hepatocellular hypertrophy

Panlobular hepatocellular hypertrophy was observed in all but 5 of the rats given the test substance and the incidence and severity were dose related. Hypertrophy was present in 0/10, 5/10, 10/10, 10/10, 10/10, and 10/10 rats given 0, 0.3, 1, 10, 30, and 30/0 mg/kg, respectively. The hypertrophy was graded as minimal in the 5 affected rats given 0.3 mg/kg, minimal in 3/10 and mild in 7/10 rats given 1 mg/kg, and moderate in all rats given ≥10 mg/kg.

The hepatocellular hypertrophy was characterized by an increase in the size of all hepatocytes due to an increase in cytoplasmic volume. The cytoplasm had a uniformly eosinophilic granular appearance consistent with peroxisome proliferation.

Hepatocellular hypertrophy correlated with increased mean liver weight parameters at all doses. Although the 30/0 mg/kg group still had moderate hypertrophy (grade 3 of 4), the decrease in mean liver weights, relative to the 30 mg/kg group suggests that there was some hepatocellular shrinkage and/or loss that was microscopically unapparent.

b. Focal necrosis

Focal necrosis was also observed in several rats given ≥10 mg/kg of the test substance. The incidence was mildly dose related. Focal necrosis was present in 0/10, 0/10, 0/10, 1/10, 4/10, and 1/10 rats given 0, 0.3, 1, 10, 30, and 30/0 mg/kg, respectively. All were graded minimal. A decrease in the incidence was apparent in the 30/0 mg/kg group, as compared to the 30 mg/kg group.

Focal necrosis was characterized by the focal or multifocal coagulative necrosis of a cluster of hepatocytes. The distribution was usually subcapsular and the pattern was non-zonal. Focal coagulative necrosis of hepatocytes clusters is a common secondary effect of hepatocellular hypertrophy and is most likely the result of secondary focal ischemia.

2. Spleen

a. Increased extramedullary hematopoiesis

An increase in the incidence of splenic extramedullary hematopoiesis (EMH) was considered test substance related only in high-dose rats allowed a recovery period (30/0 mg/kg). Minimal to mild increased EMH was observed in 0/10, 0/10, 1/10, 0/10, 0/10, and 7/10 rats given 0, 0.3, 1, 10, 30, and 30/0 mg/kg, respectively.

The increased splenic EMH in the high-dose recovery rats was erythrocytic and correlated with the hematology findings, which included decreased red cell mass parameters and increased circulating reticulocytes (see Clinical Pathology).

3. Other

All other microscopic observations in this study were consistent with normal background lesions in rats of this age and strain.

E. Anatomic Pathology Conclusions

There were no test substance-related deaths. All 60 rats on study survived until the scheduled sacrifice on test day 29.

Following 28-days of daily gavage administration of the test substance, there was a test substance-related decrease in final body weights and increase in liver weights. Mean final body weights were decreased at dose levels ≥10 mg/kg of the test substance. Mean liver weight parameters were increased at dose levels ≥0.3 mg/kg.

At the terminal sacrifice, test substance-related gross observations were limited to discoloration of the liver in a few rats at doses ≥10 mg/kg.

Microscopic examination of the liver demonstrated minimal to mild hepatocellular hypertrophy at 0.3 and 1 mg/kg and moderate hepatocellular hypertrophy at ≥10 mg/kg.

Microscopic examination of lymphohematopoietic organs (spleen, thymus, bone marrow, lymph nodes) revealed increased hematopoiesis in the spleen of rats dosed with 30/0 mg/kg.

The thymus, mesenteric lymph nodes and popliteal lymph nodes had no test substance-related effects.

Total Cell Counts

A. Spleen Cell Number

(Table 17, Appendix K)

No significant changes in total spleen cell number compared to control rats were noted in any animal treated with any dose of APFO. A 10% increase was observed at 10 mg/kg and a 16% decrease was observed at 30 mg/kg, but neither value was statistically different than vehicle control.

B. Thymus Cell Number

(Table 17, Appendix K)

No significant changes in total thymocyte number compared to control rats were noted in any animals treated with any dose of APFO. For rats in the 30/0 mg/kg group, an increase in thymocyte number was observed, which was statistically greater than rats who continued to receive 30 mg/kg APFO, but not greater when compared to vehicle control.

CONCLUSIONS

Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for APFO for systemic toxicity in male rats was less than 0.3 mg/kg, whereas the NOAEL for immunotoxicity was 30 mg/kg.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

Laboratory-specific raw data such as personnel files, instrument, equipment, refrigerator and/or freezer raw data will be retained at the facility where the work was done.

REFERENCES

1. Vanden Heuvel, J., Kuslikis, B., and Van Rafelghem, M. (1991). Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *Biochem. Toxicol.* **6**, 83-92.

2. Taves, D., Guy, W., and Brey, W. (1976). Organic fluorocarbons in human plasma: prevalence and characterization. *Biochemistry Involving Carbon-Fluorine Bonds* (R. Filler, Ed.), pp. 117-134. American Chemical Society, Washington, D.C.

3. Hansen, K.J., Clemen, L.A., Ellefson, M.E., and Johnson, H.O. (2001). Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environ. Sci. Technol.* **35**, 766-770.

4. Olsen, G.W., Burris, J.M., Burlew, M.M., and Mandel, J.H. (2000). Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. *Drug Chem. Toxicol.* **23**, 603 620.

5. Yang, Q., Abedi-Valugerdi, M., Xie, Y., Zhao, X., Möller, G., Nelson, B.D., and DePierre, J.W. (2002). Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferators, perfluorooctanoic acid. *International Immunopharmacology*, **2 (2002)**, 389-397.

6. DuPont Haskell Laboratory (2005). APFO (Linear/Branched), APFO (Linear), and APFO (Branched): 14-Day Oral Gavage Study in Male Rats and Mice. Unpublished report, DuPont-14162.

7. Temple, L., Kawabata, T.T., Munson, A.E., and White, K.L., Jr. Comparison of ELISA and plaque-forming cell assays for measuring the humoral immune response to SRBC in rats and mice treated with benzo(a)pyrene or cyclophosphamide. *Fundam. Appl. Toxicol.* **1993:21**, 412-419.

8. Levene, H. (1960). Robust test for equality of variances. *Contributions to Probability and Statistics* (J. Olkin, ed.), pp 278-292. Stanford University Press, Palo Alto.

9. Shapiro, S.S. and Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). *Biometrika* **52**, 591-611.

10. Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*, 6th edition, pp 246-248 and 349-352. The Iowa State University Press, Iowa.

11. Dunnett, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics* **20**, 482-491.

12. Dunnett, C.W. (1980). Pairwise multiple comparisons in the unequal variance case. *J. Amer. Statist. Assoc.* **75**, 796-800.

13. Tamhane, A.C. (1979). A comparison of procedures for multiple comparison of means with unequal variances. *J. Amer. Statist. Assoc.* **74**, 471-480.

14. Kruskal, W.H. and Wallis, W.A. (1952). Use of ranks in one-criterion analysis of variance. *J. Amer. Statist. Assoc.* **47**, 583-621.

15. Dunn, O.J. (1964). Multiple contrasts using rank sums. *Technometrics* **6**, 241-252.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

Summary of Hematology Values

RBC - red blood cell count
HGB - hemoglobin
HCT - hematocrit
MCV - mean corpuscular (cell) volume
MCH - mean corpuscular (cell) hemoglobin
MCHC - mean corpuscular (cell) hemoglobin concentration
RDW - red cell distribution width
ARET - absolute reticulocyte count
PLT - platelet count
WBC - white blood cell count
ANEU - absolute neutrophil (all forms)
ALYM - absolute lymphocyte
AMON - absolute monocyte
AEOS - absolute eosinophil
ABAS - absolute basophil
ALUC - absolute large unstained cell

Summary of Clinical Chemistry Values

CHOL - cholesterol
TRIG - triglycerides
TP - total protein
ALB - albumin
GLOB - globulin
HDL - high-density lipoprotein cholesterol
NHDL - non-high-density lipoprotein cholesterol
SCORT - serum corticosterone

NOTES:

Summary of Hematology Values
Summary of Clinical Chemistry Values

Groups with identical values may vary in statistical significance, because tabulated statistics are rounded to fewer decimal places than the values used for statistical determination.

TABLES

EXPLANATORY NOTES (Continued)

NOTES: (Continued)

Summary of Total Cell Counts

$$\text{Organ Weight as Percent of Body Weight} = \frac{\text{Organ Weight (g)}}{\text{Final Body Weight (g)}} \times 100$$

$$\text{Total Number of Organ Cells } (\times 10^8) = \frac{\text{Organ Weight (g)}}{\text{Half Organ Weight (g)}} \times \text{Organ Cell Suspension Volume (mL)} \times \text{Number of Cells in Half Organ } (\times 10^6 \text{ cells/mL}) \div 100$$

Table 1
Recovery of APFO Added to Dosing Vehicle

Sample Type	APFO (mg/mL)		Percent
	Nominal	Measured	Nominal
RECOVERY[A]	0.0302	0.0327	108.3
RECOVERY[B]	0.0300	0.0305	101.7
		Mean	***105.0 ± 5, C.V. 5%***
RECOVERY[A]	0.104	0.114	109.6
RECOVERY[B]	0.100	0.104	104.0
		Mean	***106.8 ± 4, C.V. 4%***
RECOVERY[A]	1.00	1.02	102.0
RECOVERY[B]	1.00	1.05	105.0
		Mean	***103.5 ± 2, C.V. 2%***
RECOVERY[A]	3.00	3.05	101.7
RECOVERY[B]	3.00	3.21	107.0
		Mean	***104.4 ± 4, C.V. 4%***

(A) Processed with dosing samples submitted October 17, 2005 for concentration verification, uniformity of mixing, and 5-hour room temperature stability analyses.
(B) Processed with dosing samples submitted November 15, 2005 for concentration verification and uniformity of mixing analyses.

Table 2
Concentration Verification, Uniformity of Mixing, and 5-Hour Room Temperature Stability of APFO in Dosing Solutions

Sample Date Sample Type[A]	APFO (mg/mL) Nominal		 Measured	Percent Nominal
15-November-2005				
Concentration Verification				
Control	0		ND[B]	----
#1	0.03		0.0278	92.7
#2	0.03		0.0277	92.3
		Mean:	***0.0278 ± 0.0001***	***(92.7)***
			C.V. 0.3%	
#1	0.1		0.0966	96.6
#2	0.1		0.0979	97.9
		Mean:	***0.0973 ± 0.0009***	***(97.3)***
			C.V. 0.9%	
#1	1		0.979	97.9
#1[C]	1		1.04	104.0
#2[C]	1		1.03	103.0
		Mean:	***1.02 ± 0.03***	***(102.0)***
			C.V. 3%	
#1	3		3.16	105.3
#2	3		3.06	102.0
		Mean:	***3.11 ± 0.07***	***(103.7)***
			C.V. 2%	
Stability[D]				
	0.03		0.0289	96.3
	0.1		0.0990	99.0
	1		0.969	96.9
	3		3.06	102.0

(A) Duplicate analyses per level performed for concentration verification. Mean, S.D. and C.V. calculated to verify uniformity of mixing.
(B) Denotes not detected.
(C) Duplicate analyses from the re-diluted sample.
(D) Samples held at room temperature for 5 hours.

Table 3
Concentration Verification and Uniformity of Mixing of APFO in Dosing Solutions

Sample Type[A] Sample Date	APFO (mg/mL) Nominal		Measured	Percent Nominal
Concentration Verification 11-October-2005				
Control	0		ND[B]	----
#1	0.03		0.0276	92.0
#2	0.03		0.0272	90.7
		Mean:	***0.0274 ± 0.0003***	***(91.3)***
			C.V. 1%	
#1	0.1		0.0954	95.4
#2	0.1		0.0986	98.6
		Mean:	***0.0970 ± 0.002***	***(97.0)***
			C.V. 2%	
#1	1		1.02	102.0
#2	1		1.01	101.0
		Mean:	***1.02 ± 0.008***	***(102.0)***
			C.V. 0.7%	
#1	3		3.21	107.0
#2	3		3.23	107.7
		Mean:	***3.22 ± 0.01***	***(107.3)***
			C.V. 0.4%	

(A) Duplicate analyses per level performed for concentration verification. Mean, S.D. and C.V. calculated to verify uniformity of mixing.
(B) Denotes not detected.

Table 4
Mean Body Weights of Male Rats

DAYS ON TEST	MEAN BODY WEIGHTS (g)					
	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
0	269.1 11.6(10)	270.3 11.3(10)	270.6 13.7(10)	270.5 14.1(10)	271.3 8.9(10)	268.4 8.7(10)
7	329.8 15.8(10)	328.5 15.2(10)	328.2 18.4(10)	314.7 17.9(10)	278.0@ 37.4(10)	275.0@ 47.0(10)
14	378.8 20.1(10)	375.0 20.5(10)	373.7 25.5(10)	358.0 21.2(10)	310.7@ 28.3(10)	298.5@ 49.9(10)
21	424.0 24.4(10)	419.7 24.4(10)	412.6 34.5(10)	387.5 30.1(10)	322.0* 38.0(10)	322.2* 45.4(10)
28	453.4 26.5(10)	446.6 30.7(10)	437.2 38.7(10)	407.5* 34.8(10)	339.6* 36.1(10)	359.8* 39.8(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

* Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
@ Statistically significant difference from control at $p < 0.05$ by Dunn's test.

NOTE: All data from groups III, V, VII, IX and XI were compared with the control (I) group data. In addition, data from group IX were compared with data from group XI; no significant differences between IX and XI were detected.

Table 5
Mean Body Weight Gains of Male Rats

	MEAN BODY WEIGHT GAINS (g)					
DAYS ON TEST	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
0-7	60.7 7.8(10)	58.2 9.4(10)	57.6 8.7(10)	44.2 10.2(10)	6.7@ 35.4(10)	6.6@ 45.0(10)
7-14	49.0 8.5(10)	46.5 7.2(10)	45.5 7.5(10)	43.3 10.0(10)	32.7 19.5(10)	23.5* 16.9(10)
14-21	45.3 8.6(10)	44.7 6.6(10)	38.9 10.7(10)	29.5* 9.5(10)	11.3* 13.6(10)	23.7*† 11.9(10)
21-28	29.3 3.9(10)	26.9 7.4(10)	24.7 9.5(10)	20.0 7.7(10)	17.6@ 8.8(10)	37.5† 19.0(10)
OVERALL 0-28	184.3 21.2(10)	176.3 25.7(10)	166.6 28.6(10)	137.1* 30.9(10)	68.3* 34.3(10)	91.4* 38.3(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

* Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
@ Statistically significant difference from control at $p < 0.05$ by Dunn's test.
† Statistically significant difference from Group IX at $p < 0.05$ by Dunn's test.

NOTE: All data from groups III, V, VII, IX and XI were compared with the control (I) group data. In addition, data from group IX were compared with data from group XI; significant differences between IX and XI were detected.

Table 6
Mean Daily Food Consumption by Male Rats

DAYS ON TEST	MEAN DAILY FOOD CONSUMED PER ANIMAL (g)					
	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
7	28.8 1.8(10)	28.0 2.1(10)	28.5 2.2(10)	26.4 1.6(10)	20.1@ 6.7(10)	20.9@ 6.6(10)
14	29.2 2.3(10)	28.7 2.6(10)	28.7 2.7(10)	29.1 2.0(10)	27.9 3.6(10)	25.3* 4.8(10)
21	30.0 2.1(10)	29.2 2.6(10)	28.5 3.0(10)	29.2 2.7(10)	24.0* 5.2(10)	25.5* 2.7(10)
28	30.2 1.7(10)	29.7 2.7(10)	28.5 2.7(10)	29.1 2.4(10)	26.5* 2.0(10)	27.0* 2.7(10)
OVERALL 0-28	29.5 1.8(10)	28.9 2.4(10)	28.6 2.5(10)	28.4 2.0(10)	24.6* 3.2(10)	24.7* 2.9(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

* Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
@ Statistically significant difference from control at $p < 0.05$ by Dunn's test.

NOTE: All data from groups III, V, VII, IX and XI were compared with the control (I) group data. In addition, data from group IX were compared with data from group XI; no significant differences between IX and XI were detected.

Table 7
Mean Daily Food Efficiency of Male Rats

	MEAN DAILY FOOD EFFICIENCY (g weight gain/g food consumed)					
DAYS ON TEST	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
0-7	0.301 0.029(10)	0.296 0.035(10)	0.287 0.029(10)	0.238 0.047(10)	-0.070@ 0.401(10)	-0.209@ 1.003(10)
14	0.240 0.033(10)	0.231 0.024(10)	0.226 0.025(10)	0.211 0.039(10)	0.164 0.096(10)	0.127* 0.081(10)
21	0.215 0.033(10)	0.218 0.022(10)	0.192 0.037(10)	0.143* 0.038(10)	0.054* 0.080(10)	0.135* 0.078(10)
28	0.139 0.018(10)	0.128 0.027(10)	0.122 0.038(10)	0.096@ 0.032(10)	0.096@ 0.049(10)	0.193† 0.076(10)
OVERALL 0-28	0.222 0.018(10)	0.217 0.019(10)	0.207 0.019(10)	0.171* 0.029(10)	0.096* 0.042(10)	0.129*† 0.044(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

* Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
@ Statistically significant difference from control at $p < 0.05$ by Dunn's test.
† Statistically significant difference from Group IX at $p < 0.05$ by Dunn's test.

NOTE: All data from groups III, V, VII, IX and XI were compared with the control (I) group data. In addition, data from group IX were compared with data from group XI; significant differences between IX and XI were detected.

Table 8
Summary of Daily Animal Health Observations in Male Rats

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group IX 30/0 mg/kg (Recovery)
ANIMAL COUNT:	10	10	10	10	10	10
Wet Fur	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
Feces Absent	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	1 (10%)
Decreased Feces	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (20%)	1 (10%)
Not Eating	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (30%)	2 (20%)
Stain Fur/Skin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)

Data arranged as: number of animals (percent of group) for which an observation was recorded

Table 9
Summary of Detailed Clinical Observations in Male Rats

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group IX 30/0 mg/kg (Recovery)
ANIMAL COUNT:	10	10	10	10	10	10
Wet Fur	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
Carriage, High	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
Feces Absent	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
Hair Loss	1 (10%)	0 (0%)	1 (10%)	1 (10%)	3 (30%)	0 (0%)
Lethargic	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
Not Eating	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
Stain Fur/Skin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)

Data arranged as: number of animals (percent of group) for which an observation was recorded

49

p. 48

Table 10
Summary of Hematology Values for Male Rats

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
RBC ($x10^6/\mu L$)						
DAY 29	7.66	7.61	7.60	7.28	7.47	6.75*#
	0.20(9)	0.33(10)	0.32(10)	0.50(10)	0.60(10)	0.34(10)
HGB (g/dL)						
DAY 29	14.9	14.7	14.7	13.5@	13.6@	12.8@#
	0.3(9)	0.5(10)	0.6(10)	0.7(10)	0.7(10)	0.3(10)
HCT (%)						
DAY 29	46.2	45.4	45.6	42.6*	42.7*	40.2*#
	1.0(9)	1.5(10)	1.8(10)	2.3(10)	2.0(10)	1.2(10)
MCV (fL)						
DAY 29	60.3	59.7	60.1	58.5	57.3*	59.6
	2.0(9)	2.0(10)	2.2(10)	2.3(10)	2.4(10)	2.8(10)
MCH (pg)						
DAY 29	19.5	19.3	19.4	18.6*	18.3*	19.0
	0.5(9)	0.6(10)	0.7(10)	0.8(10)	0.7(10)	0.9(10)
MCHC (g/dL)						
DAY 29	32.3	32.4	32.2	31.8	31.9	31.8
	0.5(9)	0.3(10)	0.4(10)	0.5(10)	0.5(10)	0.6(10)

Table 10
Summary of Hematology Values for Male Rats (Continued)

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
RDW (%)						
DAY 29	11.5	11.4	11.7	12.8@	13.2@	14.2@
	0.3(9)	0.5(10)	0.3(10)	0.7(10)	0.8(10)	2.1(10)
ARET ($x10^3/\mu L$)						
DAY 29	187.3	170.5	177.5	204.5	208.9	369.8@#
	16.5(9)	19.7(10)	24.1(10)	46.2(10)	40.8(10)	88.6(10)
PLT ($x10^3/\mu L$)						
DAY 29	1090	1058	1094	1044	1196	1207
	125(6)	76(10)	111(6)	344(7)	174(7)	162(8)
WBC ($x10^3/\mu L$)						
DAY 29	12.49	11.41	13.28	16.26	17.07*	13.91
	3.48(9)	2.83(10)	2.83(10)	2.69(10)	2.93(10)	4.42(10)
ANEU ($x10^3/\mu L$)						
DAY 29	1.46	1.38	1.55	1.66	1.79	1.46
	0.58(9)	0.49(10)	0.71(10)	0.53(10)	0.59(10)	0.43(10)
ALYM ($x10^3/\mu L$)						
DAY 29	10.40	9.56	11.19	13.87*	14.53*	11.81
	2.80(9)	2.54(10)	2.47(10)	2.41(10)	2.67(10)	4.07(10)

Table 10
Summary of Hematology Values for Male Rats (Continued)

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
AMON ($x10^3/\mu L$)						
DAY 29	0.25	0.22	0.26	0.33	0.35	0.29
	0.09(9)	0.09(10)	0.12(10)	0.17(10)	0.15(10)	0.11(10)
AEOS ($x10^3/\mu L$)						
DAY 29	0.17	0.08	0.09	0.13	0.11	0.10
	0.11(9)	0.04(10)	0.04(10)	0.07(10)	0.07(10)	0.06(10)
ABAS ($x10^3/\mu L$)						
DAY 29	0.05	0.05	0.07	0.07	0.07	0.06
	0.02(9)	0.03(10)	0.04(10)	0.04(10)	0.04(10)	0.05(10)
ALUC ($x10^3/\mu L$)						
DAY 29	0.15	0.11	0.13	0.20	0.22	0.19
	0.08(9)	0.03(10)	0.04(10)	0.11(10)	0.12(10)	0.17(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

* Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
@ Statistically significant difference from control at $p < 0.05$ by Dunn's test.
\# Statistically significant difference from Group IX at $p < 0.05$ by t-test.

NOTE: All data from groups III, V, VII, IX and XI were compared with the control (I) group data. In addition, data from group IX were compared with data from group XI; significant differences between IX and XI were detected.

Table 11
Summary of Clinical Chemistry Values for Male Rats

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
CHOL (mg/dL)						
DAY 29	64	41@	44@	52	54	73†
	17(10)	10(10)	8(10)	10(10)	9(10)	23(10)
TRIG (mg/dL)						
DAY 29	68	47@	51	46@	45@	47@
	19(10)	17(10)	20(10)	15(10)	11(10)	16(10)
TP (g/dL)						
DAY 29	6.1	6.1	6.2	6.1	6.2	6.5
	0.2(10)	0.2(10)	0.3(10)	0.4(10)	0.3(10)	0.5(10)
ALB (g/dL)						
DAY 29	3.3	3.4	3.5	3.7@	3.8@	3.7@
	0.1(10)	0.1(10)	0.2(10)	0.2(10)	0.1(10)	0.3(10)
GLOB (g/dL)						
DAY 29	2.8	2.8	2.7	2.5*	2.5*	2.7#
	0.1(10)	0.2(10)	0.2(10)	0.2(10)	0.3(10)	0.2(10)
HDL (mg/dL)						
DAY 29	24	18*	19*	18*	19	25#
	4(10)	3(10)	3(10)	3(10)	4(10)	5(10)

53

p. 52

Table 11
Summary of Clinical Chemistry Values for Male Rats (Continued)

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
NHDL (mg/dL)						
DAY 29	40	23@	25@	34	35	47†
	14(10)	8(10)	6(10)	7(10)	5(10)	18(10)
SCORT (ng/mL)						
DAY 29	137	167	147	185	268	131
	95(10)	73(10)	69(10)	91(10)	217(10)	90(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

* Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
@ Statistically significant difference from control at $p < 0.05$ by Dunn's test.
† Statistically significant difference from Group IX at $p < 0.05$ by Dunn's test.
\# Statistically significant difference from Group IX at $p < 0.05$ by t-test.

NOTE: All data from groups III, V, VII, IX and XI were compared with the control (I) group data. In addition, data from group IX were compared with data from group XI; significant differences between IX and XI were detected.

Table 12
Summary of Primary Humoral Immune Response to SRBC for Male Rats Dosed with APFO

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
LOG_2[a]	10.060 1.503(10)	10.368 0.466(10)	9.858 1.503(10)	9.917 1.928(10)	9.906 1.142(10)	9.519 1.198(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

a Mean log_2 of the serum IgM titer data.

There were no statistically significant differences from control at $p < 0.05$.

55

Table 13
Summary of Primary Humoral Immune Response to SRBC for Male Rats Dosed With Positive Control

	Saline[a]	Cyclophosphamide 20 mg/kg[a]	Cyclophosphamide 20 mg/kg[b]
LOG_2	9.456 1.147(10)	4.098 0.978(10)	4.241 0.872(2)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

a Mean log_2 of the SRBC-specific serum IgM titer data for individual samples.
b Log_2 of the SRBC-specific serum IgM titer data for pooled samples.

p. 54

Table 14
Mean Final Body and Organ Weights from Male Rats

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
MEAN FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams)						
LIVER	13.179 1.397(10)	14.379 1.604(10)	17.227* 2.860(10)	21.469* 2.864(10)	18.684* 2.866(10)	16.206*† 2.170(10)
SPLEEN	0.844 0.167(10)	0.872 0.209(10)	0.835 0.144(10)	0.808 0.126(10)	0.674 0.085(10)	0.780 0.182(10)
THYMUS	0.568 0.126(10)	0.604 0.123(10)	0.559 0.121(10)	0.581 0.134(10)	0.487 0.171(10)	0.639† 0.110(10)
BRAIN	2.012 0.088(10)	2.111 0.117(10)	2.086 0.087(10)	1.999 0.123(10)	1.992 0.108(10)	1.913 0.129(10)
FINAL BODY WEIGHT (grams)	423.1 26.0(10)	419.7 25.0(10)	410.0 35.2(10)	377.0* 32.8(10)	314.4* 35.1(10)	333.8* 36.1(10)

Table 14
Mean Final Body and Organ Weights from Male Rats (Continued)

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
MEAN RELATIVE ORGAN WEIGHTS (% of body weight)						
LIVER/ FINAL BODY * 100	3.113 0.229(10)	3.419 0.250(10)	4.194 0.524(10)	5.680@ 0.385(10)	5.931@ 0.503(10)	4.849@ 0.345(10)
SPLEEN/ FINAL BODY * 100	0.199 0.033(10)	0.208 0.047(10)	0.203 0.021(10)	0.215 0.030(10)	0.215 0.020(10)	0.232 0.039(10)
THYMUS/ FINAL BODY * 100	0.133 0.024(10)	0.144 0.028(10)	0.136 0.024(10)	0.153 0.029(10)	0.153 0.046(10)	0.191*† 0.019(10)
BRAIN/ FINAL BODY * 100	0.477 0.028(10)	0.504 0.030(10)	0.511 0.038(10)	0.533* 0.044(10)	0.639* 0.059(10)	0.577*† 0.052(10)

57

p. 56

Table 14
Mean Final Body and Organ Weights from Male Rats (Continued)

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
MEAN RELATIVE ORGAN WEIGHTS (% of brain weight)						
LIVER/ BRAIN * 100	654.981 61.796(10)	681.399 71.841(10)	825.207* 128.554(10)	1073.082* 119.548(10)	937.602* 129.373(10)	846.704* 95.980(10)
SPLEEN/ BRAIN * 100	41.814 7.212(10)	41.120 8.849(10)	39.940 6.035(10)	40.554 6.671(10)	33.805 3.600(10)	40.732† 8.641(10)
THYMUS/ BRAIN * 100	28.191 5.790(10)	28.563 5.440(10)	26.750 5.543(10)	29.072 6.473(10)	24.424 8.255(10)	33.319† 4.511(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

* Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
@ Statistically significant difference from control at $p < 0.05$ by Dunn's test.
† Statistically significant difference from Group IX at $p < 0.05$ by Dunn's test.

NOTE: All data from groups III, V, VII, IX and XI were compared with the control (I) group data. In addition, data from group IX were compared with data from group XI; significant differences between IX and XI were detected.

Table 15
Incidence of Gross Observations in Male Rats

LESIONS	TREATMENT per day	LESION INCIDENCE (Numeric) 0 mg/kg I	0.3 mg/kg III	1 mg/kg V	10 mg/kg VII	30 mg/kg IX	30/0 mg/kg XI Recovery
LIVER		(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	9	8	9
LARGE						1	
DISCOLORATION					1	2	1
SPLEEN		(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10	10	10
THYMUS		(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10	10	10
POPLITEAL LYMPH NODE		(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10	10	10
MESENTERIC LYMPH NODE		(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10	10	10
BRAIN		(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10	10	10

Figures in parentheses are the number of animals grossly examined for this tissue
The absence of a number indicates the finding specified was not identified

Table 15
Incidence of Gross Observations in Male Rats (Continued)

LESIONS	TREATMENT per day	LESION INCIDENCE (Numeric) 0 mg/kg I	0.3 mg/kg III	1 mg/kg V	10 mg/kg VII	30 mg/kg IX	30/0 mg/kg XI Recovery
FEMUR/KNEE JOINT		(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10	10	10
STERNUM		(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10	10	10

Figures in parentheses are the number of animals grossly examined for this tissue
The absence of a number indicates the finding specified was not identified

p. 59

Table 16
Incidences and Lesion Grades of Microscopic Observations in Male Rats

LESIONS	LESION INCIDENCE (NUMERIC)						
TREATMENT per day	0 mg/kg I	0.3 mg/kg III	1 mg/kg V	10 mg/kg VII	30 mg/kg IX	30/0 mg/kg XI Recovery
LIVER	(10)	(10)	(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED	1					
NECROSIS, FOCAL.						
minimal				1	4	1
Total observations per lesion				1	4	1
MINERALIZATION, BILE DUCT.						
minimal				1		
moderate						1
Total observations per lesion				1		1
INFLAMMATION, SUBACUTE/CHRONIC.						
minimal	9	9	9	9	9	8
mild			1	1	1	2
Total observations per lesion	9	9	10	10	10	10
HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR.						
minimal		5	3			
mild			7			
moderate				10	10	10
Total observations per lesion		5	10	10	10	10
HYPERPLASIA, BILE DUCT, FOCAL.						
minimal				1		
Total observations per lesion				1		

Figures in parentheses are the number of animals microscopically examined for this tissue
The absence of a number indicates the lesion specified was not identified

p.60

Table 16
Incidences and Lesion Grades of Microscopic Observations in Male Rats (Continued)

LESIONS	TREATMENT per day	LESION INCIDENCE (NUMERIC) 0 mg/kg I	0.3 mg/kg III	1 mg/kg V	10 mg/kg VII	30 mg/kg IX	30/0 mg/kg XI Recovery
LIVER		(10)	(10)	(10)	(10)	(10)	(10)
HEMATOPOIESIS, EXTRAMEDULLARY.							
minimal							1
Total observations per lesion							1
FIBROSIS, FOCAL.							
minimal							1
Total observations per lesion							1
FATTY CHANGE, MEDIAN CLEFT.							
minimal		2					
Total observations per lesion		2					
SPLEEN		(10)	(10)	(10)	(9)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	9	9	10	3
HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED.							
minimal				1			5
mild							2
Total observations per lesion				1			7
THYMUS		(10)				(10)	(10)
NO ABNORMALITY DETECTED		10				10	10

Figures in parentheses are the number of animals microscopically examined for this tissue
The absence of a number indicates the lesion specified was not identified

p. 61

Table 16
Incidences and Lesion Grades of Microscopic Observations in Male Rats (Continued)

LESIONS	TREATMENT per day	LESION INCIDENCE (NUMERIC) 0 mg/kg I	0.3 mg/kg III	1 mg/kg V	10 mg/kg VII	30 mg/kg IX	30/0 mg/kg XI Recovery
POPLITEAL LYMPH NODE		(10)				(10)	(10)
NO ABNORMALITY DETECTED		2				7	8
NOT PRESENT IN TISSUE SECTION.		8				3	2
MESENTERIC LYMPH NODE		(10)				(10)	(10)
NO ABNORMALITY DETECTED		10				9	10
DEPLETION/ATROPHY, LYMPHOID.							
minimal						1	
Total observations per lesion						1	
BONE MARROW		(10)				(10)	(10)
NO ABNORMALITY DETECTED		9				10	9
FIBROSIS, FOCAL.							
minimal		1					1
Total observations per lesion		1					1
BRAIN		(10)				(10)	(10)
NO ABNORMALITY DETECTED		9				10	10
PIGMENT, FOCAL.							
minimal		1					
Total observations per lesion		1					

Figures in parentheses are the number of animals microscopically examined for this tissue
The absence of a number indicates the lesion specified was not identified

p. 62

Table 16
Incidences and Lesion Grades of Microscopic Observations in Male Rats (Continued)

LESIONS	LESION INCIDENCE (NUMERIC)					
TREATMENT per day	0 mg/kg I	0.3 mg/kg III	1 mg/kg V	10 mg/kg VII	30 mg/kg IX	30/0 mg/kg XI Recovery
FEMUR/KNEE JOINT						
NO ABNORMALITY DETECTED	(10) 10				(10) 10	(10) 10
STERNUM						
NO ABNORMALITY DETECTED	(10) 10				(10) 10	(10) 10

Figures in parentheses are the number of animals microscopically examined for this tissue
The absence of a number indicates the lesion specified was not identified

69

p. 63

Table 17
Summary of Total Cell Counts

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
Final Body Weight (g)	423.12 26.05(10)	419.74 24.95(10)	410.03 35.20(10)	376.95 32.76(10)	314.42 35.14(10)	333.79 36.15(10)
SPLEEN Absolute Weight (g)	0.844 0.167(10)	0.872 0.209(10)	0.835 0.144(10)	0.808 0.126(10)	0.674 0.085(10)	0.780 0.182(10)
Weight Ratio (% Body Weight)	0.1988 0.0330(10)	0.2078 0.0469(10)	0.2026 0.0210(10)	0.2146 0.0296(10)	0.2147 0.0198(10)	0.2323 0.0390(10)
Half Weight (g)	0.435 0.089(10)	0.443 0.110(10)	0.421 0.081(10)	0.416 0.062(10)	0.348 0.038(10)	0.401 0.095(10)
Cell Suspension Volume (mL)	6.9 0.5(10)	7.1 0.3(10)	7.4 0.9(10)	7.1 0.5(10)	7.5 1.4(10)	6.8 0.3(10)
Number of Cells in Half (x 10^6 cells/mL)	41.58 17.41(10)	44.22 16.99(10)	43.89 24.34(10)	45.43 16.31(10)	34.32 17.07(10)	44.44 16.55(10)
Total Number of Cells (x 10^8)	5.65 2.60(10)	6.23 2.65(10)	6.45 3.29(10)	6.24 2.10(10)	4.72 1.97(10)	5.79 2.05(10)

59

p. 64

Table 17
Summary of Total Cell Counts (Continued)

	Group I 0 mg/kg	Group III 0.3 mg/kg	Group V 1 mg/kg	Group VII 10 mg/kg	Group IX 30 mg/kg	Group XI 30/0 mg/kg (Recovery)
THYMUS						
Absolute Weight (g)	0.568 0.126(10)	0.604 0.123(10)	0.559 0.121(10)	0.581 0.134(10)	0.487 0.171(10)	0.639 0.110(10)
Weight Ratio (% Body Weight)	0.1335 0.0238(10)	0.1439 0.0281(10)	0.1357 0.0238(10)	0.1531 0.0290(10)	0.1529 0.0463(10)	0.1909 0.0190(10)
Half Weight (g)	0.284 0.069(10)	0.292 0.063(10)	0.272 0.057(10)	0.289 0.066(10)	0.247 0.086(10)	0.325 0.055(10)
Cell Suspension Volume (mL)	7.2 0.3(10)	7.0 0.3(10)	7.2 0.4(10)	7.2 0.4(10)	7.2 0.3(10)	7.3 0.2(10)
Number of Cells in Half (x 10^6 cells/mL)	85.03 23.22(10)	83.16 36.67(10)	88.66 28.23(10)	96.80 40.05(10)	80.30 39.10(10)	120.95 38.87(10)
Total Number of Cells (x 10^8)	12.34 3.34(10)	12.44 5.92(10)	13.18 4.49(10)	14.03 5.81(10)	11.67 6.26(10)	17.47† 6.40(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

† Statistically significant difference from Group IX at $p < 0.05$ by Dunn's test.

NOTE: All data from groups III, V, VII, IX and XI were compared with the control (I) group data. In addition, data from group IX were compared with data from group XI; significant differences between IX and XI were detected.

p. 65

FIGURES

Figure 1
Representative Analytical Calibration Curve

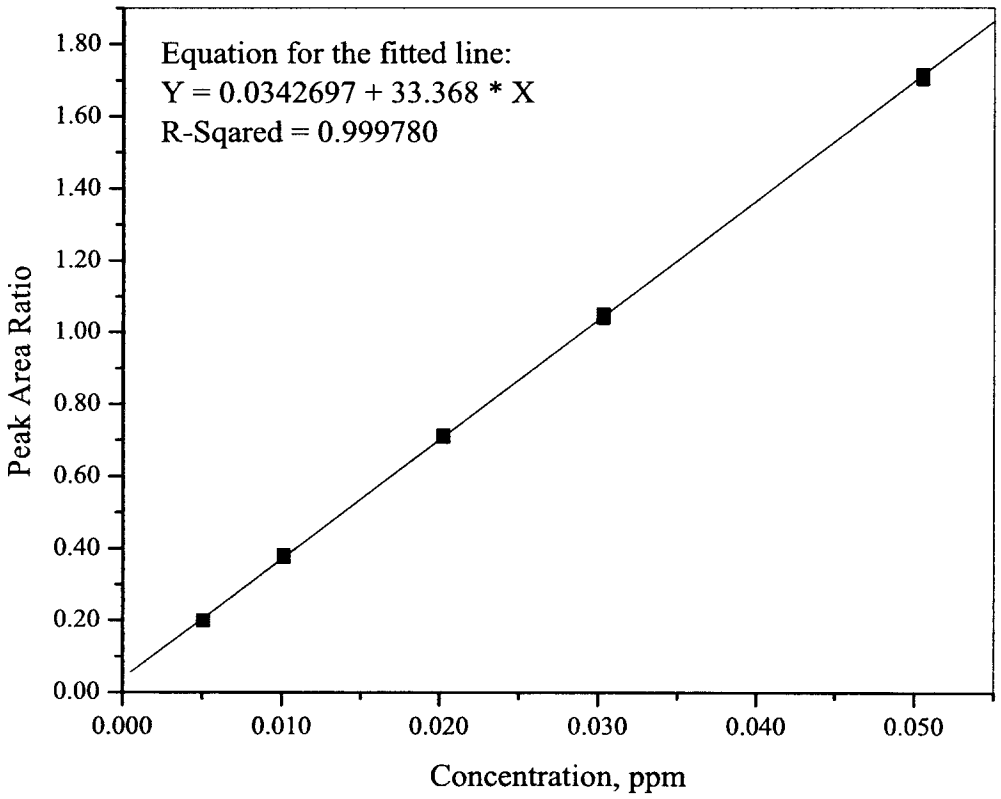


Figure 1: Calibration curve showing linear fit (line) to replicate peak area ratio measurements (squares) for matrix matched calibration solutions of APFO diluted over a concentration range of 0.00505 to 0.0505 ppm.

Figure 2
Representative LC/MS/MS Chromatograms

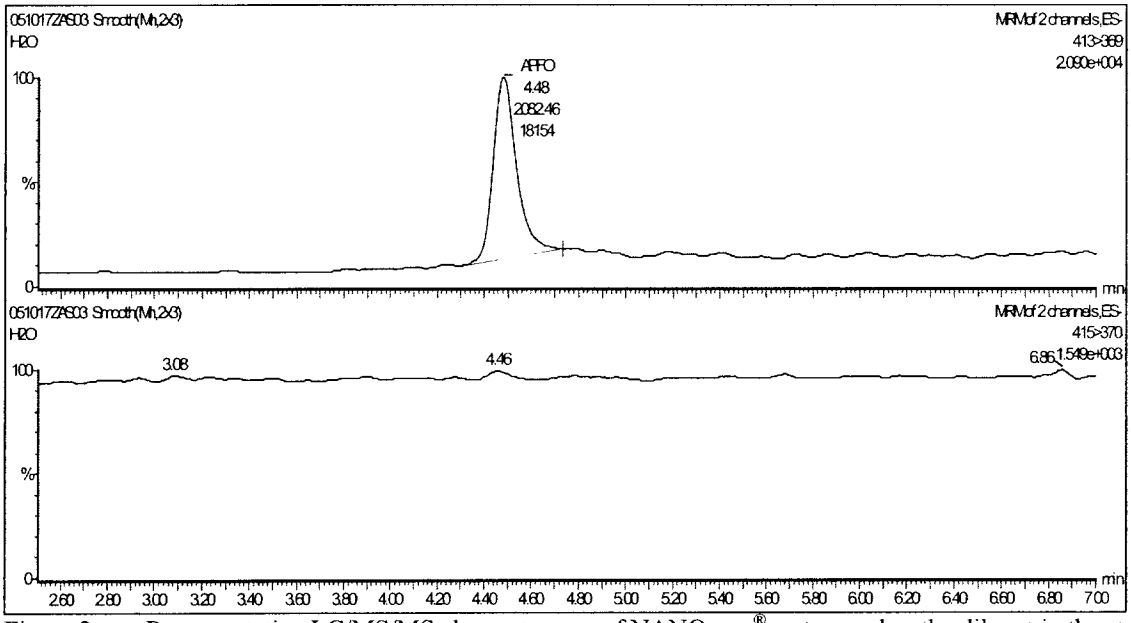


Figure 2a: Representative LC/MS/MS chromatogram of NANOpure® water used as the diluent in the study. Retention time for PFOA is approximately 4.5 minutes.

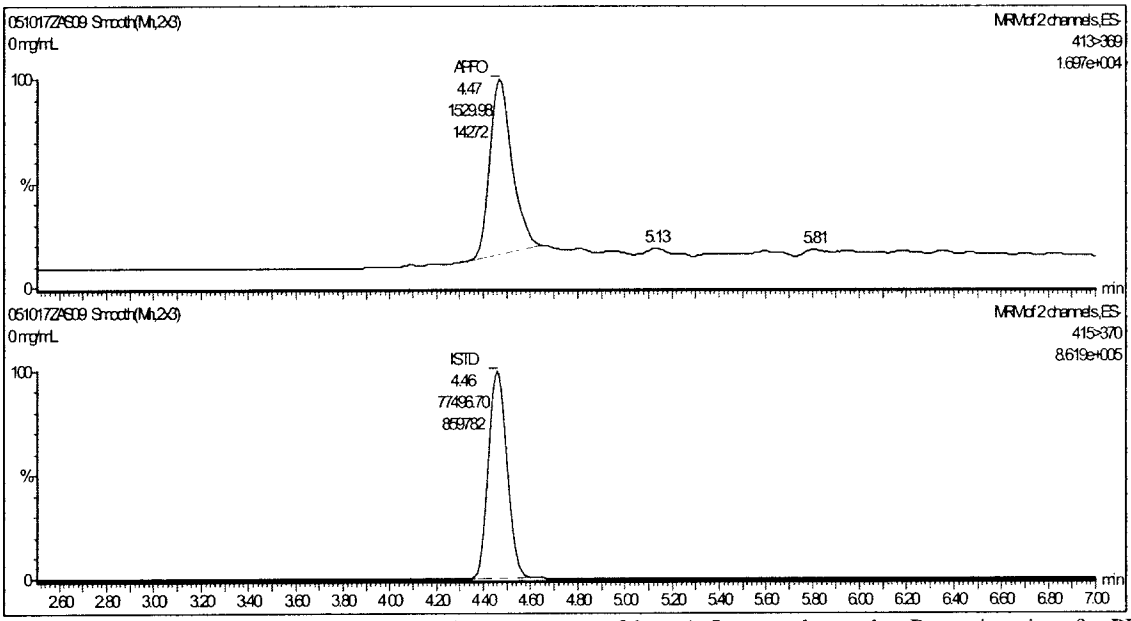


Figure 2b: Representative LC/MS/MS chromatogram of 0 mg/mL control sample. Retention time for PFOA is approximately 4.5 minutes.

Figure 2
Representative LC/MS/MS Chromatograms (Continued)

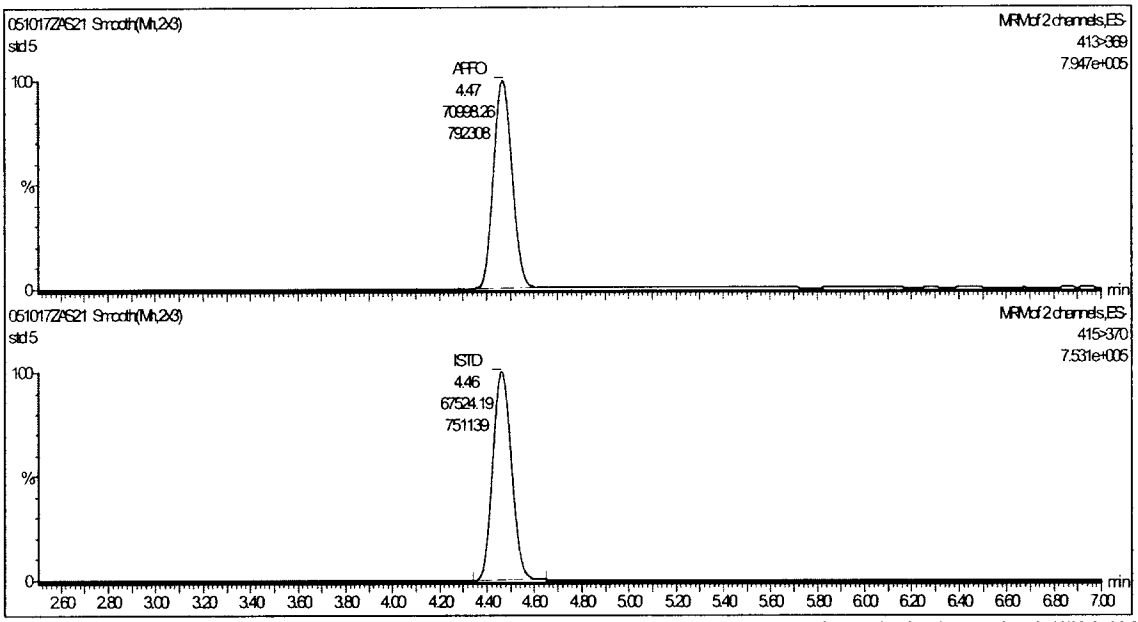


Figure 2c: Representative LC/MS/MS chromatogram of 0.0303 ppm APFO analytical standard (H22703-376) diluted with NANOpure® water after matrix correction.

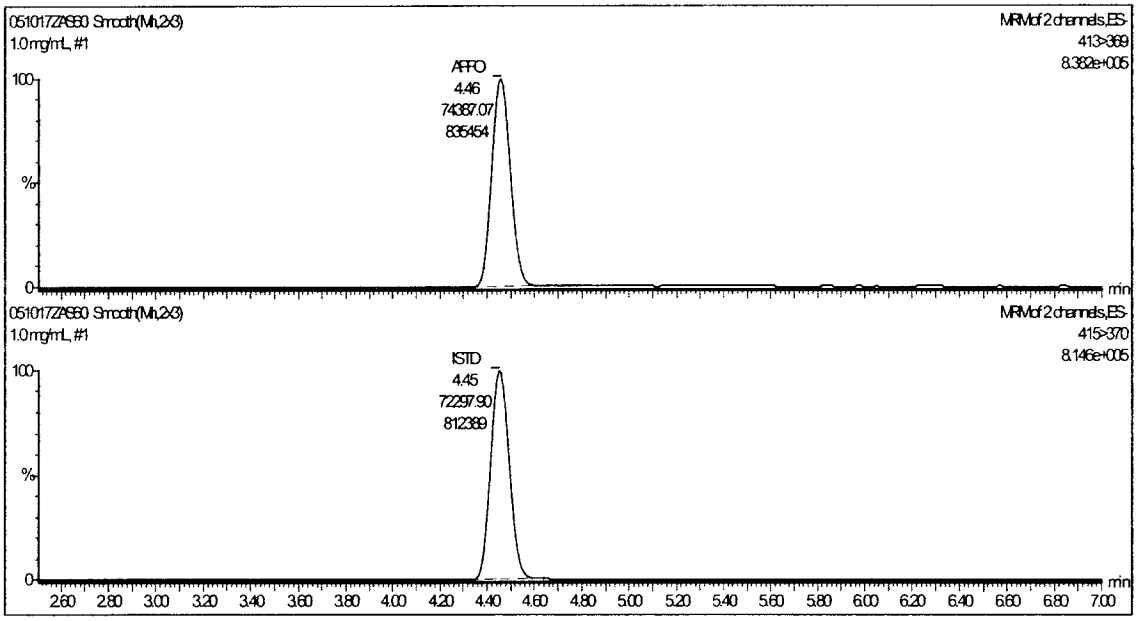


Figure 2d: Representative LC/MS/MS chromatogram of 1 mg/mL APFO dosing solution diluted to a nominal concentration of 0.03 mg/mL. The measured concentration of the representative solution is 0.979 mg/mL.

Figure 2
Representative LC/MS/MS Chromatograms (Continued)

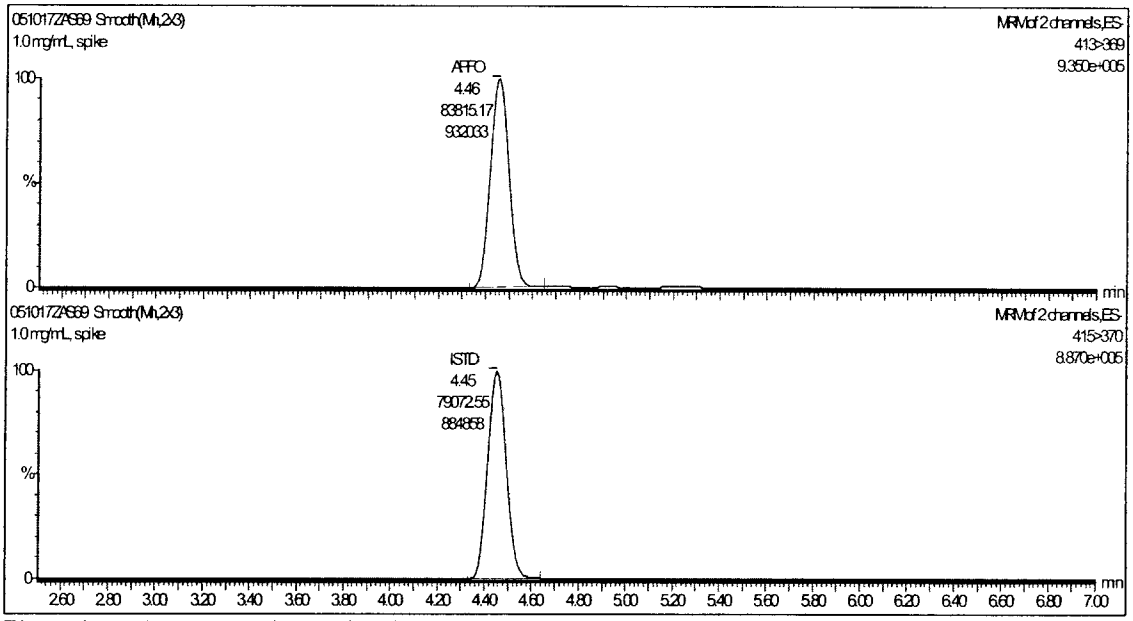
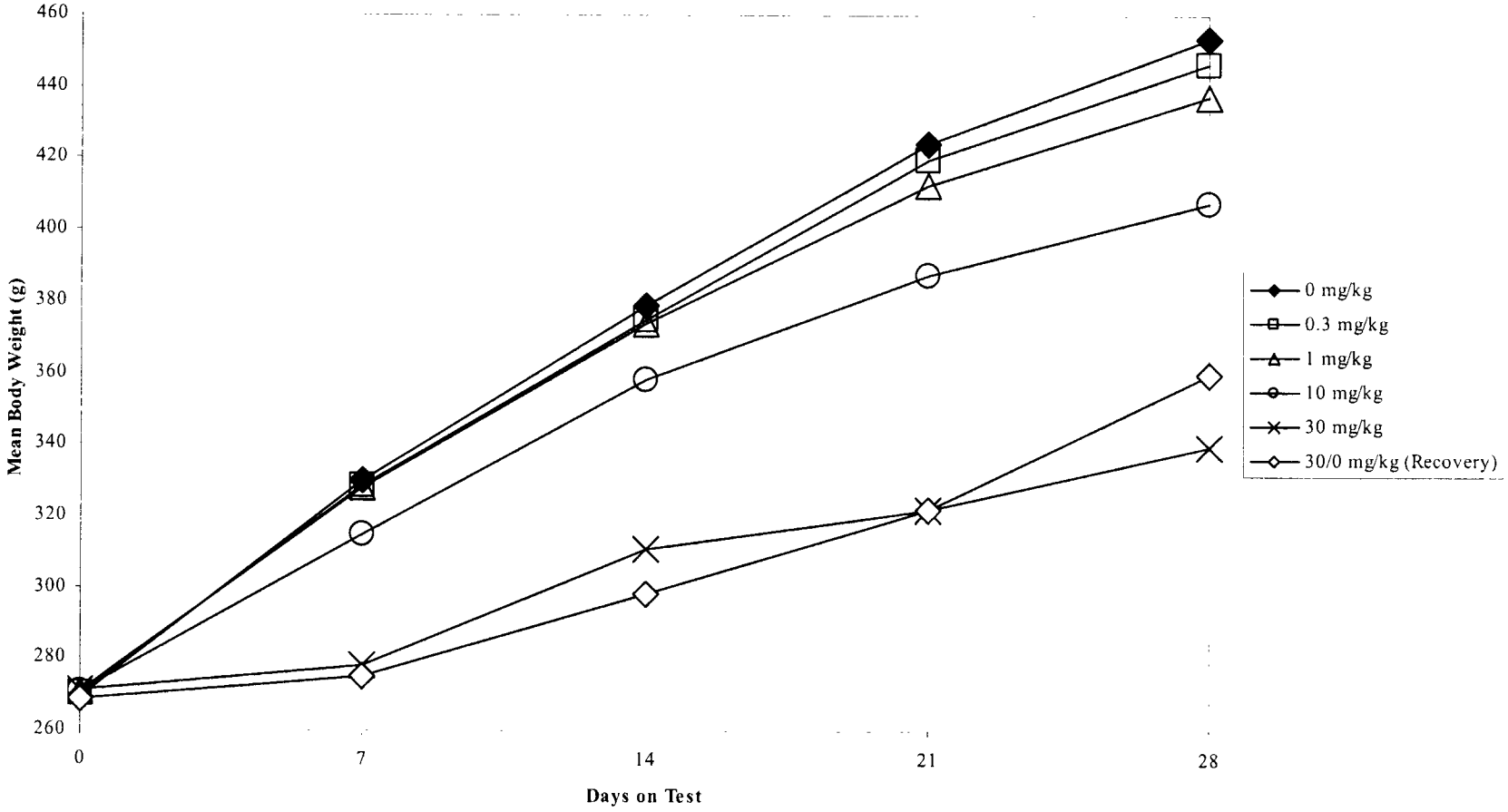


Figure 2e: Representative LC/MS/MS chromatogram of the 1.00 mg/mL level recovery sample of APFO diluted with NANOpure® water after matrix correction to a nominal concentration of 0.0300 ppm. The measured concentration of the representative recovery sample is 1.02 mg/mL.

Figure 3
Mean Body Weights of Male Rats



APPENDICES

Appendix A
Certificate of Analysis

Exygen RESEARCH
Precise Research. Proven Results.

3058 Research Drive
State College, PA 16801
T: 814.272.1039
exygen.com

Certificate of Analysis

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to the GLP regulations. It documents the purity of the test substance. This work was conducted under TSCA Good Laboratory Practice Standards (40 CFR 792) and FIFRA Good Laboratory Practice Standards (40 CFR 160).

Designation of the Certified Material:

Compound:	APFO (Linear)
Haskell Number:	H27308

Analytical Data:

The Purity of the Certified Material was Established by LC/MS/MS

Purity:	19.5%
Last Date of Analysis:	07-November-2005
Re-certification Date:	07-November-2006

Origin of Certified Material:
E.I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

Testing Facility/Performing Laboratory:
Exygen Research
3058 Research Drive
State College, PA 16801

Prepared By: _____ 11/15/05
Charles Simons — Date
Study Director, Exygen Research

Facility Management: _____ for John Flaherty 15-Nov-05
John Flaherty — Date
Vice-President, Exygen Research

Appendix B
Individual Body Weights

INDIVIDUAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

g - grams

Individual Body Weights

	Body Weight g Day 0	Body Weight g Day 3	Body Weight g Day 6	Body Weight g Day 7	Body Weight g Day 8	Body Weight g Day 9	Body Weight g Day 10	Body Weight g Day 11	Body Weight g Day 12
Male, I 0 mg/kg									
101	274.9	299.7	336.0	343.3	351.4	361.0	372.4	375.7	382.1
102	258.4	282.9	323.9	333.2	342.2	354.6	366.8	374.5	382.0
103	276.7	299.4	329.7	339.5	344.2	354.4	364.3	369.9	376.8
104	280.8	301.2	327.2	340.5	342.7	353.8	357.9	358.7	371.5
105	248.1	262.3	290.3	295.5	301.2	311.9	316.7	322.2	331.1
106	270.4	287.8	319.2	325.8	333.3	341.5	348.0	348.8	362.2
107	267.3	282.8	305.9	320.2	325.2	331.7	336.0	343.0	352.2
108	264.3	289.0	319.8	324.2	331.6	338.2	341.6	351.2	352.9
109	287.6	304.9	342.0	351.9	358.0	372.1	376.7	381.9	388.8
110	262.2	281.0	313.0	323.7	328.6	340.3	346.7	353.1	360.1
Male, III 0.3 mg/kg									
301	275.5	300.8	336.1	342.5	350.5	361.0	364.7	374.1	380.6
302	262.8	288.5	321.6	333.3	338.7	353.3	360.7	366.9	376.2
303	283.5	304.9	338.8	350.9	358.6	371.9	373.1	386.4	397.7
304	277.4	293.9	321.9	324.0	326.9	340.3	339.9	347.4	353.8
305	247.4	269.0	298.6	306.5	309.4	323.1	328.4	333.6	340.1
306	272.3	297.1	333.1	339.0	340.8	356.7	361.6	371.4	377.8
307	272.5	286.8	315.2	321.2	324.1	336.0	340.1	340.7	353.9
308	265.4	275.1	308.6	311.1	317.6	328.0	332.0	333.9	339.5
309	284.9	304.4	339.4	342.2	351.9	366.2	365.6	373.2	389.0
310	261.5	276.8	307.2	314.0	316.7	329.2	330.6	336.8	346.7
Male, V 1 mg/kg									
501	266.9	284.9	320.1	331.2	337.0	349.5	349.4	360.1	375.9
502	259.7	278.2	308.9	317.5	327.4	332.2	341.4	341.8	353.6
503	276.2	302.9	336.9	346.3	354.1	365.1	369.1	375.2	385.5
504	283.6	297.6	336.0	346.2	354.8	367.5	372.7	376.6	385.7
505	245.0	264.5	296.4	300.5	300.8	311.7	316.0	318.6	326.1
506	271.9	287.1	320.2	326.5	326.4	341.7	343.6	347.8	356.4
507	274.5	284.0	309.4	314.1	322.5	327.7	332.7	336.1	342.3
508	271.3	292.0	321.6	329.3	335.9	347.4	352.0	359.4	366.9
509	295.2	313.7	353.3	359.6	370.1	379.8	386.1	396.5	406.1
510	261.8	277.2	305.1	310.6	317.3	327.4	327.2	334.2	340.9

Individual Body Weights

	Body Weight g Day 0	Body Weight g Day 3	Body Weight g Day 6	Body Weight g Day 7	Body Weight g Day 8	Body Weight g Day 9	Body Weight g Day 10	Body Weight g Day 11	Body Weight g Day 12
Male, VII 10 mg/kg									
701	270.1	284.6	305.9	308.8	313.9	320.2	325.7	328.0	326.7
702	252.6	274.2	305.9	306.3	313.8	323.6	333.8	340.4	348.1
703	278.4	298.0	322.2	323.0	334.5	342.6	349.9	346.9	354.6
704	285.3	302.5	306.3	314.0	324.5	333.5	330.2	327.1	332.2
705	243.3	257.6	270.4	273.3	273.9	289.0	298.6	302.3	313.9
706	273.1	296.0	321.9	325.2	334.5	346.2	353.1	358.2	372.8
707	275.2	295.5	325.0	334.7	338.4	349.9	356.4	360.0	370.6
708	270.7	287.1	307.0	309.3	320.3	323.8	336.5	340.9	345.3
709	290.6	307.5	328.1	336.3	346.7	354.0	364.9	369.0	377.1
710	265.4	280.3	315.3	316.0	317.9	331.5	337.9	340.6	349.6
Male, IX 30 mg/kg									
901	269.5	233.8	201.9	223.0	242.9	250.8	244.5	264.6	275.1
902	261.2	225.3	186.8	212.2	231.4	241.0	254.4	257.3	263.3
903	278.1	268.2	304.0	307.6	315.5	328.1	335.8	337.0	349.3
904	277.8	270.6	293.9	293.0	295.7	299.6	298.6	296.1	296.9
905	254.4	262.0	298.4	301.6	305.7	312.9	315.1	315.4	322.0
906	277.7	261.0	217.9	246.5	257.5	267.4	276.0	280.8	291.5
907	274.6	261.7	294.5	309.4	312.0	318.5	320.5	326.8	330.7
908	267.2	249.8	279.2	284.0	288.4	290.6	297.2	306.4	310.8
909	284.0	278.1	315.6	317.1	316.6	322.1	327.5	335.8	339.5
910	268.0	249.2	281.7	285.2	291.4	300.8	303.1	297.8	304.0
Male, XI 30/0 mg/kg (Recovery)									
1101	263.1	254.2	284.3	287.6	286.5	273.9	275.1	287.5	293.6
1102	257.0	238.0	225.8	243.3	255.1	260.8	267.2	286.6	289.9
1103	275.3	274.1	307.1	313.2	315.4	316.0	322.5	324.1	326.7
1104	286.3	280.8	313.0	325.2	324.4	320.2	322.3	329.8	342.8
1105	259.9	248.8	270.9	276.6	277.0	284.0	287.7	293.4	301.8
1106	269.4	272.4	314.6	319.2	327.1	335.7	346.0	346.0	354.8
1107	267.3	254.7	287.1	284.2	285.1	290.0	290.4	285.6	287.4
1108	272.9	259.5	279.5	273.0	270.9	274.6	267.8	261.2	266.8
1109	271.7	227.5	173.0	162.8	171.5	193.5	213.0	228.1	225.8
1110	260.9	256.7	263.3	264.9	275.9	283.0	280.9	276.2	283.0

79

p. 78

Individual Body Weights

	Body Weight g Day 13	Body Weight g Day 14	Body Weight g Day 15	Body Weight g Day 16	Body Weight g Day 17	Body Weight g Day 18	Body Weight g Day 19	Body Weight g Day 20	Body Weight g Day 21
Male, I 0 mg/kg									
101	393.0	392.2	404.3	410.1	420.4	430.5	438.9	442.1	453.1
102	388.3	396.2	405.5	419.6	418.2	433.8	439.8	441.8	445.2
103	384.8	385.3	396.5	403.5	410.8	428.0	436.6	440.8	445.8
104	373.9	385.4	386.0	390.9	405.7	404.9	415.8	418.4	425.1
105	337.0	346.4	351.7	357.3	361.6	379.4	380.4	383.9	386.2
106	364.0	369.2	374.3	380.9	383.8	392.5	396.2	403.0	412.1
107	354.3	360.2	366.4	366.3	375.7	386.9	390.5	394.8	401.1
108	357.3	362.5	371.3	375.4	381.4	391.6	397.0	398.9	402.5
109	403.8	414.7	417.8	421.8	431.3	443.6	446.6	450.5	454.4
110	372.1	375.5	381.7	382.0	386.8	396.9	402.3	409.1	414.7
Male, III 0.3 mg/kg									
301	381.3	385.8	393.5	400.8	406.3	416.6	421.7	424.6	425.7
302	379.9	392.0	393.0	405.6	402.9	419.1	418.5	429.7	430.2
303	397.1	404.2	414.6	421.6	429.6	438.9	447.5	454.3	461.8
304	352.9	365.7	373.4	375.0	378.5	393.2	397.6	401.8	405.7
305	341.7	353.9	360.2	360.8	370.2	381.5	384.9	387.9	396.5
306	382.0	388.4	402.1	402.7	409.8	420.1	424.7	432.1	436.8
307	359.3	362.6	374.6	374.1	380.2	389.8	397.1	399.5	408.7
308	346.1	348.6	354.9	354.9	357.6	374.0	371.1	381.1	384.5
309	389.3	396.3	402.7	410.9	418.2	427.3	437.5	440.9	446.0
310	347.8	352.1	364.4	366.6	371.0	387.2	392.2	392.6	400.7
Male, V 1 mg/kg									
501	375.0	375.8	389.8	393.8	404.5	408.3	414.9	421.0	424.4
502	357.8	359.7	362.9	369.1	370.9	386.4	390.0	390.3	390.1
503	390.9	402.0	407.0	411.1	416.8	444.0	433.6	432.9	446.5
504	390.7	399.7	402.1	406.9	413.8	423.7	426.5	434.6	440.6
505	328.1	334.9	336.8	339.6	346.3	353.6	360.0	363.0	368.2
506	367.5	367.0	377.3	376.1	383.4	396.0	399.3	409.2	408.6
507	350.5	355.4	357.7	360.2	360.3	374.4	374.3	377.6	376.0
508	372.6	380.6	383.8	390.1	391.9	405.6	410.6	416.5	424.3
509	409.4	413.5	424.7	436.2	437.1	459.5	467.4	466.1	470.4
510	337.2	348.2	356.1	355.7	362.8	368.7	379.1	377.0	376.5

p. 79

Individual Body Weights

	Body Weight g Day 13	Body Weight g Day 14	Body Weight g Day 15	Body Weight g Day 16	Body Weight g Day 17	Body Weight g Day 18	Body Weight g Day 19	Body Weight g Day 20	Body Weight g Day 21
Male, VII 10 mg/kg									
701	328.8	337.9	342.3	345.0	345.0	355.0	353.6	361.0	358.6
702	350.8	364.0	364.9	370.4	375.8	382.4	385.1	391.4	392.6
703	362.0	371.0	374.5	375.9	378.9	396.9	395.8	397.0	398.4
704	336.3	342.4	344.1	348.4	350.4	358.2	364.6	369.3	369.2
705	319.0	321.1	331.6	330.7	331.2	337.1	334.1	338.0	333.2
706	375.3	378.1	381.4	396.1	395.8	407.9	410.5	423.8	419.0
707	372.1	380.6	385.3	387.9	394.5	409.7	418.9	413.0	424.3
708	342.8	342.2	354.2	350.5	355.4	362.3	365.6	366.7	367.9
709	380.4	384.1	388.4	399.0	402.1	403.6	408.7	417.8	421.5
710	360.2	358.7	364.6	371.9	364.9	379.6	381.1	389.2	390.6
Male, IX 30 mg/kg									
901	281.1	261.5	244.9	229.4	215.8	208.4	234.3	248.5	253.1
902	272.9	282.5	279.2	288.5	292.8	298.1	302.0	296.6	297.4
903	348.9	355.4	357.9	367.0	368.8	378.4	381.1	383.5	392.5
904	296.8	297.9	298.1	294.3	289.1	301.6	301.5	301.9	294.6
905	324.3	320.1	321.5	328.3	326.0	336.9	339.1	336.4	337.2
906	297.6	299.4	312.2	304.5	307.4	315.1	315.5	321.0	325.1
907	329.0	331.1	340.9	340.9	340.2	349.1	344.0	339.4	341.7
908	309.1	307.8	309.8	311.0	312.7	317.1	318.9	317.0	319.1
909	341.1	345.2	350.3	355.9	363.2	365.2	366.8	359.9	353.5
910	304.6	306.0	308.9	303.6	306.6	306.7	304.4	306.9	305.3
Male, XI 30/0 mg/kg (Recovery)									
1101	300.9	298.5	312.5	312.9	320.4	317.4	324.8	329.0	334.0
1102	296.9	298.4	312.8	309.5	312.3	328.8	327.1	338.9	333.1
1103	329.2	329.4	339.3	332.2	334.9	341.7	342.6	344.6	343.0
1104	353.9	355.8	354.0	359.6	360.9	375.8	374.9	375.0	370.5
1105	307.4	312.0	309.0	319.2	319.4	321.3	324.1	328.0	324.4
1106	358.8	355.9	368.7	369.1	374.8	383.1	384.9	387.4	388.6
1107	290.6	291.7	297.2	300.7	297.9	311.2	308.6	314.3	308.7
1108	263.9	270.1	277.7	272.3	279.6	280.8	283.2	284.4	282.7
1109	197.7	181.2	173.9	199.9	211.0	219.1	213.0	197.3	225.9
1110	285.2	292.0	293.0	296.5	297.0	307.0	309.1	315.4	311.3

Individual Body Weights

	Body Weight g Day 22	Body Weight g Day 23	Body Weight g Day 24	Body Weight g Day 25	Body Weight g Day 26	Body Weight g Day 27	Body Weight g Day 28
Male, I 0 mg/kg							
101	454.7	464.8	463.6	478.2	480.1	479.0	483.9
102	452.1	457.7	462.8	472.2	476.8	471.6	475.9
103	446.2	455.6	454.3	468.4	470.7	474.3	476.3
104	430.5	426.4	438.0	448.7	451.7	451.9	454.4
105	393.4	399.0	405.5	405.5	411.0	412.2	417.4
106	416.6	417.8	422.2	429.9	433.7	430.4	440.5
107	400.3	408.8	409.5	413.6	413.7	418.1	420.6
108	409.6	414.9	416.3	425.6	430.4	429.6	432.4
109	457.0	463.1	470.0	475.8	479.8	481.2	489.3
110	420.5	425.2	430.3	435.7	440.4	442.5	442.8
Male, III 0.3 mg/kg							
301	431.6	438.7	437.1	444.3	447.7	454.2	452.7
302	435.2	440.5	447.1	454.8	453.7	456.3	463.7
303	467.3	471.0	480.3	489.7	492.7	496.0	502.4
304	407.5	407.6	417.4	420.1	426.9	423.2	419.8
305	397.9	400.6	408.6	416.9	415.4	416.5	421.4
306	439.8	443.4	447.5	461.1	462.0	465.6	467.1
307	411.1	415.9	422.5	425.5	432.7	436.3	434.4
308	388.1	392.9	394.8	401.0	405.8	404.0	402.9
309	448.5	456.4	460.4	473.8	473.8	476.0	475.1
310	406.3	409.6	416.6	421.0	421.6	423.6	426.3
Male, V 1 mg/kg							
501	429.4	433.2	431.8	438.3	442.8	443.9	450.2
502	401.3	409.4	406.2	413.1	415.9	414.9	418.0
503	445.7	453.2	455.6	459.9	463.9	464.8	464.6
504	448.3	451.5	458.3	466.7	470.4	467.6	472.4
505	372.3	373.9	376.6	384.5	385.5	384.6	389.0
506	412.6	416.8	421.0	424.9	428.9	430.3	433.3
507	380.5	383.5	380.2	388.9	390.3	387.8	390.6
508	427.5	425.2	423.1	426.7	429.5	433.0	433.4
509	478.7	481.8	488.6	502.7	505.4	507.0	512.1
510	389.4	391.0	390.2	402.3	405.6	406.7	408.6

Individual Body Weights

	Body Weight g Day 22	Body Weight g Day 23	Body Weight g Day 24	Body Weight g Day 25	Body Weight g Day 26	Body Weight g Day 27	Body Weight g Day 28
Male, VII 10 mg/kg							
701	363.8	368.4	366.0	375.7	378.7	378.7	380.2
702	392.4	404.6	399.6	413.5	422.9	422.8	420.3
703	398.0	408.1	403.1	406.3	403.6	401.1	406.9
704	374.6	373.9	377.2	375.3	372.5	381.9	382.2
705	339.6	343.7	341.7	344.3	345.5	345.1	345.9
706	425.8	429.4	426.5	433.3	451.8	445.7	452.5
707	434.2	437.4	436.5	443.4	447.2	446.6	450.6
708	372.0	378.0	375.1	376.1	377.2	377.1	384.7
709	424.6	431.8	423.8	434.6	440.7	441.5	441.5
710	393.5	400.9	403.5	411.0	409.4	415.9	410.4
Male, IX 30 mg/kg							
901	261.0	271.7	281.9	292.3	295.4	296.7	292.6
902	302.8	300.6	302.0	307.6	307.4	313.9	313.7
903	395.3	399.1	401.2	406.7	416.8	413.4	418.0
904	296.8	299.4	301.0	310.5	311.4	308.0	309.7
905	344.1	346.2	337.8	350.2	353.1	351.8	353.2
906	327.5	329.9	323.0	334.2	338.1	337.3	333.2
907	342.1	347.8	348.2	350.7	353.3	352.3	356.9
908	318.0	321.3	326.1	327.3	333.3	331.1	333.4
909	358.7	353.8	350.5	363.6	363.1	366.0	367.1
910	306.3	306.3	309.5	316.0	316.0	312.3	317.7
Male, XI 30/0 mg/kg (Recovery)							
1101	338.3	338.0	338.7	352.6	353.1	352.0	352.5
1102	338.5	338.4	346.3	356.6	360.6	358.9	366.7
1103	339.3	348.0	351.0	357.5	361.7	364.8	366.1
1104	378.3	389.7	394.4	408.2	417.1	418.4	424.4
1105	330.7	333.2	341.9	347.9	352.9	359.8	361.0
1106	399.3	400.2	409.4	416.3	421.5	418.4	421.9
1107	314.3	313.3	323.2	327.9	337.6	334.7	342.1
1108	284.1	288.0	286.1	294.3	299.4	300.6	304.0
1109	233.5	247.7	258.8	274.6	289.0	297.4	309.1
1110	315.9	316.0	322.7	332.6	339.3	342.0	349.8

83

p. 82

Appendix C
Individual Food Consumption

INDIVIDUAL FOOD CONSUMPTION

EXPLANATORY NOTES

ABBREVIATIONS:

Cons.	-	consumption
g/anm/day	-	grams of food consumed per animal per day

Individual Food Consumption

	Food Cons. g/anm/day Day 7	Food Cons. g/anm/day Day 14	Food Cons. g/anm/day Day 21	Food Cons. g/anm/day Day 28
Male, I 0 mg/kg				
101	30.2	30.6	31.7	31.1
102	29.3	31.5	32.1	30.5
103	30.2	28.9	32.2	31.0
104	27.6	27.9	28.1	30.8
105	24.7	25.2	26.1	26.8
106	30.0	29.1	30.8	29.9
107	27.6	27.6	28.3	28.0
108	29.6	28.1	29.1	30.2
109	30.3	33.5	32.0	31.9
110	28.1	29.2	30.1	32.2
Male, III 0.3 mg/kg				
301	29.8	29.3	29.2	31.2
302	29.0	30.9	29.5	30.5
303	30.1	31.0	33.6	34.9
304	27.5	26.8	27.7	27.6
305	25.7	26.1	27.3	26.6
306	30.2	32.9	32.8	30.6
307	25.5	26.1	27.5	27.9
308	25.3	26.2	25.4	26.6
309	30.0	31.1	31.1	32.3
310	26.9	26.9	28.0	28.8
Male, V 1 mg/kg				
501	28.5	28.9	29.9	28.6
502	28.3	28.8	27.8	28.4
503	29.6	30.1	29.8	27.0
504	31.4	32.9	30.6	31.8
505	27.5	26.6	25.1	26.4
506	27.4	28.4	29.3	29.1
507	24.1	24.1	22.9	23.7
508	30.1	28.8	30.0	28.2
509	31.2	32.5	33.2	33.6
510	27.3	26.2	26.7	28.0

Individual Food Consumption

	Food Cons. g/anm/day Day 7	Food Cons. g/anm/day Day 14	Food Cons. g/anm/day Day 21	Food Cons. g/anm/day Day 28
Male, VII 10 mg/kg				
701	27.8	26.6	27.2	28.8
702	25.5	29.8	30.2	30.1
703	26.9	30.0	30.9	27.1
704	25.3	25.9	26.6	26.1
705	24.0	29.0	26.1	26.7
706	27.2	31.1	31.2	30.9
707	29.0	31.8	34.7	33.9
708	24.4	26.8	26.9	27.3
709	26.0	29.7	28.1	29.3
710	27.4	30.3	30.0	31.0
Male, IX 30 mg/kg				
901	9.2	24.6	11.9	25.7
902	8.6	27.0	23.5	24.8
903	23.4	30.5	29.2	29.7
904	24.7	23.4	22.2	23.9
905	24.7	25.3	22.9	24.0
906	14.5	35.3	30.3	29.0
907	25.0	26.2	24.2	26.4
908	22.2	29.3	25.0	28.5
909	24.5	30.9	28.7	26.5
910	23.9	26.8	22.3	26.6
Male, XI 30/0 mg/kg (Recovery)				
1101	24.2	23.2	26.2	24.4
1102	13.9	31.9	27.2	26.1
1103	25.8	28.6	26.8	25.8
1104	23.7	28.6	27.0	29.7
1105	23.0	25.1	22.3	25.1
1106	26.9	29.2	28.9	29.2
1107	22.1	22.2	24.7	25.9
1108	22.8	21.0	23.5	23.1
1109	5.1	15.8	20.4	32.0
1110	21.4	27.1	27.9	28.2

Appendix D
Individual Daily Animal Health Observations

Individual Daily Animal Health Observations

Sex	Group	Animal	Observation	Days
M	I	101	General observation, No Abnormality Detected	0-28
M	I	102	General observation, No Abnormality Detected	0-28
M	I	103	General observation, No Abnormality Detected	0-28
M	I	104	General observation, No Abnormality Detected	0-28
M	I	105	General observation, No Abnormality Detected	0-28
M	I	106	General observation, No Abnormality Detected	0-28
M	I	107	General observation, No Abnormality Detected	0-28
M	I	108	General observation, No Abnormality Detected	0-28
M	I	109	General observation, No Abnormality Detected	0-28
M	I	110	General observation, No Abnormality Detected	0-28
M	III	301	General observation, No Abnormality Detected	0-28
M	III	302	General observation, No Abnormality Detected	0-28
M	III	303	General observation, No Abnormality Detected	0-28
M	III	304	General observation, No Abnormality Detected	0-28
M	III	305	General observation, No Abnormality Detected	0-28
M	III	306	General observation, No Abnormality Detected	0-28
M	III	307	General observation, No Abnormality Detected	0-28
M	III	308	General observation, No Abnormality Detected	0-28
M	III	309	General observation, No Abnormality Detected	0-28
M	III	310	General observation, No Abnormality Detected	0-28
M	V	501	General observation, No Abnormality Detected	0-28
M	V	502	General observation, No Abnormality Detected	0-28
M	V	503	General observation, No Abnormality Detected	0-28
M	V	504	General observation, No Abnormality Detected	0-28
M	V	505	General observation, No Abnormality Detected	0-28
M	V	506	General observation, No Abnormality Detected	0-28
M	V	507	General observation, No Abnormality Detected	0-28
M	V	508	General observation, No Abnormality Detected	0-28
M	V	509	General observation, No Abnormality Detected	0-28
M	V	510	General observation, No Abnormality Detected	0-28

Individual Daily Animal Health Observations

Sex	Group	Animal	Observation	Days
M	VII	701	General observation, No Abnormality Detected	0-28
M	VII	702	General observation, No Abnormality Detected	0-28
M	VII	703	General observation, No Abnormality Detected	0-28
M	VII	704	General observation, No Abnormality Detected	0-28
M	VII	705	General observation, No Abnormality Detected	0-28
M	VII	706	General observation, No Abnormality Detected	0-28
M	VII	707	General observation, No Abnormality Detected	0-28
M	VII	708	General observation, No Abnormality Detected	0-28
M	VII	709	General observation, No Abnormality Detected	0-28
M	VII	710	General observation, No Abnormality Detected	0-28
M	IX	901	General observation, No Abnormality Detected	0-3,8-17,19-28
			Feces, Absent	18
			Comments, decreased feces	4-7
			Not Eating	18
M	IX	902	General observation, No Abnormality Detected	0-3,8-28
			Comments, decreased feces	4-7
			Not Eating	4-7
M	IX	903	General observation, No Abnormality Detected	0-3,5-28
			Not Eating	4
M	IX	904	General observation, No Abnormality Detected	0-28
M	IX	905	General observation, No Abnormality Detected	0-28
M	IX	906	General observation, No Abnormality Detected	0-28
M	IX	907	General observation, No Abnormality Detected	0-28
M	IX	908	General observation, No Abnormality Detected	0-28
M	IX	909	General observation, No Abnormality Detected	0-28
M	IX	910	General observation, No Abnormality Detected	0-28
M	XI	1101	General observation, No Abnormality Detected	0-3,5-28
			Not Eating	4
M	XI	1102	General observation, No Abnormality Detected	0-4,8-28
			Comments, decreased feces	5-7
M	XI	1103	General observation, No Abnormality Detected	0-28
M	XI	1104	General observation, No Abnormality Detected	0-28
M	XI	1105	General observation, No Abnormality Detected	0-28
M	XI	1106	General observation, No Abnormality Detected	0-28
M	XI	1107	General observation, No Abnormality Detected	0-28
M	XI	1108	General observation, No Abnormality Detected	0-28
M	XI	1109	General observation, No Abnormality Detected	0-3,11-28
			Feces, Absent	4-5
			Stain Fur/Skin, Inguen, Brown	9-10
			Stain Fur/Skin, Inguen, Red	5
			Wet Fur, Inguen	5
			Not Eating	4-5
M	XI	1110	General observation, No Abnormality Detected	0-28

Appendix E
Individual Detailed Clinical Observations and Mortality Records

Individual Detailed Clinical Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	I	101	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	I	102	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	I	103	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	I	104	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	I	105	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	I	106	General observation, No Abnormality Detected	0-7
			Hair Loss, Forelimb, Bilateral	14-29
			Sacrificed by design	29
M	I	107	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	I	108	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	I	109	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	I	110	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	301	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	302	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	303	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	304	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	305	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	306	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	307	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	308	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	309	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	III	310	General observation, No Abnormality Detected	0-29
M			Sacrificed by design	29
M	V	501	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	502	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	503	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	504	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	505	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	506	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	507	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	508	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	509	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	V	510	General observation, No Abnormality Detected	0
			Hair Loss, Forelimb, Bilateral	7-29
			Sacrificed by design	29

Individual Detailed Clinical Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	VII	701	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	VII	702	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	VII	703	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	VII	704	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	VII	705	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	VII	706	General observation, No Abnormality Detected	0-14
			Hair Loss, Forepaw, Bilateral	21-29
			Sacrificed by design	29
M	VII	707	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	VII	708	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	VII	709	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	VII	710	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	IX	901	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	IX	902	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	IX	903	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	IX	904	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	IX	905	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	IX	906	General observation, No Abnormality Detected	0
			Hair Loss, Abdomen, Bilateral	7-29
			Hair Loss, Forelimb, Bilateral	21-29
			Hair Loss, Hindlimb, Bilateral	21-29
			Sacrificed by design	29
M	IX	907	General observation, No Abnormality Detected	0-7
			Hair Loss, Forepaw, Bilateral	14-29
			Sacrificed by design	29
M	IX	908	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	IX	909	General observation, No Abnormality Detected	0-14
			Hair Loss, Forelimb, Bilateral	21-29
			Hair Loss, Forepaw, Bilateral	21-29
			Sacrificed by design	29
M	IX	910	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29

Individual Detailed Clinical Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	XI	1101	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	XI	1102	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	XI	1103	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	XI	1104	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	XI	1105	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	XI	1106	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	XI	1107	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	XI	1108	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29
M	XI	1109	General observation, No Abnormality Detected	0,11-29
			Lethargic	6-7
			Carriage, High	6-7
			Feces, Absent	6-8
			Stain Fur/Skin, Abdomen, Red	8
			Stain Fur/Skin, Forepaw, Bilateral, Red	6-7
			Stain Fur/Skin, Inguen, Red	8
			Stain Fur/Skin, Perineum, Red	8
			Stain Fur/Skin, Ventral body, Red	6-7
			Stain Fur/Skin, Perinasal, Red	6-7
			Stain Fur/Skin, Perioral, Red	6-7
			Wet Fur, Ventral body, Ventral	6
			Not Eating	6-7
			Sacrificed by design	29
M	XI	1110	General observation, No Abnormality Detected	0-29
			Sacrificed by design	29

Appendix F
Individual Animal Clinical Pathology Data

INDIVIDUAL ANIMAL CLINICAL PATHOLOGY DATA

EXPLANATORY NOTES

ABBREVIATIONS:

General:

Adeq	-	adequate
CLOT or Clot	-	sample clotted
Decr	-	decreased
Mod	-	moderate
NP	-	not taken, not performed, or results not valid
OK	-	sample condition OK for testing

Individual Hematology Values:

COND	-	sample condition
RBC	-	red blood cell count
HGB	-	hemoglobin
HCT	-	hematocrit
MCV	-	mean corpuscular (cell) volume
MCH	-	mean corpuscular (cell) hemoglobin
MCHC	-	mean corpuscular (cell) hemoglobin concentration
RDW	-	red cell distribution width
ARET	-	absolute reticulocyte count
PLT	-	platelet count
WBC	-	white blood cell count
ANEU	-	absolute neutrophil (all forms)
ALYM	-	absolute lymphocyte
AMON	-	absolute monocyte
AEOS	-	absolute eosinophil
ABAS	-	absolute basophil
ALUC	-	absolute large unstained cell

Individual Red Blood Cell Morphology Values:

ANIS	-	anisocytosis
MIC	-	microcytes
MAC	-	macrocytes
POLY	-	polychromasia
HYPO	-	hypochromasia
ECHI	-	echinocytes
ACAN	-	acanthocytes
TARG	-	target cells
RX	-	rouleaux
HJB	-	Howell-Jolly body
-	-	not observed

Individual White Blood Cell / Platelet Morphology Values:

SM	-	smudge white blood cells
TOX	-	toxic neutrophils
DB	-	Döhle bodies
VC	-	vacuolated cytoplasm
BC	-	basophilic cytoplasm
PCE	-	platelet clumps / estimate
GP	-	giant platelets
BP	-	bizarre platelets
-	-	not observed

INDIVIDUAL ANIMAL CLINICAL PATHOLOGY DATA

EXPLANATORY NOTES (Continued)

ABBREVIATIONS: (Continued)

Individual Clinical Chemistry Values:

HEM	-	hemolysis
LIP	-	lipemia
ICT	-	icterus
CHOL	-	cholesterol
TRIG	-	triglycerides
TP	-	total protein
ALB	-	albumin
GLOB	-	globulin
HDL	-	high-density lipoprotein cholesterol
NHDL	-	non-high-density lipoprotein cholesterol
SCORT	-	serum corticosterone

NOTES:

When individual animal data are not reported, it may be due to one of the following reasons or other reasons, all of which are explained in the study records:
the sample was clotted (CLOT)
there was insufficient sample for testing (QNS)
a valid result could not be obtained (RNV)
the sample was not suitable for testing
the animal died prior to sample collection
no sample was available for testing (NSR)

Only positive findings were recorded for special observations (e.g., additional cell types) or observations marked other.

Individual Animal Clinical Pathology Data

Male,	Group	I	0	mg/kg	Day	29				
Animal	COND	RBC $x10^6/\mu L$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	ARET $x10^3/\mu L$	PLT $x10^3/\mu L$
101	OK	7.77	14.6	45.6	58.7	18.8	32.0	11.5	200.3	NP
102	OK	7.53	15.0	47.1	62.6	19.9	31.8	11.8	196.6	1006
103	OK	7.85	15.4	48.3	61.6	19.6	31.9	11.6	208.2	NP
104	OK	7.84	15.1	45.5	58.0	19.2	33.1	11.0	194.3	1119
105	OK	7.32	14.8	46.4	63.4	20.3	32.0	11.0	160.5	1261
106	OK	7.48	14.8	45.8	61.2	19.8	32.3	11.8	198.6	1207
107	CLOT	NP	NP	NP	NP	NP	NP	NP	NP	NP
108	OK	7.90	15.1	45.9	58.1	19.2	33.0	11.9	164.5	NP
109	OK	7.59	14.6	45.1	59.5	19.3	32.4	11.7	182.1	968
110	OK	7.67	14.8	45.9	59.9	19.4	32.3	11.6	180.9	976

Male,	Group	III	0.3	mg/kg	Day	29				
Animal	COND	RBC $x10^6/\mu L$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	ARET $x10^3/\mu L$	PLT $x10^3/\mu L$
301	OK	7.87	15.6	48.3	61.4	19.8	32.2	11.9	169.0	1042
302	OK	7.40	14.5	44.7	60.3	19.6	32.5	12.1	152.6	940
303	OK	8.15	15.4	47.7	58.5	18.9	32.2	11.4	166.7	1104
304	OK	8.11	14.9	45.9	56.6	18.4	32.5	11.8	189.9	1114
305	OK	7.50	14.6	45.0	59.9	19.4	32.4	11.2	148.8	1064
306	OK	7.31	14.5	44.6	61.0	19.9	32.6	11.0	158.0	1080
307	OK	7.57	13.9	43.5	57.5	18.4	31.9	10.8	147.4	1096
308	OK	7.61	14.6	44.6	58.6	19.2	32.8	10.7	180.3	1052
309	OK	7.45	14.5	44.4	59.5	19.4	32.6	11.8	185.7	1167
310	OK	7.17	14.7	45.6	63.5	20.4	32.2	11.6	206.3	923

96

p. 97

Individual Animal Clinical Pathology Data

Male,	Group	V	1	mg/kg	Day	29				
Animal	COND	RBC $x10^6/\mu L$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	ARET $x10^3/\mu L$	PLT $x10^3/\mu L$
501	OK	7.96	15.2	48.3	60.7	19.1	31.4	12.1	163.1	NP
502	OK	8.05	15.8	48.3	59.9	19.7	32.8	12.3	207.6	872
503	OK	7.23	13.9	42.9	59.4	19.2	32.3	11.3	158.2	1112
504	OK	7.33	15.0	46.1	62.9	20.5	32.5	11.5	140.3	1166
505	OK	7.30	14.5	45.7	62.6	19.8	31.7	11.6	197.9	NP
506	OK	7.68	14.3	44.3	57.7	18.6	32.2	11.8	161.5	1159
507	OK	7.58	14.6	44.7	59.0	19.3	32.6	11.6	166.7	1120
508	OK	7.54	15.0	47.1	62.4	19.8	31.8	11.3	170.6	NP
509	OK	7.31	14.2	44.1	60.3	19.5	32.3	12.0	210.8	1135
510	OK	8.03	14.6	44.9	55.9	18.2	32.5	11.7	197.9	NP

Male,	Group	VII	10	mg/kg	Day	29				
Animal	COND	RBC $x10^6/\mu L$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	ARET $x10^3/\mu L$	PLT $x10^3/\mu L$
701	OK	7.98	13.7	43.6	54.7	17.2	31.4	12.7	210.5	981
702	OK	7.44	13.9	44.0	59.2	18.6	31.5	12.2	235.8	1038
703	OK	7.09	13.1	40.4	56.9	18.5	32.5	13.8	233.8	NP
704	OK	6.10	12.0	37.4	61.3	19.6	32.0	12.3	152.7	352
705	OK	7.48	13.5	43.1	57.6	18.1	31.4	12.4	123.0	NP
706	OK	7.24	13.5	42.7	59.0	18.7	31.7	13.6	257.1	1182
707	OK	7.31	14.7	45.9	62.7	20.1	32.1	11.9	195.8	NP
708	OK	7.65	14.2	43.8	57.2	18.6	32.5	12.2	154.9	1022
709	OK	7.54	13.5	43.3	57.4	17.9	31.2	13.7	248.5	1350
710	OK	7.00	13.3	41.6	59.4	18.9	31.9	12.7	232.4	1380

99

p. 98

Individual Animal Clinical Pathology Data

Male,	Group	IX	30	mg/kg	Day	29				
Animal	COND	RBC $x10^6/\mu L$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	ARET $x10^3/\mu L$	PLT $x10^3/\mu L$
901	OK	7.44	13.3	42.6	57.2	17.9	31.2	14.6	280.3	NP
902	OK	7.22	13.5	41.1	56.9	18.7	32.8	13.2	206.0	1027
903	OK	6.85	13.3	42.0	61.3	19.4	31.7	12.1	178.4	1081
904	OK	8.72	14.8	45.8	52.5	17.0	32.4	12.7	152.4	NP
905	OK	7.13	13.5	42.6	59.8	19.0	31.8	12.4	198.0	1280
906	OK	8.08	14.4	44.9	55.6	17.8	32.1	12.8	156.9	998
907	OK	6.75	12.1	38.7	57.4	18.0	31.4	13.8	213.6	1278
908	OK	7.81	14.2	44.2	56.6	18.2	32.2	13.1	222.3	1217
909	OK	7.22	13.4	42.1	58.3	18.5	31.8	12.8	221.9	NP
910	OK	7.47	13.5	42.8	57.3	18.0	31.5	14.1	259.3	1491

Male,	Group	XI	30/0	mg/kg	(Recovery)	Day	29			
Animal	COND	RBC $x10^6/\mu L$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	ARET $x10^3/\mu L$	PLT $x10^3/\mu L$
1101	OK	6.98	13.0	42.0	60.2	18.7	31.1	13.7	366.7	1410
1102	OK	6.80	13.3	41.1	60.5	19.5	32.2	14.3	396.5	1156
1103	OK	7.22	13.0	40.5	56.0	18.0	32.2	12.9	285.1	1179
1104	OK	6.52	12.8	40.7	62.4	19.6	31.4	14.6	406.3	963
1105	OK	6.68	12.4	39.4	59.0	18.6	31.5	15.7	422.5	NP
1106	OK	6.22	12.8	39.4	63.4	20.7	32.6	11.9	241.4	1278
1107	OK	6.67	12.3	37.7	56.5	18.4	32.6	13.5	369.9	1264
1108	OK	7.00	13.1	40.9	58.4	18.7	31.9	12.8	330.6	1391
1109	OK	6.28	12.3	39.6	63.1	19.5	31.0	19.3	563.7	NP
1110	OK	7.15	12.8	40.3	56.3	17.9	31.8	13.0	315.5	1015

Individual Animal Clinical Pathology Data

Male, Group I 0 mg/kg Day 29

Animal	WBC x10³/µL	ANEU x10³/µL	ALYM x10³/µL	AMON x10³/µL	AEOS x10³/µL	ABAS x10³/µL	ALUC x10³/µL
101	16.73	2.54	13.11	0.42	0.34	0.06	0.26
102	12.13	1.03	10.69	0.22	0.05	0.05	0.09
103	17.69	1.59	15.23	0.28	0.31	0.08	0.21
104	10.15	1.22	8.63	0.15	0.06	0.03	0.06
105	6.35	0.45	5.65	0.12	0.06	0.02	0.05
106	10.92	1.27	9.16	0.20	0.12	0.06	0.11
107	NP	NP	NP	NP	NP	NP	NP
108	11.56	1.61	9.14	0.28	0.28	0.06	0.19
109	14.79	1.86	12.13	0.32	0.16	0.07	0.24
110	12.06	1.58	9.83	0.28	0.16	0.05	0.15

Male, Group III 0.3 mg/kg Day 29

Animal	WBC x10³/µL	ANEU x10³/µL	ALYM x10³/µL	AMON x10³/µL	AEOS x10³/µL	ABAS x10³/µL	ALUC x10³/µL
301	12.65	1.84	10.21	0.19	0.16	0.08	0.17
302	12.13	1.78	9.90	0.26	0.05	0.02	0.12
303	15.31	1.87	12.73	0.41	0.12	0.05	0.13
304	16.29	1.14	14.70	0.17	0.11	0.05	0.13
305	11.09	1.30	9.29	0.24	0.03	0.10	0.13
306	8.30	0.67	7.33	0.11	0.08	0.03	0.07
307	7.40	1.01	6.10	0.12	0.06	0.04	0.07
308	11.29	2.14	8.65	0.29	0.07	0.06	0.09
309	10.11	1.04	8.64	0.20	0.10	0.02	0.11
310	9.52	1.04	8.07	0.21	0.06	0.03	0.11

191

p. 100

Individual Animal Clinical Pathology Data

Male,	Group	V	1	mg/kg	Day	29	
Animal	WBC $x10^3/\mu L$	ANEU $x10^3/\mu L$	ALYM $x10^3/\mu L$	AMON $x10^3/\mu L$	AEOS $x10^3/\mu L$	ABAS $x10^3/\mu L$	ALUC $x10^3/\mu L$
501	17.11	1.54	14.87	0.37	0.08	0.12	0.13
502	14.81	2.13	12.15	0.24	0.08	0.08	0.14
503	10.66	0.95	9.24	0.28	0.07	0.04	0.10
504	9.63	0.99	8.26	0.20	0.02	0.03	0.13
505	12.87	1.29	11.07	0.24	0.05	0.06	0.14
506	11.28	1.42	9.39	0.11	0.13	0.13	0.10
507	14.91	1.38	13.15	0.14	0.06	0.05	0.11
508	16.44	1.28	14.54	0.31	0.09	0.05	0.16
509	9.61	1.18	8.04	0.20	0.09	0.02	0.08
510	15.45	3.33	11.14	0.52	0.18	0.07	0.21

Male,	Group	VII	10	mg/kg	Day	29	
Animal	WBC $x10^3/\mu L$	ANEU $x10^3/\mu L$	ALYM $x10^3/\mu L$	AMON $x10^3/\mu L$	AEOS $x10^3/\mu L$	ABAS $x10^3/\mu L$	ALUC $x10^3/\mu L$
701	16.43	1.44	14.29	0.26	0.16	0.08	0.20
702	15.57	1.66	13.11	0.34	0.11	0.10	0.24
703	15.38	1.85	13.38	0.00	0.15	0.00	0.00
704	13.51	0.84	12.30	0.19	0.04	0.04	0.11
705	12.35	1.70	9.95	0.34	0.12	0.08	0.16
706	14.85	2.43	11.64	0.38	0.29	0.03	0.09
707	21.39	2.30	17.99	0.62	0.10	0.10	0.28
708	15.73	1.03	13.84	0.39	0.05	0.12	0.29
709	19.09	1.25	16.99	0.26	0.13	0.11	0.34
710	18.32	2.13	15.20	0.47	0.11	0.07	0.33

701 702

Individual Animal Clinical Pathology Data

Male,	Group	IX	30	mg/kg	Day	29	
Animal	WBC $x10^3/\mu L$	ANEU $x10^3/\mu L$	ALYM $x10^3/\mu L$	AMON $x10^3/\mu L$	AEOS $x10^3/\mu L$	ABAS $x10^3/\mu L$	ALUC $x10^3/\mu L$
901	13.64	1.76	11.35	0.32	0.02	0.07	0.12
902	18.92	0.76	17.59	0.57	0.00	0.00	0.00
903	17.38	2.24	14.57	0.28	0.12	0.05	0.12
904	15.26	1.58	12.86	0.32	0.16	0.05	0.29
905	13.29	1.15	11.61	0.22	0.08	0.06	0.17
906	18.04	2.60	14.51	0.35	0.25	0.08	0.26
907	18.41	1.54	16.05	0.24	0.07	0.10	0.41
908	22.08	2.53	18.42	0.51	0.15	0.13	0.34
909	19.68	1.67	16.97	0.57	0.11	0.11	0.25
910	13.97	2.10	11.35	0.16	0.12	0.05	0.19

Male,	Group	XI	30/0	mg/kg	(Recovery)	Day	29
Animal	WBC $x10^3/\mu L$	ANEU $x10^3/\mu L$	ALYM $x10^3/\mu L$	AMON $x10^3/\mu L$	AEOS $x10^3/\mu L$	ABAS $x10^3/\mu L$	ALUC $x10^3/\mu L$
1101	13.64	1.09	12.14	0.27	0.14	0.00	0.00
1102	10.03	1.31	8.31	0.23	0.06	0.03	0.09
1103	15.43	0.93	13.41	0.29	0.17	0.07	0.56
1104	11.94	1.68	9.78	0.26	0.04	0.03	0.14
1105	11.44	2.30	8.74	0.17	0.09	0.05	0.08
1106	18.93	1.38	16.78	0.31	0.09	0.12	0.26
1107	23.15	1.89	19.98	0.53	0.21	0.16	0.39
1108	14.53	1.64	12.20	0.42	0.10	0.05	0.12
1109	8.10	0.99	6.71	0.25	0.03	0.04	0.09
1110	11.89	1.37	10.02	0.21	0.02	0.07	0.20

103

Individual Animal Clinical Pathology Data

Male,	Group	I	0	mg/kg	Day	29				
Animal	ANIS	MIC	MAC	POLY	HYPO	ECHI	ACAN	TARG	RX	HJB
101	-	-	-	Trace	-	Few	Mod	-	-	-
102	Trace	-	Trace	Trace	Few	Trace	Trace	-	-	-
103	-	-	-	Trace	Trace	Few	Few	-	-	-
104	Trace	-	Trace	Trace	-	-	Trace	-	-	-
105	Few	-	Few	-	-	-	-	-	-	-
106	-	-	-	Trace	-	Mod	Few	-	-	-
107	CLOT	NP	NP	NP	NP	NP	NP	NP	NP	NP
108	-	-	-	-	-	-	-	-	-	-
109	-	-	-	-	-	Mod	Mod	-	-	-
110	-	-	-	-	-	-	-	-	-	-

Male,	Group	III	0.3	mg/kg	Day	29				
Animal	ANIS	MIC	MAC	POLY	HYPO	ECHI	ACAN	TARG	RX	HJB
301	Trace	-	Trace	-	Few	-	-	-	-	-
302	-	-	-	-	-	-	-	-	-	-
303	-	-	-	-	-	-	-	-	-	-
304	Trace	-	Trace	Trace	-	-	Trace	-	-	-
305	-	-	-	-	-	-	-	-	-	-
306	-	-	-	-	-	-	-	-	-	-
307	-	-	-	-	Few	-	-	-	-	-
308	-	-	-	-	-	-	-	-	-	-
309	Few	-	Few	Trace	-	Few	Few	-	-	-
310	Trace	-	Trace	Trace	-	-	-	-	-	-

104

Individual Animal Clinical Pathology Data

Male,	Group	V	1	mg/kg	Day	29				
Animal	ANIS	MIC	MAC	POLY	HYPO	ECHI	ACAN	TARG	RX	HJB
501	Trace	-	Trace	-	Few	Few	Trace	-	-	-
502	Trace	-	Trace	Trace	-	-	-	-	-	-
503	Trace	-	Trace	-	Mod	-	Trace	-	-	-
504	Trace	-	Trace	-	-	-	-	-	-	-
505	Trace	-	Trace	Trace	Mod	Trace	Trace	-	-	-
506	Trace	-	Trace	-	Trace	-	Trace	-	-	-
507	-	-	-	-	-	-	-	-	-	-
508	Trace	-	Trace	-	Few	-	-	-	-	-
509	Trace	-	Trace	Trace	Few	-	Few	-	-	-
510	-	-	-	Trace	-	-	-	-	-	-

Male,	Group	VII	10	mg/kg	Day	29				
Animal	ANIS	MIC	MAC	POLY	HYPO	ECHI	ACAN	TARG	RX	HJB
701	Trace	-	Trace	Trace	Mod	-	Few	-	-	-
702	Trace	-	Trace	Few	Mod	-	Trace	-	-	-
703	Trace	-	Trace	Trace	Many	-	Trace	-	-	-
704	Trace	-	Trace	Trace	Mod	-	Trace	-	-	-
705	Trace	-	Trace	-	Many	-	-	-	-	-
706	Trace	-	Trace	Few	Few	-	Few	-	-	-
707	Trace	-	Trace	Trace	Few	-	-	-	-	-
708	Trace	-	Trace	-	Trace	-	-	-	-	-
709	Trace	-	Trace	Trace	Few	-	Few	-	-	-
710	Trace	-	Trace	Trace	Few	-	-	-	-	-

105

Individual Animal Clinical Pathology Data

Male,	Group	IX	30	mg/kg	Day	29				
Animal	ANIS	MIC	MAC	POLY	HYPO	ECHI	ACAN	TARG	RX	HJB
901	Trace	-	Trace	Few	Mod	-	Trace	-	-	-
902	Trace	-	Trace	Trace	Trace	-	Trace	-	-	-
903	Trace	-	Trace	Trace	Many	-	Trace	-	-	-
904	Trace	-	Trace	-	-	-	-	-	-	-
905	Trace	-	Trace	Trace	Few	-	Trace	-	-	-
906	-	-	-	-	-	-	-	-	-	
907	Trace	-	Trace	Trace	Few	-	Few	-	-	-
908	Trace	Trace	Trace	Trace	Trace	-	-	-	-	-
909	-	-	-	Trace	Mod	-	-	-	-	-
910	Trace	Trace	Trace	Few	Few	-	Few	-	-	-

Male,	Group	XI	30/0	mg/kg	(Recovery)	Day	29			
Animal	ANIS	MIC	MAC	POLY	HYPO	ECHI	ACAN	TARG	RX	HJB
1101	Trace	-	Trace	Mod	Few	Trace	Few	-	-	-
1102	Few	-	Few	Few	Mod	-	Trace	-	-	-
1103	Trace	-	Trace	Few	Mod	Trace	Few	-	-	-
1104	Few	-	Few	Mod	Mod	-	Trace	-	-	-
1105	Few	-	Few	Mod	Many	-	Few	-	-	-
1106	Trace	-	Trace	Few	Few	-	Few	-	-	-
1107	Trace	-	Trace	Few	Few	-	Few	-	-	-
1108	Trace	Trace	Trace	Few	Few	-	Trace	-	-	-
1109	Few	Trace	Few	Mod	Many	-	Few	-	-	-
1110	Trace	Trace	Trace	Few	Mod	Few	Few	-	-	-

106

p. 105

Individual Animal Clinical Pathology Data

Male, Group I 0 mg/kg Day 29

Animal	SM	TOX	DB	VC	BC	PCE	GP	BP
101	-	-	-	-	-	Decr	-	-
102	-	-	-	-	-	-	-	-
103	-	-	-	-	-	Decr	-	-
104	-	-	-	-	-	-	-	-
105	-	-	-	-	-	-	-	-
106	-	-	-	-	-	-	-	-
107	CLOT	NP	NP	NP	NP	NP	NP	NP
108	-	-	-	-	-	Decr	-	-
109	-	-	-	-	-	-	-	-
110	-	-	-	-	-	-	-	-

Male, Group III 0.3 mg/kg Day 29

Animal	SM	TOX	DB	VC	BC	PCE	GP	BP
301	-	-	-	-	-	-	-	-
302	-	-	-	-	-	-	-	-
303	-	-	-	-	-	-	-	-
304	-	-	-	-	-	-	-	-
305	-	-	-	-	-	-	-	-
306	-	-	-	-	-	-	-	-
307	-	-	-	-	-	-	-	-
308	-	-	-	-	-	-	-	-
309	-	-	-	-	-	-	-	-
310	-	-	-	-	-	-	-	-

107

p. 106

Individual Animal Clinical Pathology Data

Male,	Group	V	1	mg/kg	Day	29		
Animal	SM	TOX	DB	VC	BC	PCE	GP	BP
501	-	-	-	-	-	Adeq	-	-
502	-	-	-	-	-	-	-	-
503	-	-	-	-	-	-	-	-
504	-	-	-	-	-	-	-	-
505	-	-	-	-	-	Adeq	-	-
506	-	-	-	-	-	-	-	-
507	-	-	-	-	-	-	-	-
508	-	-	-	-	-	Adeq	-	-
509	-	-	-	-	-	-	-	-
510	-	-	-	-	-	Adeq	-	-

Male,	Group	VII	10	mg/kg	Day	29		
Animal	SM	TOX	DB	VC	BC	PCE	GP	BP
701	-	-	-	-	-	-	-	-
702	-	-	-	-	-	-	-	-
703	-	-	-	-	-	Decr	-	-
704	-	-	-	-	-	-	-	-
705	-	-	-	-	-	Decr	-	-
706	-	-	-	-	-	-	-	-
707	-	-	-	-	-	Adeq	-	-
708	-	-	-	-	-	-	-	-
709	-	-	-	-	-	-	-	-
710	-	-	-	-	-	-	-	-

201

p. 107

Individual Animal Clinical Pathology Data

Male,	Group	IX	30	mg/kg	Day	29		
Animal	SM	TOX	DB	VC	BC	PCE	GP	BP
901	-	-	-	-	-	Adeq	-	-
902	-	-	-	-	-	-	-	-
903	-	-	-	-	-	-	-	-
904	-	-	-	-	-	Decr	-	-
905	-	-	-	-	-	-	-	-
906	-	-	-	-	-	-	-	-
907	-	-	-	-	-	-	-	-
908	-	-	-	-	-	-	-	-
909	-	-	-	-	-	Adeq	-	-
910	-	-	-	-	-	-	-	-

Male,	Group	XI	30/0	mg/kg	(Recovery)	Day	29	
Animal	SM	TOX	DB	VC	BC	PCE	GP	BP
1101	-	-	-	-	-	-	-	-
1102	-	-	-	-	-	-	-	-
1103	-	-	-	-	-	-	-	-
1104	-	-	-	-	-	-	-	-
1105	-	-	-	-	-	Adeq	-	-
1106	-	-	-	-	-	-	-	-
1107	-	-	-	-	-	-	-	-
1108	-	-	-	-	-	-	-	-
1109	-	-	-	-	-	Adeq	-	-
1110	-	-	-	-	-	-	-	-

107

p. 108

Individual Animal Clinical Pathology Data

Male, Group I 0 mg/kg Day 29

Animal	HEM	LIP	ICT	CHOL mg/dL	TRIG mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	HDL mg/dL	NHDL mg/dL	SCORT ng/mL
101	Small	None	None	95	57	6.4	3.4	3.0	29	66	213
102	None	None	None	67	92	6.1	3.3	2.8	27	40	32
103	None	None	None	70	65	6.4	3.4	3.0	25	45	104
104	None	None	None	58	50	5.9	3.3	2.6	22	36	60
105	None	None	None	46	36	5.9	3.3	2.6	19	27	109
106	None	None	None	56	59	6.1	3.2	2.9	21	35	218
107	None	None	None	91	88	6.4	3.5	2.9	31	60	50
108	None	None	None	55	66	6.2	3.3	2.9	23	32	251
109	None	None	None	57	94	5.9	3.1	2.8	22	35	283
110	None	None	None	43	69	6.1	3.3	2.8	19	24	46

Male, Group III 0.3 mg/kg Day 29

Animal	HEM	LIP	ICT	CHOL mg/dL	TRIG mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	HDL mg/dL	NHDL mg/dL	SCORT ng/mL
301	None	None	None	32	48	6.7	3.5	3.2	16	16	64
302	None	None	None	51	86	6.2	3.4	2.8	21	30	190
303	None	None	None	37	40	6.3	3.3	3.0	18	19	106
304	None	None	None	27	54	5.9	3.4	2.5	14	13	88
305	None	None	None	29	26	6.0	3.4	2.6	15	14	113
306	None	None	None	54	51	6.2	3.4	2.8	21	33	227
307	None	None	None	36	29	6.1	3.4	2.7	17	19	214
308	None	None	None	43	34	5.9	3.3	2.6	17	26	258
309	None	None	None	53	53	5.9	3.2	2.7	22	31	262
310	None	None	None	49	46	6.0	3.3	2.7	20	29	148

Individual Animal Clinical Pathology Data

Male, Group V 1 mg/kg Day 29

Animal	HEM	LIP	ICT	CHOL mg/dL	TRIG mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	HDL mg/dL	NHDL mg/dL	SCORT ng/mL
501	None	None	None	64	99	6.4	3.6	2.8	26	38	221
502	None	None	None	43	54	6.7	3.7	3.0	22	21	70
503	None	None	None	34	44	6.0	3.5	2.5	15	19	107
504	None	None	None	44	47	5.8	3.5	2.3	19	25	155
505	None	None	None	48	51	6.4	3.6	2.8	21	27	128
506	Trace	None	None	42	43	6.5	3.6	2.9	18	24	50
507	Small	None	None	46	26	6.0	3.4	2.6	18	28	271
508	Trace	None	None	34	30	5.7	3.2	2.5	15	19	208
509	None	None	None	42	56	6.5	3.6	2.9	19	23	116
510	None	None	None	42	56	6.3	3.7	2.6	18	24	147

Male, Group VII 10 mg/kg Day 29

Animal	HEM	LIP	ICT	CHOL mg/dL	TRIG mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	HDL mg/dL	NHDL mg/dL	SCORT ng/mL
701	Trace	None	None	55	47	5.9	3.5	2.4	20	35	174
702	Trace	None	None	48	47	6.5	3.8	2.7	18	30	354
703	Small	None	None	74	59	5.8	3.4	2.4	24	50	176
704	Small	None	None	52	25	5.7	3.5	2.2	19	33	158
705	None	None	None	53	42	6.0	3.5	2.5	19	34	302
706	Small	None	None	46	44	6.3	3.7	2.6	15	31	60
707	Small	None	None	55	81	6.8	4.0	2.8	21	34	88
708	Trace	None	None	41	32	6.3	3.8	2.5	16	25	119
709	Small	None	None	36	42	6.5	3.8	2.7	12	24	236
710	Small	None	None	59	40	5.6	3.5	2.1	20	39	182

Individual Animal Clinical Pathology Data

Male,	Group	IX	30	mg/kg	Day	29					
Animal	HEM	LIP	ICT	CHOL mg/dL	TRIG mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	HDL mg/dL	NHDL mg/dL	SCORT ng/mL
901	Small	None	None	46	55	5.8	3.7	2.1	16	30	436
902	None	None	None	70	48	6.3	3.7	2.6	26	44	736
903	Trace	None	None	50	55	6.4	3.8	2.6	18	32	370
904	Small	None	None	47	35	6.3	3.7	2.6	17	30	238
905	None	None	None	64	37	6.5	3.9	2.6	25	39	76
906	Trace	None	None	47	47	6.4	3.7	2.7	18	29	43
907	Small	None	None	64	26	6.0	3.8	2.2	22	42	331
908	Trace	None	None	47	42	5.8	3.6	2.2	16	31	38
909	Trace	None	None	53	44	5.9	3.7	2.2	16	37	287
910	None	None	None	55	62	6.7	3.9	2.8	20	35	124

Male,	Group	XI	30/0	mg/kg	(Recovery)	Day	29				
Animal	HEM	LIP	ICT	CHOL mg/dL	TRIG mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	HDL mg/dL	NHDL mg/dL	SCORT ng/mL
1101	Small	None	None	88	45	6.8	3.9	2.9	29	59	71
1102	Trace	None	None	61	79	6.3	3.6	2.7	23	38	317
1103	None	None	None	122	57	7.0	3.9	3.1	35	87	90
1104	Trace	None	None	67	39	6.6	3.9	2.7	24	43	198
1105	None	None	None	84	64	7.0	4.2	2.8	28	56	68
1106	Small	None	None	64	25	6.4	3.7	2.7	25	39	54
1107	Small	None	None	36	35	6.3	3.6	2.7	15	21	89
1108	Trace	None	None	82	37	6.6	3.7	2.9	30	52	95
1109	None	None	None	62	44	5.4	3.2	2.2	22	40	245
1110	None	None	None	59	42	6.2	3.6	2.6	22	37	85

112

p. 111

Appendix G
Individual Primary Humoral Immune Response Data

Individual Primary Humoral Immune Response Data

Animal Number	SLOPE	X	Log_2
Male, Group I - 0 mg/kg			
101	-0.8772	1648	10.687
102	-0.9165	872	9.768
103	-0.9442	5777	12.496
104	-1.0021	790	9.626
105	-0.9721	1355	10.404
106	-1.0214	2414	11.237
107	-1.0063	122	6.928
108	-1.0053	1586	10.631
109	-1.0377	394	8.622
110	-1.0022	1175	10.198
Male, Group III - 0.3 mg/kg			
301	-1.0141	1590	10.635
302	-0.9706	695	9.441
303	-1.0180	1237	10.273
304	-0.9477	1878	10.875
305	-1.0010	1854	10.856
306	-0.9884	1618	10.660
307	-0.9671	1605	10.648
308	-0.9972	1312	10.358
309	-0.9972	1095	10.097
310	-0.8896	914	9.836
Male, Group V - 1 mg/kg			
501	-0.9758	1307	10.352
502	-0.9001	2679	11.387
503	-0.9786	1559	10.606
504	-0.9388	111	6.794
505	-0.9980	915	9.838
506	-1.0325	1048	10.033
507	-0.9969	3228	11.656
508	-0.9742	791	9.628
509	-0.9732	227	7.826
510	-0.9843	1409	10.460

Individual Primary Humoral Immune Response Data

Animal Number	SLOPE	X	Log_2
Male, Group VII - 10 mg/kg			
701	-1.0199	788	9.622
702	-0.9756	956	9.901
703	-0.9847	201	7.650
704	-1.0187	855	9.740
705	-0.9605	2116	11.047
706	-0.9560	7340	12.842
707	-0.9836	494	8.948
708	-0.9532	86	6.426
709	-0.9399	3660	11.838
710	-0.9843	2287	11.159
Male, Group IX - 30 mg/kg			
901	-1.0014	1068	10.061
902	-1.0035	342	8.418
903	-1.0215	760	9.570
904	-0.9123	294	8.200
905	-1.0340	1788	10.804
906	-0.9742	2755	11.428
907	-0.9052	2078	11.021
908	-0.9060	457	8.836
909	-0.9504	1893	10.886
910	-1.0107	912	9.833
Male, Group XI - 30/0 mg/kg (Recovery)			
1101	-1.0040	543	9.085
1102	-0.9456	370	8.531
1103	-0.9969	392	8.615
1104	-0.9935	460	8.845
1105	-0.7713	1009	9.979
1106	-0.9712	2410	11.235
1107	-0.9856	314	8.295
1108	-0.9682	2447	11.257
1109	-0.9463	362	8.500
1110	-0.9972	1844	10.849

Appendix H
Individual Primary Humoral Immune Response Positive Control Data

Individual Primary Humoral Immune Response Positive Control Data

Animal Number	SLOPE	X	Log_2
Male, Group CIX - Saline			
C901	-1.0282	1215	10.247
C902	-1.0269	1400	10.451
C903	-0.9992	1249	10.287
C904	-1.0116	977	9.932
C905	-0.9398	820	9.679
C906	-0.9457	109	6.768
C907	-0.9708	554	9.114
C908	-0.9619	1255	10.293
C909	-0.9601	692	9.435
C910	-1.0013	328	8.358
Male, Group CXI - 20 mg/kg Cyclophosphamide			
C1101	-0.6474	4	2.000
C1102	-0.9816	14	3.807
C1103	-0.9912	18	4.170
C1104	-0.9556	12	3.585
C1105	-0.9067	23	4.524
C1106	-0.8857	26	4.700
C1107	-1.0035	55	5.781
C1108	-1.0031	15	3.907
C1109	-0.9962	14	3.807
C1110	-0.9898	26	4.700
Male, Pooled Samples - 20 mg/kg Cyclophosphamide			
	-0.9859	29	4.858
	-0.9832	12	3.625

Appendix I
Individual Animal Final Body and Organ Weights

Individual Animal Final Body and Organ Weights

Group: I Treatment: 0 mg/kg Sex: MALES

ANIMAL	FBW	BRAIN		LIVER			SPLEEN			THYMUS		
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN
101	455.90	2.163	0.4744	16.208	3.5552	749.33	1.143	0.2507	52.843	0.691	0.1516	31.946
102	441.60	1.995	0.4518	13.249	3.0002	664.11	0.761	0.1723	38.145	0.651	0.1474	32.632
103	445.00	2.122	0.4769	14.415	3.2393	679.31	0.976	0.2193	45.994	0.775	0.1742	36.522
104	427.00	1.959	0.4588	13.245	3.1019	676.11	0.771	0.1806	39.357	0.690	0.1616	35.222
105	390.80	2.072	0.5302	11.388	2.9140	549.61	0.806	0.2062	38.900	0.425	0.1088	20.512
106	404.70	1.949	0.4816	12.348	3.0511	633.56	0.669	0.1653	34.325	0.499	0.1233	25.603
107	387.90	2.004	0.5166	12.113	3.1227	604.44	0.617	0.1591	30.788	0.429	0.1106	21.407
108	405.90	1.948	0.4799	12.243	3.0163	628.49	1.008	0.2483	51.745	0.435	0.1072	22.331
109	457.80	2.038	0.4452	12.617	2.7560	619.09	0.944	0.2062	46.320	0.544	0.1188	26.693
110	414.60	1.873	0.4518	13.968	3.3690	745.76	0.744	0.1795	39.722	0.544	0.1312	29.044
Mean	423.12	2.012	0.4767	13.179	3.1126	654.98	0.844	0.1988	41.814	0.568	0.1335	28.191
S.D.	26.047	0.088	0.0280	1.397	0.2286	61.796	0.167	0.0330	7.2116	0.126	0.0238	5.7896

Group: III Treatment: 0.3 mg/kg Sex: MALES

ANIMAL	FBW	BRAIN		LIVER			SPLEEN			THYMUS		
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN
301	420.90	2.301	0.5467	15.614	3.7097	678.57	0.990	0.2352	43.025	0.704	0.1673	30.595
302	434.90	2.100	0.4829	16.174	3.7190	770.19	0.692	0.1591	32.952	0.828	0.1904	39.429
303	458.60	2.196	0.4788	15.448	3.3685	703.46	1.254	0.2734	57.104	0.504	0.1099	22.951
304	403.80	2.021	0.5005	14.184	3.5126	701.83	1.073	0.2657	53.093	0.652	0.1615	32.261
305	395.90	2.140	0.5405	12.639	3.1925	590.61	0.880	0.2223	41.121	0.619	0.1564	28.925
306	437.60	2.286	0.5224	14.947	3.4157	653.85	1.018	0.2326	44.532	0.689	0.1574	30.140
307	414.00	2.062	0.4981	13.090	3.1618	634.82	0.683	0.1650	33.123	0.421	0.1017	20.417
308	380.00	1.977	0.5203	11.256	2.9621	569.35	0.763	0.2008	38.594	0.457	0.1203	23.116
309	448.60	2.009	0.4478	15.946	3.5546	793.73	0.582	0.1297	28.970	0.577	0.1286	28.721
310	403.10	2.019	0.5009	14.488	3.5941	717.58	0.781	0.1937	38.683	0.587	0.1456	29.074
Mean	419.74	2.111	0.5039	14.379	3.4191	681.40	0.872	0.2078	41.120	0.604	0.1439	28.563
S.D.	24.955	0.117	0.0299	1.604	0.2497	71.841	0.209	0.0469	8.8488	0.123	0.0281	5.4395

FBW - Final Body Weight

Individual Animal Final Body and Organ Weights

Group: V Treatment: 1 mg/kg Sex: MALES

ANIMAL	FBW	BRAIN		LIVER			SPLEEN			THYMUS		
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN
501	419.60	2.036	0.4852	23.052	5.4938	1132.2	0.725	0.1728	35.609	0.427	0.1018	20.972
502	395.30	2.090	0.5287	16.936	4.2843	810.33	0.762	0.1928	36.459	0.561	0.1419	26.842
503	439.20	2.031	0.4624	16.885	3.8445	831.36	0.907	0.2065	44.658	0.634	0.1444	31.216
504	441.30	2.108	0.4777	16.810	3.8092	797.44	1.004	0.2275	47.628	0.745	0.1688	35.342
505	364.70	2.086	0.5720	14.998	4.1124	718.98	0.673	0.1845	32.263	0.410	0.1124	19.655
506	408.80	1.987	0.4861	15.668	3.8327	788.53	0.836	0.2045	42.073	0.569	0.1392	28.636
507	369.90	2.096	0.5666	14.125	3.8186	673.90	0.660	0.1784	31.489	0.521	0.1408	24.857
508	406.10	2.093	0.5154	15.830	3.8981	756.33	0.938	0.2310	44.816	0.691	0.1702	33.015
509	476.20	2.307	0.4845	21.644	4.5451	938.19	1.085	0.2278	47.031	0.633	0.1329	27.438
510	379.20	2.028	0.5348	16.321	4.3041	804.78	0.758	0.1999	37.377	0.396	0.1044	19.527
Mean	410.03	2.086	0.5113	17.227	4.1943	825.21	0.835	0.2026	39.940	0.559	0.1357	26.750
S.D.	35.195	0.087	0.0383	2.860	0.5238	128.55	0.144	0.0210	6.0349	0.121	0.0238	5.5431

Group: VII Treatment: 10 mg/kg Sex: MALES

ANIMAL	FBW	BRAIN		LIVER			SPLEEN			THYMUS		
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN
701	354.30	1.933	0.5456	20.606	5.8160	1066.0	0.776	0.2190	40.145	0.679	0.1916	35.127
702	393.60	1.826	0.4639	22.561	5.7320	1235.5	0.760	0.1931	41.621	0.547	0.1390	29.956
703	382.60	2.053	0.5366	21.889	5.7211	1066.2	0.750	0.1960	36.532	0.674	0.1762	32.830
704	355.70	1.910	0.5370	17.564	4.9379	919.58	0.927	0.2606	48.534	0.420	0.1181	21.990
705	317.70	1.965	0.6185	16.749	5.2720	852.37	0.652	0.2052	33.181	0.319	0.1004	16.234
706	409.10	2.285	0.5585	25.831	6.3141	1130.5	0.767	0.1875	33.567	0.569	0.1391	24.902
707	421.10	2.029	0.4818	23.567	5.5965	1161.5	0.912	0.2166	44.948	0.717	0.1703	35.338
708	347.60	1.949	0.5607	19.401	5.5814	995.43	0.625	0.1798	32.068	0.528	0.1519	27.091
709	409.00	2.057	0.5029	23.440	5.7311	1139.5	0.898	0.2196	43.656	0.734	0.1795	35.683
710	378.80	1.983	0.5235	23.086	6.0945	1164.2	1.017	0.2685	51.286	0.626	0.1653	31.568
Mean	376.95	1.999	0.5329	21.469	5.6797	1073.1	0.808	0.2146	40.554	0.581	0.1531	29.072
S.D.	32.760	0.123	0.0438	2.864	0.3849	119.55	0.126	0.0296	6.6706	0.134	0.0290	6.4726

FBW - Final Body Weight

120

p. 119

Individual Animal Final Body and Organ Weights

Group: IX Treatment: 30 mg/kg Sex: MALES

ANIMAL	FBW	BRAIN		LIVER			SPLEEN			THYMUS		
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN
901	272.60	1.814	0.6654	15.544	5.7021	856.89	0.498	0.1827	27.453	0.299	0.1097	16.483
902	294.00	1.946	0.6619	15.920	5.4150	818.09	0.633	0.2153	32.528	0.668	0.2272	34.327
903	394.10	2.086	0.5293	22.466	5.7006	1077.0	0.779	0.1977	37.344	0.765	0.1941	36.673
904	284.00	2.135	0.7518	15.315	5.3926	717.33	0.673	0.2370	31.522	0.191	0.0673	8.9461
905	329.20	1.961	0.5957	18.034	5.4781	919.63	0.673	0.2044	34.319	0.563	0.1710	28.710
906	303.40	2.043	0.6734	18.366	6.0534	898.97	0.713	0.2350	34.900	0.582	0.1918	28.488
907	334.00	1.979	0.5925	23.114	6.9204	1168.0	0.762	0.2281	38.504	0.487	0.1458	24.608
908	303.80	1.908	0.6280	18.002	5.9256	943.50	0.734	0.2416	38.470	0.372	0.1224	19.497
909	335.00	2.144	0.6400	21.838	6.5188	1018.6	0.691	0.2063	32.229	0.487	0.1454	22.715
910	294.10	1.904	0.6474	18.242	6.2027	958.09	0.586	0.1993	30.777	0.453	0.1540	23.792
Mean	314.42	1.992	0.6385	18.684	5.9309	937.60	0.674	0.2147	33.805	0.487	0.1529	24.424
S.D.	35.139	0.108	0.0590	2.866	0.5033	129.37	0.085	0.0198	3.6004	0.171	0.0463	8.2546

Group: XI Treatment: 30/0 mg/kg (Recovery) Sex: MALES

ANIMAL	FBW	BRAIN		LIVER			SPLEEN			THYMUS		
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN	(Gms)	%FBW	%BRAIN
1101	329.40	1.808	0.5489	17.005	5.1624	940.54	0.596	0.1809	32.965	0.607	0.1843	33.573
1102	343.20	1.964	0.5723	17.783	5.1815	905.45	0.782	0.2279	39.817	0.619	0.1804	31.517
1103	344.00	1.993	0.5794	16.745	4.8677	840.19	0.722	0.2099	36.227	0.739	0.2148	37.080
1104	393.00	2.056	0.5232	19.117	4.8644	929.82	1.076	0.2738	52.335	0.834	0.2122	40.564
1105	336.60	1.999	0.5939	17.024	5.0576	851.63	0.665	0.1976	33.267	0.571	0.1696	28.564
1106	386.50	1.914	0.4952	17.552	4.5413	917.03	1.013	0.2621	52.926	0.776	0.2008	40.543
1107	312.50	1.782	0.5702	16.575	5.3040	930.13	0.841	0.2691	47.194	0.534	0.1709	29.966
1108	283.60	1.641	0.5786	11.859	4.1816	722.67	0.624	0.2200	38.026	0.483	0.1703	29.433
1109	287.60	1.969	0.6846	13.520	4.7010	686.64	0.548	0.1905	27.831	0.624	0.2170	31.691
1110	321.50	2.003	0.6230	14.881	4.6286	742.94	0.936	0.2911	46.730	0.606	0.1885	30.255
Mean	333.79	1.913	0.5769	16.206	4.8490	846.70	0.780	0.2323	40.732	0.639	0.1909	33.319
S.D.	36.149	0.129	0.0521	2.170	0.3449	95.980	0.182	0.0390	8.6409	0.110	0.0190	4.5108

FBW - Final Body Weight

121

p. 120

Appendix J
Individual Animal Pathology Data

INDIVIDUAL ANIMAL PATHOLOGY DATA

KEY TO APPENDIX

LESION GRADING:

Histopathology changes are described according to their morphologic character, distribution and severity. The distribution (extent of tissue involvement) is indicated, where appropriate, by modifiers such as focal, multifocal, diffuse, unilateral, bilateral, etc. A severity score, if appropriate, is also assigned as follows:

MINIMAL: The amount of change present barely exceeds that which is considered to be within normal limits.

MILD: In general, the lesion is easily identified but of limited severity. The lesion probably does not produce any functional impairment.

MODERATE: The lesion is prominent but there is significant potential for increased severity. Limited tissue or organ dysfunction is possible.

SEVERE: The degree of change is either as complete as considered possible or great enough in intensity or extent to expect significant tissue or organ dysfunction.

COMMENT:

Grades minimal through severe represent progressive involvement/severity along a continuum with minimal lesions being the least severe and severe lesions being the most severe. While the grades refer to the morphologic characteristics of lesions, they also indicate their relative biologic significance.

Gross observations listing multiple masses for a tissue are distinguished with letters (i.e., a, b, c, d, etc.).

Individual Animal Pathology Data

Dose Group: I Treatment: 0 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
101	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. CAUSE OF DEATH : SACRIFICE BY DESIGN. POPLITEAL LYMPH NODE : NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed : SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
102	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	CAUSE OF DEATH : SACRIFICE BY DESIGN. POPLITEAL LYMPH NODE : NOT PRESENT IN TISSUE SECTION.

102 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: I Treatment: 0 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
102	Continued from previous page
	Histopathology :
	No Microscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
103	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. FATTY CHANGE, MEDIAN CLEFT, minimal. CAUSE OF DEATH : SACRIFICE BY DESIGN. POPLITEAL LYMPH NODE : NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed : SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
104	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

104 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: I Treatment: 0 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
104	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE
105	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	POPLITEAL LYMPH NODE :
	NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
106	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

106 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: I Treatment: 0 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
106	Continued from previous page

Histopathology :

LIVER :
INFLAMMATION, SUBACUTE/CHRONIC, minimal.
CAUSE OF DEATH :
SACRIFICE BY DESIGN.

No Microscopic Abnormality Observed :
SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE

107 Terminal Sacrifice
Killed on Day : 29
Animal is signed off from necropsy

Gross Pathology :

No Macroscopic Abnormality Observed :
LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

Histopathology :

LIVER :
INFLAMMATION, SUBACUTE/CHRONIC, minimal.
BONE MARROW :
FIBROSIS, FOCAL, minimal, (femur).
CAUSE OF DEATH :
SACRIFICE BY DESIGN.
POPLITEAL LYMPH NODE :
NOT PRESENT IN TISSUE SECTION.

No Microscopic Abnormality Observed :
SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM

Individual Animal Pathology Data

Dose Group: I Treatment: 0 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
108	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. BRAIN : PIGMENT, FOCAL, minimal, hemosiderin (cerebellum). CAUSE OF DEATH : SACRIFICE BY DESIGN. POPLITEAL LYMPH NODE : NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed : SPLEEN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
109	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. FATTY CHANGE, MEDIAN CLEFT, minimal. CAUSE OF DEATH : SACRIFICE BY DESIGN.

109 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: I Treatment: 0 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
109	Continued from previous page
	Histopathology :
	POPLITEAL LYMPH NODE :
	NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
110	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	POPLITEAL LYMPH NODE :
	NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW

Individual Animal Pathology Data

Dose Group: III Treatment: 0.3 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
301	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, minimal, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN
302	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, minimal, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.

302 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: III Treatment: 0.3 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
302	Continued from previous page
	Histopathology :
	No Microscopic Abnormality Observed : SPLEEN
303	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, minimal, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN
304	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

304 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: III Treatment: 0.3 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
304	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
305	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS,
	FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, minimal, with
	cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
306	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS,
	FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

306 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: III Treatment: 0.3 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
306	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
307	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS,
	FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
308	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS,
	FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

308 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: III Treatment: 0.3 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
308	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
309	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS,
	FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, minimal, with
	cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
310	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS,
	FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

310 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: III Treatment: 0.3 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
310	Continued from previous page

Histopathology :

LIVER :
INFLAMMATION, SUBACUTE/CHRONIC, minimal.
CAUSE OF DEATH :
SACRIFICE BY DESIGN.

No Microscopic Abnormality Observed :
SPLEEN

Individual Animal Pathology Data

Dose Group: V Treatment: 1 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
501	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, minimal, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN
502	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, mild, with cytoplasmic eosinophilic stippling. SPLEEN : HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED, minimal. CAUSE OF DEATH : SACRIFICE BY DESIGN.

Individual Animal Pathology Data

Dose Group: V Treatment: 1 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
503	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology : No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology : LIVER : INFLAMMATION, SUBACUTE/CHRONIC, mild. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, mild, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN. No Microscopic Abnormality Observed : SPLEEN
504	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology : No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology : LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, minimal, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.

504 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: V Treatment: 1 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
504	Continued from previous page
	Histopathology :
	No Microscopic Abnormality Observed : SPLEEN
505	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, mild, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN
506	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

506 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: V Treatment: 1 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
506	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, mild, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
507	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, mild, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
508	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

508 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: V Treatment: 1 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
508	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, minimal, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
509	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, mild, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
510	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

510 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: V Treatment: 1 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
510	Continued from previous page

Histopathology :

LIVER :
INFLAMMATION, SUBACUTE/CHRONIC, minimal.
HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, mild, with cytoplasmic eosinophilic stippling.
CAUSE OF DEATH :
SACRIFICE BY DESIGN.

No Microscopic Abnormality Observed :
SPLEEN

Individual Animal Pathology Data

Dose Group: VII Treatment: 10 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
701	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN
702	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.

702 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: VII Treatment: 10 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
702	Continued from previous page
	Histopathology :
	No Microscopic Abnormality Observed : SPLEEN
703	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERPLASIA, BILE DUCT, FOCAL, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN
704	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

704 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: VII Treatment: 10 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
704	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
705	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	LIVER :
	DISCOLORATION, TAN, LEFT, LINEAR <3MM .
	No Macroscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN

Individual Animal Pathology Data

Dose Group: VII Treatment: 10 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
706	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology : No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology : LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN. No Microscopic Abnormality Observed : SPLEEN
707	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology : No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology : LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. NECROSIS, FOCAL, minimal, coagulative. MINERALIZATION, BILE DUCT, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.

707 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: VII Treatment: 10 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
707	Continued from previous page
	Histopathology :
	No Microscopic Abnormality Observed : SPLEEN
708	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN
709	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

709 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: VII Treatment: 10 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
709	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, mild.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN
710	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.

Individual Animal Pathology Data

Dose Group: IX Treatment: 30 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
901	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. INFLAMMATION, SUBACUTE/CHRONIC, minimal. MESENTERIC LYMPH NODE : DEPLETION/ATROPHY, LYMPHOID, minimal, (inner cortex, outer cortex, and follicles). CAUSE OF DEATH : SACRIFICE BY DESIGN. POPLITEAL LYMPH NODE : NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed : SPLEEN, BRAIN, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
902	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal.

902 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: IX Treatment: 30 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
902	Continued from previous page
	Histopathology :
	LIVER :
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	POPLITEAL LYMPH NODE :
	NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
903	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE

Individual Animal Pathology Data

Dose Group: IX Treatment: 30 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
904	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy

Gross Pathology :

No Macroscopic Abnormality Observed :
LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

Histopathology :

LIVER :
INFLAMMATION, SUBACUTE/CHRONIC, mild.
HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
NECROSIS, FOCAL, minimal, coagulative, subcapsular.
CAUSE OF DEATH :
SACRIFICE BY DESIGN.
POPLITEAL LYMPH NODE :
NOT PRESENT IN TISSUE SECTION.

No Microscopic Abnormality Observed :
SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW

Animal Ref	Microscopic & Macroscopic Findings
905	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy

Gross Pathology :

LIVER :
DISCOLORATION, TAN, MOTTLED.

No Macroscopic Abnormality Observed :
SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

905 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: IX Treatment: 30 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
905	Continued from previous page
	Histopathology :
	LIVER :
	NECROSIS, FOCAL, minimal, coagulative, subcapsular.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE
906	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE

Individual Animal Pathology Data

Dose Group: IX Treatment: 30 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
907	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	LIVER : LARGE DISCOLORATION, PALE.
	No Macroscopic Abnormality Observed : SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. NECROSIS, FOCAL, minimal, coagulative, subcapsular. INFLAMMATION, SUBACUTE/CHRONIC, minimal. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE
908	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal.

908 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: IX Treatment: 30 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
908	Continued from previous page
	Histopathology :
	LIVER :
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE
909	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	NECROSIS, FOCAL, minimal, coagulative.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE

Individual Animal Pathology Data

Dose Group: IX Treatment: 30 mg/kg Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
910	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy

Gross Pathology :

No Macroscopic Abnormality Observed :
LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

Histopathology :

LIVER :
INFLAMMATION, SUBACUTE/CHRONIC, minimal.
HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
CAUSE OF DEATH :
SACRIFICE BY DESIGN.

No Microscopic Abnormality Observed :
SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE

Individual Animal Pathology Data

Dose Group: XI Treatment: 30/0 mg/kg (Recovery) Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
1101	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	LIVER : DISCOLORATION, TAN, MOTTLED, LEFT.
	No Macroscopic Abnormality Observed : SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. SPLEEN : HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED, minimal. BONE MARROW : FIBROSIS, FOCAL, minimal, (femur). CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
1102	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

1102 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: XI Treatment: 30/0 mg/kg (Recovery) Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
1102	Continued from previous page

Histopathology :

LIVER :
MINERALIZATION, BILE DUCT, moderate, with fibrosis.
HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
INFLAMMATION, SUBACUTE/CHRONIC, minimal.
HEMATOPOIESIS, EXTRAMEDULLARY, minimal.
SPLEEN :
HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED, mild.
CAUSE OF DEATH :
SACRIFICE BY DESIGN.

No Microscopic Abnormality Observed :
BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE

1103 Terminal Sacrifice
Killed on Day : 29
Animal is signed off from necropsy

Gross Pathology :

No Macroscopic Abnormality Observed :
LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

Histopathology :

LIVER :
INFLAMMATION, SUBACUTE/CHRONIC, mild.
HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
CAUSE OF DEATH :
SACRIFICE BY DESIGN.

No Microscopic Abnormality Observed :
SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE

Individual Animal Pathology Data

Dose Group: XI Treatment: 30/0 mg/kg (Recovery) Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
1104	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. SPLEEN : HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED, minimal. CAUSE OF DEATH : SACRIFICE BY DESIGN. POPLITEAL LYMPH NODE : NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed : BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW
1105	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.

1105 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: XI Treatment: 30/0 mg/kg (Recovery) Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
1105	Continued from previous page
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	SPLEEN :
	HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed :
	BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE
1106	Terminal Sacrifice
	Killed on Day : 29
	Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed :
	LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER :
	INFLAMMATION, SUBACUTE/CHRONIC, minimal.
	HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
	SPLEEN :
	HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED, minimal.
	CAUSE OF DEATH :
	SACRIFICE BY DESIGN.
	POPLITEAL LYMPH NODE :
	NOT PRESENT IN TISSUE SECTION.
	No Microscopic Abnormality Observed :
	BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW

Individual Animal Pathology Data

Dose Group: XI Treatment: 30/0 mg/kg (Recovery) Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
1107	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. INFLAMMATION, SUBACUTE/CHRONIC, minimal. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE
1108	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : INFLAMMATION, SUBACUTE/CHRONIC, minimal. HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. CAUSE OF DEATH : SACRIFICE BY DESIGN.

1108 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: XI Treatment: 30/0 mg/kg (Recovery) Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
1108	Continued from previous page
	Histopathology :
	No Microscopic Abnormality Observed : SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE
1109	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE
	Histopathology :
	LIVER : HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling. INFLAMMATION, SUBACUTE/CHRONIC, minimal. FIBROSIS, FOCAL, minimal, subcapsular. SPLEEN : HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED, mild. CAUSE OF DEATH : SACRIFICE BY DESIGN.
	No Microscopic Abnormality Observed : BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE
1110	Terminal Sacrifice Killed on Day : 29 Animal is signed off from necropsy
	Gross Pathology :
	No Macroscopic Abnormality Observed : LIVER, SPLEEN, BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, POPLITEAL LYMPH NODE

1110 Continued on the next page ....

Individual Animal Pathology Data

Dose Group: XI Treatment: 30/0 mg/kg (Recovery) Sex: Males

Animal Ref	Microscopic & Macroscopic Findings
1110	Continued from previous page

Histopathology :

LIVER :
INFLAMMATION, SUBACUTE/CHRONIC, mild.
HYPERTROPHY, PANLOBULAR, HEPATOCELLULAR, moderate, with cytoplasmic eosinophilic stippling.
NECROSIS, FOCAL, minimal, coagulative.
SPLEEN :
HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED, minimal.
CAUSE OF DEATH :
SACRIFICE BY DESIGN.

No Microscopic Abnormality Observed :
BRAIN, MESENTERIC LYMPH NODE, THYMUS, FEMUR/KNEE JOINT, STERNUM, BONE MARROW, POPLITEAL LYMPH NODE

Appendix K
Individual Total Cell Counts

INDIVIDUAL TOTAL CELL COUNTS

EXPLANATORY NOTES

NOTES:

$$\text{Organ Weight as Percent of Body Weight} = \frac{\text{Organ Weight (g)}}{\text{Final Body Weight (g)}} \times 100$$

$$\text{Total Number of Organ Cells } (\times 10^3) = \frac{\text{Organ Weight (g)}}{\text{Half Organ Weight (g)}} \times \text{Organ Cell Suspension Volume (mL)} \times \text{Number of Cells in Half Organ } (\times 10^4 \text{ cells/mL}) \div 100$$

Individual Total Cell Counts

Animal Number	Final Body Weight (g)	Spleen Weight (g)	Spleen Weight (% Body Weight)	Half Spleen Weight (g)	Spleen Cell Suspension Volume (mL)	Number of Cells in Half Spleen (x 10^6 cells/mL)	Total Number of Spleen Cells (x10^8)
Male, Group I - 0 mg/kg							
101	455.90	1.143	0.2507	0.589	7.8	71.50	10.82
102	441.60	0.761	0.1723	0.379	7.0	27.50	3.87
103	445.00	0.976	0.2193	0.511	7.0	53.90	7.21
104	427.00	0.771	0.1806	0.411	7.2	58.30	7.87
105	390.80	0.806	0.2062	0.439	6.5	22.00	2.63
106	404.70	0.669	0.1653	0.346	6.3	26.40	3.22
107	387.90	0.617	0.1591	0.303	7.2	35.20	5.16
108	405.90	1.008	0.2483	0.506	7.2	22.00	3.16
109	457.80	0.944	0.2062	0.499	6.5	45.10	5.55
110	414.60	0.744	0.1795	0.370	6.5	53.90	7.04
Male, Group III - 0.3 mg/kg							
301	420.90	0.990	0.2352	0.485	7.0	66.00	9.43
302	434.90	0.692	0.1591	0.365	7.0	26.40	3.50
303	458.60	1.254	0.2734	0.645	7.8	71.50	10.84
304	403.80	1.073	0.2657	0.556	7.4	66.00	9.43
305	395.90	0.880	0.2223	0.468	7.0	27.50	3.62
306	437.60	1.018	0.2326	0.514	7.0	35.20	4.88
307	414.00	0.683	0.1650	0.349	7.0	42.90	5.88
308	380.00	0.763	0.2008	0.384	7.0	35.20	4.90
309	448.60	0.582	0.1297	0.281	6.8	36.30	5.11
310	403.10	0.781	0.1937	0.380	6.5	35.20	4.70

Individual Total Cell Counts

Animal Number	Final Body Weight (g)	Spleen Weight (g)	Spleen Weight (% Body Weight)	Half Spleen Weight (g)	Spleen Cell Suspension Volume (mL)	Number of Cells in Half Spleen (x 10^6 cells/mL)	Total Number of Spleen Cells (x10^8)
Male, Group V - 1 mg/kg							
501	419.60	0.725	0.1728	0.366	9.9	38.50	7.55
502	395.30	0.762	0.1928	0.364	7.5	42.90	6.74
503	439.20	0.907	0.2065	0.459	7.5	18.70	2.77
504	441.30	1.004	0.2275	0.545	7.2	105.60	14.01
505	364.70	0.673	0.1845	0.366	7.0	22.00	2.83
506	408.80	0.836	0.2045	0.416	6.5	30.80	4.02
507	369.90	0.660	0.1784	0.301	7.0	52.80	8.10
508	406.10	0.938	0.2310	0.497	7.2	34.10	4.63
509	476.20	1.085	0.2278	0.521	7.5	44.00	6.87
510	379.20	0.758	0.1999	0.375	7.0	49.50	7.00
Male, Group VII - 10 mg/kg							
701	354.30	0.776	0.2190	0.397	8.0	52.80	8.26
702	393.60	0.760	0.1931	0.399	7.0	16.50	2.20
703	382.60	0.750	0.1960	0.430	6.5	79.20	8.98
704	355.70	0.927	0.2606	0.453	6.8	36.30	5.05
705	317.70	0.652	0.2052	0.374	7.0	45.10	5.50
706	409.10	0.767	0.1875	0.368	7.8	48.40	7.87
707	421.10	0.912	0.2166	0.455	6.9	39.60	5.48
708	347.60	0.625	0.1798	0.304	7.0	50.60	7.28
709	409.00	0.898	0.2196	0.448	6.5	33.00	4.30
710	378.80	1.017	0.2685	0.530	7.4	52.80	7.50

165

p. 164

Individual Total Cell Counts

Animal Number	Final Body Weight (g)	Spleen Weight (g)	Spleen Weight (% Body Weight)	Half Spleen Weight (g)	Spleen Cell Suspension Volume (mL)	Number of Cells in Half Spleen (x 10^6 cells/mL)	Total Number of Spleen Cells (x10^8)
Male, Group IX - 30 mg/kg							
901	272.60	0.498	0.1827	0.293	10.0	25.30	4.30
902	294.00	0.633	0.2153	0.297	10.2	12.10	2.63
903	394.10	0.779	0.1977	0.367	7.5	46.20	7.35
904	284.00	0.673	0.2370	0.340	6.8	26.40	3.55
905	329.20	0.673	0.2044	0.368	7.3	36.30	4.85
906	303.40	0.713	0.2350	0.334	7.0	23.10	3.45
907	334.00	0.762	0.2281	0.416	6.3	33.00	3.81
908	303.80	0.734	0.2416	0.388	6.5	70.40	8.66
909	335.00	0.691	0.2063	0.346	7.0	20.90	2.92
910	294.10	0.586	0.1993	0.330	6.5	49.50	5.71
Male, Group XI - 30/0 mg/kg (Recovery)							
1101	329.40	0.596	0.1809	0.317	7.1	58.30	7.78
1102	343.20	0.782	0.2279	0.392	6.5	29.70	3.85
1103	344.00	0.722	0.2099	0.357	6.8	33.00	4.54
1104	393.00	1.076	0.2738	0.529	6.8	66.00	9.13
1105	336.60	0.665	0.1976	0.353	7.0	36.30	4.79
1106	386.50	1.013	0.2621	0.552	7.0	58.30	7.49
1107	312.50	0.841	0.2691	0.436	6.5	48.40	6.07
1108	283.60	0.624	0.2200	0.360	6.5	61.60	6.94
1109	287.60	0.548	0.1905	0.248	7.2	15.40	2.45
1110	321.50	0.936	0.2911	0.470	6.5	37.40	4.84

Individual Total Cell Counts

Animal Number	Thymus Weight (g)	Thymus Weight (% Body Weight)	Half Thymus Weight (g)	Thymus Cell Suspension Volume (mL)	Number of Cells in Half Thymus (x 10^6 cells/mL)	Total Number of Thymus Cells (x10^6)
Male, Group I - 0 mg/kg						
101	0.691	0.1516	0.343	7.5	77.00	11.63
102	0.651	0.1474	0.331	7.5	112.75	16.63
103	0.775	0.1742	0.388	7.5	121.00	18.13
104	0.690	0.1616	0.371	7.0	111.10	14.46
105	0.425	0.1088	0.219	6.5	65.45	8.26
106	0.499	0.1233	0.266	7.0	72.05	9.46
107	0.429	0.1106	0.197	7.2	85.25	13.37
108	0.435	0.1072	0.223	7.5	78.10	11.43
109	0.544	0.1188	0.269	7.2	80.30	11.69
110	0.544	0.1312	0.232	7.5	47.30	8.32
Male, Group III - 0.3 mg/kg						
301	0.704	0.1673	0.367	6.5	18.70	2.33
302	0.828	0.1904	0.388	7.5	137.50	22.01
303	0.504	0.1099	0.266	7.5	68.75	9.77
304	0.652	0.1615	0.330	7.0	84.15	11.64
305	0.619	0.1564	0.293	7.0	116.60	17.24
306	0.689	0.1574	0.324	7.0	132.00	19.65
307	0.421	0.1017	0.225	6.8	72.05	9.17
308	0.457	0.1203	0.192	7.0	72.60	12.10
309	0.577	0.1286	0.238	7.0	78.10	13.25
310	0.587	0.1456	0.292	7.0	51.15	7.20

1677

Individual Total Cell Counts

Animal Number	Thymus Weight (g)	Thymus Weight (% Body Weight)	Half Thymus Weight (g)	Thymus Cell Suspension Volume (mL)	Number of Cells in Half Thymus (x 10^6 cells/mL)	Total Number of Thymus Cells (x10^9)
Male, Group V - 1 mg/kg						
501	0.427	0.1018	0.237	6.6	67.10	7.98
502	0.561	0.1419	0.277	7.5	70.40	10.69
503	0.634	0.1444	0.307	7.8	135.85	21.88
504	0.745	0.1688	0.341	7.0	89.10	13.63
505	0.410	0.1124	0.199	7.0	59.40	8.57
506	0.569	0.1392	0.278	7.0	111.65	16.00
507	0.521	0.1408	0.264	7.0	108.90	15.04
508	0.691	0.1702	0.354	7.5	112.75	16.51
509	0.633	0.1329	0.291	7.4	84.70	13.63
510	0.396	0.1044	0.172	7.3	46.75	7.86
Male, Group VII - 10 mg/kg						
701	0.679	0.1916	0.341	7.7	123.20	18.89
702	0.547	0.1390	0.268	7.2	81.40	11.96
703	0.674	0.1762	0.353	7.5	107.25	15.36
704	0.420	0.1181	0.237	6.8	39.05	4.71
705	0.319	0.1004	0.143	7.5	49.50	8.28
706	0.569	0.1391	0.295	6.5	157.30	19.72
707	0.717	0.1703	0.303	6.8	145.20	23.36
708	0.528	0.1519	0.260	7.3	81.95	12.15
709	0.734	0.1795	0.366	7.0	119.35	16.75
710	0.626	0.1653	0.323	7.4	63.80	9.15

Individual Total Cell Counts

Animal Number	Thymus Weight (g)	Thymus Weight (% Body Weight)	Half Thymus Weight (g)	Thymus Cell Suspension Volume (mL)	Number of Cells in Half Thymus (x 10^6 cells/mL)	Total Number of Thymus Cells (x10^8)
Male, Group IX - 30 mg/kg						
901	0.299	0.1097	0.153	7.4	46.20	6.68
902	0.668	0.2272	0.333	7.5	110.50	16.62
903	0.765	0.1941	0.401	7.6	102.85	14.91
904	0.191	0.0673	0.116	6.8	21.45	2.40
905	0.563	0.1710	0.280	7.0	105.05	14.79
906	0.582	0.1918	0.269	7.5	80.30	13.03
907	0.487	0.1458	0.227	7.3	152.90	23.95
908	0.372	0.1224	0.167	7.0	39.05	6.09
909	0.487	0.1454	0.269	7.0	72.60	9.20
910	0.453	0.1540	0.254	7.0	72.05	8.99
Male, Group XI - 30/0 mg/kg (Recovery)						
1101	0.607	0.1843	0.307	7.4	127.60	18.67
1102	0.619	0.1804	0.330	7.5	112.20	15.78
1103	0.739	0.2148	0.378	7.0	148.50	20.32
1104	0.834	0.2122	0.365	7.5	177.65	30.44
1105	0.571	0.1696	0.296	7.3	121.00	17.04
1106	0.776	0.2008	0.428	7.3	169.40	22.42
1107	0.534	0.1709	0.268	7.3	130.35	18.96
1108	0.483	0.1703	0.238	7.0	66.00	9.38
1109	0.624	0.2170	0.315	7.0	92.95	12.89
1110	0.606	0.1885	0.320	7.3	63.80	8.82

691

p. 168

Appendix L
Electron Microscopy Report from Experimental Pathology Laboratories, Inc.

EPL® Experimental Pathology Laboratories, Inc.

DUPONT/HASKELL LABORATORY

DUPONT STUDY NUMBER: 18317
WORK REQUEST NUMBER: 16160
SERVICE CODE: 1545

AMMONIUM PERFLUOROOCTANOATE: 28-DAY IMMUNOTOXICITY STUDY IN MALE RATS

ELECTRON MICROSCOPY

PATHOLOGY REPORT
EPL PROJECT NO. 129-077

Submitted to:

DuPont/Haskell Laboratory for Health
and Environmental Science
Stine Haskell Research Center
1090 Elkton Road
Newark, DE 19711

Submitted by:

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

October 25, 2006

EPL® Experimental Pathology Laboratories, Inc.

TABLE OF CONTENTS

EPL® Experimental Pathology Laboratories, Inc.

DuPont-18317

DUPONT/HASKELL LABORATORY

DUPONT STUDY NUMBER: 18317
WORK REQUEST NUMBER: 16160
SERVICE CODE: 1545

EPL PROJECT NO.: 129-077

AMMONIUM PERFLUOROOCTANOATE: 28-DAY IMMUNOTOXICITY STUDY IN MALE RATS

ELECTRON MICROSCOPY

PATHOLOGY SUMMARY

The in-life phase of this study was conducted at Haskell Laboratory for Health and Environmental Sciences, E.I. duPont de Nemours and Company, Newark, Delaware. The objective of this study is to evaluate the potential of ammonium perfluorooctanoate to suppress the primary humoral immune response to sheep red blood cells (SRBC) when administered by oral gavage to male rats for at least 28 days. The table below summarizes the experimental design:

Experimental Design

Group	Number/Group	Daily Dosage (mg/kg)[a]	Dose Solution Concentration (mg/mL)[b]
I	10	0 (Control)	0
III	10	0.3	0.03
V	10	1	0.1
VII	10	10	1
IX	10	30	3
XI	10	30 (Recovery)[c]	3

[a] Weight of test substance/kg of animal body weight.
[b] Solutions will be adjusted for purity (20%)
[c] The recovery group (XI) will be dosed with 30 mg/kg of test substance through test day 22. Following injection of SRBC on test day 23, group XI will be dosed with NANOpure® water, at a volume of 10 mL/kg of body weight, until sacrifice.

Electron microscopic evaluation of samples of liver from designated animals was added to clarify light microscopic histopathological findings in the liver. Samples of liver from two male

EPL® Experimental Pathology Laboratories, Inc.

rats in Group I (Control) and two male rats in Group IX (30 mg/kg) that were fixed in formalin were submitted for transmission electron microscopy. The samples that were processed and evaluated are listed in the following table:

TEM Number	Tissue	Animal ID	Group	TEM Negative Number (evaluated)
G06-399	Liver	105	I (Control)	06-1894 to 06-1896
G06-400	Liver	106	I (Control)	06-1897 to 06-1899
G06-401	Liver	905	IX (30 mg/kg)	06-1900 to 06-1902
G06-402	Liver	906	IX (30 mg/kg)	06-1903 to 06-1905

Samples, cut into small cubes, were preserved in formalin and shipped to Experimental Pathology Laboratories, Inc (EPL®), Research Triangle Park, NC. The samples were transferred to the Laboratory for Advanced Electron and Light Optical Methods (LAELOM) at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC for further processing and examination by transmission electron microscopy.

The samples were washed in buffer, post-fixed in 1% osmium tetroxide in the phosphate buffer, dehydrated in an ethanolic series culminating in acetone, and infiltrated with Spurr epoxide resin. The resulting blocks were trimmed and semithin sections (approximately 0.5 µm thick) were cut, mounted on glass slides, and stained with 1% toluidine blue O in 1% sodium borate prior to being examined with a light microscope. The slides of semithin sections were sent to Experimental Pathology Laboratories for evaluation by the Pathologist, Dr. Henry Wall. When the slides were returned to the LAELOM, areas of interest for ultrathin sectioning were trimmed in the corresponding tissue blocks.

Ultrathin (80-90 nm thick) sections were cut from the selected trimmed blocks and placed on 200 mesh copper grids before being stained with uranyl acetate and lead citrate. For each sample, two survey photographs (final print magnification 5,600x) were taken. One higher magnification (final print magnification 22,400x) was taken of each sample to show more cellular detail.

RESULTS

TEM #G06-399 (Animal 105, Control, Liver, TEM Neg # 06-1894 to 06-1896)

Two low magnification images (06-1894 and 06-1895; 5,600X) depict portions of multiple hepatocytes. A few clear to moderately electron-lucent smooth-contoured lipid droplets are in the cytoplasm of most hepatocytes. The cytoplasm of all hepatocytes contain numerous well-formed mitochondria as the predominant cytoplasmic organelles. The higher magnification image (06-1896; 22,400X) shows greater detail of the hepatocytic mitochondria, lipid droplets, cisternae of rough endoplasmic reticulum, a few electron-dense membrane-bound peroxisomes, and electron dense clusters of cytoplasmic glycogen. No cell injury is apparent.

TEM #G06-400 (Animal 106, Control, Liver, TEM Neg # 06-1897 to 06-1899)

Both low magnification images (06-1897 and 06-1898; 5,600X) show multiple hepatocytes that have numerous mitochondria as their predominant cytoplasmic organelles. Aggregates of linearly arrayed rough endoplasmic reticulum are scattered in the cytoplasm of hepatocytes. The high magnification image (06-1899; 22,400X) shows greater detail of the several mitochondria, rough endoplasmic reticulum, and a few slightly electron-dense lysosomes. A few of the smaller diameter electron-dense bodies may be peroxisomes, however, their structure is not optically resolved to the extent that their identity as peroxisomes can be confirmed. Irregular profiles of translucent smooth endoplasmic reticulum are interspersed between other organelles in the cytoplasm. A portion of a well formed nucleus is at the lower right of the image. No cell injury is present.

TEM #G06-401 (Animal 905, Group IX/30mg/kg, Liver, TEM Neg # 06-1900 to 06-1902)

Both low magnification images (06-1900 and 06-1901; 5,600X) depict multiple hepatocytes with abundant densely arranged cytoplasmic mitochondria. Peroxisomes which appear as uniformly electron-dense bodies are prominent among the mitochondria. A few small clear smooth-contoured vacuoles are in most cells. These vacuoles are considered to be lipid vacuoles that lost their content during tissue processing. A few lysosomes are in some hepatocytes. Most lysosomes contain either lipid droplets or electron-dense residual bodies. In one image (06-1901) a cross section of a blood vessel contains electron-dense erythrocytes and shows the nucleus of an endothelial cell. The higher magnification image (06-1902;

EPL® Experimental Pathology Laboratories, Inc.

22,400X) shows more detail of clear lipid vacuoles, numerous mitochondria, a lipid-laden lysosome and a few electron-dense peroxisomes. The peroxisomes are along the right border of the image and in the upper left quadrant of the image.

TEM #G06-402 (Animal 906, Group IX/30mg/kg, Liver, TEM Neg # 06-1903 to 06-1905)

The low magnification images (06-1903 and 06-1904; 5,600X) show numerous mitochondria as the predominant organelles in the cytoplasm of adjacent hepatocytes. Several uniformly electron-dense peroxisomes are scattered in the cells but are somewhat difficult to discern in the low magnification images. The high magnification image (06-1905; 22,400X) shows the detail of several peroxisomes in hepatocytic cytoplasm at the periphery of the endothelium of a blood vessel along the left border of the image. The peroxisomes are generally smaller in diameter than the more numerous mitochondria in the image. The peroxisomes are lined by a single membrane and have relatively uniform electron-dense matrix in this high magnification image.

CONCLUSIONS

Compared to hepatocytes from the two control rats, the hepatocytes from the two rats that received ammonium perfluorooctanoate have more abundant peroxisomes in their cytoplasm.

Henry G. Wall

HENRY G.WALL, DVM, PhD
Diplomate, ACVP
Veterinary Pathologist

25 October 2006
Date

HGW/dc

EPL® Experimental Pathology Laboratories, Inc.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: Ammonium Perfluorooctanoate: 28-Day Immunotoxicity Study in Male Rats

Client Study: DuPont-18317; Service Code 1545; Work Request 16160

EPL Project Coordinator: Dr. Henry Wall

EPL Project Number: 129-077

EPL Pathologist: Dr. Henry Wall

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

	Dates	
Area Inspected	Inspection	Reporting
EPL Project Sheets	May 30, 2006	May 30, 2006
Data Review	June 14, 2006	June 14, 2006
Draft Pathology Report	June 27, 2006	June 27, 2006
Final Pathology Report	October 25, 2006	October 25, 2006

Date reported to Study Director/Management: October 25, 2006

Date of last quarterly facility inspection: October 2006

Jane J Hollingsworth
EPL Quality Assurance Unit

October 25, 2006
Date

5

Form No. 6-2 (October 23, 2006)