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John Butenhoff 1/14/04
SLG/no

Study Title

PFOA: Lactational and Placental Transport Pharmacokinetic
Study in Rats

Laboratory Project ID: DuPont-13309

7-6889.11

AUTHOR: Eve Mylchreest, Ph.D.

STUDY COMPLETED ON: December 19, 2003

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
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SPONSOR: 3M Company
3M Center
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Wilmington, Delaware 19898

WORK REQUEST NUMBER: 14787

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

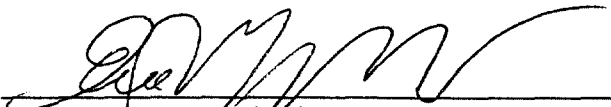
This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850).

Applicant / Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
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Study Director:



Eve Mylchreest, Ph.D.
Senior Research Toxicologist

19-Dec-2003

Date

Applicant / Sponsor: _____

Applicant/Sponsor Representative

Date

QUALITY ASSURANCE STATEMENT

Haskell Sample Number(s):

24921

Dates of Inspections:

Protocol: July 11, 2003

Conduct: July 11,28,31, 2003; August 15, 2003; September 5,30 2003

Records, Reports: October 2,3,6-8,13-15,30-31 2003; November 3-7, 2003

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Study Director: July 11,29,31, 2003; August 18, 2003; September 10,30, 2003;
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October 10,16,17, 2003; November 6,7,10,12, 2003

Reported by:

Robert C. Rhea for KBB

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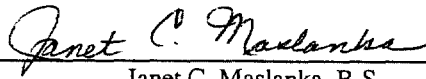
19-DEC-2003

Date

CERTIFICATION

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

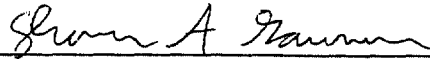
Analytical Evaluations by:



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Senior Staff Chemist

18-Dec-2003
Date

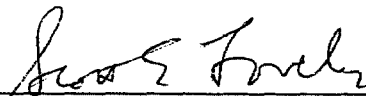
Biochemical Evaluations by:



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18-Dec-2003
Date

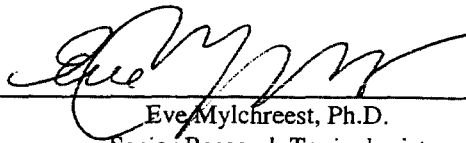
Approved by:



Scott E. Loveless, Ph.D.
Research Manager and Director

19-DEC-2003
Date

Issued by Study Director:



Eve Mylchreest, Ph.D.
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19-Dec-2003
Date

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STUDY INFORMATION

9th Collective Nomenclature: Octanoic acid, pentadecafluoro-, ammonium salt

Synonyms/Codes:

- Ammonium perfluorooctanoate
- FC-143 FLUORAD Brand Fluorochemical Surfactant (3M Company, Specialty Materials)
- C-8
- Perfluorooctanoate, ammonium salt
- PFOA
- H-24921
- Lot 332 (3M Specialty Materials) (Lot No.)

Haskell Number: 24921

CAS Registry Number: 3825-26-1

Purity: 95.2% – 97.99%
Straight chain: 77.6%
Branched: 12.6% internal monomethyl (non-alpha)
9% isopropyl
0.2 % tert-butyl
0.1% gem-dimethyl
0.1% alpha monomethyl

Known Impurities: C₄ (C₃F₇CO₂⁻ NH₄⁺), 0.01%
C₅ (C₄F₉CO₂⁻ NH₄⁺), 0.03%
C₆ (C₅F₁₁CO₂⁻ NH₄⁺), 0.43%
C₇ (C₆F₁₃CO₂⁻ NH₄⁺), 0.57%
C₉ (C₈F₁₇CO₂⁻ NH₄⁺), 0.16%
Monohydro APFO, 0.09%
Monounsaturated APFO, 0.72%
Undefined (possibly) substituted perfluorocyclo species, 0.2%
cyclopentyl, and 0.1% cyclohexyl

Physical Characteristics: White solid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.
and
3M Company
3M Center
St. Paul, MN 55144-1000

Study Initiated/Completed: July 8, 2003 / (see report cover page)

In-Life Initiated/Completed: July 11, 2003 / August 22, 2003

STUDY PERSONNEL

Study Director: Eve Mylchreest, Ph.D.
Management: Scott E. Loveless, Ph.D.

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Analytical Chemist: Janet C. Maslanka, B.S.
Management: S. Mark Kennedy, Ph.D.

Biochemical Toxicologist: Shawn A. Gannon, B.S.
Management: Gary W. Jepson, Ph.D.

Toxicology Report Preparation: Mary K. LaRoe
Lisa G. Burchfield, A.A.
Management: Nancy S. Selzer, M.S.

Laboratory Veterinarian: Thomas W. Mayer, D.V.M., A.C.L.A.M.
Management: Janice L. Connell, M.S., B.A.

SUMMARY

Twenty time-mated female CrI:CD[®](SD)IGS BR rats were dosed by oral gavage once daily at concentrations of 0, 3, 10, or 30 mg/kg/day of test substance on days 4-10, 4-15, or 4-21 of gestation (G), or from day 4G to day 21 postpartum (PP). Clinical observations and body weights were recorded on the day after rats arrived and daily until the end of the study.

On days 10, 15, and 21G, 5 rats per group per time point were euthanized. For each female with visible implantation sites, the intrauterine location of each embryo/fetus and implantation type was recorded. Maternal viscera were examined grossly and blood samples were collected approximately 2 hours ($\pm$ 30 minutes) post-dose and were processed to obtain plasma samples. Embryos were collected on day 10G, and amniotic fluid, placentas, and embryos/fetuses were collected on days 15 and 21G. Fetuses were euthanized and fetal blood samples were collected on day 21G.

The last 5 animals per group were designated for the lactational evaluation and were allowed to deliver and nurse their litters. On days 0, 3, 7, 14, and 21PP, offspring were individually handled and examined for abnormal behavior and appearance. Live pups in each litter were counted and weighed (sexes separate). On days 3, 7, 14, and 21PP, dams were anesthetized and milk and blood samples were collected. Maternal blood samples were collected approximately 2 hours ($\pm$ 30 minutes) post-dose. Randomly selected pups were weighed, euthanized and blood samples were collected on days 3, 7, 14, and 21PP.

Plasma, milk, amniotic fluid extract, and tissue homogenate (placenta, embryo, and fetus) supernatants were analyzed by high performance liquid chromatography – mass spectrometry (HPLC-MS).

Clinical Observations and Mortality

- There were no test substance-related clinical observations in dams or litters.
- There was no mortality in maternal rats at any level tested.

Body Weight and Body Weight Gain

- Mean body weight gain at 30 mg/kg/day was lower than control during gestation and was similar to control during lactation.
- Mean daily body weights at 30 mg/kg/day were lower than control throughout gestation and lactation.
- Mean body weights and body weight gain at 3 and 10 mg/kg/day during gestation and lactation were similar to the control group.

Reproductive Data

- All animals were pregnant at scheduled sacrifice.
- All dams delivered a live litter.
- The number of implantation sites, resorptions, and live fetuses were comparable across groups that were sacrificed on either day 10, 15, or 21G.
- There were 2 small litters in the 30 mg/kg/day lactation group. Due to the intrinsic variability in litter size and the small number of litters evaluated (5 per group) the relationship of this finding to test substance administration is equivocal.
- Pup survival and pup weights during lactation were comparable across groups.

Biochemical Toxicology

- The concentration of PFOA in maternal plasma appeared to be at steady state (means of 11.2, 26.8, and 66.6 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg dose levels, respectively) over the range of time points sampled.
- The mean concentrations of PFOA in fetal plasma collected on day 21G were 5.9, 14.5, and 33.1 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg dose levels, respectively.
- Pup plasma concentration decreased from day 3PP (means of 2.9, 5.9, and 12.0 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively) to day 7PP (0.7, 2.8, and 4.9 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg/day, respectively, and were similar on days 7, 14, and 21PP at all dose levels.
- The concentration of PFOA in milk was at steady state (means of 1.1, 2.8, and 6.2 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg dose levels, respectively) from day 3PP to day 21PP at all dose levels.
- The concentration of PFOA in amniotic fluid was approximately four times higher on day 21G (means of 1.5, 3.8, and 8.1 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively) than on day 15G (means of 0.6, 0.7, and 1.7 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively).
- The concentration of PFOA in placenta was approximately two times higher on day 21G (means of 3.6, 9.4, and 24.4 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively) than on day 15G (means of 2.2, 5.1, and 13.2 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively).
- The day 10G embryo had the highest concentration of PFOA per gram of tissue (means of 1.4, 3.3, and 12.5 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively) and the day 21G fetus had slightly lower levels (means of 1.3, 2.6, and 8.8 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively). The day 15G embryo had a lower tissue concentration than tissue from day 10G or day 21G (means of 0.2, 0.5, and 1.2 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively).

In conclusion, under the conditions of this study, there was maternal toxicity (reduced body weight and weight gain) at 30 mg/kg/day. There were 2 small litters in the 30 mg/kg/day lactation group, a finding that was possibly test substance-related. Concentrations of PFOA in maternal plasma and milk were at steady state during the sampling interval. Steady state concentration in milk was approximately ten times less than the steady state concentration in maternal plasma. The concentration of PFOA in fetal plasma on day 21G was approximately half the steady state concentration in maternal plasma. The milk concentrations appeared to be generally comparable to the concentrations in pup plasma. Pup plasma concentrations decreased from day 3PP to day 7PP, and were similar on days 7, 14, and 21PP at all dose levels. PFOA was detected in placenta (days 15 and 21G), amniotic fluid (days 15 and 21G), embryo (days 10 and 15G), and fetus (day 21G).

INTRODUCTION

The subject test substance, ammonium PFOA, is a surfactant used in industrial processes. In pharmacokinetic studies in adult rats, whole body elimination of the test substance was found to be much more rapid in females compared to males following oral dosing. Available data on the transport concentrations of the test substance when administered to pregnant females is limited and there is no existing data on the concentration of the test substance transported via the milk from lactating females.^(1,2)

OBJECTIVE

The objective of this study was to determine the concentration of the test substance in maternal milk and blood plasma, placenta, amniotic fluid, embryo, fetus, pup, and fetal and pup plasma following repeated oral dosing of the dam using 3 graded dose levels during gestation and lactation.

SPONSOR AND TEST FACILITY

This study was co-sponsored by 3M Company, St. Paul, Minnesota, and E.I. du Pont de Nemours and Company, Wilmington, Delaware. The sponsor's approval was effective the date the sponsor authorized the work on the contract. The study was conducted at DuPont Haskell Laboratory for Health and Environmental Sciences, E.I. du Pont de Nemours and Company, Newark, Delaware.

STUDY DESIGN

A. Experimental Design

Group	Dose (mg/kg/day) ^a	Test Formulation	
		Concentration (mg/mL) ^b	Time-Mated Females
I	0 ^c	0	20
II	3	0.6	20
III	10	2.0	20
IV	30	6.0	20

a Formulations of test substance in deionized water (NANOpure deionized water) were administered once daily by oral gavage at a dosing volume of 5 mL/kg.

b To achieve these concentrations of active ingredient, the formulations were adjusted for sample purity (%).

c The control group animals received NANOpure deionized water only at 5 mL/kg.

Dose Group Subsets And Dosing Schedules

Subset ^a	Number of Animals	Dosing Period ^b (days)	Terminal Sacrifice (days)
A	5	4-10G	10G
B	5	4-15G	15G
C	5	4-21G	21G
D	5	4G-21PP	21PP

a Animals were randomly assigned to each subset.

b G=gestation; PP=postpartum

B. Selection of Dose Levels

Dose levels selected for this study were based upon the results of a two-generation reproduction study in rats.⁽³⁾ Groups of 30 male and 30 female rats were dosed by oral gavage at 0, 1, 3, 10, or 30 mg/kg/day for approximately 70 days before cohabitation. Dosing of the P₀ females continued throughout mating, gestation, and lactation or approximately 112 days total. While there was no test substance-related maternal toxicity observed at doses up to 30 mg/kg/day, a slight increase in offspring mortality, attributed to failure to thrive, was observed in F₁ generation weanlings at 30 mg/kg/day.

MATERIALS AND METHODS

ANALYTICAL

A. Vehicle

The test formulation vehicle was NANOpure deionized water.

B. Test Substance

1. Identification

The test substance was obtained from 3M, St. Paul, Minnesota, and was assigned Haskell Laboratory Number PFOA upon receipt. Available information on the purity, composition, contaminants, synonyms, hazards, and hazardous material classification(s) was provided by the vendor and documented in the study records and report.

2. Purity

The vendor reported purity was 95.2% - 97.99%.

3. Test Substance Stability

The stability of the test substance over the course of the study was confirmed by purity analyses conducted near the beginning and end of the study.

4. Test Substance Administration, Preparation, and Sampling

a. Administration

The test substance was administered by oral gavage once daily at a dose volume of 5 mL/kg. Animals assigned to gestation subsets A, B, and C, were dosed for 7, 12, and 18 days respectively. Animals assigned to the lactation subset (D) were dosed for 40 days. Dose volumes were adjusted daily based on body weights.

b. Preparation

Test formulations of the test substance in the vehicle were prepared daily and stored at room temperature until used. The method of mixing the test substance with the vehicle is documented in the study records.

c. Sampling

Test formulations at concentrations of 0.6, 2.0, and 6.0 mg/mL of PFOA were prepared and collected for uniformity of mixing/concentration verification and stability analysis (5-hour room temperature) on July 11, 2003. On July 31, 2003 and August 21, 2003, test formulations at all levels were collected for concentration verification analysis. In addition, 0 mg/mL (control) samples were submitted for analysis with each set of samples.

Samples submitted for analysis were analyzed the day they were received and/or when re-analysis was indicated.

5. Analytical Methods

a. Recovery Sample Analysis

Concurrent with test formulations analyses, recovery of PFOA from spiked vehicle (NANOpure deionized water) was tested at the low level (0.6 mg/mL), at the mid level (2.0 mg/mL) and at the high level (6.0 mg/mL) to confirm the analytical method. A stock solution of PFOA was prepared in NANOpure deionized water. For all concentration levels, an appropriate aliquot of this solution to obtain 0.3 mg (low), 1 mg (mid), and 3 mg (high) of the test substance was added to 100 mL of NANOpure deionized water. All recovery samples were then mixed for dispersion of the test substance in the vehicle. The samples were then processed and analyzed in the same manner as the dosing samples at similar concentrations.

b. Dosing Test Formulation Treatment

Each dosing sample (0.5 mL) was diluted to 100 mL with NANOpure deionized water and mixed. The dosing samples were further diluted with NANOpure deionized water to an expected

concentration of approximately 0.000003 mg/mL (50 μ L injections) or 0.00003 mg/mL (5 μ L injections) prior to analysis. Before all final dilutions, the internal standard and the 0 mg/mL sample (initial dilution) was added to each test sample to give an equivalent final concentration of the internal standard and the matrix in all samples.

Samples submitted for analysis were analyzed on the day of receipt and/or when re-analysis was indicated.

c. Chromatographic Conditions

LC parameters

Instrument:	Hewlett Packard Model 1100 HPLC
Column:	Zorbax [®] RX-C8, 2.1 mm x 150 mm, 5 μ m
Flow Rate:	0.4 mL/min
Injection Volume:	5 or 50 μ L
Column Temperature:	30°C
Column Switch:	4.0 minutes
Mobile Phase:	0.15% Acetic Acid/Acetonitrile
Gradient:	

Time (min.)	% Acetonitrile
0.0	5
0.9	5
1.0	80
6.0	80
6.1	5
7.0	5

MS parameters

Instrument:	Micromass Quattro Micro Tandem MS
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:	perfluorooctanoic acid (PFOA): 413 m/z (parent) to 369 m/z (daughter) [1,2-di- ¹³ C] PFOA: 415 m/z (parent) to 369 m/z (daughter)

Retention time of PFOA and [1,2-di-¹³C] PFOA: approximately 4.9 to 5.2 min (5 or 50 μ L injections).

d. Calibration and Quantitation

A stock solution of the PFOA (separate sample used as analytical reference) was made in NANOpure deionized water. Appropriate aliquots of the stock were diluted with NANOpure deionized water to make calibration standards that bracketed the target concentration of the diluted sample solutions. Before these aliquots were brought to volume, an appropriate amount of internal standard ([1,2-di-¹³C] PFOA) was added.

Analysis of PFOA was by high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS). Negative-ion electrospray was used to generate negatively charged ions of PFOA. The ions were selected with the first MS quadrupole, collisionally dissociated using argon, and a fragment ion was monitored. [1,2-di-¹³C] PFOA is used as an internal standard. Triplicate injections of the sample solutions and calibration standards solutions were made and peak areas were calculated electronically.

The calibration curve was generated by regression analysis using the peak area ratio from the PFOA and the internal standard. Data for test formulations were compared to the calibration curves to evaluate the concentrations of PFOA.

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

The mean result of the concentration verification samples (n = 2) for each dosing level was used to determine the concentration of the test substance for the respective dosing levels.

Stability was evaluated by using the mean result of the uniformity of mixing/concentration verification samples as the baseline for comparing the corresponding stability results.

IN-LIFE

A. Test Species

The rat was selected for this study as it is a preferred species for reproductive toxicity studies and was the species used in a previous 2-generation reproduction study.⁽³⁾ The CrI:CD[®](SD)IGS BR strain was selected based on consistently acceptable health status and on extensive experience with this strain at Haskell Laboratory.

Eighty nulliparous, time-mated females were received on July 8, 2003 (60 female rats) and July 11, 2003 (20 female rats) from Charles River Laboratories, Inc., Raleigh, North Carolina, with an assigned birth date of May 5, 2003. The rats for this study were approximately 63 days old and were at day 1G upon arrival. Body weights on the day rats were mated (day 0G) were supplied by the vendor and are documented in the study records.

B. Animal Husbandry

1. Identification

Each rat was assigned a unique number and identified with an AVID Microchip implant by the supplier prior to shipping. Upon receipt, each animal was assigned a Haskell animal number. Both the Haskell animal number and unique vendor animal number were recorded on each cage

card. A master list of unique vendor numbers, Haskell animal numbers, and corresponding unique AVID Microchip implant numbers are maintained with the study records.

2. Environmental Conditions

Animal rooms were maintained at an acceptable temperature of 18-26°C (targeted at 22°-24°C) and maintained at an acceptable relative humidity of 30%-70% (targeted at 40%-60%). Animal rooms were artificially illuminated (fluorescent light) on a 12-hour light/dark cycle (approximately 0600-1800 hours).

3. Housing

All rats were housed individually in stainless steel, wire-mesh cages, suspended above cageboard until sacrifice or day 19G. Females selected for the lactation subset (D) were housed in polycarbonate pans containing bedding material on day 20G through day 21PP.

4. Feed and Water

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

All rats were provided with tap water from United Water Delaware *ad libitum*.

5. Health 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.
- Feed samples are analyzed for total bacterial, spore and fungal counts.
- 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.

C. Quarantine and Pretest Period

Rats were quarantined according to procedures outlined in Haskell Laboratory SOP LA003-P, and then released for the study upon approval of the Laboratory Animal Veterinarian or a designee.

D. Assignment to Groups – Randomization

Upon arrival, dams were ranked on the basis of day 0G body weights and assigned to control and experimental groups by random sampling from the ranked list. The distribution resulted in mean body weights for all groups that were not statistically different ($p > 0.05$). Animals in each group were then randomly assigned to each subset (A,B,C or D).

E. In-life Observations

1. Clinical Observations

Clinical observations were recorded on the day after arrival and daily until the end of the study.

2. Body Weights

Body weights were recorded on the day after arrival and daily until the end of the study.

3. Lactation Procedures – Subset D

The day when delivery was complete was designated day 0 postpartum. At each examination period (days 0, 3, 7, 14, and 21PP), offspring were individually handled and examined for abnormal behavior and appearance; any dead or abnormal pups were recorded. Live and dead pups in each litter were counted by sex as soon as possible after delivery was complete and litter weights were recorded. Dead pups were discarded.

F. Terminal Sample Collection and Sacrifice

1. Gestation Subsets A, B, and C

On days 10, 15, and 21G, animals assigned to Subset A, B, and C, respectively, were euthanized by carbon dioxide inhalation. For each female with visible implantation sites, the intrauterine location of each embryo/fetus and implantation type (live or early resorption) were recorded. A gross examination of maternal viscera was done and blood samples from each dam were collected from the *vena cava* approximately 2 hours (± 30 minutes) post-dose and processed to obtain plasma samples. On day 21G, fetuses were euthanized with an overdose injection (i.p.) of sodium pentobarbital and fetal blood samples were taken from a transverse cut made to the carotid artery.

Gestation Subset Sample Collection and Sacrifice Schedule

Subset	Terminal Sacrifice	Samples Collected
A	10G	Maternal Blood, ^a Whole Embryos ^c
B	15G	Maternal Blood, ^a Amniotic Fluid, ^c Placentas, ^b Whole Embryos ^b
C	21G	Maternal Blood, ^a Amniotic Fluid, ^c Placentas, ^b Whole Fetuses, ^b Fetal Blood ^d

a Maternal blood samples (volume 0.5 mL per animal) in tubes containing heparin, were collected 2 hours ($\pm$ 30 minutes) post-dose; other tissues were collected as soon as possible after maternal blood collection.

b All embryos, fetuses, and placentas were pooled by litter.

c Amniotic Fluid sample minimum volume approximately 100 μ L was pooled by litter.

d Fetal blood sample minimum volume approximately 100 μ L collected in tubes containing heparin was pooled by litter.

2. Lactation Subset D

a. Anesthesia Procedure

Dams were removed from their litters approximately 1-2 hours before blood and milk samples were collected. Dams were placed in charged induction chambers (FLUOVAC® anesthesia machine) and were anesthetized by isoflurane inhalation. When the dams appeared to be unconscious from the anesthesia, they were removed from the induction chambers and were checked for response to toe pinch.

After samples were collected, dams were returned to holding cages or their home pen where they were monitored while recovering from anesthesia. Dams were returned to their litters after recovery from anesthesia.

b. Blood Sample Collection

Sample Collection – Subset D

Day	Maternal Samples ^a	Number of Pups for Terminal Sacrifice ^b	Pup Samples
3PP	Milk, ^c Blood ^d	1 per litter	Blood ^e
7PP	Milk, ^c Blood ^d	1 per litter	Blood ^e
14PP	Milk, ^c Blood ^d	1 per litter	Blood ^e
21PP ^f	Milk, ^c Blood ^d	2 per litter	Blood ^e

a Samples were collected 2 hours ($\pm$ 30 minutes) post-dose; other samples were collected as soon as possible after maternal blood collection.

b Litter size permitting.

c Maternal milk samples (minimum volume approximately 100 μ L).

d Maternal blood sample volume 0.5 mL per animal collected in tubes containing heparin.

e Pup blood sample minimum volume approximately 100 μ L collected in tubes containing heparin.

f Maternal Terminal Sacrifice

Maternal

Blood samples were collected from the orbital sinus (days 3, 7, and 14 PP) or vena cava (day 21PP) approximately 2 hours ($\pm$ 30 minutes) post-dose.

Fetal

Randomly selected pups from these dams were weighed and euthanized on the same postpartum day with an overdose injection (i.p.) of sodium pentobarbital. Pup blood samples were taken from a transverse cut made to the carotid artery on days 3 and 7PP or from the *vena cava* on days 14 and 21PP.

Maternal and pup blood samples were processed to obtain plasma samples.

c. Milk Sample Collection

Milk samples were collected after blood sample collection. Oxytocin was administered by intraperitoneal injection to promote milk ejection from the mammary gland. Approximately 2 to 5 minutes after injection, the dam's nipple area was washed with warm saline or water. Milk was collected from more than one mammary gland; mammary glands were changed frequently and were repeated as necessary. Milk was collected by applying pressure to the entire base of the gland, pushing milk toward the nipple and into a test tube.

Pups were kept in polycarbonate pans with bedding during the milk collection procedure. On day 3PP litters were kept warm during the absence of the dam by the use of either a circulating hot water pad, heated gel packs, or heat lamp.

d. Euthanasia

After milk collection but before recovery from anesthesia, dams were euthanized by exsanguination. The remaining pups were euthanized with an overdose injection (i.p.) of sodium pentobarbital. Dam and pup carcasses were discarded.

BIOCHEMICAL TOXICOLOGY SAMPLE ANALYSES

A. Tissue and Plasma Analysis

1. Amniotic Fluid, Milk, and Plasma

Plasma, milk, and amniotic fluid samples (pooled by litter), were processed by protein precipitation (PPT) using Isolute Array protein precipitation columns (Jones Chromatography, Lakewood, CO). A 0.5 $\mu\text{g/mL}$ solution of perfluorononanoic acid (Aldrich Chemicals, Milwaukee, WI) in acetonitrile (ACN) was used as an internal standard. Samples were thawed and a 20 μL aliquot of each sample was applied to the PPT array. Samples were precipitated by adding appropriate dilution rate volumes of ACN/internal standard solution. Dilution rates ranging from 1:4 (60 μL of internal standard solution) to 1:80 (1580 μL of internal standard solution) were utilized to capture the sample concentrations within the range of the standard

curve concentrations. The array was slowly eluted under vacuum into a 96-well receiver plate, centrifuged at ~3000 rpm for 10 minutes, and the extracts were analyzed by high performance liquid chromatography – mass spectrometry (HPLC-MS).

2. Placenta and Embryo

Placenta samples (pooled by litter) were coarsely chopped using scissors; embryo samples (pooled by litter) did not require this step. Aliquots (approximately 0.5 g) of the embryo samples or of the placenta coarse homogenate were transferred to another tube and homogenized further using a Branson ultrasonic probe sonifier in the presence of ACN in a ratio of approximately 4:1 (v:w) ACN:tissue. The homogenized sample was then centrifuged at ~3000 rpm for 10 minutes. The supernatant was removed and taken to dryness by nitrogen convection, dissolved in internal standard solution (0.5 µg/mL perfluorononanoic acid in ACN), and analyzed by HPLC-MS.

3. Fetus

Fetus samples were pooled by litter and homogenized using a Waring blender in the presence of liquid nitrogen. Aliquots (approximately 0.5 g) of the homogenized samples were then transferred to another tube and homogenized further using a Branson ultrasonic probe sonifier in the presence of ACN in a ratio of approximately 4:1 (v:w) ACN:tissue. The homogenized sample was then centrifuged at ~3000 rpm for 10 minutes. The supernatant was removed and taken to dryness by nitrogen convection, dissolved in internal standard solution (0.5 µg/mL perfluorononanoic acid in ACN), and analyzed by HPLC-MS.

4. High performance liquid chromatography – mass spectrometry (HPLC-MS)

a. Chromatographic Method

Method: Plasma concentration analysis
Column: Waters Xterra MS C18, 2.1x30 mm, 2.5 µm
Column temperature: Ambient
Mobile phases: A: 50 mM Ammonium Acetate
B: ACN

Gradient:

Time (min)	%B
0.0	10
0.5	10
10.0	100
10.9	100
11.0	10

Flow rate: 0.25 mL/min
Stop time: 11.0 min
Injection volume: 5 µL

b. Mass Spectrometer Method

System: Waters 2790 Liquid Chromatograph, equipped with quaternary pump, column heater, and autosampler
Detector: Quattro Micro Mass Spectrometer
Mode: Multiple Reaction Monitoring (MRM)
Source: Negative Electrospray
LM 1 resolution: 10.0
HM 1 resolution: 10.0
Ion energy 1: 1.0
Entrance: 5
Collision: 2
Exit: 5
LM 2 resolution: 14
HM 2 resolution: 14
Ion energy 2: 2.0
Multiplier (V): 650
Capillary (kV): 2.0
Cone (V): -15
Extractor (V): 0
RF lens (V): 0
Source temperature: 130°C
Desolvation temperature: 350°C

MS Method:
Mode: MRM, 2 transitions
Time: 0-11 min
Ch1: 413.00 → 369.00
Dwell: 0.25 sec
Collision energy: 15 eV
Ch2: 469.00 → 419.00
Dwell: 0.25 sec
Collision energy: 15 eV
Limit of Quantitation 0.05 µg/mL

STATISTICAL ANALYSES

For all quantitative data presented in the report, descriptive statistics were performed that included calculation of mean and standard deviation.

RESULTS AND CONCLUSIONS

ANALYTICAL DATA

A. Test Substance Stability Analyses

Samples of the test substance were analyzed near the beginning and end of the study. These analyses indicated that PFOA was stable over the course of the study.

The average of the active ingredient was $91.9 \pm 1.0\%$ and $96.6 \pm 2.1\%$ for samples analyzed July 14, 2003 and September 4, 2003.⁽⁴⁾ The PFOA was reported by the sponsor to be 95.2-97.99% pure. The difference between the sponsor reported purity and the experimental data are most likely due to analytical variability.

B. Test Substance Formulation Analyses (Table 1, Appendix A)

1. Chromatography

PFOA eluted from the HPLC column as a resolved peak with a retention time of approximately 4.9 to 5.2 minutes (5 μ L or 50 μ L injections) for the negative ion. Representative LC-MS/MS chromatograms are shown in Appendix A, Figures 2 (a - f). Test substance was not detected in the 0 mg/mL control formulation. [1,2-di-¹³C] perfluorooctanoic acid used as an internal standard introduced a detectable peak in the control (0 mg/mL sample) in the study. This was determined by comparison of the NANOpure deionized water (diluent) used as a wash in the LC/MS analysis and the NANOpure deionized water (diluent) with internal standard along with the 0 mg/mL sample (control) with internal standard. Refer to Appendix A, Figures 2 (a - c).

2. Recovery Samples

Detailed analytical results of recovery samples are summarized in Appendix A, Table I. The variability of the analytical method was demonstrated by the coefficients of variation of the recovery results at each targeted dosing concentration (approximately 0.6, 2.0, 6.0 mg/mL) over the course of the study. The measured concentrations of PFOA for the 0.6 mg/mL level were 92.0% to 106.2% of nominal (mean percent recovery = $97.0 \pm 8.0\%$, C.V. = 8%). The measured concentrations of PFOA for the 2.0 mg/mL level were 83.0% to 100.2% of nominal (mean percent recovery = $92.6 \pm 8.8\%$, C.V. = 9%). The measured concentrations of PFOA for the 6.0 mg/mL level were 90.3% to 108.5% of nominal (mean percent recovery = $99.2 \pm 9.1\%$, C.V. = 9%). Based on this data, the analytical method performed satisfactorily over the entire concentration range for the study.

3. Uniformity of Mixing/Concentration and Stability Samples

Analytical results from dosing solutions collected on July 11, 2003 and analyzed for uniformity of mixing/concentration verification and 5-hour room temperature stability are shown in Appendix A, Table II and Summary Table 1.

The following table summarizes the results for homogeneity/concentration verification and stability analyses for this sampling day of the PFOA preparation.

Sample Day Sample Type	Nominal mg/mL	Measured ^a mg/mL	Mean % Nominal	C.V. (%)	Stability ^b % Nominal
11-July-2003					
Uniformity/Concentration	0	ND ^c	---	---	---
	0.60	0.613, 0.558	97.7	7	102.0
	2.0	2.21, 2.07	107.0	5	102.0
	6.0	6.68, 6.30	108.2	4	104.0

a Mean results for the analysis of the duplicate samples.

b Samples held 5 hours at room temperature.

c Denotes none detected. Reported results are based on showing that the internal standard addition to the control caused a detectable peak for the test substance in the control. Shown in Figures 2 (a - c).

The results for PFOA samples prepared and collected on July 11, 2003 indicated that the test substance was at the targeted concentrations ($\pm 8.2\%$ of nominal), adequately mixed (CV less than 10) and stable in the vehicle when held 5 hours at room temperature for all levels. Test substance was not detected in the 0 mg/mL samples.

4. Concentration Verification Samples

Analytical results from dosing solutions collected on July 31, 2003 and August 21, 2003 and analyzed for concentration verification are shown in Appendix A, Table III and Summary Table 1.

The following table summarizes the results for concentration verification analyses for both sampling days of the PFOA preparation.

Preparation Day	Nominal mg/mL	Measured ^a mg/mL	Average % Nominal	CV %
31-July-2003 ^b	0.60	0.497, 0.493	82.5	1
	2.0	1.72, 1.69	85.5	1
	6.0	4.88, 5.07	83.0	3
21-Aug-2003 ^c	0.6	0.596, 0.613	100.7	2
	2.0	1.90, 1.99	97.5	3
	6.0	5.45, 5.04	87.5	5

a Duplicate samples per level were analyzed. C.V. calculated to verify uniformity of mixture.

b Reported results are the mean of duplicate re-sampling of the original submitted samples because the original sample analysis was not acceptable due to standard curve preparation.

c Reported results are from re-sampling of the original submitted samples and dilution to a higher level for analysis (5 μ L injection) because the original sample analysis was not acceptable due to standard curve preparation.

The results for samples prepared and collected on July 31, 2003 indicated that the test substance was at the targeted levels ($\pm 17.5\%$ of nominal) and adequately mixed (CV less than 10) for all PFOA samples. Test substance was not detected in the 0 mg/mL samples.

The results for samples prepared and collected on August 21, 2003 indicated that the test substance was at the targeted levels ($\pm 12.5\%$ of nominal) and adequately mixed (CV less than 10) for all PFOA samples. Test substance was not detected in the 0 mg/mL samples.

C. Analytical Conclusions

Results from the analysis of the PFOA dosing solutions during the study indicate that the test substance was mixed properly (CV less than 10), at the targeted levels ($\pm 20\%$ of nominal) and stable under the conditions of the study. Test substance was not found in the 0 mg/mL samples.

REPRODUCTIVE TOXICITY EVALUATIONS

IN-LIFE TOXICOLOGY

A. Maternal Observations

(Figures 1 and 2, Appendices B, C, and D)

There was no mortality at any level tested; all animals on study survived to scheduled sacrifice. There were no test substance-related clinical observations.

Means and standard deviations for body weight and body weight gain data are in Appendices C and D.

Mean body weight gain at 30 mg/kg/day was lower than control during days 4-10G (24.9g vs 34.5g for the control group) and days 10-15G (28.7g vs 33.8g in the control group). These reductions resulted in overall weight gain during gestation that was 10% lower than the control group (138.1g vs 152.7g for the control group) for days 4-21G. Mean body weight gain at 30 mg/kg/day was similar to control during lactation (11.2g vs 7.8g for the control group). However, mean daily body weights remained approximately 4% lower than control throughout gestation and lactation for this group. Body weights and body weight gain at 3 and 10 mg/kg/day during gestation and lactation were similar to the control group.

Mean Body Weight Gain (grams) for Selected Intervals During Gestation

Gestation Days	Dose (mg/kg/day)			
	0	3	10	30
4-10	34.5	32.7	36.4	24.9
	8.28	10.57	10.49	7.93
10-15	33.8	33.5	35.3	28.7
	7.16	9.21	5.83	8.49
4-21	152.7	144.4	159.7	138.1
	15.76	24.66	20.16	33.71

Data presented: Means
Standard Deviation

B. Reproductive Data

1. Gestation Subsets
(Appendix E)

All animals were pregnant at scheduled sacrifice.

The number of implantation sites, resorptions, and live fetuses were comparable across groups that were sacrificed on either day 10G, 15G, or 21G.

2. Lactation Subset
(Appendices F, G, and H)

All animals delivered a live litter.

There were no test substance-related clinical observations in litters during lactation.

Pup survival and pup weights during lactation were comparable across groups; there was no test substance-related pup mortality during lactation. There were 2 small litters in the 30 mg/kg/day lactation group. Due to the intrinsic variability in litter size and the small number of litters evaluated (5 per group) the relationship of this finding to test substance administration is equivocal.

Number of Pups Born for Individual Litters

	Dose (mg/kg/day)			
	0	3	10	30
	13	15	12	13
	14	4	14	3
	19	14	12	6
	16	16	14	11
	12	13	14	13
Mean	14.8	12.4	13.2	9.2
Standard Deviation	2.77	4.83	1.10	4.49

BIOCHEMICAL TOXICOLOGY

A. Maternal, Fetal, and Pup Plasma

(Tables 2-3, Figures 3-5, Appendix I)

The concentration of PFOA in maternal plasma appeared to be at steady state over the range of time points sampled. The mean concentrations at steady state were 11.2, 26.8, and 66.6 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg dose levels, respectively. The mean concentrations of PFOA in fetal plasma collected on day 21G were 5.9, 14.5, and 33.1 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg dose levels, respectively. Pup plasma concentration decreased from day 3PP (means of 2.9, 5.9, and 12.0 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively) to day 7PP (0.7, 2.8, and 4.9 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg/day, respectively), and were similar on days 7, 14, and 21PP at all dose levels.

B. Milk

(Table 4, Figure 6, Appendix I)

The concentration of PFOA in milk was at steady state from day 3PP to day 21PP at all dose levels. The mean concentrations at steady state were 1.1, 2.8, and 6.2 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg dose levels, respectively. These concentrations are approximately ten times less than the steady state concentrations observed in maternal plasma. The milk concentrations appeared to be generally comparable to the concentrations in pup plasma.

C. Amniotic Fluid

(Table 5, Figure 7, Appendix I)

The concentration of PFOA in amniotic fluid was approximately four times higher on day 21G (means of 1.5, 3.8, and 8.1 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively) than on day 15G (means of 0.6, 0.7, and 1.7 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively).

D. Placenta

(Table 6, Figure 8, Appendix I)

The concentration of PFOA in placenta was approximately two times higher on day 21G (means of 3.6, 9.4, and 24.4 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively) than on day 15G (means of 2.2, 5.1, and 13.2 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively).

E. Embryo and Fetus

(Table 7, Figure 9, Appendix I)

The day 10G embryo had the highest concentration of PFOA per gram of tissue (1.4, 3.3, and 12.5 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively), and the day 21G fetus had slightly lower levels (1.3, 2.6, and 8.8 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively). The day 15G embryo had a lower tissue concentration than tissue from day 10G or day 21G (0.2, 0.5, and 1.2 $\mu\text{g/mL}$ at 3, 10, and 30 mg/kg, respectively).

CONCLUSIONS

Under the conditions of this study, there was maternal toxicity (reduced body weight and weight gain) at 30 mg/kg/day. The mean number of pups born was lower than control at 30 mg/kg/day, a finding that was considered possibly test substance-related. Concentrations of PFOA in maternal plasma and milk were at steady state during the sampling interval. Steady state concentration in milk was approximately ten times less than the steady state concentration in maternal plasma. The concentration of PFOA in fetal plasma on day 21G was approximately half the steady state concentration in maternal plasma. The milk concentrations appeared to be generally comparable to the concentrations in pup plasma. Pup plasma concentrations decreased from days 3 to 7PP, and were similar on days 7, 14, and 21PP at all dose levels. PFOA was detected in placenta (days 15 and 21G), amniotic fluid (days 15 and 21G), embryo (days 10 and 15G) and fetus (day 21G).

RECORDS AND SAMPLE STORAGE

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

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TABLES

TABLES

EXPLANATORY NOTES

Abbreviations

G	=	Gestation
LOQ	=	Limit of Quantitation, 0.05 µg/mL.
<LOQ	=	Less than limit of quantitation
N.A.	=	Not applicable
PP	=	Postpartum
S.D.	=	Standard Deviation

TABLE 1

SUMMARY OF DOSING TEST FORMULATION ANALYSES

Sample Type	Dosing Concentrations and Stability of PFOA (mg/mL)			
	Nominal:	0.6	2.0	6.0
<u>Uniformity/Concentration</u>				
July 11, 2003				
#1		0.613 (102.2) ^a	2.21 (110.5)	6.68 (111.3)
#2		0.558 (93.0)	2.07 (103.5)	6.30 (105.0)
Average Measured Conc. ^b		0.586	2.14	6.49
Average Percent Nominal ^b		(97.7)	(107.0)	(108.2)
Standard Deviation ^b		± 0.04	± 0.10	± 0.27
Coefficient of Variation ^b		7%	5%	4%
<u>Stability</u>				
5-hour Room Temperature				
		0.612 (102.0)	2.04 (102.0)	6.24 (104.0)
<u>Concentration Verification</u>				
July 31, 2003				
#1		0.497 (82.8)	1.72 (86.0)	4.88 (81.3)
#2		0.493 (82.2)	1.69 (84.5)	5.07 (84.5)
Average Measured Conc. ^c		0.495	1.71	4.98
Average Percent Nominal ^c		(82.5)	(85.5)	(83.0)
Standard Deviation ^c		± 0.003	± 0.02	± 0.13
Coefficient of Variation ^c		1%	1%	3%
August 21, 2003				
#1		0.596 (99.3)	1.90 (95.0)	5.45 (90.8)
#2		0.613 (102.2)	1.99 (99.5)	5.04 (84.0)
Average Measured Conc. ^d		0.604	1.95	5.25
Average Percent Nominal ^d		(100.7)	(97.5)	(87.5)
Standard Deviation ^d		± 0.01	± 0.06	± 0.29
Coefficient of Variation ^d		2%	3%	5%

a Numbers in parentheses are the respective percent of nominal values.

b The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of duplicate samples.

c The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of the mean of duplicate re-dilution of the original samples.

d The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of the mean of re-dilution of the original samples to higher level for analysis (5µL injection).

TABLE 2
CONCENTRATION ($\mu\text{g/mL}$) OF PFOA IN MATERNAL PLASMA
DURING GESTATION AND POSTPARTUM

Day	0 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
10G	<LOQ	NA	8.53	1.06	23.32	2.15	70.49	8.94
15G	<LOQ	NA	15.92	12.96	29.40	14.19	79.55	3.11
21G	<LOQ	NA	14.04	2.27	34.20	6.68	76.36	14.76
3PP	<LOQ	NA	11.01	2.11	22.47	2.74	54.39	17.86
7PP	<LOQ	NA	10.09	2.90	25.83	2.07	66.91	11.82
14PP	<LOQ	NA	9.69	0.92	23.79	2.81	54.65	11.63
21PP	<LOQ	NA	9.04	1.01	28.84	5.15	64.13	1.45
Average	<LOQ		11.19		26.84		66.64	
S.D.	NA		2.76		4.21		9.80	

TABLE 3
CONCENTRATION ($\mu\text{g/mL}$) OF PFOA IN FETAL AND PUP PLASMA

Sample	Day	0 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
		Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
Fetal	21G	<LOQ	NA	5.88	0.69	14.48	1.51	33.11	4.64
Pup	3PP	<LOQ	NA	2.89	0.70	5.94	1.44	11.96	1.66
Pup	7PP	<LOQ	NA	0.65	0.20	2.77	0.58	4.92	1.28
Pup	14PP	<LOQ	NA	0.77	0.10	2.22	0.38	4.91	1.12
Pup	21PP	<LOQ	NA	1.28	0.72	3.25	0.52	7.36	2.17

TABLE 4
CONCENTRATION ($\mu\text{g/mL}$) OF PFOA IN MILK

Day	0 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
3PP	<LOQ	NA	1.07	0.26	2.03	0.33	4.97	1.20
7PP	<LOQ	NA	0.94	0.22	2.74	0.91	5.76	1.26
14PP	0.21	NA	1.15	0.06	3.45	1.18	6.45	1.38
21PP	<LOQ	NA	1.13	0.08	3.07	0.51	7.48	1.63
Average	NA ^a		1.07		2.82		6.16	
S.D.	NA		0.09		0.60		1.06	

a Average not calculated because 3 of 4 values <LOQ.

TABLE 5
CONCENTRATION ($\mu\text{g/mL}$) OF PFOA IN AMNIOTIC FLUID

Day	0 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
15G	<LOQ	NA	0.60	0.69	0.70	0.15	1.70	0.91
21G	<LOQ	NA	1.50	0.32	3.76	0.81	8.13	0.86

TABLE 6
CONCENTRATION ($\mu\text{g/g}$) OF PFOA IN PLACENTA

Day	0 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
15G	<LOQ	NA	2.22	1.79	5.10	1.70	13.22	1.03
21G	<LOQ	NA	3.55	0.57	9.37	1.76	24.37	4.13

TABLE 7
CONCENTRATION ($\mu\text{g/g}$) OF PFOA IN EMBRYO/FETUS

Tissue	Day	0 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
		Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
Embryo	10G	<LOQ	NA	1.40	0.30	3.33	0.81	12.49	3.50
Embryo	15G	<LOQ	NA	0.24	0.19	0.53	0.18	1.24	0.22
Fetus	21G	<LOQ	NA	1.27	0.26	2.61	0.37	8.77	2.36

FIGURES

FIGURES

EXPLANATORY NOTES

Abbreviations

G	=	Gestation
LOQ	=	Limit of Quantitation, 0.05 µg/mL.
<LOQ	=	Less than limit of quantitation
PP	=	Postpartum

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FIGURE 1

MEAN BODY WEIGHTS OF FEMALE RATS DURING GESTATION

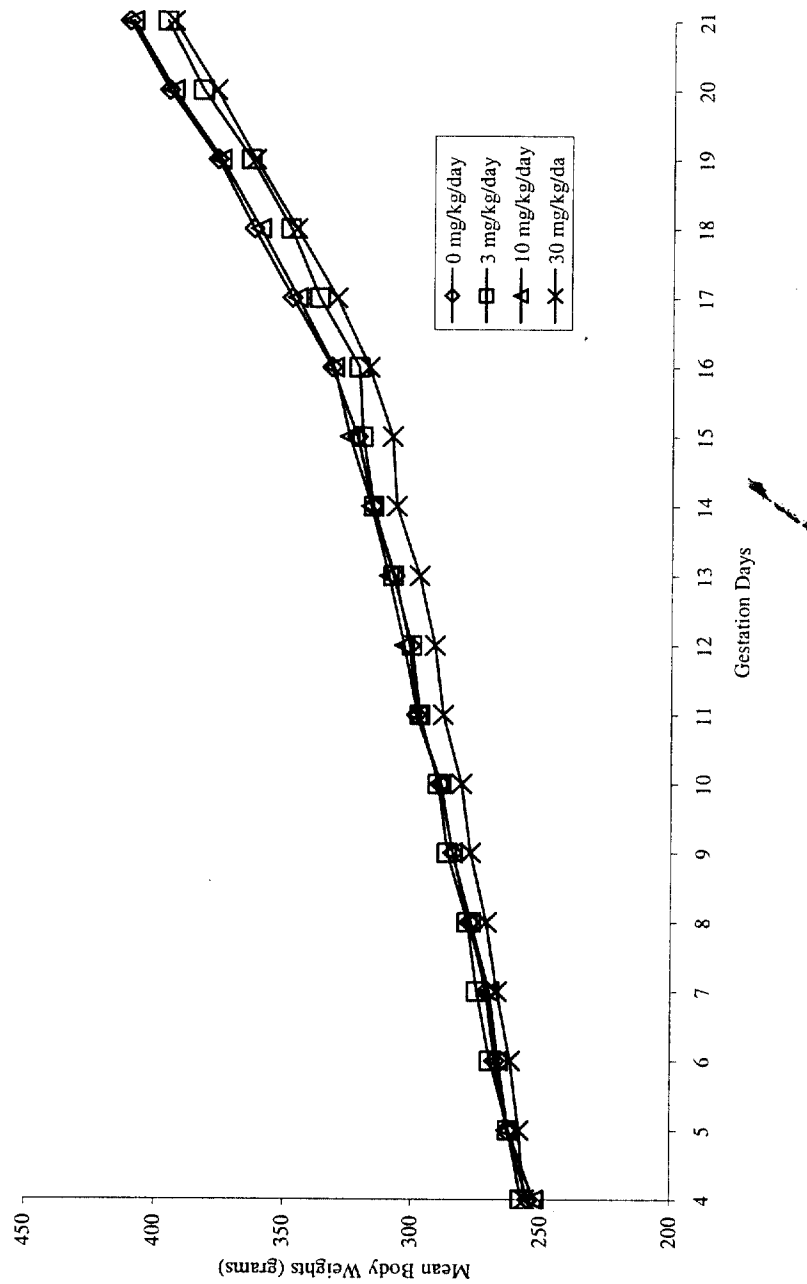


FIGURE 2
MEAN BODY WEIGHTS OF FEMALE RATS DURING LACTATION

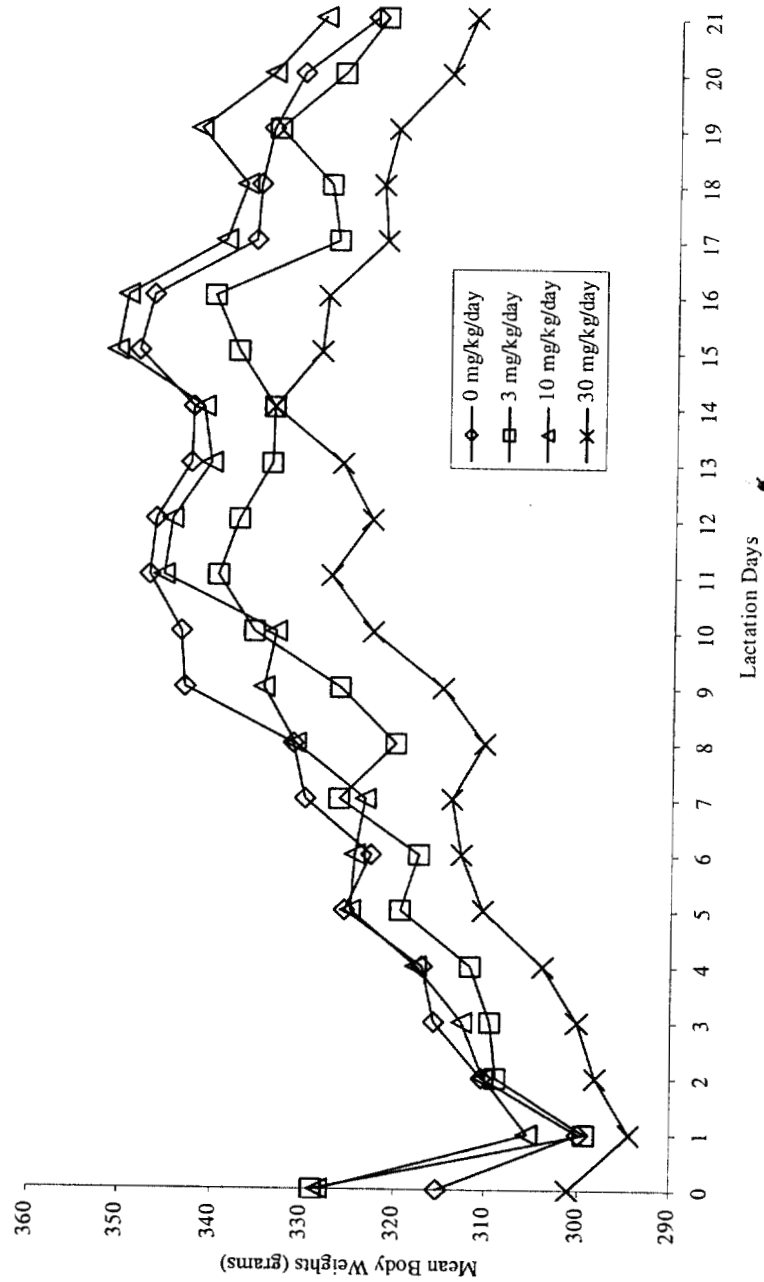
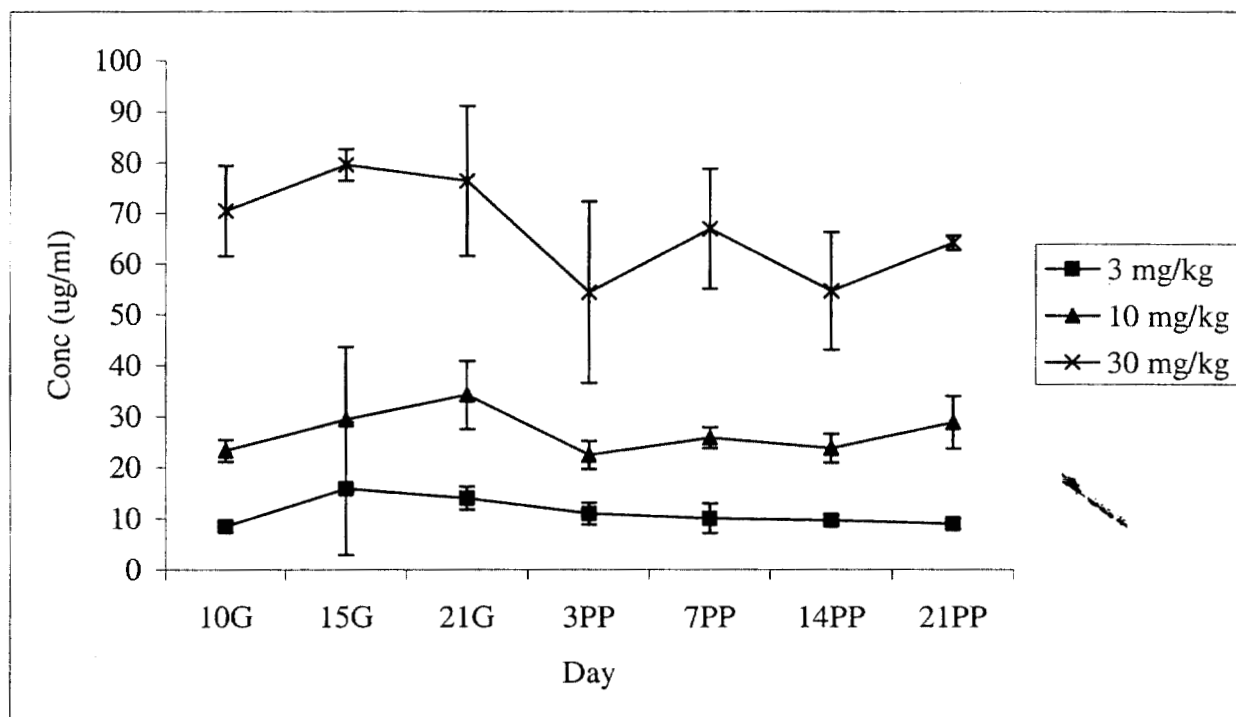
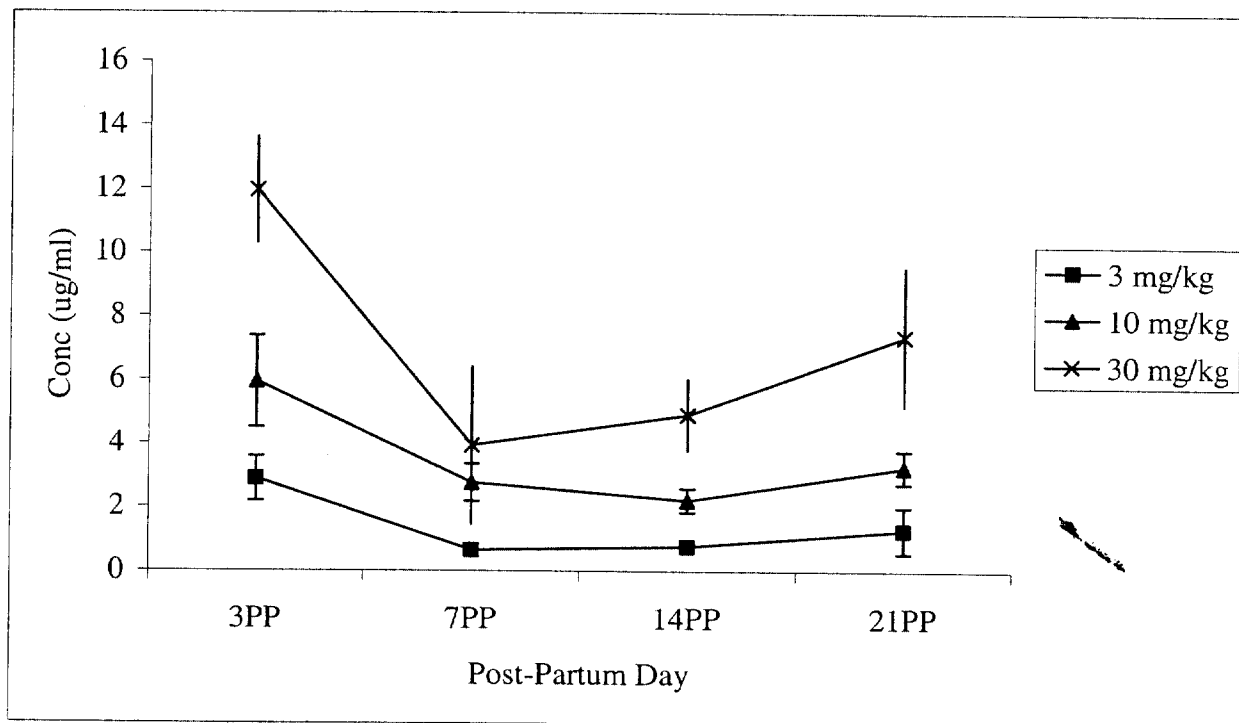


FIGURE 3

CONCENTRATION OF PFOA IN MATERNAL PLASMA
DURING GESTATION AND POSTPARTUM

Note: Concentration in the 0 mg/kg group = <LOQ (0.05 $\mu\text{g/mL}$).

FIGURE 4
CONCENTRATION OF PFOA IN PUP PLASMA



Note: Concentration in the 0 mg/kg group = <LOQ (0.05 $\mu\text{g/mL}$).

FIGURE 5

CONCENTRATION OF PFOA IN FETAL PLASMA ON GESTATION DAY 21

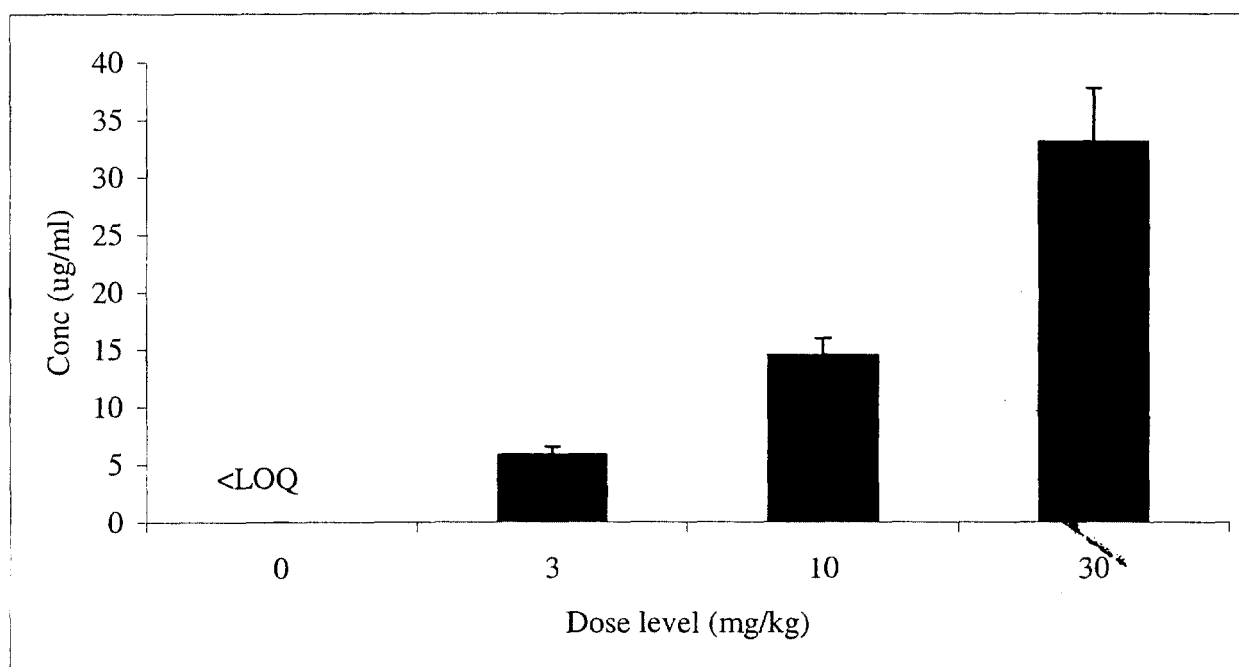
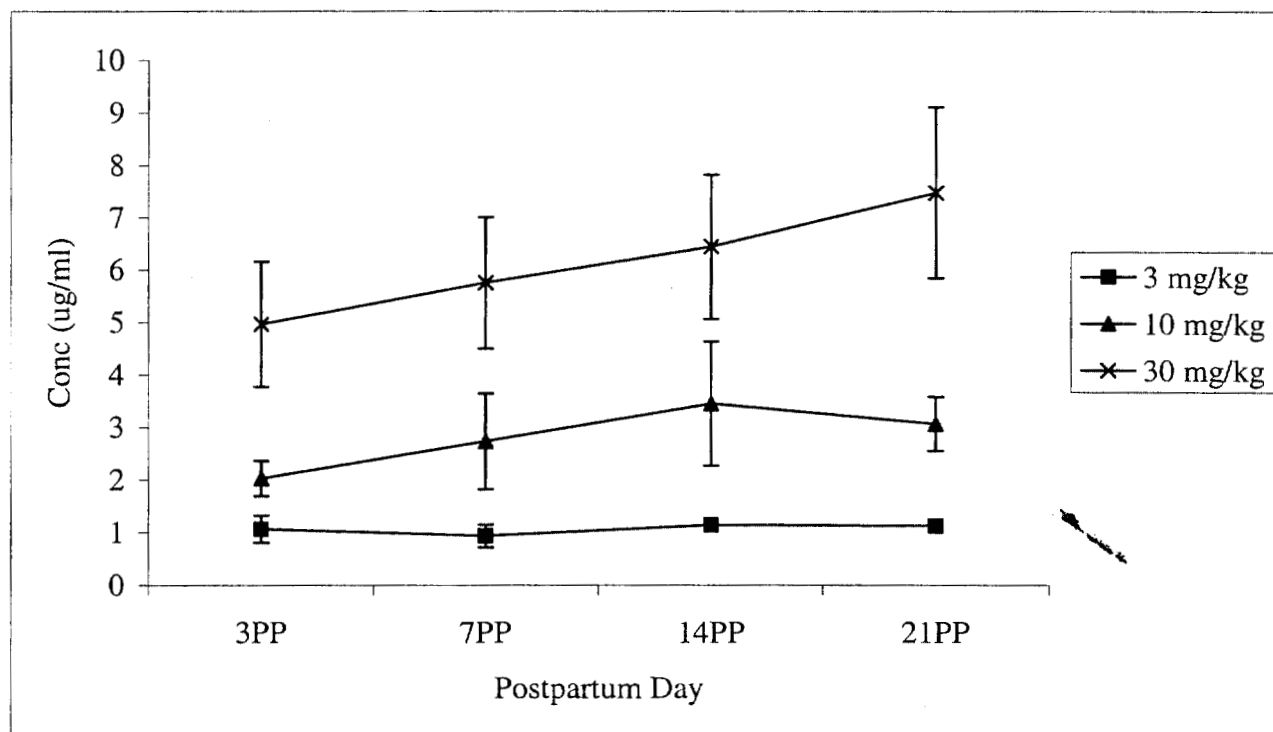
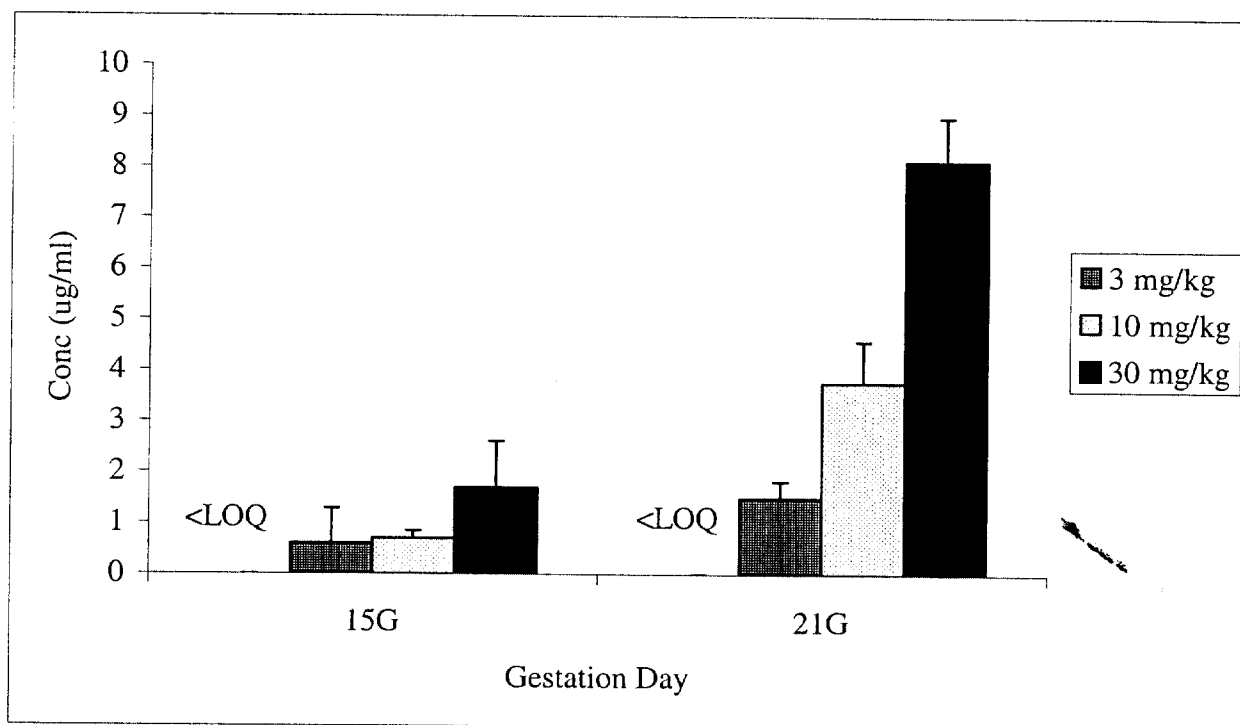


FIGURE 6
CONCENTRATION OF PFOA IN MILK



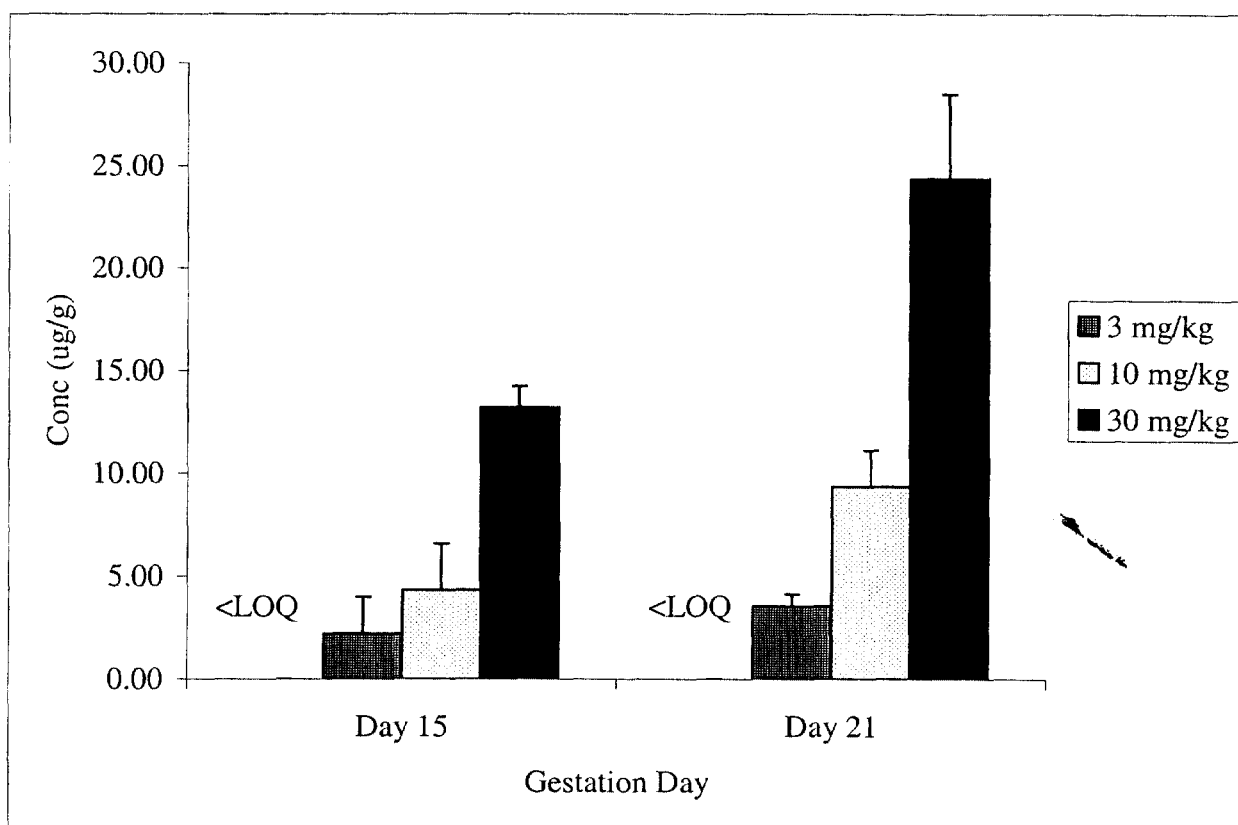
Note: Concentration in the 0 mg/kg group = <LOQ (0.05 $\mu\text{g/mL}$).

FIGURE 7
CONCENTRATION OF PFOA IN AMNIOTIC FLUID



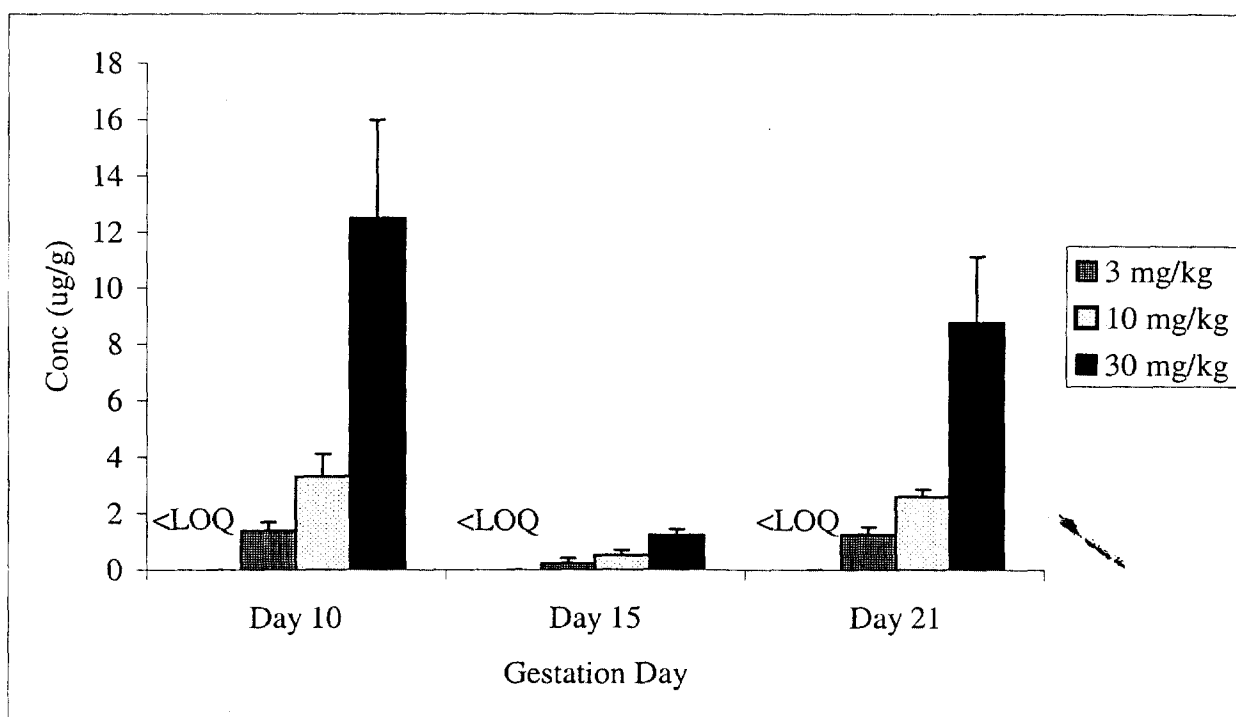
Note: Concentration in the 0 mg/kg group = <LOQ (0.05 $\mu\text{g/mL}$).

FIGURE 8
CONCENTRATION ($\mu\text{g/g}$) OF PFOA IN PLACENTA



Note: Concentration in the 0 mg/kg group = <LOQ (0.05 $\mu\text{g/mL}$).

FIGURE 9
CONCENTRATION ($\mu\text{g/g}$) OF PFOA IN EMBRYO/FETUS



Note: Concentration in the 0 mg/kg group = <LOQ (0.05 $\mu\text{g/mL}$).

APPENDICES

APPENDIX A
ANALYTICAL DATA

Table I. Recovery of PFOA Added to Dosing Vehicle

Sample Type	mg/ml PFOA		Percent Nominal
	Nominal	Measured	
RECOVERY ^(A)	0.584	0.620	106.2
RECOVERY ^(B)	0.585	0.542	92.6
RECOVERY ^(C)	0.587	0.540	<u>92.0</u>
			Mean: 97.0 ± 8.0, C.V. 8%
RECOVERY ^(A)	1.95	1.953	100.2
RECOVERY ^(B)	1.95	1.618	83.0
RECOVERY ^(C)	1.96	1.854	<u>94.6</u>
			Mean: 92.6 ± 8.8, C.V. 9%
RECOVERY ^(A)	5.84	6.336	108.5
RECOVERY ^(B)	5.85	5.283	90.3
RECOVERY ^(C)	5.87	5.799	<u>98.8</u>
			Mean: 99.2 ± 9.1, C.V. 9%

(A) Processed with uniformity of mixing/concentration verification samples from dosing prepared on July 11, 2003.

(B) Processed with concentration verification samples from dosing prepared on July 31, 2003.

(C) Processed with concentration verification samples from dosing prepared on August 21, 2003.

Table II. Uniformity of Mixing/Concentration Verification and Stability of PFOA in Dosing Solutions

Sample Type	mg/mL PFOA		Percent
	Nominal	Measured	Nominal
11-July-2003			
<u>Uniformity/Concentration</u>			
CONTROL	0.00	ND ^(A)	----
#1	0.60	0.613	102.2
#2	0.60	<u>0.558</u>	93.0
		<i>Mean^(B): 0.586 ± 0.04</i>	<i>(97.7%)</i>
		<i>C.V. 7%</i>	
#1	2.0	2.21	110.5
#2	2.0	<u>2.07</u>	103.5
		<i>Mean^(B): 2.14 ± 0.10</i>	<i>(107.0%)</i>
		<i>C.V. 5%</i>	
#1	6.0	6.68	111.3
#2	6.0	<u>6.30</u>	105.0
		<i>Mean^(B): 6.49 ± 0.27</i>	<i>(108.2%)</i>
		<i>C.V. 4%</i>	
5 HOUR ^(C)	0.60	0.612	102.0
	2.0	2.04	102.0
	6.0	6.24	104.0

(A) Denotes none detected. Reported results are based on showing that the internal standard addition to the control caused a detectable peak for the test substance in the control. Shown in Figures 2 (a - c).

(B) The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of duplicate samples.

(C) Stability samples held 5 hours a room temperature.

Table III. Concentration Verification of PFOA in Dosing solutions

Preparation Day	mg/mL PFOA		Percent
Sample Type	Nominal	Measured	Nominal
31-July-2003			
<u>Concentration Verification</u> ^(A)			
Control	0.0	ND ^(B)	----
#1	0.60	0.497	82.8
#2	0.60	<u>0.493</u>	82.2
		<i>Mean</i> ^(C) : 0.495 ± 0.003	(82.5)
		<i>C.V.</i> 1%	
#1	2.0	1.72	86.0
#2	2.0	<u>1.69</u>	84.5
		<i>Mean</i> ^(C) : 1.71 ± 0.02	(85.5)
		<i>C.V.</i> 1%	
#1	6.0	4.88	81.3
#2	6.0	<u>5.07</u>	84.5
		<i>Mean</i> ^(C) : 4.98 ± 0.13	(83.0)
		<i>C.V.</i> 3%	
21-Aug-2003			
<u>Concentration Verification</u> ^(A)			
Control	0.0	ND ^(B)	----
#1	0.60	0.596	99.3
#2	0.60	<u>0.613</u>	102.2
		<i>Mean</i> ^(D) : 0.604 ± 0.01	(100.7)
		<i>C.V.</i> 2%	
#1	2.0	1.90	95.0
#2	2.0	<u>1.99</u>	99.5
		<i>Mean</i> ^(D) : 1.95 ± 0.06	(97.5)
		<i>C.V.</i> 3%	
#1	6.0	5.45	90.8
#2	6.0	<u>5.04</u>	84.0
		<i>Mean</i> ^(D) : 5.25 ± 0.29	(87.5)
		<i>C.V.</i> 5%	

(A) The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of duplicate samples.

(B) Denotes none detected. Reported results are based on showing that the internal standard addition to the control caused a detectable peak for the test substance in the control. Shown in Figures 2 (a - c).

(C) Mean result for analyses for duplicate re-sampling of the original samples. Original analysis of samples was not evaluated due to error in standard curve preparation.

(D) Mean result for analyses for re-sampling of the original samples and dilution to a higher level (5µL injection). Original analysis of samples was not evaluated due to error in standard curve preparation.

Figure 1

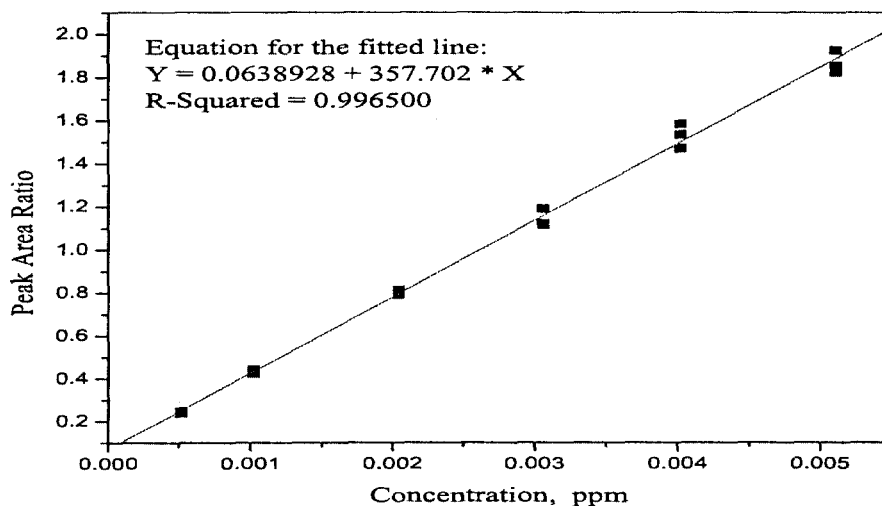
Representative Analytical Calibration Curves

Figure 1a: Calibration curve showing linear fit (line) to replicate peak area ratio (squares) for calibration solutions of PFOA diluted with NANOpure deionized water and matrix matched over a concentration range of 0.00051 to 0.00511 ppm.

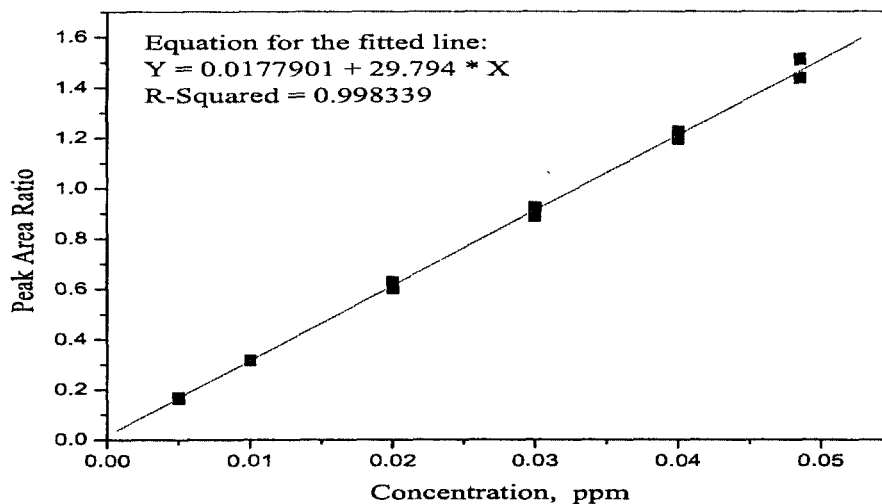


Figure 1b: Calibration curve showing linear fit (line) to replicate peak area ratio (squares) for calibration solutions of PFOA diluted with NANOpure deionized water and matrix matched over a concentration range of 0.0050 to 0.0485 ppm.

Figure 2
Representative LC/MS/MS Chromatography Chromatograms

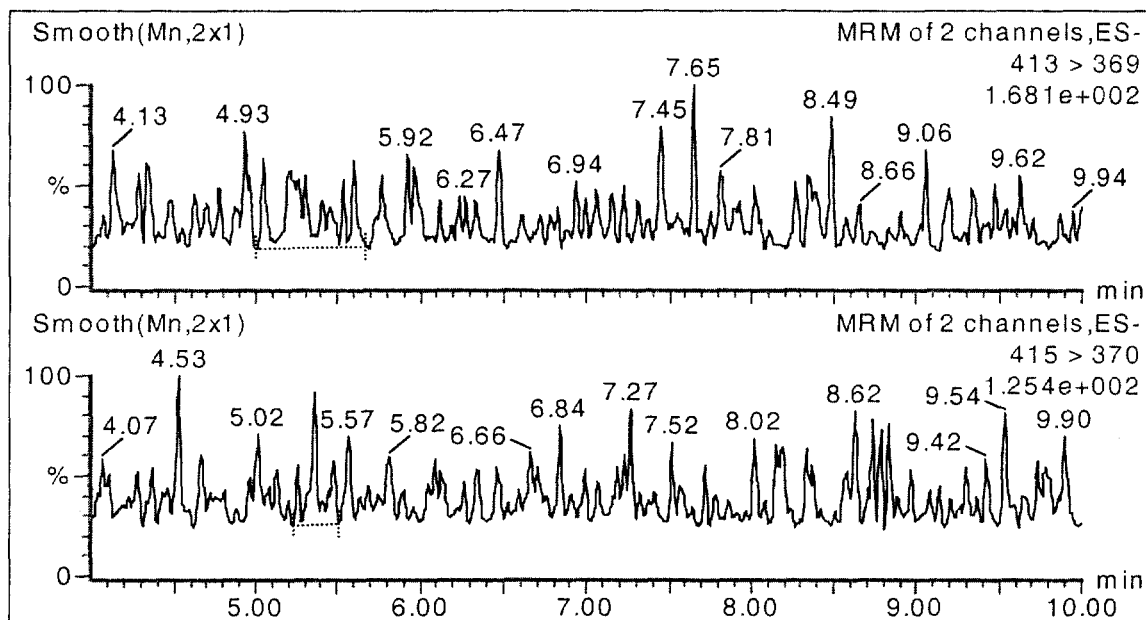


Figure 2a: Representative LC/MS chromatogram of NANOpure deionized water (sample diluent) used in the study. Negative ion/ perfluorooctanoic acid (PFOA) retention time is approximately 4.9 to 5.2 minutes.

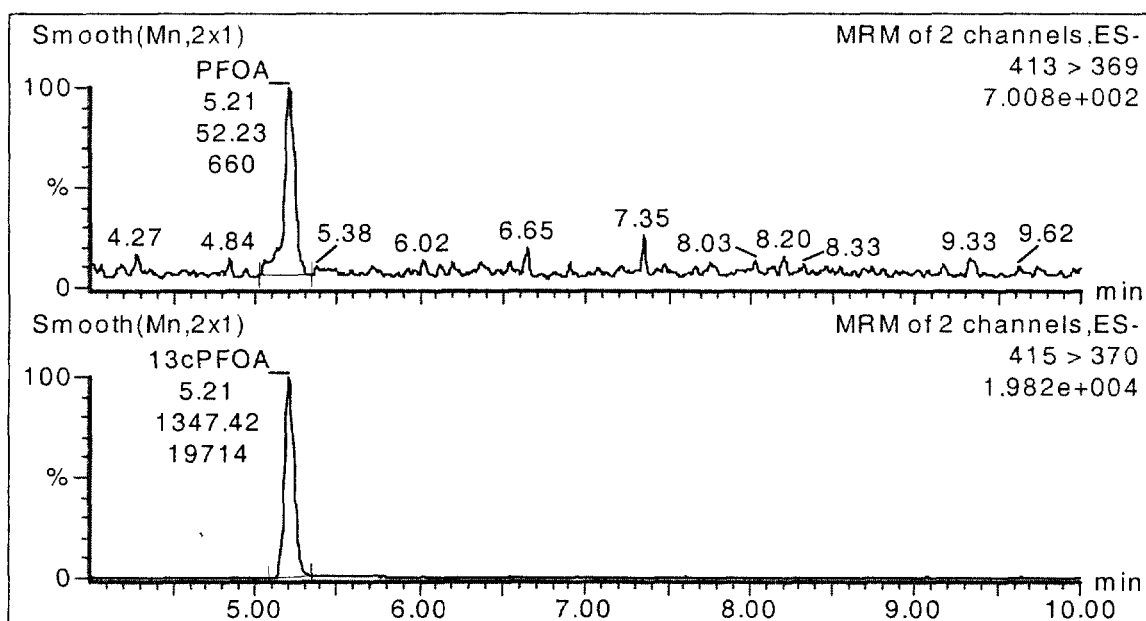


Figure 2b: Representative LC/MS chromatogram of NANOpure deionized water (sample diluent) used in the study with the internal standard. Negative ion/PFOA retention time is approximately 4.9 to 5.2 minutes.

Figure 2 (continued)

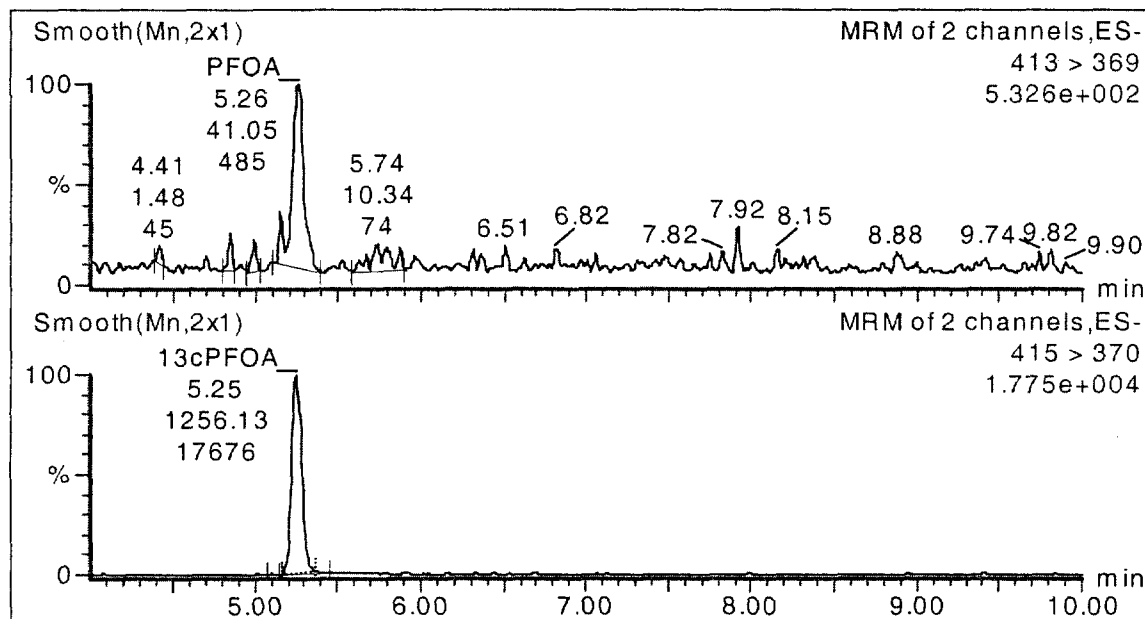
Representative LC/MS/MS Chromatography Chromatograms

Figure 2c: Representative LC/MS chromatogram of 0.00 mg/mL control solution of PFOA with the internal standard. Negative ion/PFOA retention time is approximately 4.9 to 5.2 minutes.

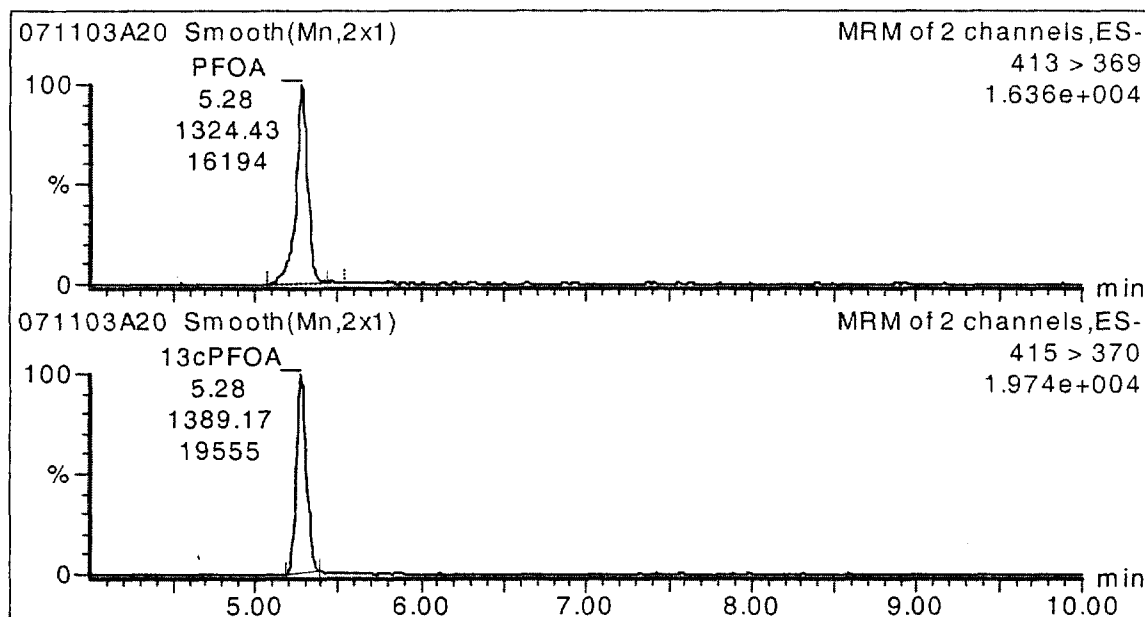


Figure 2d: Representative LC/MS chromatogram of PFOA analytical reference standard (0.00306 ppm) with the internal standard (0.0032 ppm). Negative ion/PFOA retention time is approximately 4.9 to 5.2 minutes.

Figure 2 (continued)

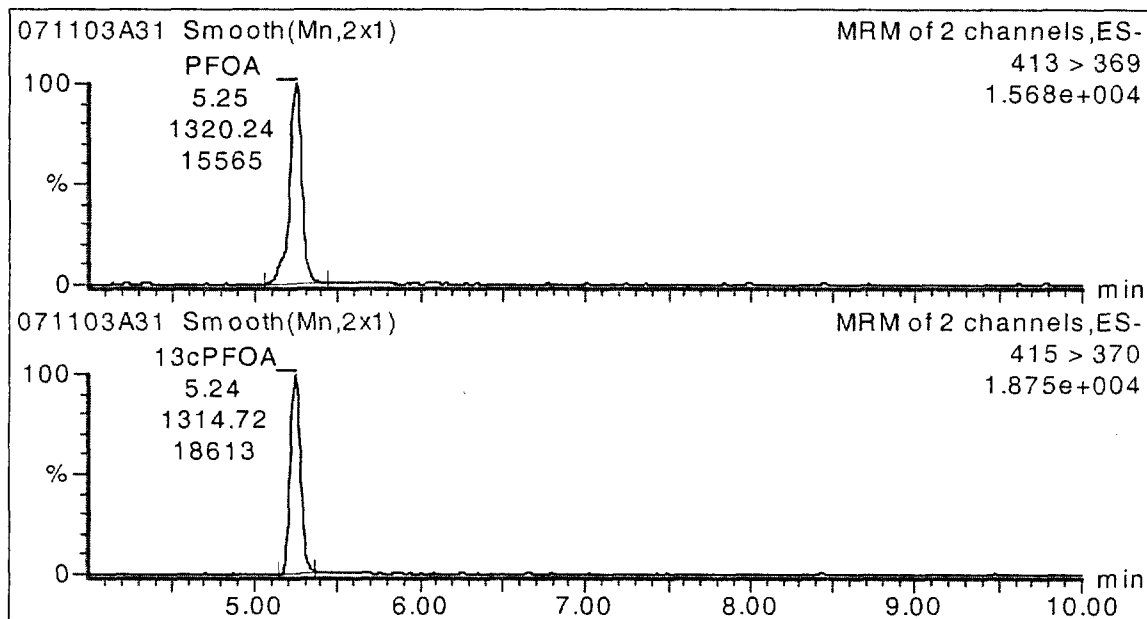
Representative LC/MS/MS Chromatography Chromatograms

Figure 2e: Representative LC/MS chromatogram of 0.6 mg/mL dosing solution diluted to nominal concentration of 0.003 ppm of PFOA for negative ion /PFOA with retention time 5.2 minutes. The measured concentration of the representative solution is 0.613 mg/mL.

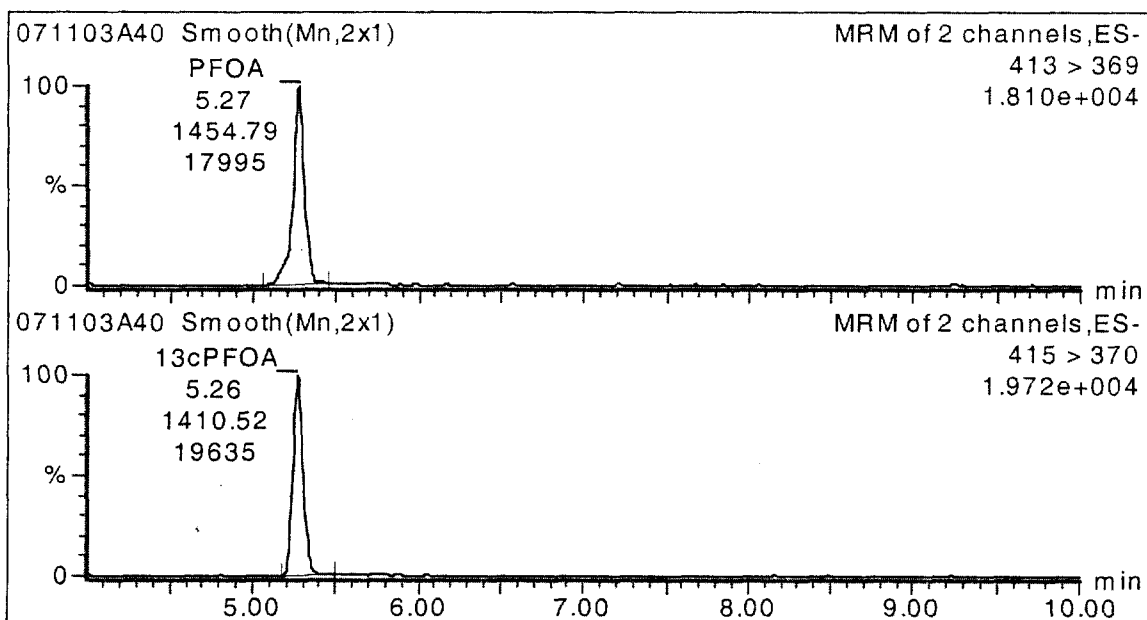


Figure 2f: Representative LC/MS chromatogram of low recovery dosing solution (0.584 mg/mL) diluted to nominal concentration of 0.003 ppm of PFOA for negative ion /PFOA with retention time approximately 5.2 minutes. The measured concentration of the representative recovery solution is 0.620 mg/mL.

APPENDIX B

**INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA
DURING GESTATION AND LACTATION**

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA
DURING GESTATION AND LACTATION

EXPLANATORY NOTES

Note

Day 21 = Gestation Day 21.

Day 22 = Lactation Day 0.

Day 43 = Lactation Day 21.

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21
7	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , , ,
9	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21
12	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21
15	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , 15, , , , , ,
17	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , 15, , , , , ,
20	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , 15, , , , , ,
23	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , 15, , , , , ,
31	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , 15, , , , , ,
32	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , , ,
34	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21
36	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , , ,
38	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21
47	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , , ,

GROUP I: 0 MG/KG/DAY

66

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43
62	ALOPECIA BOTH FRONT PAW	a: 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43
	ALOPECIA BOTH FRONT LEG	a: , , , , , , , , , , , , , 33,34,35,36,37,38,39,40,41,42,43
	SACRIFICED BY DESIGN 08/22/03	a: , 43
66	ALOPECIA BOTH FRONT PAW	a: 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43
	ALOPECIA BOTH FRONT LEG	a: , , , , , , , , , , , , , 33,34,35,36,37,38,39,40,41,42,43
	SACRIFICED BY DESIGN 08/22/03	a: , 43
67	NO ABNORMALITIES DETECTED	
	SACRIFICED BY DESIGN 08/22/03	a: , 43
75	NO ABNORMALITIES DETECTED	
	SACRIFICED BY DESIGN 08/22/03	a: , 43
80	NO ABNORMALITIES DETECTED	
	SACRIFICED BY DESIGN 08/22/03	a: , 43

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21
2	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , , , , , 21
4	SORE TOE BOTH FRONT PAW SACRIFICED BY DESIGN 07/28/03	a: 4, 5, , , , , , , , , , , , , , , , , , , 21
22	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , , , , , 21
24	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , , , , , 21
25	SACRIFICED BY DESIGN 07/17/03	a: , , , , , , 10, , , , , , , , , , , , ,
30	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , , 15, , , , , ,
40	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , , , , , 21
41	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , , , 15, , , , , ,
44	SACRIFICED BY DESIGN 07/17/03	a: , , , , , , 10, , , , , , , , , , , , ,
45	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , , , 15, , , , , ,
50	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , , , 15, , , , , ,
51	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , , , 15, , , , , ,
54	SACRIFICED BY DESIGN 07/17/03	a: , , , , , , 10, , , , , , , , , , , , ,

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21
57	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , ,
58	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , ,
63	NO ABNORMALITIES DETECTED	
69	NO ABNORMALITIES DETECTED	
72	NO ABNORMALITIES DETECTED	
74	ALOPECIA BOTH FRONT PAW	a: , , , , , , , , , , , , , 18,19,20,21
76	NO ABNORMALITIES DETECTED	

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED:
=====		
63	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN 08/22/03	a: , 43
69	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN 08/22/03	a: , 43
72	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN 08/22/03	a: , 43
74	ALOPECIA BOTH FRONT PAW SACRIFICED BY DESIGN 08/22/03	a: 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43 a: , 43
76	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN 08/22/03	a: , 43

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21
5	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , 15, , , , , ,
8	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , ,
13	SORE TOE LEFT FRONT PAW	a: 4, , , , , , , , , , , , , , ,
	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , 15, , , , , ,
14	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21
16	SORE TOE BOTH REAR PAW	a: 4,5,6, , , , , , , , , , , , , ,
	SORE TOE BOTH FRONT PAW	a: 4,5,6, , , , , , , , , , , , , ,
	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , ,
18	ALOPECIA BOTH FRONT PAW	a: 4,5,6,7,8,9,10,11,12,13,14,15, , , , , ,
	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , 15, , , , , ,
19	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , ,
21	ALOPECIA LEFT FRONT PAW	a: , , , , , 10,11,12,13,14,15,16,17,18,19,20,21
	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21
28	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21
33	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , ,
35	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21
39	SACRIFICED BY DESIGN 07/17/03	a: , , , , , 10, , , , , , , , , , ,
43	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , 15, , , , , ,
46	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , 15, , , , , ,
60	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , 21
64	NO ABNORMALITIES DETECTED	
65	NO ABNORMALITIES DETECTED	
68	NO ABNORMALITIES DETECTED	
77	NO ABNORMALITIES DETECTED	
79	NO ABNORMALITIES DETECTED	

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43
64	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN 08/22/03	a: , 43
65	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN 08/22/03	a: , 43
68	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN 08/22/03	a: , 43
77	ALOPECIA NECK ALOPECIA BOTH FRONT PAW ALOPECIA BOTH FRONT LEG SACRIFICED BY DESIGN 08/22/03	a: , , , , , , , , , , 32,33, , , , , , , , , , , a: , , , , , , , , , , , , , , , 35,36,37,38,39,40,41,42,43 a: , 43 a: , 43
79	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN 08/22/03	a: , 43

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21
1	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , , , , , 21
3	SACRIFICED BY DESIGN 07/17/03	a: , , , , , , 10, , , , , , , , , , , , ,
6	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , 15, , , , , , ,
10	SACRIFICED BY DESIGN 07/17/03	a: , , , , , , 10, , , , , , , , , , , , ,
11	SACRIFICED BY DESIGN 07/17/03	a: , , , , , , 10, , , , , , , , , , , , ,
26	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , 15, , , , , , ,
27	SORE TOE BOTH FRONT PAW SACRIFICED BY DESIGN 07/28/03	a: 4,5,6, , , , , , , , , , , , , , , , , , a: , , , , , , , , , , , , , , , , , , , 21
29	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , 15, , , , , , ,
37	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , , , , , 21
42	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , 15, , , , , , ,
48	SACRIFICED BY DESIGN 07/22/03	a: , , , , , , , , , , , , 15, , , , , , ,
49	SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , , , , , , , , , , , , 21
52	ALOPECIA BOTH FRONT LEG	a: , , , , , 9,10,11,12,13,14,15,16,17,18,19,20,21

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21
52	ALOPECIA BOTH FRONT PAW SACRIFICED BY DESIGN 07/28/03	a: , , , , , , , , ,13,14,15,16,17,18,19,20,21 a: , , , , , , , , , , , , , , , , , ,21
55	SACRIFICED BY DESIGN 07/17/03	a: , , , , , ,10, , , , , , , , , , ,
56	SACRIFICED BY DESIGN 07/17/03	a: , , , , , ,10, , , , , , , , , , ,
61	ALOPECIA BOTH FRONT PAW ALOPECIA BOTH FRONT LEG	a: , , , , , , , , , , , , ,16,17,18,19,20,21 a: , , , , , , , , , , , , , , ,19,20,21
70	NO ABNORMALITIES DETECTED	
71	NO ABNORMALITIES DETECTED	
73	NO ABNORMALITIES DETECTED	
78	NO ABNORMALITIES DETECTED	

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY DATA DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER:	OBSERVATION	DAYS ON WHICH SIGN WAS OBSERVED: 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43
61	ALOPECIA BOTH FRONT PAW	a: 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43
	ALOPECIA BOTH FRONT LEG	a: 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43
	SACRIFICED BY DESIGN 08/22/03	a: ,43
70	NO ABNORMALITIES DETECTED	
	SACRIFICED BY DESIGN 08/22/03	a: ,43
71	NO ABNORMALITIES DETECTED	
	SACRIFICED BY DESIGN 08/22/03	a: ,43
73	NO ABNORMALITIES DETECTED	
	SACRIFICED BY DESIGN 08/22/03	a: ,43
78	NO ABNORMALITIES DETECTED	
	SACRIFICED BY DESIGN 08/22/03	a: ,43

APPENDIX C

INDIVIDUAL BODY WEIGHTS DURING GESTATION AND LACTATION

INDIVIDUAL BODY WEIGHTS DURING GESTATION AND LACTATION

EXPLANATORY NOTES

Note

Day 21 = Gestation Day 21.

Day 22 = Lactation Day 0.

Day 43 = Lactation Day 21.

Abbreviation

- = No data

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
7	249.1	263.8	271.7	272.4	281.3	285.0	297.1
9	274.7	284.8	285.3	290.9	291.5	300.0	301.0
12	255.5	266.1	264.6	259.8	271.2	276.0	281.0
15	247.8	263.8	265.1	268.8	278.4	287.3	283.5
17	257.9	266.0	265.1	273.3	273.9	281.9	287.9
20	266.5	274.6	281.7	288.2	291.3	299.6	304.4
23	233.0	250.8	259.9	263.5	262.8	268.8	271.0
31	256.9	263.9	268.7	275.8	278.3	284.4	289.4
32	266.1	272.6	275.8	281.7	284.2	295.3	304.0
34	262.8	269.3	273.8	276.1	282.0	288.8	293.5
36	246.2	250.4	256.2	260.9	271.3	275.7	284.3
38	250.6	257.2	260.0	267.1	271.7	281.2	283.7
47	231.7	248.2	238.0	242.1	263.2	278.5	285.9
53	260.4	267.5	272.1	279.7	283.8	290.1	294.5
59	257.5	261.8	264.8	266.3	271.4	274.9	288.6
62	266.5	271.0	276.2	274.7	282.2	285.5	288.5
66	245.4	243.8	268.7	276.0	279.7	285.6	291.6
67	270.5	277.2	286.2	283.2	295.4	299.0	301.8
75	249.1	258.4	267.1	267.0	277.4	282.5	285.9
80	246.3	251.4	254.4	256.2	265.8	266.8	267.6
GROUP MEAN	254.7	263.1	267.8	271.2	277.8	284.3	289.3
S.D.	11.55	10.60	11.25	11.52	9.09	9.50	9.84
S.E.	2.58	2.37	2.52	2.58	2.03	2.12	2.20
N	20	20	20	20	20	20	20

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	DAY 16	DAY 17
7	-	-	-	-	-	-	-
9	311.2	316.1	324.8	331.9	338.0	357.3	369.9
12	292.1	294.0	293.8	306.4	311.4	318.1	335.9
15	295.1	306.0	307.3	320.9	337.3	-	-
17	294.7	297.4	303.5	310.7	320.0	-	-
20	317.1	323.3	328.7	341.0	348.1	-	-
23	280.1	281.7	285.4	293.5	299.9	-	-
31	302.0	303.6	307.0	313.6	319.8	-	-
32	-	-	-	-	-	-	-
34	306.9	308.3	317.3	323.2	327.9	342.1	359.1
36	-	-	-	-	-	-	-
38	292.8	295.5	300.6	308.6	313.5	326.4	335.8
47	-	-	-	-	-	-	-
53	306.2	310.8	313.1	322.1	320.2	336.8	346.8
59	-	-	-	-	-	-	-
62	296.7	299.5	306.6	315.1	321.9	327.0	342.8
66	300.4	297.5	308.0	313.7	319.1	328.6	348.6
67	312.1	312.8	322.5	329.7	338.8	352.1	373.9
75	293.3	293.3	302.5	313.9	314.4	324.2	343.0
80	277.6	278.8	287.4	296.2	301.4	308.9	320.8
GROUP MEAN	298.6	301.2	307.2	316.0	322.1	332.1	347.7
S.D.	11.16	12.18	12.73	12.73	13.79	15.00	16.20
S.E.	2.88	3.14	3.29	3.29	3.56	4.74	5.12
N	15	15	15	15	15	10	10

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 18	DAY 19	DAY 20	DAY 21	DAY 22	DAY 23	DAY 24
7	-	-	-	-	-	-	-
9	390.1	406.1	426.6	450.8	-	-	-
12	345.7	355.7	381.8	407.1	-	-	-
15	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-
34	374.8	382.9	404.1	433.2	-	-	-
36	-	-	-	-	-	-	-
38	349.7	358.7	375.9	399.1	-	-	-
47	-	-	-	-	-	-	-
53	351.5	359.0	373.5	396.9	-	-	-
59	-	-	-	-	-	-	-
62	356.3	370.6	391.2	400.8	284.6	297.0	293.1
66	359.7	382.6	397.3	410.3	426.5	305.5	322.2
67	396.2	416.5	434.1	414.6	293.0	317.9	328.7
75	367.7	381.7	399.8	415.6	295.1	300.0	316.9
80	335.0	355.0	373.7	380.5	277.2	278.5	291.5
GROUP MEAN	362.7	376.9	395.8	410.9	315.3	299.8	310.5
S.D.	19.56	21.43	21.35	19.72	62.58	14.33	17.12
S.E.	6.19	6.78	6.75	6.24	27.99	6.41	7.66
N	10	10	10	10	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 25	DAY 26	DAY 27	DAY 28	DAY 29	DAY 30	DAY 31
7	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-
38	-	-	-	-	-	-	-
47	-	-	-	-	-	-	-
53	-	-	-	-	-	-	-
59	-	-	-	-	-	-	-
62	307.4	307.4	311.5	308.6	322.4	320.6	335.0
66	331.1	320.0	333.2	328.6	338.3	338.9	351.2
67	331.9	343.2	356.6	349.9	347.8	351.6	358.6
75	311.1	315.7	316.6	323.7	321.1	326.9	341.8
80	297.1	298.7	310.8	303.9	320.5	318.4	329.4
GROUP MEAN	315.7	317.0	325.7	322.9	330.0	331.3	343.2
S.D.	15.29	16.76	19.48	18.22	12.38	13.88	11.84
S.E.	6.84	7.50	8.71	8.15	5.54	6.21	5.30
N	5	5	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 32	DAY 33	DAY 34	DAY 35	DAY 36	DAY 37	DAY 38
7	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-
38	-	-	-	-	-	-	-
47	-	-	-	-	-	-	-
53	-	-	-	-	-	-	-
59	-	-	-	-	-	-	-
62	334.7	331.8	329.9	321.8	322.1	326.8	324.7
66	347.2	356.0	353.7	361.1	357.0	354.4	361.5
67	366.1	367.2	369.2	362.0	356.7	369.7	365.5
75	341.1	343.7	333.5	336.3	334.7	343.5	332.2
80	329.6	337.3	346.2	333.5	343.0	348.3	351.0
GROUP MEAN	343.7	347.2	346.5	342.9	342.7	348.5	347.0
S.D.	14.15	14.36	15.90	17.84	14.91	15.65	17.92
S.E.	6.33	6.42	7.11	7.98	6.67	7.00	8.02
N	5	5	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST				
	DAY 39	DAY 40	DAY 41	DAY 42	DAY 43
7	-	-	-	-	-
9	-	-	-	-	-
12	-	-	-	-	-
15	-	-	-	-	-
17	-	-	-	-	-
20	-	-	-	-	-
23	-	-	-	-	-
31	-	-	-	-	-
32	-	-	-	-	-
34	-	-	-	-	-
36	-	-	-	-	-
38	-	-	-	-	-
47	-	-	-	-	-
53	-	-	-	-	-
59	-	-	-	-	-
62	321.0	322.4	320.3	316.2	306.9
66	355.1	355.6	352.8	345.6	341.7
67	349.7	350.0	343.3	338.5	336.2
75	315.5	317.0	318.2	320.5	312.0
80	338.7	332.3	336.3	334.1	318.4
GROUP MEAN	336.0	335.5	334.2	331.0	323.0
S.D.	17.36	16.87	14.85	12.33	15.21
S.E.	7.76	7.54	6.64	5.52	6.80
N	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
2	266.8	271.1	276.8	283.0	289.4	294.7	296.5
4	248.3	244.2	257.0	263.4	265.5	272.9	271.8
22	243.1	247.6	256.0	263.9	247.3	269.6	254.4
24	233.6	244.8	253.6	261.0	269.6	279.6	284.6
25	266.0	271.8	276.0	284.5	290.0	294.8	315.1
30	252.1	254.8	260.0	264.8	266.9	274.8	276.8
40	245.8	244.2	250.9	255.2	261.7	268.7	270.9
41	286.4	291.5	296.6	306.8	305.8	321.6	325.6
44	259.7	261.7	272.9	276.5	276.4	288.3	294.9
45	253.2	266.5	271.8	275.0	278.2	287.4	291.4
50	247.8	261.7	267.7	274.0	276.3	285.4	288.1
51	269.3	278.3	281.1	293.7	297.4	313.2	313.4
54	263.9	267.7	271.5	274.8	278.0	285.8	288.0
57	263.0	269.6	278.6	280.1	283.1	289.6	297.1
58	262.5	274.7	281.4	286.5	290.3	301.0	308.2
63	248.5	255.6	262.5	266.5	278.7	279.8	287.7
69	268.5	275.7	277.7	279.0	288.2	287.3	293.4
72	277.2	279.5	288.5	290.2	295.3	296.6	306.0
74	255.7	258.3	266.0	266.0	271.7	271.1	279.6
76	234.7	231.7	244.4	253.5	260.8	265.3	255.7
GROUP MEAN	257.3	262.5	269.6	274.9	278.5	286.4	290.0
S.D.	13.55	14.93	13.30	13.55	14.32	14.71	18.68
S.E.	3.03	3.34	2.97	3.03	3.20	3.29	4.18
N	20	20	20	20	20	20	20

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	DAY 16	DAY 17
2	308.2	307.7	313.6	324.1	327.2	337.6	356.3
4	280.5	285.6	289.8	297.3	297.6	311.8	327.4
22	284.7	290.2	288.9	301.5	305.9	304.9	327.6
24	297.3	300.9	303.8	316.3	314.9	332.0	342.6
25	-	-	-	-	-	-	-
30	290.1	298.4	299.6	313.4	326.0	-	-
40	285.3	284.9	290.1	296.2	299.3	312.7	322.0
41	336.4	339.7	340.6	348.8	365.5	-	-
44	-	-	-	-	-	-	-
45	300.5	307.7	310.2	318.4	324.3	-	-
50	299.9	299.5	309.8	315.7	321.7	-	-
51	324.2	333.9	339.1	350.2	357.4	-	-
54	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-
58	-	-	-	-	-	-	-
63	295.7	301.9	312.3	321.9	308.4	330.8	347.8
69	299.2	299.6	314.9	312.7	317.9	320.4	332.2
72	317.3	313.6	331.0	329.5	331.1	338.5	361.3
74	286.7	290.1	300.5	305.0	307.6	319.1	334.3
76	252.3	252.6	272.1	280.8	293.4	306.6	318.2
GROUP MEAN	297.2	300.4	307.8	315.5	319.9	321.4	337.0
S.D.	19.94	20.55	19.11	18.63	20.45	12.57	14.53
S.E.	5.15	5.30	4.93	4.81	5.28	3.97	4.59
N	15	15	15	15	15	10	10

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 18	DAY 19	DAY 20	DAY 21	DAY 22	DAY 23	DAY 24
2	372.6	387.4	405.8	423.0	-	-	-
4	337.1	353.0	374.7	389.5	-	-	-
22	329.6	344.4	361.1	385.8	-	-	-
24	353.9	369.0	385.8	405.7	-	-	-
25	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-
40	333.3	351.1	369.8	385.3	-	-	-
41	-	-	-	-	-	-	-
44	-	-	-	-	-	-	-
45	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-
51	-	-	-	-	-	-	-
54	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-
58	-	-	-	-	-	-	-
63	373.0	385.3	415.1	429.9	295.4	306.5	325.5
69	336.9	349.5	355.2	357.2	355.3	301.3	305.6
72	369.5	386.5	409.2	418.8	429.3	313.2	322.2
74	349.1	368.5	391.4	398.7	289.5	297.5	306.3
76	329.0	343.9	357.7	372.3	275.2	277.9	285.2
GROUP MEAN	348.4	363.9	382.6	396.6	328.9	299.3	309.0
S.D.	17.90	17.76	22.23	23.15	63.91	13.33	16.06
S.E.	5.66	5.62	7.03	7.32	28.58	5.96	7.18
N	10	10	10	10	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 25	DAY 26	DAY 27	DAY 28	DAY 29	DAY 30	DAY 31
2	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-
41	-	-	-	-	-	-	-
44	-	-	-	-	-	-	-
45	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-
51	-	-	-	-	-	-	-
54	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-
58	-	-	-	-	-	-	-
63	323.8	322.0	335.4	335.0	356.1	341.9	346.5
69	307.8	300.0	304.7	297.0	305.8	297.5	304.8
72	320.5	328.8	338.4	342.6	351.0	344.0	351.7
74	309.2	306.3	317.3	311.5	312.5	318.3	322.7
76	287.0	301.3	302.3	301.7	305.9	299.0	306.4
GROUP MEAN	309.7	311.7	319.6	317.6	326.3	320.1	325.4
S.D.	14.44	12.97	16.81	20.26	25.12	22.39	21.93
S.E.	6.46	5.80	7.52	9.06	11.24	10.01	9.81
N	5	5	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 32	DAY 33	DAY 34	DAY 35	DAY 36	DAY 37	DAY 38
2	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-
41	-	-	-	-	-	-	-
44	-	-	-	-	-	-	-
45	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-
51	-	-	-	-	-	-	-
54	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-
58	-	-	-	-	-	-	-
63	364.6	371.0	369.9	359.2	359.4	356.8	359.4
69	307.5	313.4	312.9	305.3	297.1	297.8	303.8
72	350.8	356.3	357.2	346.8	358.5	366.8	373.4
74	336.0	337.1	334.3	342.0	333.1	344.0	343.3
76	319.4	320.7	313.5	317.3	321.6	323.2	321.4
GROUP MEAN	335.7	339.7	337.6	334.1	333.9	337.7	340.3
S.D.	23.04	24.06	25.64	22.17	26.28	27.63	28.10
S.E.	10.31	10.76	11.47	9.91	11.75	12.36	12.57
N	5	5	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST				
	DAY 39	DAY 40	DAY 41	DAY 42	DAY 43
2	-	-	-	-	-
4	-	-	-	-	-
22	-	-	-	-	-
24	-	-	-	-	-
25	-	-	-	-	-
30	-	-	-	-	-
40	-	-	-	-	-
41	-	-	-	-	-
44	-	-	-	-	-
45	-	-	-	-	-
50	-	-	-	-	-
51	-	-	-	-	-
54	-	-	-	-	-
57	-	-	-	-	-
58	-	-	-	-	-
63	348.2	352.3	360.4	351.1	345.3
69	291.3	292.2	299.1	297.4	300.2
72	357.9	357.3	365.0	351.1	342.9
74	324.7	324.9	331.7	323.7	315.3
76	312.7	312.5	311.9	309.7	305.8
GROUP MEAN	327.0	327.8	333.6	326.6	321.9
S.D.	26.87	27.30	29.02	24.22	20.99
S.E.	12.02	12.21	12.98	10.83	9.39
N	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
5	260.1	262.6	265.3	268.1	269.4	277.1	284.1
8	249.3	261.7	265.3	271.8	280.5	288.0	295.7
13	259.5	266.7	273.7	277.6	284.7	291.9	297.0
14	264.8	265.1	272.9	278.3	278.5	282.8	290.4
16	247.3	258.5	259.7	270.9	280.4	283.3	294.9
18	269.0	283.5	288.0	295.8	298.3	309.4	317.6
19	263.9	273.0	277.3	280.6	284.3	295.1	300.9
21	236.1	243.0	247.7	251.2	253.7	264.9	263.7
28	217.6	246.0	225.3	238.6	254.7	270.1	274.9
33	248.3	255.3	257.6	262.9	264.2	270.9	270.9
35	261.4	266.7	273.8	278.7	283.1	292.1	294.7
39	240.8	247.8	249.9	253.7	255.0	262.0	268.9
43	250.4	263.5	268.4	276.0	279.9	289.5	289.8
46	261.0	267.4	273.1	275.0	283.5	289.7	293.8
60	260.1	279.8	285.0	290.7	300.8	309.3	313.4
64	256.0	258.9	268.7	269.2	279.6	279.1	284.6
65	222.4	247.7	251.5	253.0	258.6	266.5	269.4
68	250.0	256.3	267.6	266.7	274.2	273.9	274.6
77	271.3	277.4	287.0	266.8	296.8	301.8	301.2
79	260.0	266.3	274.0	275.3	285.4	290.3	297.2
GROUP MEAN	252.5	262.4	266.6	270.0	277.3	284.4	288.9
S.D.	14.30	11.15	15.00	13.52	14.15	13.89	14.83
S.E.	3.20	2.49	3.35	3.02	3.17	3.11	3.32
N	20	20	20	20	20	20	20

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	DAY 16	DAY 17
5	293.1	299.9	300.0	304.5	315.6	-	-
8	-	-	-	-	-	-	-
13	302.0	308.2	313.9	325.4	334.2	-	-
14	296.3	297.7	303.5	308.6	319.2	328.9	334.3
16	-	-	-	-	-	-	-
18	328.2	328.8	338.4	341.2	360.3	-	-
19	-	-	-	-	-	-	-
21	274.9	277.6	287.0	292.3	292.7	308.0	319.3
28	282.2	287.2	290.6	297.9	302.6	316.9	329.1
33	-	-	-	-	-	-	-
35	307.1	311.3	314.1	328.5	335.0	352.1	365.6
39	-	-	-	-	-	-	-
43	304.5	309.0	313.8	322.9	335.3	-	-
46	306.1	306.5	313.6	326.6	328.4	-	-
60	315.1	330.3	331.3	340.9	348.0	366.0	376.9
64	291.3	297.8	309.1	309.0	318.7	321.9	342.3
65	276.5	278.5	290.2	293.2	300.3	307.7	328.8
68	281.6	284.7	291.1	299.0	305.0	310.0	323.3
77	311.4	315.9	321.9	326.7	339.3	350.5	366.8
79	302.9	318.3	325.5	332.8	341.5	351.4	369.5
GROUP MEAN	298.2	303.4	309.6	316.6	325.1	331.3	345.6
S.D.	15.12	16.58	15.80	16.87	19.40	21.77	21.81
S.E.	3.90	4.28	4.08	4.36	5.01	6.88	6.90
N	15	15	15	15	15	10	10

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 18	DAY 19	DAY 20	DAY 21	DAY 22	DAY 23	DAY 24
5	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-
14	349.7	359.7	380.1	401.9	-	-	-
16	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
21	332.2	339.4	360.7	378.0	-	-	-
28	339.9	352.8	373.4	388.9	-	-	-
33	-	-	-	-	-	-	-
35	382.3	406.2	426.1	457.2	-	-	-
39	-	-	-	-	-	-	-
43	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-
60	390.9	404.4	424.6	434.6	-	-	-
64	357.7	374.4	389.4	402.7	419.2	306.6	312.2
65	345.7	357.2	379.6	394.1	302.5	280.5	285.3
68	339.6	357.1	372.3	383.7	276.4	285.4	293.2
77	381.0	395.9	408.8	424.3	316.0	322.5	319.0
79	384.2	410.0	427.4	431.5	326.8	331.6	341.2
GROUP MEAN	360.3	375.7	394.2	409.7	328.2	305.3	310.2
S.D.	22.08	26.09	25.22	25.89	54.26	22.36	22.09
S.E.	6.98	8.25	7.98	8.19	24.26	10.00	9.88
N	10	10	10	10	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 25	DAY 26	DAY 27	DAY 28	DAY 29	DAY 30	DAY 31
5	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-
39	-	-	-	-	-	-	-
43	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-
64	322.3	321.1	331.7	340.7	331.6	344.5	346.9
65	286.1	288.0	297.3	293.0	299.3	300.2	298.3
68	294.2	297.5	301.7	304.4	304.6	302.9	315.5
77	317.8	332.4	334.3	329.5	331.8	342.5	346.6
79	343.3	349.3	359.7	355.1	350.2	365.6	365.7
GROUP MEAN	312.7	317.7	324.9	324.5	323.5	331.1	334.6
S.D.	22.94	25.08	25.72	25.59	21.16	28.50	27.13
S.E.	10.26	11.22	11.50	11.45	9.46	12.75	12.13
N	5	5	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 32	DAY 33	DAY 34	DAY 35	DAY 36	DAY 37	DAY 38
5	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-
39	-	-	-	-	-	-	-
43	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-
64	354.7	365.8	359.4	362.9	352.4	364.3	366.5
65	258.0	298.2	300.9	311.2	305.6	317.0	318.7
68	321.9	325.8	323.0	310.7	315.5	323.6	322.8
77	356.5	360.7	363.0	349.0	359.7	368.0	364.4
79	375.6	378.5	378.3	369.0	374.8	381.9	376.8
GROUP MEAN	333.3	345.8	344.9	340.6	341.6	351.0	349.8
S.D.	46.33	33.00	31.89	27.99	29.68	28.84	27.01
S.E.	20.72	14.76	14.26	12.52	13.27	12.90	12.08
N	5	5	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST				
	DAY 39	DAY 40	DAY 41	DAY 42	DAY 43
5	-	-	-	-	-
8	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-
16	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
21	-	-	-	-	-
28	-	-	-	-	-
33	-	-	-	-	-
35	-	-	-	-	-
39	-	-	-	-	-
43	-	-	-	-	-
46	-	-	-	-	-
60	-	-	-	-	-
64	348.7	340.5	357.9	348.6	358.4
65	321.4	317.8	316.4	323.1	310.0
68	312.5	306.2	308.3	299.5	295.6
77	354.4	356.4	355.2	340.9	330.2
79	359.7	364.7	372.7	359.2	350.0
GROUP MEAN	339.3	337.1	342.1	334.3	328.8
S.D.	21.04	24.87	28.11	23.48	26.38
S.E.	9.41	11.12	12.57	10.50	11.80
N	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
1	263.4	265.6	267.4	272.7	278.5	282.8	288.6
3	271.9	279.6	281.1	291.6	285.4	299.0	298.1
6	256.9	262.6	268.5	268.9	271.5	279.3	275.9
10	259.2	260.0	265.5	273.3	273.7	278.2	279.9
11	269.7	273.6	274.2	274.9	278.9	283.7	280.3
26	234.1	243.8	243.6	250.5	248.0	255.8	259.5
27	248.3	231.9	217.3	245.7	246.2	262.0	263.5
29	248.3	251.4	259.0	264.5	265.7	277.5	278.9
37	253.8	255.3	263.0	269.5	269.4	279.7	283.9
42	253.8	243.7	245.2	247.0	253.3	262.6	266.8
48	268.2	274.3	275.2	281.3	287.7	295.3	296.4
49	272.8	277.0	276.6	283.6	286.4	289.7	295.2
52	257.1	260.9	257.6	266.6	269.8	275.4	286.2
55	254.4	260.8	266.9	272.4	275.4	286.5	290.9
56	246.5	253.0	255.7	263.1	257.5	269.0	277.4
61	241.4	254.4	268.5	257.9	289.5	287.0	286.4
70	256.5	256.9	262.2	263.6	274.2	276.0	280.4
71	250.6	250.1	259.9	259.6	265.7	266.7	271.9
73	261.3	262.2	266.4	267.8	274.7	276.5	285.8
78	253.9	251.4	264.3	266.9	272.9	267.6	274.5
GROUP MEAN	256.1	258.4	261.9	267.1	271.2	277.5	281.0
S.D.	10.04	11.99	14.08	11.60	12.43	11.24	10.55
S.E.	2.24	2.68	3.15	2.59	2.78	2.51	2.36
N	20	20	20	20	20	20	20

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	DAY 16	DAY 17
1	297.8	300.8	305.0	317.4	319.2	334.3	346.3
3	-	-	-	-	-	-	-
6	285.0	284.1	291.0	299.5	310.5	-	-
10	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-
26	273.6	276.7	277.1	282.6	293.7	-	-
27	277.0	281.3	286.8	297.8	299.6	315.5	326.5
29	293.9	297.2	301.3	309.0	316.2	-	-
37	283.9	292.3	296.0	306.9	309.0	327.2	337.5
42	277.9	277.1	282.6	291.5	296.8	-	-
48	307.5	314.3	316.4	330.1	336.7	-	-
49	306.2	305.4	309.6	317.8	319.6	328.8	335.7
52	291.5	300.2	300.6	316.3	324.7	337.5	349.9
55	-	-	-	-	-	-	-
56	-	-	-	-	-	-	-
61	301.0	297.0	324.9	324.0	297.8	296.7	336.2
70	281.2	285.2	293.3	298.8	296.3	300.2	307.4
71	277.5	282.5	291.1	298.2	294.3	304.8	309.6
73	283.1	293.0	292.1	304.2	308.0	317.1	325.8
78	283.8	284.2	294.0	303.1	301.5	313.9	327.0
GROUP MEAN	288.1	291.4	297.5	306.5	308.3	317.6	330.2
S.D.	10.95	11.02	12.63	12.76	12.84	14.20	13.96
S.E.	2.83	2.85	3.26	3.29	3.31	4.49	4.42
N	15	15	15	15	15	10	10

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 18	DAY 19	DAY 20	DAY 21	DAY 22	DAY 23	DAY 24
1	360.9	373.8	395.0	414.2	-	-	-
3	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-
27	347.1	363.6	379.3	413.6	-	-	-
29	-	-	-	-	-	-	-
37	360.8	368.4	391.8	421.5	-	-	-
42	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-
49	348.3	364.5	375.8	393.0	-	-	-
52	367.9	383.3	410.9	430.2	-	-	-
55	-	-	-	-	-	-	-
56	-	-	-	-	-	-	-
61	357.4	383.3	399.7	417.3	291.9	305.1	314.1
70	314.4	326.4	329.1	335.7	339.1	296.8	297.1
71	321.3	335.9	347.1	349.0	307.4	290.8	299.7
73	340.5	359.5	370.0	379.5	284.1	288.8	291.0
78	341.8	363.2	376.3	386.1	282.9	290.6	288.7
GROUP MEAN	346.0	362.2	377.5	394.0	301.1	294.4	298.1
S.D.	17.36	18.42	24.61	31.80	23.39	6.69	9.98
S.E.	5.49	5.82	7.78	10.06	10.46	2.99	4.46
N	10	10	10	10	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 25	DAY 26	DAY 27	DAY 28	DAY 29	DAY 30	DAY 31
1	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-
37	-	-	-	-	-	-	-
42	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-
52	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-
56	-	-	-	-	-	-	-
61	311.1	319.2	332.2	331.9	330.7	323.2	342.6
70	300.6	293.4	305.7	305.3	309.1	296.4	298.9
71	300.5	297.6	301.4	304.7	306.9	298.7	303.5
73	301.3	305.3	312.0	319.1	323.3	327.8	328.0
78	286.8	303.6	300.9	303.9	299.9	305.8	302.2
GROUP MEAN	300.1	303.8	310.4	313.0	314.0	310.4	315.0
S.D.	8.66	9.82	12.95	12.30	12.64	14.32	19.27
S.E.	3.87	4.39	5.79	5.50	5.65	6.41	8.62
N	5	5	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	DAY 32	DAY 33	DAY 34	DAY 35	DAY 36	DAY 37	DAY 38
1	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-
37	-	-	-	-	-	-	-
42	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-
52	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-
56	-	-	-	-	-	-	-
61	356.1	363.4	353.9	343.5	366.1	358.8	371.2
70	306.0	313.8	311.5	316.2	323.9	312.6	301.3
71	305.2	311.4	307.8	321.3	320.0	309.2	305.0
73	334.3	331.8	331.1	337.1	346.4	341.9	337.4
78	311.7	317.3	311.0	313.6	313.2	321.3	325.4
GROUP MEAN	322.7	327.5	323.1	326.3	333.9	328.8	328.1
S.D.	22.11	21.55	19.54	13.24	21.88	21.06	28.30
S.E.	9.89	9.64	8.74	5.92	9.78	9.42	12.66
N	5	5	5	5	5	5	5

INDIVIDUAL BODY WEIGHTS (grams) DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST				
	DAY 39	DAY 40	DAY 41	DAY 42	DAY 43
1	-	-	-	-	-
3	-	-	-	-	-
6	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
26	-	-	-	-	-
27	-	-	-	-	-
29	-	-	-	-	-
37	-	-	-	-	-
42	-	-	-	-	-
48	-	-	-	-	-
49	-	-	-	-	-
52	-	-	-	-	-
55	-	-	-	-	-
56	-	-	-	-	-
61	358.4	355.3	355.2	342.5	345.0
70	296.5	299.4	302.6	298.9	301.1
71	307.2	307.8	304.8	307.2	298.8
73	332.8	333.6	330.7	321.3	312.4
78	314.1	315.1	309.4	304.6	303.9
GROUP MEAN	321.8	322.2	320.5	314.9	312.2
S.D.	24.36	22.38	22.35	17.49	19.02
S.E.	10.89	10.01	10.00	7.82	8.51
N	5	5	5	5	5

APPENDIX D

INDIVIDUAL BODY WEIGHT CHANGES DURING GESTATION AND LACTATION

INDIVIDUAL BODY WEIGHT CHANGES DURING GESTATION AND LACTATION
EXPLANATORY NOTES

Note

Day 21 = Gestation Day 21.
Day 22 = Lactation Day 0.
Day 43 = Lactation Day 21.

Abbreviation

- = No data

INDIVIDUAL BODY WEIGHT CHANGES (grams) DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	4-10	10-15	15-21	22-25	25-29	29-36	36-43
7	48.0	-	-	-	-	-	-
9	26.3	37.0	112.8	-	-	-	-
12	25.5	30.4	95.7	-	-	-	-
15	35.7	53.8	-	-	-	-	-
17	30.0	32.1	-	-	-	-	-
20	37.9	43.7	-	-	-	-	-
23	38.0	28.9	-	-	-	-	-
31	32.5	30.4	-	-	-	-	-
32	37.9	-	-	-	-	-	-
34	30.7	34.4	105.3	-	-	-	-
36	38.1	-	-	-	-	-	-
38	33.1	29.8	85.6	-	-	-	-
47	54.2	-	-	-	-	-	-
53	34.1	25.7	76.7	-	-	-	-
59	31.1	-	-	-	-	-	-
62	22.0	33.4	78.9	22.8	15.0	-0.3	-15.2
66	46.2	27.5	91.2	-95.4	7.2	18.7	-15.3
67	31.3	37.0	75.8	38.9	15.9	8.9	-20.5
75	36.8	28.5	101.2	16.0	10.0	13.6	-22.7
80	21.3	33.8	79.1	19.9	23.4	22.5	-24.6
GROUP MEAN	34.5	33.8	90.2	0.4	14.3	12.7	-19.7
S.D.	8.28	7.16	13.13	54.28	6.22	8.89	4.28
S.E.	1.85	1.85	4.15	24.27	2.78	3.98	1.91
N	20	15	10	5	5	5	5

INDIVIDUAL BODY WEIGHT CHANGES (grams) DURING GESTATION AND LACTATION

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST	
	4-21	22-43

7	-	-
9	176.1	-
12	151.6	-
15	-	-
17	-	-
20	-	-
23	-	-
31	-	-
32	-	-
34	170.4	-
36	-	-
38	148.5	-
47	-	-
53	136.5	-
59	-	-
62	134.3	22.3
66	164.9	-84.8
67	144.1	43.2
75	166.5	16.9
80	134.2	41.2

GROUP MEAN	152.7	7.8
S.D.	15.76	53.00
S.E.	4.98	23.70
N	10	5

INDIVIDUAL BODY WEIGHT CHANGES (grams) DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	4-10	10-15	15-21	22-25	25-29	29-36	36-43
2	29.7	30.7	95.8	-	-	-	-
4	23.5	25.8	91.9	-	-	-	-
22	11.3	51.5	79.9	-	-	-	-
24	51.0	30.3	90.8	-	-	-	-
25	49.1	-	-	-	-	-	-
30	24.7	49.2	-	-	-	-	-
40	25.1	28.4	86.0	-	-	-	-
41	39.2	39.9	-	-	-	-	-
44	35.2	-	-	-	-	-	-
45	38.2	32.9	-	-	-	-	-
50	40.3	33.6	-	-	-	-	-
51	44.1	44.0	-	-	-	-	-
54	24.1	-	-	-	-	-	-
57	34.1	-	-	-	-	-	-
58	45.7	-	-	-	-	-	-
63	39.2	20.7	121.5	28.4	32.3	3.3	-14.1
69	24.9	24.5	39.3	-47.5	-2.0	-8.7	3.1
72	28.8	25.1	87.7	-108.8	30.5	7.5	-15.6
74	23.9	28.0	91.1	19.7	3.3	20.6	-17.8
76	21.0	37.7	78.9	11.8	18.9	15.7	-15.8
GROUP MEAN	32.7	33.5	86.3	-19.3	16.6	7.7	12.0
S.D.	10.57	9.21	20.31	58.24	15.56	11.39	8.57
S.E.	2.36	2.38	6.42	26.05	6.96	5.09	3.83
N	20	15	10	5	5	5	5

INDIVIDUAL BODY WEIGHT CHANGES (grams) DURING GESTATION AND LACTATION

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST	
	4-21	22-43
2	156.2	-
4	141.2	-
22	142.7	-
24	172.1	-
25	-	-
30	-	-
40	139.5	-
41	-	-
44	-	-
45	-	-
50	-	-
51	-	-
54	-	-
57	-	-
58	-	-
63	181.4	49.9
69	88.7	-55.1
72	141.6	-86.4
74	143.0	25.8
76	137.6	30.6
GROUP MEAN	144.4	-7.0
S.D.	24.66	59.89
S.E.	7.80	26.78
N	10	5

INDIVIDUAL BODY WEIGHT CHANGES (grams) DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	4-10	10-15	15-21	22-25	25-29	29-36	36-43
5	24.0	31.5	-	-	-	-	-
8	46.4	-	-	-	-	-	-
13	37.5	37.2	-	-	-	-	-
14	25.6	28.8	82.7	-	-	-	-
16	47.6	-	-	-	-	-	-
18	48.6	42.7	-	-	-	-	-
19	37.0	-	-	-	-	-	-
21	27.6	29.0	85.3	-	-	-	-
28	57.3	27.7	86.3	-	-	-	-
33	22.6	-	-	-	-	-	-
35	33.3	40.3	122.2	-	-	-	-
39	28.1	-	-	-	-	-	-
43	39.4	45.5	-	-	-	-	-
46	32.8	34.6	-	-	-	-	-
60	53.3	34.6	86.6	-	-	-	-
64	28.6	34.1	84.0	-96.9	9.3	20.8	6.0
65	47.0	30.9	93.8	-16.4	13.2	6.3	4.4
68	24.6	30.4	78.7	17.8	10.4	10.9	-19.9
77	29.9	38.1	85.0	1.8	14.0	27.9	-29.5
79	37.2	44.3	90.0	16.5	6.9	24.6	-24.8
GROUP MEAN	36.4	35.3	89.5	-15.4	10.8	18.1	12.8
S.D.	10.49	5.83	12.19	47.59	2.90	9.17	16.75
S.E.	2.35	1.51	3.86	21.28	1.30	4.10	7.49
N	20	15	10	5	5	5	5

INDIVIDUAL BODY WEIGHT CHANGES (grams) DURING GESTATION AND LACTATION

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST	
	4-21	22-43
5	-	-
8	-	-
13	-	-
14	137.1	-
16	-	-
18	-	-
19	-	-
21	141.9	-
28	171.3	-
33	-	-
35	195.8	-
39	-	-
43	-	-
46	-	-
60	174.5	-
64	146.7	-60.8
65	171.7	7.5
68	133.7	19.2
77	153.0	14.2
79	171.5	23.2
GROUP MEAN	159.7	0.7
S.D.	20.16	34.85
S.E.	6.37	15.59
N	10	5

INDIVIDUAL BODY WEIGHT CHANGES (grams) DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST						
	4-10	10-15	15-21	22-25	25-29	29-36	36-43
1	25.2	30.6	95.0	-	-	-	-
3	26.2	-	-	-	-	-	-
6	19.0	34.6	-	-	-	-	-
10	20.7	-	-	-	-	-	-
11	10.6	-	-	-	-	-	-
26	25.4	34.2	-	-	-	-	-
27	15.2	36.1	114.0	-	-	-	-
29	30.6	37.3	-	-	-	-	-
37	30.1	25.1	112.5	-	-	-	-
42	13.0	30.0	-	-	-	-	-
48	28.2	40.3	-	-	-	-	-
49	22.4	24.4	73.4	-	-	-	-
52	29.1	38.5	105.5	-	-	-	-
55	36.5	-	-	-	-	-	-
56	30.9	-	-	-	-	-	-
61	45.0	11.4	119.5	19.2	19.6	35.4	-21.1
70	23.9	15.9	39.4	-38.5	8.5	14.8	-22.8
71	21.3	22.4	54.7	-6.9	6.4	13.1	-21.2
73	24.5	22.2	71.5	17.2	22.0	23.1	-34.0
78	20.6	27.0	84.6	3.9	13.1	13.3	-9.3
GROUP MEAN	24.9	28.7	87.0	-1.0	13.9	19.9	-21.7
S.D.	7.93	8.49	27.04	23.48	6.79	9.57	8.76
S.E.	1.77	2.19	8.55	10.50	3.03	4.28	3.92
N	20	15	10	5	5	5	5

INDIVIDUAL BODY WEIGHT CHANGES (grams) DURING GESTATION AND LACTATION

GROUP IV: 30 MG/KG/DAY

ANIMAL NUMBER	DAYS ON TEST	
	4-21	22-43

1	150.8	-
3	-	-
6	-	-
10	-	-
11	-	-
26	-	-
27	165.3	-
29	-	-
37	167.7	-
42	-	-
48	-	-
49	120.2	-
52	173.1	-
55	-	-
56	-	-
61	175.9	53.1
70	79.2	-38.0
71	98.4	-8.6
73	118.2	28.3
78	132.2	21.0
GROUP MEAN	138.1	11.2
S.D.	33.71	35.20
S.E.	10.66	15.74
N	10	5

APPENDIX E
INDIVIDUAL REPRODUCTIVE DATA

Individual Reproductive Data

Explanatory Notes

Notes

Nidations = Implantations

Five dams per group were sacrificed on day 10, 15, or 21G.

Abbreviations

S.E. = Standard Error

N = Number in Group

... = No Data

INDIVIDUAL REPRODUCTIVE DATA

GROUP I: 0 MG/KG/DAY

ANIMAL NUMBER	CORPORA LUTEA	NIDA- TIONS	RESORPTIONS		DEAD FETUSES	LIVE FETUSES TOTAL
			TOTAL	EARLY LATE		
7	...	10	0	0	0	10
9	...	18	0	0	0	18
12	...	12	0	0	0	12
15	...	16	0	0	0	16
17	...	13	0	0	0	13
20	...	18	0	0	0	18
23	...	14	0	0	0	14
31	...	12	0	0	0	12
32	...	15	0	0	0	15
34	...	14	0	0	0	14
36	...	16	0	0	0	16
38	...	12	0	0	0	12
47	...	15	0	0	0	15
53	...	8	0	0	0	8
59	...	13	0	0	0	13

INDIVIDUAL REPRODUCTIVE DATA

GROUP II: 3 MG/KG/DAY

ANIMAL NUMBER	CORPORA LUTEA	NIDA- TIONS	RESORPTIONS			DEAD FETUSES	LIVE FETUSES TOTAL
			TOTAL	EARLY	LATE		
2	...	15	2	2	0	0	13
4	...	13	0	0	0	0	13
22	...	13	0	0	0	0	13
24	...	13	0	0	0	0	13
25	...	13	0	0	0	0	13
30	...	13	0	0	0	0	13
40	...	13	0	0	0	0	13
41	...	17	0	0	0	0	17
44	...	14	0	0	0	0	14
45	...	14	0	0	0	0	14
50	...	14	0	0	0	0	14
51	...	16	1	1	0	0	15
54	...	14	0	0	0	0	14
57	...	17	0	0	0	0	17
58	...	14	0	0	0	0	14

INDIVIDUAL REPRODUCTIVE DATA

GROUP III: 10 MG/KG/DAY

ANIMAL NUMBER	CORPORA LUTEA	NIDA- TIONS	RESORPTIONS			DEAD FETUSES	LIVE FETUSES TOTAL
			TOTAL	EARLY	LATE		
5	...	17	0	0	0	0	17
8	...	15	0	0	0	0	15
13	...	14	0	0	0	0	14
14	...	12	0	0	0	0	12
16	...	14	0	0	0	0	14
18	...	16	0	0	0	0	16
19	...	16	0	0	0	0	16
21	...	13	2	2	0	0	11
28	...	12	0	0	0	0	12
33	...	15	0	0	0	0	15
35	...	16	0	0	0	0	16
39	...	12	0	0	0	0	12
43	...	15	0	0	0	0	15
46	...	14	0	0	0	0	14
60	...	14	0	0	0	0	14

INDIVIDUAL REPRODUCTIVE DATA

GROUP IV: 30 MG/KG/DAY						
ANIMAL NUMBER	CORPORA LUTEA	NIDA- TIONS	TOTAL	RESORPTIONS EARLY LATE	DEAD FETUSES	LIVE FETUSES TOTAL
1	...	13	0	0	0	13
3	...	15	0	0	0	15
6	...	12	0	0	0	12
10	...	14	0	0	0	14
11	...	17	0	0	0	17
26	...	13	0	0	0	13
27	...	13	0	0	0	13
29	...	14	0	0	0	14
37	...	13	0	0	0	13
42	...	13	0	0	0	13
48	...	12	0	0	0	12
49	...	13	0	0	0	13
52	...	15	0	0	0	15
55	...	9	0	0	0	9
56	...	16	0	0	0	16

APPENDIX F

NUMBER OF PUPS DURING LACTATION

Number of Pups During Lactation

Explanatory Notes

Note

One pup per litter was sacrificed on days 3, 7, and 14 for blood collection.
Two pups per litter (litter size permitting) were sacrificed on day 21 for blood collection.

Abbreviations

S.D. = Standard Error
S.E. = Standard Error
N = Number of Litters in Group

NUMBER OF PUPS DURING LACTATION

GROUP: I DOSE: 0 MG/KG/DAY

ANIMAL #	BORN	BORN ALIVE	DAY 3	DAY 7	DAY 14	DAY 21
62	13	13	13	12	11	10
66	14	14	14	13	12	11
67	19	19	19	18	17	16
75	16	16	16	15	14	12
80	12	12	12	11	10	9
Mean	14.8	14.8	14.8	13.8	12.8	11.6
S.D.	2.77	2.77	2.77	2.77	2.77	2.70
S.E.	1.24	1.24	1.24	1.24	1.24	1.21
N	5	5	5	5	5	5

GROUP: II DOSE: 3 MG/KG/DAY

ANIMAL #	BORN	BORN ALIVE	DAY 3	DAY 7	DAY 14	DAY 21
63	15	15	15	13	12	11
69	4	4	4	3	2	1
72	14	13	13	12	11	10
74	16	16	16	14	13	11
76	13	13	13	12	11	10
Mean	12.4	12.2	12.2	10.8	9.8	8.6
S.D.	4.83	4.76	4.76	4.44	4.44	4.28
S.E.	2.16	2.13	2.13	1.98	1.98	1.91
N	5	5	5	5	5	5

GROUP: III DOSE: 10 MG/KG/DAY

ANIMAL #	BORN	BORN ALIVE	DAY 3	DAY 7	DAY 14	DAY 21
64	12	12	12	11	10	9
65	14	13	13	12	11	10
68	12	12	12	11	10	9
77	14	14	14	13	12	11
79	14	14	14	13	12	11
Mean	13.2	13.0	13.0	12.0	11.0	10.0
S.D.	1.10	1.00	1.00	1.00	1.00	1.00
S.E.	0.49	0.45	0.45	0.45	0.45	0.45
N	5	5	5	5	5	5

GROUP: IV DOSE: 30 MG/KG/DAY

ANIMAL #	BORN	BORN ALIVE	DAY 3	DAY 7	DAY 14	DAY 21
61	13	13	13	12	11	10
70	3	3	3	2	1	-a
71	6	6	6	5	4	3
73	11	11	11	10	9	8
78	13	13	13	12	11	10
Mean	9.2	9.2	9.2	8.2	7.2	7.8
S.D.	4.49	4.49	4.49	4.49	4.49	3.30
S.E.	2.01	2.01	2.01	2.01	2.01	1.48
N	5	5	5	5	5	4

a No pups.

APPENDIX G

INDIVIDUAL LITTER CLINICAL OBSERVATIONS

INDIVIDUAL LITTER CLINICAL OBSERVATIONS

GROUP: I DOSE: 0 MG/KG/DAY

ANIMAL NUMBER	LACTATION DAY	SIGN INCIDENCE	OBSERVATIONS
------------------	------------------	-------------------	--------------

62	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

66	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

67	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

75	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

80	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

GROUP: II DOSE: 3 MG/KG/DAY

ANIMAL NUMBER	LACTATION DAY	SIGN INCIDENCE	OBSERVATIONS
------------------	------------------	-------------------	--------------

63	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

69	DAY 21	1 OF 1	SMALL WHOLE BODY
----	--------	--------	------------------

LITTER SUMMARY:	1		SMALL WHOLE BODY
-----------------	---	--	------------------

72	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

74	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

76	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

GROUP: III DOSE: 10 MG/KG/DAY

ANIMAL NUMBER	LACTATION DAY	SIGN INCIDENCE	OBSERVATIONS
------------------	------------------	-------------------	--------------

64	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

65	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

68	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

77	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

79	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

GROUP: IV DOSE: 30 MG/KG/DAY

ANIMAL NUMBER	LACTATION DAY	SIGN INCIDENCE	OBSERVATIONS
------------------	------------------	-------------------	--------------

61	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

70	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

71	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

73	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

78	NO ABNORMALITIES DETECTED		
----	---------------------------	--	--

APPENDIX H

MEAN PUP WEIGHTS PER LITTER

Mean Pup Weights Per Litter

Explanatory Notes

Note

One pup per litter was sacrificed on days 3, 7, and 14 for blood collection.
Two pups per litter (litter size permitting) were sacrificed on day 21 for blood collection.

Abbreviations

S.D. = Standard Error
S.E. = Standard Error
N = Number of Litters in Group

MEAN PUP WEIGHTS (grams) PER LITTER

		GROUP: I		DOSE: 0 MG/KG/DAY		
ANIMAL #	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	
62	6.7	9.4	14.3	26.4	43.6	
66	6.8	9.3	13.7	27.1	37.9	
67	5.8	8.3	13.6	22.8	38.7	
75	6.3	8.7	13.9	24.1	41.3	
80	6.9	10.4	15.7	26.5	46.1	
Mean	6.5	9.2	14.2	25.4	41.5	
S.D.	0.45	0.80	0.87	1.84	3.40	
S.E.	0.20	0.36	0.39	0.82	1.52	
N	5	5	5	5	5	

		GROUP: II		DOSE: 3 MG/KG/DAY		
ANIMAL #	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	
63	6.9	9.7	16.0	31.3	50.6	
69	7.3	11.7	15.9	21.4	17.7	
72	6.7	9.6	14.8	30.0	45.5	
74	6.1	8.6	13.1	21.3	35.1	
76	6.7	9.7	14.0	26.5	44.5	
Mean	6.7	9.9	14.8	26.1	38.7	
S.D.	0.43	1.13	1.27	4.67	13.00	
S.E.	0.19	0.50	0.57	2.09	5.81	
N	5	5	5	5	5	

		GROUP: III		DOSE: 10 MG/KG/DAY		
ANIMAL #	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	
64	7.0	10.6	17.0	33.6	54.9	
65	6.5	9.4	14.2	27.6	41.8	
68	6.5	9.5	15.5	29.7	44.5	
77	6.9	10.1	13.8	25.4	41.2	
79	7.0	11.3	17.3	32.9	53.0	
Mean	6.8	10.2	15.6	29.8	47.1	
S.D.	0.23	0.79	1.59	3.48	6.42	
S.E.	0.10	0.35	0.71	1.56	2.87	
N	5	5	5	5	5	

		GROUP: IV		DOSE: 30 MG/KG/DAY		
ANIMAL #	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	
61	6.6	9.8	15.3	24.2	44.4	
70	7.1	11.2	15.6	15.1	-a	
71	7.6	12.3	19.9	38.8	59.4	
73	6.9	10.3	16.9	30.4	36.5	
78	6.8	9.7	14.1	27.4	43.2	
Mean	7.0	10.7	16.3	27.2	45.9	
S.D.	0.38	1.09	2.23	8.69	9.64	
S.E.	0.17	0.49	1.00	3.89	4.82	
N	5	5	5	5	4	

a No pups.

APPENDIX I
PLASMA AND TISSUE ANALYSIS

PLASMA AND TISSUE ANALYSIS

EXPLANATORY NOTES

Abbreviations

G	=	Gestation
N.S.	=	No Sample
LOQ	=	Limit of Quantitation, 0.05 µg/mL.
<LOQ	=	Less than limit of quantitation
PP	=	Postpartum
S.D.	=	Standard Deviation
*	=	Insufficient for sample reanalysis

Concentration ($\mu\text{g/mL}$) of PFOA in Maternal Plasma During Gestation and Postpartum

Day	Dose (mg/kg)	Concentration	Average	S.D.
10G	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	7.76	8.53	1.06
		10.07		
		8.54		
		7.38		
		8.93		
	10	24.05	23.32	2.15
		25.66		
		23.88		
		23.20		
		19.83		
	30	57.09	70.49	8.94
		71.53		
		68.65		
		81.78		
		73.41		
15G	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	10.61	15.92	12.96
		8.71		
		39.04		
		11.32		
		9.94		
	10	47.27	29.40	14.19
		30.22		
		9.15		
		24.08		
		36.26		
	30	81.42	79.55	3.11
		81.62		
		79.10		
		81.33		
		74.29		

Concentration ($\mu\text{g/mL}$) PFOA in Maternal Plasma During Gestation and Postpartum (cont'd)

Day	Dose (mg/kg)	Concentration	Average	S.D.
21G	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	14.15	14.04	2.27
		13.61		
		12.71		
		11.93		
		17.80		
	10	26.28	34.20	6.68
		30.52		
		38.53		
		32.51		
		43.18		
	30	78.50	76.36	14.76
		74.02		
		65.60		
		63.32		
		100.35		
3PP	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	8.65	11.01	2.11
		10.62		
		13.45		
		9.42		
		12.92		
	10	*	22.47	2.74
		19.82		
		25.30		
		22.31		
		*		
	30	53.25	54.39	17.86
		69.57		
		51.50		
		70.90		
		26.76		

Concentration ($\mu\text{g/mL}$) PFOA in Maternal Plasma During Gestation and Postpartum (cont'd)

Day	Dose (mg/kg)	Concentration	Average	S.D.
7PP	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	9.34	10.09	2.90
		7.79		
		10.37		
		8.02		
		14.93		
	10	23.38	25.83	2.07
		24.45		
		25.89		
		26.72		
		28.72		
	30	64.97	66.91	11.82
		*		
		84.10		
		60.58		
		57.98		
14PP	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	9.68	9.69	0.92
		10.22		
		8.38		
		9.35		
		10.82		
	10	19.52	23.79	2.81
		26.27		
		23.61		
		23.16		
		26.38		
	30	50.73	54.65	11.63
		42.05		
		56.48		
		50.69		
		73.30		

Concentration ($\mu\text{g/mL}$) PFOA in Maternal Plasma During Gestation and Postpartum (cont'd)

Day	Dose (mg/kg)	Concentration	Average	S.D.
21PP	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	10.35	9.04	1.01
		7.99		
		9.68		
		9.05		
		8.12		
	10	24.34	28.84	5.15
		23.45		
		28.76		
		35.68		
		31.97		
	30	63.27	64.13	1.45
		62.86		
		*		
		64.30		
		66.10		

Concentration ($\mu\text{g/mL}$) PFOA in Fetal Plasma on Gestation Day 21

Day	Dose (mg/kg)	Concentration	Average	S.D.
21G	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	5.52	5.88	0.69
		7.06		
		5.53		
		5.38		
		5.91		
	10	12.19	14.48	1.51
		14.90		
		15.96		
		15.51		
		13.84		
	30	37.32	33.11	4.64
		37.09		
		32.27		
		25.90		
		32.96		

Concentration ($\mu\text{g/mL}$) PFOA in Pup Plasma

Day	Dose (mg/kg)	Concentration	Average	S.D.
3PP	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	3.24	2.89	0.70
		1.89		
		3.33		
		2.45		
		3.56		
	10	5.62	5.94	1.44
		5.01		
		8.06		
		5.08		
		*		
	30	11.46	11.96	1.66
		11.18		
		13.43		
		9.87		
		13.87		
7PP	0	*	<LOQ	NA
		<LOQ		
		38.85 ^a		
		<LOQ		
		<LOQ		
	3	0.78	0.65	0.20
		0.41		
		0.84		
		0.57		
		*		
	10	3.38	2.77	0.58
		2.28		
		3.02		
		2.03		
		3.13		
	30	4.22	4.92	1.28
		<LOQ		
		5.15		
		6.61		
		3.70		

^a Sample not used in calculation. Extraordinary high value is likely the result of sample contamination.

Concentration ($\mu\text{g/mL}$) PFOA in Pup Plasma (cont'd)

Day	Dose (mg/kg)	Concentration	Average	S.D.
14PP	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	0.87	0.77	0.10
		0.72		
		0.74		
		0.65		
		0.87		
	10	2.50	2.22	0.38
		2.47		
		2.06		
		1.62		
		2.44		
	30	4.84	4.91	1.12
		3.23		
		5.10		
		5.01		
		6.38		
21PP	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	1.31	1.28	0.72
		*		
		1.00		
		0.56		
		2.27		
	10	3.55	3.25	0.52
		2.67		
		3.40		
		3.89		
		2.76		
	30	6.18	7.36	2.17
		7.06		
		10.50		
		5.70		

Concentration ($\mu\text{g/mL}$) of PFOA in Milk

Dose (mg/kg)	Postpartum Day			
	3	7	14	21
0	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	0.21	<LOQ
Average	<LOQ	<LOQ	NA ^a	<LOQ
S.D.	NA	NA	NA	NA
3	0.81	0.75	1.10	1.14
	0.92	0.90	1.20	N.S.
	1.41	0.90	1.12	1.01
	0.93	0.84	1.09	1.17
	1.28	1.31	1.22	1.18
Average	1.07	0.94	1.15	1.13
S.D.	0.26	0.22	0.06	0.08
10	2.16	2.32	3.24	2.80
	1.97	4.04	5.34	3.72
	2.21	3.32	3.65	2.80
	1.49	2.01	2.21	2.52
	2.34	1.98	2.83	3.49
Average	2.03	2.74	3.45	3.07
S.D.	0.33	0.91	1.18	0.51
30	5.32	6.55	5.95	7.36
	6.33	4.91	4.55	N.S.
	4.97	7.47	7.73	9.83
	5.20	5.52	6.14	6.36
	3.04	4.34	7.87	6.36
Average	4.97	5.76	6.45	7.48
S.D.	1.20	1.26	1.38	1.63

a Average not calculated because 3 of 4 values <LOQ.

Concentration ($\mu\text{g/mL}$) of PFOA in Amniotic Fluid

Dose (mg/kg)	Day 15 G			Day 21 G		
	Concentration	Average	S.D.	Concentration	Average	S.D.
0	<LOQ	<LOQ	NA	<LOQ	<LOQ	NA
	<LOQ			<LOQ		
	<LOQ			<LOQ		
	<LOQ			<LOQ		
	<LOQ			<LOQ		
3	0.25	0.60	0.69	1.28	1.50	0.32
	0.22			2.00		
	1.63			1.29		
	0.32			1.29		
	<LOQ			1.66		
10	0.73	0.70	0.15	2.97	3.76	0.81
	0.60			4.00		
	0.49			4.89		
	0.88			2.98		
	0.79			3.98		
30	0.99	1.70	0.91	7.73	8.13	0.86
	1.24			8.67		
	3.25			7.54		
	1.29			7.32		
	1.73			9.37		

Concentration ($\mu\text{g/mL}$) of PFOA in Placenta

Day	Dose (mg/kg)	Tissue Concentration	Average	S.D.
15G	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	1.48	2.22	1.79
		1.33		
		5.41		
		1.61		
		1.28		
	10	7.45	5.10	1.70
		4.52		
		<LOQ		
		3.43		
		5.01		
	30	12.01	13.22	1.03
		13.17		
		12.95		
		14.86		
		13.14		
21G	0 ^a	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	3.58	3.55	0.57
		2.88		
		3.65		
		3.21		
		4.45		
	10	6.90	9.37	1.76
		8.49		
		11.14		
		10.91		
		9.40		
	30	29.85	24.37	4.13
		22.28		
		27.16		
		19.43		
		23.12		

a One control sample was used for method development.

Concentration ($\mu\text{g/mL}$) of PFOA in Embryo

Day	Dose (mg/kg)	Tissue Concentration	Average	S.D.
10G	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	1.12	1.40	0.30
		1.58		
		1.49		
		1.05		
		1.76		
	10	2.95	3.33	0.81
		4.49		
		3.59		
		3.29		
		2.31		
	30	11.51	12.49	3.50
		12.33		
		10.15		
		18.50		
		9.95		
15G	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	0.19	0.24	0.19
		0.13		
		0.57		
		0.17		
		0.14		
	10	0.55	0.53	0.18
		0.61		
		0.27		
		0.48		
		0.76		
	30	1.39	1.24	0.22
		0.97		
		1.48		
		1.31		
		1.06		

Concentration ($\mu\text{g/mL}$) of PFOA in Fetus

Day	Dose (mg/kg)	Tissue Concentration	Average	S.D.
21G	0	<LOQ	<LOQ	NA
		<LOQ		
		<LOQ		
		<LOQ		
		<LOQ		
	3	0.95	1.27	0.26
		1.65		
		1.15		
		1.26		
		1.35		
	10	--- ^a	2.61	0.37
		2.69		
		2.79		
		2.06		
		2.89		
	30	7.23	8.77	2.36
		12.28		
		--- ^a		
		7.52		
		8.07		

^a Sample lost due to misinjection.