AR226_3316

TRADE SECRET

Study Title

H-26137, H-26138, and H-26139: Repeated Dose Dermal Toxicity 28-Day Study in Male Rats

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines OPPTS 870.3200 (1998)

OECD Guideline for the Testing of Chemicals Section 4: Health Effects, Number 410 (1981)

AUTHOR: Carol Finlay, B.A.

STUDY COMPLETED ON: May 10, 2004

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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LABORATORY PROJECT ID: DuPont-13882

WORK REQUEST NUMBER: [redacted]

SERVICE CODE NUMBER: [redacted]

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

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 except for the item documented below. The item listed does not impact the validity of the study.

The test fabrics were characterized prior to the initiation of this study by determining the quantity of the melt additive incorporated into the fabric layers. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the composition of the melt additive at concentrations documented in this report is considered sufficient for the purpose of this study.

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

Study Director: Carol Finlay 10-May-2004
Carol Finlay, B.A.
Staff Toxicologist
Date

Applicant / Sponsor: _____ _____
DuPont Representative Date

QUALITY ASSURANCE DOCUMENTATION

Haskell Sample Number(s):

26136, 26137, 26138, 26139

Dates of Inspections:

Protocol: November 17,19, 2003
Conduct: November 21, 2003
Records, Reports: February 6,11, 2004; April 19-20, 2004

Dates Findings Reported to:

Study Director: November 21, 2003; February 6,11, 2004; April 20, 2004

Management: November 21, 2003; February 6, 11, 2004; April 20, 2004

Reported by: Kimberly B. Brebner 07-May-2004
Kimberly B. Brebner Date
Staff Quality Assurance Auditor

CERTIFICATION

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

Clinical Pathology Evaluation by: _____ 10-May-2004
Nancy E. Everds, D.V.M. Date
Diplomate A.C.V.P.
Principal Research Scientist

Anatomic Pathology Evaluation by: Scott E Loveless for GPS 07-May-2004
Greg P. Sykes, V.M.D. Date
Diplomate A.C.V.P., A.C.L.A.M., A.B.T.
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Anatomic Pathology Evaluation Peer Review by: _____ 07-May-2004
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Issued by Study Director: _____ 10-May-2004
Carol Finlay, B.A. Date
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STUDY INFORMATION

Control Fabric

Substance Tested: [redacted]

Synonyms/Codes:
- [redacted]
- [redacted]
- H-26136

Submitter's Notebook Number(s): [redacted]

Haskell Number: 26136

Composition: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Off-white to bluish-white fabric

Test Fabric #1

Substance Tested: [redacted]

Synonyms/Codes:
- [redacted]
- [redacted]
- H-26137

Submitter's Notebook Number(s): [redacted]

Haskell Number: 26137

Composition: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Off-white to bluish-white fabric

Test Fabric #2

Substance Tested: [redacted]

Synonyms/Codes:
- [redacted]
- [redacted]
- H-26138

Submitter's Notebook Number(s): [redacted]

Haskell Number: 26138

Composition: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Off-white to bluish-white [redacted]

Test Fabric #3

Substance Tested: [redacted]

Synonyms/Codes:
- [redacted]
- [redacted]
- H-26139

Submitter's Notebook Number(s): [redacted]

Haskell Number: 26139

Composition: [redacted]

Known Impurities: None

Physical Characteristics: Off-white to bluish-white [redacted]

Study Initiated/Completed: November 18, 2003 / (see report cover page)

In-Life Initiated/Completed: November 24, 2003 / December 22, 2003

STUDY PERSONNEL

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SUMMARY

The objective of this study was to determine if the effects that occurred in a previously conducted 28-day dermal toxicity study with the active ingredient would be observed when the active material was incorporated into a polypropylene fabric. Of specific interest were dermal and clinical chemistry effects and the levels of p[redacted]n blood plasma. The fabric samples were identified as H-26137, H-26138, and H-26139. Each test fabric was applied to the shaved, intact skin of 10 male Crl:CD®(SD)IGS BR rats for 6 hours/day for 28 consecutive days. A group of 10 male rats was similarly treated with the control fabric H-26136. The rats were observed for mortality, clinical signs, dermal effects, and body weight effects during the study. Food consumption was determined weekly during the study. Blood and urine samples were collected for clinical pathology prior to sacrifice on day 28. Blood samples were collected at necropsy for analysis of plasma concentration of perfluorooctanoic acid. All rats underwent gross necropsy and select tissues were weighed and/or collected. No microscopic pathology evaluations were conducted.

There were no effects on body weight, food consumption, food efficiency, or mortality. No clinical signs attributable to systemic toxicity occurred. No dermal irritation was observed during the study.

There were no test substance-related or adverse effects on organ weights or gross observations at necropsy. The clinical chemistry effects seen previously with the active ingredient were not confirmed in this study. However, rats treated with H-26138 and H-26139 had minimally decreased cholesterol and minimally increased urine flouride; these two effects were of uncertain relationship to treatment, but were similar to effects observed during the previous dermal study with this compound.

The mean levels of [redacted]in plasma were 35.7 ppb, 98.3 ppb, and 159.3 ppb for rats exposed to test fabrics H-26137, H-26138, and H-26139, respectively. The levels of [redacted]in the control group H-25136 were below the limit of quantitation (10 ppb). The source of the [redacted]in the plasma, whether from direct application or metabolism of chemical in the test material, is unknown.

Under the conditions of this study, application of the test fabrics, H-26137, H-26138, and H-25139, caused no adverse effects in male rats.

INTRODUCTION

The objective of this study was to determine if the effects that occurred in a previously conducted 28-day dermal toxicity study with the active ingredient (a fluorotelomer melt additive) would be observed when the active material was incorporated into a polypropylene fabric. Three fabrics were tested in this study, H-26137, H-26138, and H-26139. In the previous study, mildly decreased cholesterol and triglycerides occurred in rats treated at 500 mg/kg/day. Urine fluoride was increased in rats treated at 50 and 500 mg/kg/day and fluorine was present in the day 27 blood. [redacted] was present in the plasma of rats treated with the melt additive. No test substance-related dermal irritation occurred.[1] Of specific interest in conducting the present study were dermal and clinical chemistry effects and the levels of [redacted] in blood plasma.

STUDY DESIGN

Group	Number/Group	Haskell Laboratory Number
I	10	H-26136 (Control)
III	10	H-26137
V	10	H-26138
VII	10	H-26139

Study Parameters	Frequency
Body Weight	Test day 0 and weekly thereafter
Food Consumption	Test day 0 and weekly thereafter
Clinical Observations	Daily
Observations for dermal effects	Daily
Mortality/Moribundity Checks	Twice daily
Clinical Pathology	Week 4
[redacted] blood analysis	Week 4
Necropsy	Week 4

MATERIALS AND METHODS

A. Test Guidelines

The study design complies with the following test guidelines except as noted below:

- Office of Prevention, Pesticides and Toxic Substances (OPPTS) U.S. Environmental Protection Agency (EPA) (1998). OPPTS 870.3200 21/28-Day Dermal Toxicity. *Health Effects Test Guidelines*.

- Organisation for Economic Co-Operation and Development (OECD) (1981). 410 Repeated Dose Dermal Toxicity: 21/28-Day Study. *Guideline for the Testing of Chemicals*.

– The wrappings of some rats became dislodged during the exposure period. Therefore, on these occasions they may not have been exposed for a full 6 hours.

This exception did not affect the objectives or the validity of the study because, considering that no significant toxicological effects were observed, it is not likely that an occasional exposure period of less than 6 hours for some animals would have changed the study results.

– Only male rats were treated.

This exception did not affect the objectives or validity of the study because in previous studies with other fluorotelomers males are more sensitive to the toxicological effects compared to females. In addition, the comparison 28-day dermal study with the melt additive was conducted with male rats.

B. Test Substances

The test substances were supplied by the sponsor as off-white to bluish-white fabrics. H-26136 contained 0.03% melt additive and served as control. The test fabrics, H-26137, H-26138, and H-26139, contained 0.05%, 0.78%, and 1.46% melt additive, respectively. The sponsor marked the side of the fabric not treated with melt additive to distinguish it from the side to be placed against the animal.

The test substances appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color, was observed.

C. Test Species

On November 18, 2003, 44 male Crl:CD®(SD)IGS BR rats, with an assigned birth date of October 6, 2003, were received from Charles River Laboratories, Inc., Raleigh, North Carolina.

The Crl:CD®(SD)IGS BR rat was selected based on consistently acceptable health status and on extensive experience with the strain at Haskell Laboratory.

D. Animal Husbandry

1. Housing

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

2. Environmental Conditions

Animal rooms were maintained at a temperature of 19-25°C and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

Tap water was provided *ad libitum*. PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 meal was available *ad libitum* except when the rats were fasted.

4. Identification

Prior to assignment to groups, each rat was temporarily identified by a cage card affixed to the outside of its cage. After assignment to groups, each rat was assigned an animal number which was tattooed on the tail of each rat. The information on the cage labels included the animal number assigned to each rat.

5. Animal Health and Environmental Monitoring Program

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

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- 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.

E. Quarantine and Pretest

Upon arrival at Haskell Laboratory, the rats were removed from shipping cartons and quarantined for 6 days. The rats were weighed 3 times during the quarantine period and examined daily for

any clinically apparent signs of disease or injury. The rats were observed daily for mortality and signs of illness, injury, or abnormal behavior.

On the bases of acceptable body weight gains and freedom from clinically apparent signs of disease or injury, the rats were released from quarantine on test day 0 (prior to dosing) by the designee of the laboratory animal veterinarian.

F. Assignment to Groups and Study Start

Forty rats were selected for study use on the bases of adequate body weight gain, freedom from clinical signs of disease or injury, and a body weight within ± 20% of the mean. The selected rats were divided by computerized, stratified randomization into 4 groups of 10 rats, so that there were no statistically significant differences among group body weight means.

Dosing of the control and test fabrics began on test day 0 when the rats were approximately 8 weeks of age.

G. Dosing Procedure

On the day prior to the first treatment, the fur of each rat was closely shaved to expose the skin from the back and trunk. On each day of treatment, the rats wore plastic collars during the exposure period to prevent oral exposure to the fabrics. The rats were treated at approximately the same time each day. The rats were treated for 28 consecutive days. The exposure period was approximately 6 hours per day. The control and test fabrics were slightly moistened with deionized water, wrapped around the animal, and secured. The side of the fabric treated with melt additive was placed against the skin. The approximate shaved total body surface area covered was 20%. After the exposure period, the collars and fabrics were removed and the shaved area was washed with warm tap water. Each rat was then gently patted dry and returned to the cage.

The rats were reshaved as needed during the study. The entire area that was originally shaved was reshaved. The animals were shaved only after an evaluation.

H. Body Weights

All rats were weighed weekly throughout the study. Additionally, they were weighed on the day of sacrifice (test day 28).

I. Food Consumption and Food Efficiency

The amount of food consumed by each rat was determined once per week throughout the study. Individual food consumption was determined by weighing the amount of diet in each feeder at the beginning and end of the week and subtracting the final weight and the amount of spillage from the feeder during the week from the initial weight. From these measurements, mean daily

food consumption was determined. From the food consumption and body weight data, mean daily food efficiency was calculated.

J. Observations

The rats were observed for clinical signs and dermal effects after removal of the fabrics. The rats were also observed for clinical signs at each weighing. The Draize Scale was used to score skin irritation.

The rats were checked twice daily for mortality and for signs of illness, injury, or abnormal behavior.

K. [redacted] Level Evaluation

Blood was collected from the *vena cava* of all rats at necropsy into a tube containing EDTA. Plasma was prepared and stored frozen at -80°C to -20°C until analyzed. [redacted] was extracted from the plasma by protein precipitation in acetonitrile. The compound was quantified by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using selected reaction monitoring (SRM). Quantification was performed using extracted calibration standards containing an internal standard. The lower limit of quantitation (LLOQ) for this method is 10 ppb.

L. Clinical Pathology Evaluation

A clinical pathology evaluation was conducted on all animals 28 days after initiation of the study. The day before collection of samples for the clinical pathology evaluation, the animals were placed in metabolism cages. These animals were fasted after 3 p.m. (overnight) and urine was collected from each animal. Blood samples for clinical chemistry measurements were collected from the orbital sinus of each animal while the animal was under carbon dioxide anesthesia. Blood for [redacted] analysis was collected from the *vena cava*. Additional blood collected from the *vena cava* was placed in a serum tube, processed to serum, and frozen at -80°C. Samples for clinical chemistry and for frozen serum were evaluated for quality by visual examination prior to analysis. Results were maintained in the study records and reported only if the sample was analyzed.

1. Clinical Chemistry

Serum clinical chemistry parameters were determined on an Olympus AU640 clinical chemistry analyzer. The following parameters were determined:

cholesterol
triglycerides

2. Urinalysis

Urine volume was measured. Urine fluoride concentrations were determined using a Beckman® Φ12 digital pH/ISE meter and a fluoride-selective electrode, and total urine fluorides were calculated (volume × concentration). The following parameters were determined:

volume
fluoride

M. Anatomic Pathology

After 28 days of exposure to the control and three test fabrics (test day 28), groups of 10 male rats from Groups I, III, V, and VII were sacrificed and necropsied.

The rats were fasted overnight on the afternoon before their scheduled sacrifice. On the day of necropsy, the fur was shaved from an untreated area and the untreated area was outlined. The order of sacrifice for scheduled deaths was random among all treatment groups. Rats were euthanized by carbon dioxide anesthesia and exsanguination. Gross examinations were performed on all rats.

The following tissues were collected from all rats.

liver	skin (treated)
kidneys	skin (untreated)
thyroid gland	gross observations

The following tissues were weighed from all rats: liver, kidneys, and thyroid gland (after fixation). Organ weight/final body weight ratios were calculated.

Gross lesions which were diagnosed at necropsy and for which microscopic examination was not appropriate (e.g., fluid accumulation, ruffled fur, missing anatomic parts) were generally not collected. Gross lesions for which a microscopic diagnosis would not be additive (e.g., osteoarthritis, pododermatitis, chronic dermatitis of the tail, urinary calculi, and deformity of the teeth, toe, tail, or pinna) were saved but were generally not processed for microscopic evaluation.

All tissues were fixed in 10% neutral buffered formalin. Tissues were not processed to slides and were not evaluated microscopically.

N. Statistical Analyses

Significance was judged at $p < 0.05$.

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight Body Weight Gain Food Consumption Food Efficiency Organ Weight	Test for lack of trend[2]	Sequential application[3] of the Jonckheere-Terpstra trend test[4]	Preliminary tests for pairwise comparison
	OR[a]		
	Levene's test for homogeneity[5] and Shapiro-Wilk test[6] for normality[b]	One-way analysis of variance[7] followed with Dunnett's test[8,9,10]	Kruskal-Wallis test[11] followed with Dunn's test[12]
Clinical Pathology[c] Survival Incidence of Clinical Observations	Levene's test for homogeneity[5] and Shapiro-Wilk test[6] for normality[b]	One-way analysis of variance[7] followed with Dunnett's test[8,9,10]	Kruskal-Wallis test[11] followed with Dunn's test[12]

a Pairwise comparisons and associated preliminary tests were only conducted if the test for lack of trend was significant.

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

c When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for any calculations performed with that bilirubin data.

RESULTS AND DISCUSSION

In-Life Toxicology

A. Mean Body Weights and Body Weight Gains
(Tables 1-2, Figure 1, Appendix A)

No effects on body weights or body weight gains occurred in any group of rats.

B. Food Consumption and Food Efficiency
(Tables 3-4, Appendix B)

No effects on food consumption or food efficiency occurred in any group of rats.

C. Clinical Observations, Dermal Effects, and Mortality
(Tables 5-6, Appendices C-D)

No clinical signs attributable to systemic toxicity occurred. Ocular and nasal discharge were observed in most rats treated with the control and test fabrics. These clinical signs are considered to be due to the wrapping/collar application procedure. One rat treated with the control fabric, H-26136, exhibited swollen face. This finding was considered due to the collar being too tight. One rat treated with test fabric H-26137 and three rats treated with test fabric H-26138 exhibited swollen forelimbs. This finding was probably a result of the wrapping procedure. Hair loss present in one rat treated with test fabric H-26137 was considered incidental and not due to the test fabric. Rats treated with both the control and test fabrics exhibited superficial wounds during the study. Stained perineum observed in one rat treated with test fabric H-26137 was not considered due to the fabric because it was only observed in one rat.

No dermal irritation was observed during the study.

No deaths occurred.

D. In-Life Conclusions

Under the conditions of this study and for the in-life parameters evaluated, there were no adverse effects on in-life parameters for any of the test substances.

Clinical Pathology Evaluation

A. Clinical Chemistry
(Table 8, Appendix G)

There were no statistically significant or adverse changes in clinical chemistry parameters in the male rats.

Cholesterol was minimally lower in animals treated with H-26138 and H-26139 (means were 80% and 86% of control group means, respectively). However, these changes were not statistically significant and were very small compared to the variability of the parameter; thus they were of uncertain relationship to treatment.

B. Urinalysis
(Table 9, Appendix G)

There were no statistically significant or adverse changes in urinalysis parameters in the male rats. Urine volume was mildly increased in four treated rats (two treated with H-26137 and two treated with H-26138). These changes were considered to be unrelated to treatment due to the variability of urine volumes.

C. Urine Fluoride
(Table 9, Appendix G)

There were no statistically significant changes in mean excreted fluoride (urine fluoride) at the end of the dosing period. A few individual rats treated with H-26138 and H-26139 had minimally higher urine fluoride amounts compared to rats in other groups. However, these changes were very small and of uncertain relationship to treatment.

D. Clinical Pathology Conclusions

Under the conditions of this study and for the clinical pathology parameters measured, there were no adverse effects on clinical pathology parameters for any of the test substances.

Anatomic Pathology Evaluation

A. Cause of Death
(Appendix I)

There were no deaths. All 40 rats survived until the scheduled 28-day sacrifice.

B. Organ Weights
(Table 11, Appendix H)

There were no test substance-related effects on absolute or relative organ weights. All individual and mean organ weight differences were considered to be spurious and unrelated to test substance administration.

C. Gross Observations
(Table 12, Appendix I)

There were no test substance-related gross observations. The three gross observations made at necropsy were interpreted to be incidental lesions unrelated to test substance exposure.

D. Microscopic Findings

Microscopic examination of tissues was not performed.

E. Anatomic Pathology Conclusions

Under the conditions of this study and for anatomic pathology parameters measured, there were no test substance-related effects on pathology parameters for any of the substances.

[redacted] Analysis
(Appendix F, Table 7)

[redacted] was present in plasma from rats treated with the test fabrics. The mean [redacted] levels in plasma collected at terminal sacrifice were 35.7 ppb, 98.3 ppb, and 159.3 ppb for test fabrics H-26137, H-26138, and H-26139, respectively. The levels of [redacted] in the plasma from control rats were below the limit of quantitation (10 ppb). It was not determined if the source of the [redacted] in the blood came from a [redacted] source in the test fabrics or resulted from metabolism of a chemical ingredient in the test material.

Comparison To Study With The Melt Additive

A 28-day dermal toxicity study was previously conducted with the neat melt additive at dosages of 0, 5, 50, and 500 mg/kg/day.[1] There were no adverse effects on body weights, clinical observations, nutritional parameters, pathology parameters, or hematologic and coagulation parameters. [redacted]evels in plasma were 61.4 ppb, 155.5 ppb, 1315.9 ppb, and 14,176 ppb for dosages of 0, 5, 50, and 500 mg/kg/day, respectively. Mildly decreased cholesterol in rats treated at 500 mg/kg/day was considered treatment-related and potentially adverse. Mildly decreased triglycerides in rats treated at 500 mg/kg/day was considered treatment-related but not adverse. Increased urine fluoride at 50 or 500 mg/kg/day and the presence of fluorine in day 27 blood indicated exposure to and metabolism of a fluoride-containing test substance and was considered treatment-related but not adverse. The no-observed-effect level was 50 mg/kg/day based on the presence of decreased cholesterol at 500 mg/kg/day.

In the present study, cholesterol was minimally lower (although not statistically significant) in rats treated with test fabrics H-26138 and H-26139 (means were 80 and 86% of control group means, respectively). Urine fluorides were increased in a few rats treated with test fabrics H-26138 and H-26139. These two effects were of uncertain relationship to treatment. There were no effects on triglycerides. [redacted]levels in plasma were 35.7 ppb, 98.3 ppb, and 159.3 ppb for test fabrics H-26137, H-26138, and H-26139, respectively. Levels of [redacted]in the plasma from control rats were below the limit of quantitation.

CONCLUSIONS

No deaths occurred during the study. No test substance-related effects on body weight, food consumption, food efficiency, clinical signs, or dermal irritation were seen in male rats that received 28 applications of the test and control fabrics applied daily to the shaved skin. [redacted] was present in a dose response fashion in the plasma from rats treated with the test fabrics. There were no test substance-related or adverse effects on gross pathology, organ weights, or triglyceride parameters. In rats treated with test fabrics H-26138 and H-26139, minimally decreased cholesterol and minimally increased urine fluoride in a few rats were of uncertain relationship to treatment.

Under the conditions of this study, application of the test fabrics, H-26137, H-26138, and H-25139, caused no adverse effects in male rats.

RECORDS AND SAMPLE STORAGE

All data and records for analytical characterizations conducted by the sponsor will be retained by the sponsor.

A sample of each fabric was collected for archive purposes and retained at Haskell Laboratory, Newark, Delaware. 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:

Summary of Clinical Chemistry Values

CHOL - cholesterol
TRIG - triglycerides

Summary of Urinalysis Values

UVOL - volume
UFLU - urine fluoride

NOTES:

Summary of Urinalysis Values

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

TABLE 1

MEAN BODY WEIGHTS OF MALE RATS (g)

Group: Material	I H-26136	III H-26137	V H-26138	VII H-26139
Test DAY0	193.0 10.1(10)	191.5 8.6(10)	192.8 10.9(10)	193.0 10.6(10)
DAY7	244.8 14.4(10)	241.2 12.0(10)	245.6 13.0(10)	245.7 11.3(10)
DAY14	293.6 18.3(10)	293.3 11.1(10)	288.8 17.0(10)	294.5 13.8(10)
DAY21	329.7 22.1(10)	327.7 14.0(10)	326.1 17.1(10)	333.2 22.7(10)
DAY27	345.8 25.8(10)	347.6 16.9(10)	338.3 17.7(10)	350.7 29.5(10)
DAY28	327.0 24.9(10)	328.8 16.7(10)	320.1 18.6(10)	331.1 27.1(10)

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

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

TABLE 2

MEAN BODY WEIGHT GAINS OF MALE RATS (g)

Group: Material		I H-26136	III H-26137	V H-26138	VII H-26139
Test					
DAY0	-DAY7	51.9 8.1(10)	49.6 10.1(10)	52.7 7.0(10)	52.7 6.5(10)
DAY7	-DAY14	48.8 6.3(10)	52.1 13.0(10)	43.2 7.9(10)	48.7 8.9(10)
DAY14	-DAY21	36.1 7.4(10)	34.5 4.5(10)	37.4 6.3(10)	38.7 10.6(10)
DAY21	-DAY27	16.1 9.6(10)	19.9 7.6(10)	12.2 7.2(10)	17.5 9.0(10)
DAY27	-DAY28	-18.7 2.4(10)	-18.9 2.3(10)	-18.2 3.2(10)	-19.6 3.1(10)
DAY0	-DAY28	134.1 18.7(10)	137.2 18.6(10)	127.3 16.1(10)	138.1 26.2(10)

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

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

TABLE 3

MEAN DAILY FOOD CONSUMPTION BY MALE RATS (g/day)

Group: Material	I H-26136	III H-26137	V H-26138	VII H-26139
Test				
DAY0-DAY7	23.1 1.9(10)	22.8 1.0(10)	23.3 1.3(10)	24.0 2.0(10)
DAY7-DAY14	26.0 1.9(10)	26.8 1.1(10)	26.0 1.7(10)	26.7 1.8(10)
DAY14-DAY21	26.8 2.1(10)	27.2 1.5(10)	27.3 1.8(10)	27.7 1.9(10)
DAY21-DAY27	27.8 2.3(10)	28.4 1.3(10)	27.3 1.5(10)	28.1 1.5(10)
DAY0-DAY27	25.879 1.941(10)	26.232 0.845(10)	25.936 1.272(10)	26.568 1.588(10)

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

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

TABLE 4

MEAN DAILY FOOD EFFICIENCY OF MALE RATS

Group: Material	I H-26136	III H-26137	V H-26138	VII H-26139
Test				
DAY0-DAY7	0.319 0.030(10)	0.311 0.065(10)	0.323 0.039(10)	0.315 0.034(10)
DAY7-DAY14	0.267 0.025(10)	0.276 0.057(10)	0.237 0.040(10)	0.261 0.046(10)
DAY14-DAY21	0.192 0.032(10)	0.180 0.017(10)	0.195 0.025(10)	0.198 0.047(10)
DAY21-DAY27	0.094 0.052(10)	0.116 0.042(10)	0.074 0.043(10)	0.102 0.047(10)
DAY0-DAY27	0.218 0.014(10)	0.220 0.024(10)	0.208 0.018(10)	0.219 0.032(10)

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

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

TABLE 5

SUMMARY OF CLINICAL OBSERVATIONS FOR MALE RATS - PREDOSING

Treatment Group Material Animal Count	I H-26136 10	III H-26137 10	V H-26138 10	VII H-26139 10
Discharge				
Nose				
Incidence	0	0	0	1 (10%)
Mean onset (Days)				14
Eye bilateral				
Incidence	1 (10%)	0	0	1 (10%)
Mean onset (Days)	7			14
Hair Loss				
Shoulder				
Incidence	0	1 (10%)	0	0
Mean onset (Days)		21		
Wound				
Superficial				
Incidence	0	2 (20%)	2 (20%)	2 (20%)
Mean onset (Days)		11	21	14

Incidence - The number of animals for which an observation was recorded.
Mean onset (Days) - The mean of the first test day an observation was recorded for that group.
There were no statistically significant differences from control at $p < 0.05$.

TABLE 6

SUMMARY OF CLINICAL OBSERVATIONS FOR MALE RATS – POST DOSING

Treatment Group	I	III	V	VII
Material	H-26136	H-26137	H-26138	H-26139
Animal Count	10	10	10	10
Discharge				
Nose				
Incidence	8 (80%)	9 (90%)	9 (90%)	9 (90%)
Mean onset (Days)	13	15	13	13
Eye bilateral				
Incidence	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Mean onset (Days)	0	0	1	3
Hair Loss				
Shoulder				
Incidence	0	1 (10%)	0	0
Mean onset (Days)		21		
Wound				
Superficial				
Incidence	1 (10%)	3 (30%)	3 (30%)	3 (30%)
Mean onset (Days)	19	9	6	7
Stain Fur/Skin				
Perineum				
Incidence	0	1 (10%)	0	0
Mean onset (Days)		26		

TABLE 6 (CONTINUED)

SUMMARY OF CLINICAL OBSERVATIONS FOR MALE RATS – POST DOSING

Treatment Group Material Animal Count	I H-26136 10	III H-26137 10	V H-26138 10	VII H-26139 10
Swollen Observations				
Face				
Incidence	1 (10%)	0	0	0
Mean onset (Days)	16			
Forelimb				
Incidence	0	1 (10%)	3 (30%)	0
Mean onset (Days)		8	5	

Incidence - The number of animals for which an observation was recorded.
Mean onset (Days) - The mean of the first test day an observation was recorded for that group.
There were no statistically significant differences from control at $p < 0.05$.

TABLE 7

MEAN [redacted] LEVELS IN MALE RATS

Fabric	PLASMA [redacted] (ppb)[a] MEAN	S.D.	N
H-26136	< 10	0.0	10
H-26137	35.7	5.4	10
H-26138	98.3	26.6	10
H-26139	159.3	53.1	10

N = number of samples
S.D. = standard deviation
a limit of quantitation = 10 ppb

TABLE 8

SUMMARY OF CLINICAL CHEMISTRY VALUES FOR MALE RATS

	Group I H-26136	Group III H-26137	Group V H-26138	Group VII H-26139
CHOL (mg/dL)				
DAY 28	44	47	35	38
	9(10)	12(10)	9(10)	8(10)
TRIG (mg/dL)				
DAY 28	32	30	28	27
	11(10)	10(10)	12(10)	6(10)

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

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

TABLE 9

SUMMARY OF URINALYSIS VALUES FOR MALE RATS

	Group I H-26136	Group III H-26137	Group V H-26138	Group VII H-26139
UVOL (mL)				
DAY 28	8.9	13.0	11.2	7.9
	4.7(10)	7.1(10)	9.5(10)	2.8(10)
UFLU (µg)				
DAY 28	10.1	11.5	13.6	13.2
	2.7(10)	1.9(10)	4.4(10)	4.1(10)

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

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

TABLE 10

PERCENT SURVIVAL OF MALE RATS

Group:	I	III	V	VII
Material:	H-26136	H-26137	H-26138	H-26139
Days on Test				
0	100	100	100	100
7	100	100	100	100
14	100	100	100	100
21	100	100	100	100
28	100	100	100	100
Number at study start	10	10	10	10
Alive on test day 28	10	10	10	10

Percent Survival = (Number of rats alive/Number of rats at risk)*100
Number of rats at risk = Number at study start.

There were no statistically significant decreases in survival.

TABLE 11

MEAN FINAL BODY AND ORGAN WEIGHTS FOR MALE RATS (GRAMS)

Group:	I H-26136	III H-26137	V H-26138	VII H-26139
	MEAN FINAL BODY AND ABSOLUTE ORGAN WEIGHTS			
LIVER	10.879 0.865(10)	11.051 0.946(10)	10.003 0.614(10)	11.106 1.624(10)
KIDNEYS	3.064 0.279(10)	3.037 0.275(10)	2.968 0.159(10)	3.046 0.295(10)
THYROID GLAND	0.021 0.003(10)	0.021 0.004(10)	0.019 0.003(10)	0.019 0.003(10)
FINAL BODY WEIGHT	327.040 24.920(10)	328.760 16.747(10)	320.090 18.562(10)	331.110 27.091(10)

TABLE 11 (CONTINUED)

MEAN FINAL BODY AND ORGAN WEIGHTS FOR MALE RATS (GRAMS)

Group:	I H-26136	III H-26137	V H-26138	VII H-26139
	MEAN RELATIVE ORGAN WEIGHT (% ORGAN TO BODY)			
LIVER/ FINAL BODY * 100	3.329 0.154(10)	3.363 0.244(10)	3.127 0.130(10)	3.341 0.246(10)
KIDNEYS/ FINAL BODY * 100	0.937 0.046(10)	0.923 0.057(10)	0.930 0.067(10)	0.919 0.032(10)
THYROID GLAND/ FINAL BODY * 100	0.006 0.001(10)	0.006 0.001(10)	0.006 0.001(10)	0.006 0.001(10)

Data summarized as: Mean
Standard Deviation (n)

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

TABLE 12

INCIDENCES OF GROSS OBSERVATIONS IN MALE RATS

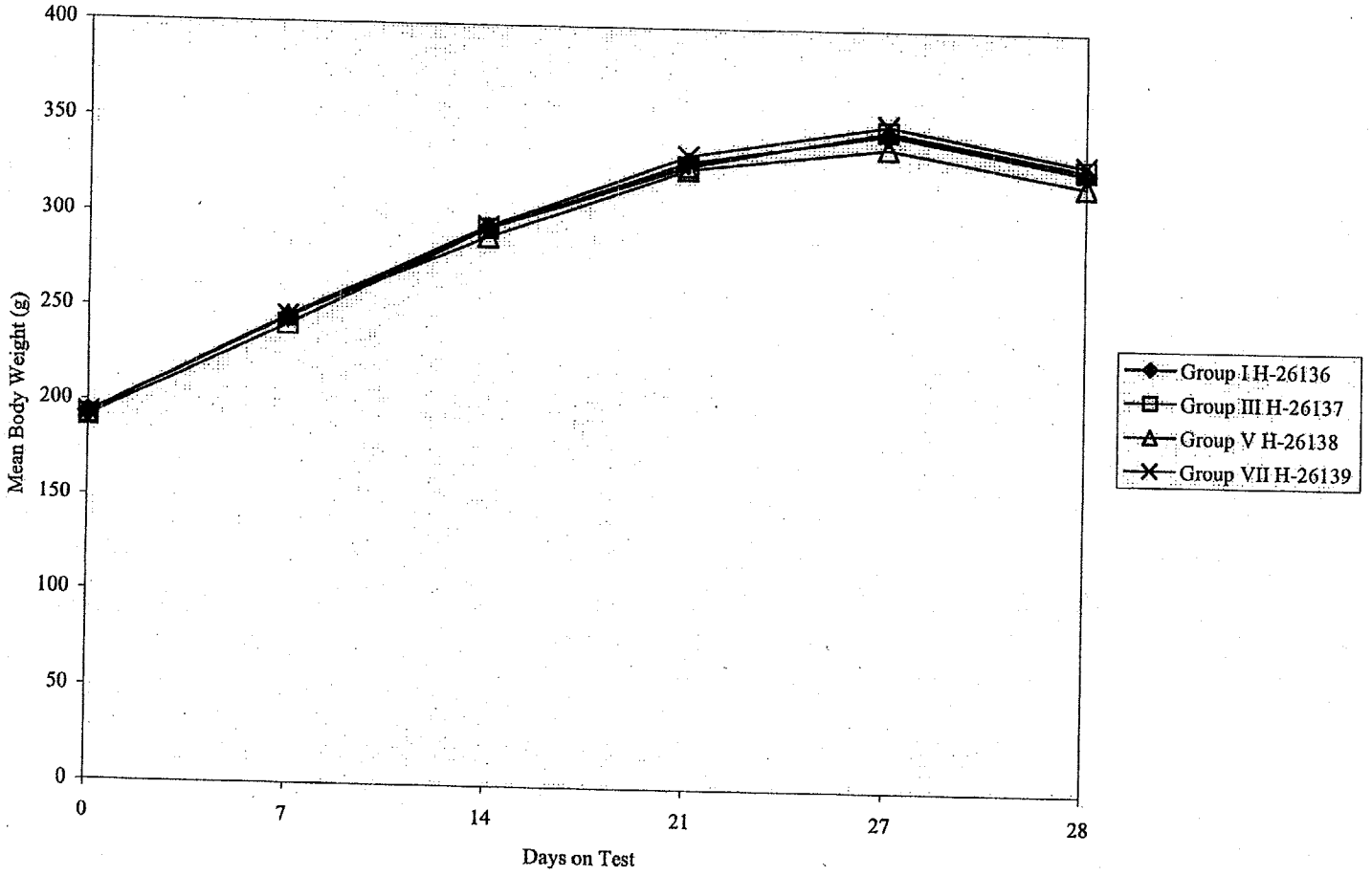
LESIONS	TREATMENT	LESION INCIDENCE (Numeric) Males I	III	V	VII
LIVER		(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10
KIDNEYS		(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	9	9	9
DILATATION, RIGHT, PELVIS.			1		1
CYST, BILATERAL, FEW, <2MM.				1	
THYROID GLAND		(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10
SKIN, TREATED		(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10
SKIN, UNTREATED		(10)	(10)	(10)	(10)
NO ABNORMALITY DETECTED		10	10	10	10

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

FIGURES

FIGURE 1

MEAN BODY WEIGHTS OF MALE RATS



APPENDICES

APPENDIX A

Individual Body Weights

Study: 14992 1012

Print Date: 14-Jan-2004
Print Time: 15:11:16

Individual Body Weights

	Body Weight g Day 0	Body Weight g Day 7	Body Weight g Day 14	Body Weight g Day 21	Body Weight g Day 27	Body Weight g Day 28
Male, I H-26136						
101	172.9	217.8	264.0	294.8	300.1	283.3
102	198.1	246.4	300.5	335.7	340.3	323.2
103	194.7	258.6	301.3	349.7	364.9	342.4
104	178.8	221.9	262.2	294.9	307.1	291.8
105	201.2	251.3	304.2	334.4	363.8	343.1
106	192.0	256.8	313.9	345.2	376.1	359.6
107	192.8	241.0	279.9	312.6	331.2	309.8
108	197.3	244.4	297.7	328.3	353.5	333.7
109	195.1	256.2	308.4	358.3	367.3	348.5
110	206.6	253.7	303.6	342.9	353.2	335.0

Study: 14992 1012

Print Date: 14-Jan-2004
Print Time: 15:11:16

Individual Body Weights

	Body Weight g Day 0	Body Weight g Day 7	Body Weight g Day 14	Body Weight g Day 21	Body Weight g Day 27	Body Weight g Day 28
Male, III H-26137						
301	176.7	229.0	277.1	303.6	329.7	307.8
302	197.4	250.3	298.6	331.4	351.8	331.5
303	192.1	216.7	299.2	340.3	371.2	351.6
304	180.8	234.3	279.9	317.2	336.6	316.9
305	195.3	240.9	289.2	325.7	345.9	324.9
306	186.3	241.9	303.1	339.6	370.8	353.3
307	197.1	256.0	311.1	345.8	357.2	336.5
308	198.0	242.4	283.8	312.1	322.0	306.7
309	187.2	246.2	301.5	339.7	357.2	340.4
310	204.5	253.9	289.1	321.8	333.9	318.0

Study: 14992 1012

Print Date: 14-Jan-2004
Print Time: 15:11:16

Individual Body Weights

	Body Weight g Day 0	Body Weight g Day 7	Body Weight g Day 14	Body Weight g Day 21	Body Weight g Day 27	Body Weight g Day 28
Male, V H-26138						
501	177.6	220.9	261.5	293.5	307.7	286.0
502	196.2	251.8	298.3	334.3	344.0	327.6
503	192.7	249.8	274.3	322.2	349.1	334.0
504	179.7	236.5	284.8	332.5	344.7	328.7
505	203.8	249.3	292.9	325.1	346.7	321.8
506	183.4	237.0	275.4	311.6	318.4	298.0
507	198.2	242.2	285.9	319.7	324.7	309.5
508	197.5	263.4	317.9	357.4	369.5	350.6
509	187.5	240.9	285.8	324.7	333.6	316.4
510	211.7	263.8	310.9	340.2	344.6	328.3

Study: 14992 1012

Print Date: 14-Jan-2004
Print Time: 15:11:16

Individual Body Weights

	Body Weight g Day 0	Body Weight g Day 7	Body Weight g Day 14	Body Weight g Day 21	Body Weight g Day 27	Body Weight g Day 28
Male, VII H-26139						
701	171.8	226.8	266.9	292.0	302.7	287.2
702	201.3	261.1	308.5	363.1	392.7	368.8
703	188.9	246.9	309.3	358.0	371.6	349.5
704	183.5	228.8	278.5	318.0	327.8	313.0
705	200.2	252.5	293.6	322.5	330.4	310.2
706	189.9	246.9	305.5	349.8	382.6	360.0
707	198.7	240.6	292.4	336.6	352.0	329.9
708	193.2	242.9	293.2	328.2	339.9	322.6
709	192.6	253.3	306.5	351.4	378.6	359.6
710	210.0	257.4	290.1	312.3	328.7	310.3

APPENDIX B

Individual Food Consumption

INDIVIDUAL FOOD CONSUMPTION

EXPLANATORY NOTES

Abbreviation

g/anm/day = grams/animal/day

Study: 14992 1012

Print Date: 14-Jan-2004
Print Time: 15:11:55

Individual Food Consumption

	Food Cons. g/anm/day Day 7	Food Cons. g/anm/day Day 14	Food Cons. g/anm/day Day 21	Food Cons. g/anm/day Day 27
Male, I H-26136				
101	20.6	23.9	24.0	24.5
102	23.1	26.6	27.0	27.0
103	25.4	27.3	29.1	27.9
104	19.7	23.7	23.8	23.8
105	23.8	28.1	28.9	30.5
106	25.5	29.0	28.1	29.8
107	22.1	23.4	23.9	26.2
108	23.6	25.2	27.2	29.8
109	24.3	26.8	28.7	29.0
110	23.3	26.3	27.9	29.0

Study: 14992 1012

Print Date: 14-Jan-2004
Print Time: 15:11:55

Individual Food Consumption

	Food Cons. g/anm/day Day 7	Food Cons. g/anm/day Day 14	Food Cons. g/anm/day Day 21	Food Cons. g/anm/day Day 27
Male, III H-26137				
301	22.2	26.2	26.7	29.2
302	23.4	26.2	26.8	27.6
303	22.1	29.0	28.8	30.0
304	21.2	25.8	28.1	28.5
305	23.6	26.8	27.9	28.9
306	21.9	26.9	26.7	28.8
307	24.0	28.1	28.3	28.2
308	23.2	25.4	23.8	25.3
309	22.5	27.4	28.4	29.0
310	24.2	26.1	26.7	28.6

Study: 14992 1012

Print Date: 14-Jan-2004
Print Time: 15:11:55

Individual Food Consumption

	Food Cons. g/anm/day Day 7	Food Cons. g/anm/day Day 14	Food Cons. g/anm/day Day 21	Food Cons. g/anm/day Day 27
Male, V H-26138				
501	21.3	23.6	24.7	25.2
502	25.4	29.1	28.9	28.6
503	23.4	24.5	30.3	27.1
504	22.4	25.9	28.9	29.3
505	24.2	25.7	26.3	28.9
506	23.1	25.9	28.4	27.2
507	21.9	24.6	24.7	25.0
508	23.7	26.6	27.0	27.8
509	22.7	25.9	26.8	27.5
510	24.9	28.3	27.3	26.2

Study: 14992 1012

Print Date: 14-Jan-2004
Print Time: 15:11:55

Individual Food Consumption

	Food Cons. g/anm/day Day 7	Food Cons. g/anm/day Day 14	Food Cons. g/anm/day Day 21	Food Cons. g/anm/day Day 27
Male, VII H-26139				
701	24.1	25.5	26.7	26.1
702	25.0	28.9	30.4	30.8
703	22.5	26.7	28.3	28.0
704	21.1	24.4	26.3	27.3
705	28.0	28.5	28.8	27.5
706	25.4	29.0	29.0	29.9
707	21.9	25.2	26.8	28.0
708	23.1	25.7	26.6	27.7
709	24.7	28.3	30.2	29.7
710	23.9	24.9	24.2	26.2

APPENDIX C

Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

Note

Clinical observations reported in this appendix were recorded during body weight collection. Clinical observations recorded post-dosing are not included.

Print Date: 30-Jan-2004
Print Time: 10:16:36

Individual Clincal Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	I	101	General observation, No Abnormality Detected	0,14-28
			Discharge, Eye bilateral, Red	7
			Sacrificed by design	28
M	I	102	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	I	103	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	I	104	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	I	105	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	I	106	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	I	107	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	I	108	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	I	109	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	I	110	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28

Print Date: 30-Jan-2004
Print Time: 10:16:36

Individual Clincal Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	III	301	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	III	302	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	III	303	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	III	304	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	III	305	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	III	306	General observation, No Abnormality Detected	0-7,28
			Hair Loss, Shoulder, Left	21
			Wound, Superficial, Shoulder, Left	14
			Sacrificed by design	28
M	III	307	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	III	308	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	III	309	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	III	310	General observation, No Abnormality Detected	0,14-28

Print Date: 30-Jan-2004
Print Time: 10:16:36

Individual Clincal Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	III	310	Wound, Superficial, Shoulder, Right	7
			Sacrificed by design	28

Print Date: 30-Jan-2004
Print Time: 10:16:36

Individual Clincal Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	V	501	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	V	502	General observation, No Abnormality Detected	0-21
			Wound, Superficial, Dorsal Body	28
			Sacrificed by design	28
M	V	503	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	V	504	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	V	505	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	V	506	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	V	507	General observation, No Abnormality Detected	0-7,21-28
			Wound, Superficial, Shoulder, Left	14
			Sacrificed by design	28
M	V	508	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	V	509	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	V	510	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28

Print Date: 30-Jan-2004
Print Time: 10:16:36

Individual Clincal Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	VII	701	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	VII	702	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	VII	703	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	VII	704	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	VII	705	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	VII	706	General observation, No Abnormality Detected	0-7
			Wound, Superficial, Shoulder, Bilateral	21-28
			Wound, Superficial, Shoulder, Left	14
			Sacrificed by design	28
M	VII	707	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	VII	708	General observation, No Abnormality Detected	0-7,21-28
			Discharge, Eye bilateral, Red	14
			Discharge, Nose, Red	14
			Sacrificed by design	28

Study: 14992 1012

Print Date: 30-Jan-2004
Print Time: 10:16:36

Individual Clincal Observations and Mortality Records

Sex	Group	Animal	Observation	Days
M	VII	709	General observation, No Abnormality Detected	0-28
			Sacrificed by design	28
M	VII	710	General observation, No Abnormality Detected	0-7,21-28
			Wound, Superficial, Shoulder, Left	14
			Sacrificed by design	28

APPENDIX D

Individual Clinical Observations - Post-Dosing

INDIVIDUAL CLINICAL OBSERVATIONS- POST-DOSING

EXPLANATORY NOTES

Clinical observations reported in this appendix were recorded daily after removal of the material (fabric). Clinical observations recorded during body weight collection are not included.

Study: 14992 1012

Print Date: 20-Jan-2004
Print Time: 12:58:37

Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	I	101	General observation, No Abnormality Detected	7-13,14-19, 23,26-27
			Discharge, Eye bilateral, Red	0-6,20,21-22, 24
			Discharge, Nose, Red	20,22,24-25
M	I	102	General observation, No Abnormality Detected	0-6,7-13, 14-20,21-27
M	I	103	General observation, No Abnormality Detected	3-5,7-8,11, 14,21,23-26
			Discharge, Eye bilateral, Red	0-2,6,9-10, 12-13,15-20, 22,27
			Discharge, Nose, Red	12-13,15-20, 22
M	I	104	General observation, No Abnormality Detected	6,7-9,14,21,23
			Discharge, Eye bilateral, Red	0-5,10-13, 15-20,22,24-27
			Discharge, Nose, Red	12-13
			Swollen Observations, Face	16
M	I	105	General observation, No Abnormality Detected	5-6,8,10,21-26
			Discharge, Eye bilateral, Red	0-4,7,9,11-13, 15-20
			Discharge, Nose, Red	12-13,14-20, 27
M	I	106	General observation, No Abnormality Detected	2-4,7-8, 12-13,14-18,21, 23-27

Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	I	106	Discharge, Eye bilateral, Red	0-1,5-6, 9-11,20
			Discharge, Nose, Red	19-20,22
M	I	107	General observation, No Abnormality Detected	0,4-6,7-9, 11,14-15,18,21-27
			Discharge, Eye bilateral, Red	1-3,10,12-13, 16-17,20
			Discharge, Nose, Red	12-13
			Wound, Superficial, Shoulder, Left	19
M	I	108	General observation, No Abnormality Detected	0-1,3-4,6, 8-9,12-13, 14-20,21-27
			Discharge, Eye bilateral, Red	2,5,7,10-11
M	I	109	General observation, No Abnormality Detected	0,2-6,7,12-13, 14-17,21-23, 27
			Discharge, Eye bilateral, Red	1
			Discharge, Nose, Red	8-11,18-20, 24-26
M	I	110	General observation, No Abnormality Detected	3,14,23,27
			Discharge, Eye bilateral, Red	0-2,4-6, 7-13,15-20, 21-22,24-26
			Discharge, Nose, Red	12-13,16-20, 22,25

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Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	III	301	General observation, No Abnormality Detected	0,2-4,6,7-8, 12-13,14,21-24
			Discharge, Eye bilateral, Red	1,5,9-11,15-20
			Discharge, Nose, Red	16-20,25-27
			Wound, Superficial, Dorsal Body	10-11
M	III	302	General observation, No Abnormality Detected	3,5-6,7,9-13, 14-15,21-27
			Discharge, Eye bilateral, Red	0-2,4,16-20
			Swollen Observations, Forelimb, Left	8
M	III	303	General observation, No Abnormality Detected	0-1,3-6, 7-10,12-13,14, 17-18,21-27
			Discharge, Eye bilateral, Red	2,11,15-16,19-20
			Discharge, Nose, Red	19-20
M	III	304	General observation, No Abnormality Detected	7,9-10,12-13, 14-15,17,21-26
			Discharge, Eye bilateral, Red	0-6,8,11,16,18-20
			Discharge, Nose, Red	16,18-20,27
M	III	305	General observation, No Abnormality Detected	2,4-6,7-13, 14-18,21-25, 27
			Discharge, Eye bilateral, Red	0-1,3
			Discharge, Nose, Red	19-20
			Stain Fur/Skin, Perineum, Yellow	26
M	III	306	General observation, No Abnormality Detected	5-6,9,26-27

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Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	III	306	Discharge, Eye bilateral, Red	0-4,7-8, 10-13,15
			Discharge, Nose, Red	12-13,15,18-20, 22
			Hair Loss, Shoulder, Left	21-25
			Wound, Superficial, Shoulder, Left	12-13,14-20
M	III	307	General observation, No Abnormality Detected	0,2,4,7,10-11, 14,21-27
			Discharge, Eye bilateral, Red	1,3,5-6,15-20
			Discharge, Eye bilateral, Red, ,	8-9
			Discharge, Nose, Red	12-13,15-20
M	III	308	General observation, No Abnormality Detected	3-5,8-10, 12-13,14,17,21,23, 27
			Discharge, Eye bilateral, Red	0-2,6,7,11, 15-16,18-20,22, 24-26
			Discharge, Nose, Red	16,18-20,22,25
M	III	309	Discharge, Eye bilateral, Red	0-6,7-13, 14-20,21-27
			Discharge, Nose, Red	12-13,15-20, 22-27
M	III	310	General observation, No Abnormality Detected	5,7-8
			Discharge, Eye bilateral, Red	0-4,6,9-13, 15,21-27
			Discharge, Nose, Red	12-13,15,17-20, 21-27

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Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	III	310	Wound, Superficial, Dorsal Body	14-20
			Wound, Superficial, Shoulder, Right	6

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Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	V	501	General observation, No Abnormality Detected	6,7-9,11-13, 14,25-27
			Discharge, Eye bilateral, Red	0-5,10,15-20, 21-24
			Discharge, Nose, Red	16-20,22-24
M	V	502	General observation, No Abnormality Detected	0,5-6,14-15, 21,23-24
			Discharge, Eye bilateral, Red	1-4,8-13, 16-20,22
			Discharge, Nose, Red	12-13,17-20, 22
			Wound, Superficial, Dorsal Body	7-10,25-27
M	V	503	General observation, No Abnormality Detected	1-3,5-6, 7-9,14-16,21-27
			Discharge, Eye bilateral, Red	0,10-11,13,18-20
			Discharge, Nose, Red	12-13,17-20
			Swollen Observations, Forelimb, Right	4
M	V	504	General observation, No Abnormality Detected	12,14,21
			Discharge, Eye bilateral, Red	0-6,7-11,13, 15-20,22-27
			Discharge, Nose, Red	13
M	V	505	General observation, No Abnormality Detected	0-1,5,9-10, 12
			Discharge, Eye bilateral, Red	2-4,6,7-8, 11,13,14-20,21-27
			Discharge, Nose, Red	13,15,17-20,22-24

Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	V	506	General observation, No Abnormality Detected	0,3-6,8,10-13, 14-15,21-27
			Discharge, Eye bilateral, Red	1-2,7,9,16-20
M	V	507	General observation, No Abnormality Detected	0,2-4,7,21-24, 26-27
			Discharge, Eye bilateral, Red	1,5-6,11-13, 15-20,25
			Discharge, Nose, Red	12-13,16-20
			Wound, Superficial, Shoulder, Left	9-13,14-19
			Swollen Observations, Forelimb, Left	8
M	V	508	General observation, No Abnormality Detected	3-5,7,9,13,14, 21-23,27
			Discharge, Eye bilateral, Red	0-2,6,8,10-12, 15-20,24-26
			Discharge, Nose, Red	12,17-20,24-25
M	V	509	General observation, No Abnormality Detected	2,6,7-10,12-13, 14-15,17-20, 21-27
			Discharge, Eye bilateral, Red	0-1,11
			Discharge, Nose, Red	16
			Wound, Superficial, Dorsal Body	3-5
			Comments, wound is from staple clip	3-5
M	V	510	General observation, No Abnormality Detected	5,14,18,21-22, 25-27

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Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	V	510	Discharge, Eye bilateral, Red	0-4,6,7-13, 15-17,19-20, 23-24
			Discharge, Nose, Red	8,16-17,24
			Swollen Observations, Forelimb, Right	2

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Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	VII	701	General observation, No Abnormality Detected	5,7,14,21,27
			Discharge, Eye bilateral, Red	0-4,6,8-13, 15-20,22-24
			Discharge, Nose, Red	12-13,17-20, 24-26
M	VII	702	General observation, No Abnormality Detected	5-6,7-9,23, 26-27
			Discharge, Eye bilateral, Red	0-4,10-13, 14-20,21-22, 24-25
			Discharge, Nose, Red	12-13,17-20, 22,24-25
M	VII	703	General observation, No Abnormality Detected	4-6,12-13, 14-20,21-27
			Discharge, Eye bilateral, Red	0-3,7-11
M	VII	704	General observation, No Abnormality Detected	0-6,7-13,21-27
			Discharge, Eye bilateral, Red	16-20
			Discharge, Nose, Red	17-20
			Wound, Superficial, Dorsal Body	14-17
M	VII	705	General observation, No Abnormality Detected	0,4,8-13,14, 17-19,21,23,26-27
			Discharge, Eye bilateral, Red	1-3,5-6,7, 15-16,20,22,24-25
			Discharge, Nose, Red	16
M	VII	706	General observation, No Abnormality Detected	7,12

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Individual Clinical Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	VII	706	Discharge, Eye bilateral, Red	0-3,9-11,13, 15-20
			Discharge, Nose, Red	13,15-20,22-25
			Wound, Superficial, Dorsal Body	0-6,8-10
			Wound, Superficial, Shoulder, Bilateral	19-20,21-27
			Wound, Superficial, Shoulder, Left	14-18
			Comments, wound is from staple clip	0-6
M	VII	707	General observation, No Abnormality Detected	5,8,14,21,25-27
			Discharge, Eye bilateral, Red	0-4,6,7,9-13, 15-20,22-24
			Discharge, Nose, Red	12-13,15-20, 24
M	VII	708	General observation, No Abnormality Detected	0-2,5-6,7, 10,14,21,25-27
			Discharge, Eye bilateral, Red	3-4,8-9, 11-13,15-20,22-24
			Discharge, Nose, Red	12-13,15-20
M	VII	709	General observation, No Abnormality Detected	0-6,7-10, 12-13,14,21-23, 25-27
			Discharge, Eye bilateral, Red	11
			Discharge, Nose, Red	15-20,24
M	VII	710	General observation, No Abnormality Detected	0,2-6,7-11, 21-22,25-27
			Discharge, Eye bilateral, Red	1,23-24

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Individual Clincal Observations - Post Dosing

Sex	Group	Animal	Observation	Days
M	VII	710	Discharge, Nose, Red	12-13,18-20, 24
			Wound, Superficial, Shoulder, Left	12-13,14-20

APPENDIX E

Individual Edema and Erythema Scores

INDIVIDUAL EDEMA AND ERYTHEMA SCORES

EXPLANATORY NOTES

DRAIZE[a] SCALE FOR SCORING SKIN IRRITATION

Evaluation of Skin Reactions	Score
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4

a Draize, J. H., "Dermal Toxicity." Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Editorial Committee of the Association of Food and Drug Officials of the United States, Austin, Texas, 1959, pp. 46-59.

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Individual Edema and Erythema Scores

	Edema Day 0	Edema Day 1	Edema Day 2	Edema Day 3	Edema Day 4	Edema Day 5	Edema Day 6	Edema Day 7	Edema Day 8	Edema Day 9	Edema Day 10	Edema Day 11
Male, I H-26136												
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 12	Edema Day 13	Edema Day 14	Edema Day 15	Edema Day 16	Edema Day 17	Edema Day 18	Edema Day 19	Edema Day 20	Edema Day 21	Edema Day 22	Edema Day 23
Male, I H-26136												
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 24	Edema Day 25	Edema Day 26	Edema Day 27
Male, I H-26136				
101	0	0	0	0
102	0	0	0	0
103	0	0	0	0
104	0	0	0	0
105	0	0	0	0
106	0	0	0	0
107	0	0	0	0
108	0	0	0	0
109	0	0	0	0
110	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 0	Edema Day 1	Edema Day 2	Edema Day 3	Edema Day 4	Edema Day 5	Edema Day 6	Edema Day 7	Edema Day 8	Edema Day 9	Edema Day 10	Edema Day 11
Male, III H-26137												
301	0	0	0	0	0	0	0	0	0	0	0	0
302	0	0	0	0	0	0	0	0	0	0	0	0
303	0	0	0	0	0	0	0	0	0	0	0	0
304	0	0	0	0	0	0	0	0	0	0	0	0
305	0	0	0	0	0	0	0	0	0	0	0	0
306	0	0	0	0	0	0	0	0	0	0	0	0
307	0	0	0	0	0	0	0	0	0	0	0	0
308	0	0	0	0	0	0	0	0	0	0	0	0
309	0	0	0	0	0	0	0	0	0	0	0	0
310	0	0	0	0	0	0	0	0	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 12	Edema Day 13	Edema Day 14	Edema Day 15	Edema Day 16	Edema Day 17	Edema Day 18	Edema Day 19	Edema Day 20	Edema Day 21	Edema Day 22	Edema Day 23
Male, III H-26137												
301	0	0	0	0	0	0	0	0	0	0	0	0
302	0	0	0	0	0	0	0	0	0	0	0	0
303	0	0	0	0	0	0	0	0	0	0	0	0
304	0	0	0	0	0	0	0	0	0	0	0	0
305	0	0	0	0	0	0	0	0	0	0	0	0
306	0	0	0	0	0	0	0	0	0	0	0	0
307	0	0	0	0	0	0	0	0	0	0	0	0
308	0	0	0	0	0	0	0	0	0	0	0	0
309	0	0	0	0	0	0	0	0	0	0	0	0
310	0	0	0	0	0	0	0	0	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 24	Edema Day 25	Edema Day 26	Edema Day 27
Male, III H-26137				
301	0	0	0	0
302	0	0	0	0
303	0	0	0	0
304	0	0	0	0
305	0	0	0	0
306	0	0	0	0
307	0	0	0	0
308	0	0	0	0
309	0	0	0	0
310	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 0	Edema Day 1	Edema Day 2	Edema Day 3	Edema Day 4	Edema Day 5	Edema Day 6	Edema Day 7	Edema Day 8	Edema Day 9	Edema Day 10	Edema Day 11
Male, V H-26138												
501	0	0	0	0	0	0	0	0	0	0	0	0
502	0	0	0	0	0	0	0	0	0	0	0	0
503	0	0	0	0	0	0	0	0	0	0	0	0
504	0	0	0	0	0	0	0	0	0	0	0	0
505	0	0	0	0	0	0	0	0	0	0	0	0
506	0	0	0	0	0	0	0	0	0	0	0	0
507	0	0	0	0	0	0	0	0	0	0	0	0
508	0	0	0	0	0	0	0	0	0	0	0	0
509	0	0	0	0	0	0	0	0	0	0	0	0
510	0	0	0	0	0	0	0	0	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 12	Edema Day 13	Edema Day 14	Edema Day 15	Edema Day 16	Edema Day 17	Edema Day 18	Edema Day 19	Edema Day 20	Edema Day 21	Edema Day 22	Edema Day 23
Male, V H-26138												
501	0	0	0	0	0	0	0	0	0	0	0	0
502	0	0	0	0	0	0	0	0	0	0	0	0
503	0	0	0	0	0	0	0	0	0	0	0	0
504	0	0	0	0	0	0	0	0	0	0	0	0
505	0	0	0	0	0	0	0	0	0	0	0	0
506	0	0	0	0	0	0	0	0	0	0	0	0
507	0	0	0	0	0	0	0	0	0	0	0	0
508	0	0	0	0	0	0	0	0	0	0	0	0
509	0	0	0	0	0	0	0	0	0	0	0	0
510	0	0	0	0	0	0	0	0	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 24	Edema Day 25	Edema Day 26	Edema Day 27
Male, V H-26138				
501	0	0	0	0
502	0	0	0	0
503	0	0	0	0
504	0	0	0	0
505	0	0	0	0
506	0	0	0	0
507	0	0	0	0
508	0	0	0	0
509	0	0	0	0
510	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 0	Edema Day 1	Edema Day 2	Edema Day 3	Edema Day 4	Edema Day 5	Edema Day 6	Edema Day 7	Edema Day 8	Edema Day 9	Edema Day 10	Edema Day 11
Male, VII H-26139												
701	0	0	0	0	0	0	0	0	0	0	0	0
702	0	0	0	0	0	0	0	0	0	0	0	0
703	0	0	0	0	0	0	0	0	0	0	0	0
704	0	0	0	0	0	0	0	0	0	0	0	0
705	0	0	0	0	0	0	0	0	0	0	0	0
706	0	0	0	0	0	0	0	0	0	0	0	0
707	0	0	0	0	0	0	0	0	0	0	0	0
708	0	0	0	0	0	0	0	0	0	0	0	0
709	0	0	0	0	0	0	0	0	0	0	0	0
710	0	0	0	0	0	0	0	0	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 12	Edema Day 13	Edema Day 14	Edema Day 15	Edema Day 16	Edema Day 17	Edema Day 18	Edema Day 19	Edema Day 20	Edema Day 21	Edema Day 22	Edema Day 23
Male, VII H-26139												
701	0	0	0	0	0	0	0	0	0	0	0	0
702	0	0	0	0	0	0	0	0	0	0	0	0
703	0	0	0	0	0	0	0	0	0	0	0	0
704	0	0	0	0	0	0	0	0	0	0	0	0
705	0	0	0	0	0	0	0	0	0	0	0	0
706	0	0	0	0	0	0	0	0	0	0	0	0
707	0	0	0	0	0	0	0	0	0	0	0	0
708	0	0	0	0	0	0	0	0	0	0	0	0
709	0	0	0	0	0	0	0	0	0	0	0	0
710	0	0	0	0	0	0	0	0	0	0	0	0

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Individual Edema and Erythema Scores

	Edema Day 24	Edema Day 25	Edema Day 26	Edema Day 27
Male, VII H-26139				
701	0	0	0	0
702	0	0	0	0
703	0	0	0	0
704	0	0	0	0
705	0	0	0	0
706	0	0	0	0
707	0	0	0	0
708	0	0	0	0
709	0	0	0	0
710	0	0	0	0

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Individual Edema and Erythema Scores

	Erythema Day 0	Erythema Day 1	Erythema Day 2	Erythema Day 3	Erythema Day 4	Erythema Day 5	Erythema Day 6	Erythema Day 7	Erythema Day 8	Erythema Day 9	Erythema Day 10	Erythema Day 11
Male, I H-26136												
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 12	Erythema Day 13	Erythema Day 14	Erythema Day 15	Erythema Day 16	Erythema Day 17	Erythema Day 18	Erythema Day 19	Erythema Day 20	Erythema Day 21	Erythema Day 22	Erythema Day 23
Male, I H-26136												
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 24	Erythema Day 25	Erythema Day 26	Erythema Day 27
Male, I H-26136				
101	0	0	0	0
102	0	0	0	0
103	0	0	0	0
104	0	0	0	0
105	0	0	0	0
106	0	0	0	0
107	0	0	0	0
108	0	0	0	0
109	0	0	0	0
110	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 0	Erythema Day 1	Erythema Day 2	Erythema Day 3	Erythema Day 4	Erythema Day 5	Erythema Day 6	Erythema Day 7	Erythema Day 8	Erythema Day 9	Erythema Day 10	Erythema Day 11
Male, III H-26137												
301	0	0	0	0	0	0	0	0	0	0	0	0
302	0	0	0	0	0	0	0	0	0	0	0	0
303	0	0	0	0	0	0	0	0	0	0	0	0
304	0	0	0	0	0	0	0	0	0	0	0	0
305	0	0	0	0	0	0	0	0	0	0	0	0
306	0	0	0	0	0	0	0	0	0	0	0	0
307	0	0	0	0	0	0	0	0	0	0	0	0
308	0	0	0	0	0	0	0	0	0	0	0	0
309	0	0	0	0	0	0	0	0	0	0	0	0
310	0	0	0	0	0	0	0	0	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 12	Erythema Day 13	Erythema Day 14	Erythema Day 15	Erythema Day 16	Erythema Day 17	Erythema Day 18	Erythema Day 19	Erythema Day 20	Erythema Day 21	Erythema Day 22	Erythema Day 23
Male, III H-26137												
301	0	0	0	0	0	0	0	0	0	0	0	0
302	0	0	0	0	0	0	0	0	0	0	0	0
303	0	0	0	0	0	0	0	0	0	0	0	0
304	0	0	0	0	0	0	0	0	0	0	0	0
305	0	0	0	0	0	0	0	0	0	0	0	0
306	0	0	0	0	0	0	0	0	0	0	0	0
307	0	0	0	0	0	0	0	0	0	0	0	0
308	0	0	0	0	0	0	0	0	0	0	0	0
309	0	0	0	0	0	0	0	0	0	0	0	0
310	0	0	0	0	0	0	0	0	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
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Individual Edema and Erythema Scores

	Erythema Day 24	Erythema Day 25	Erythema Day 26	Erythema Day 27
Male, III H-26137				
301	0	0	0	0
302	0	0	0	0
303	0	0	0	0
304	0	0	0	0
305	0	0	0	0
306	0	0	0	0
307	0	0	0	0
308	0	0	0	0
309	0	0	0	0
310	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 0	Erythema Day 1	Erythema Day 2	Erythema Day 3	Erythema Day 4	Erythema Day 5	Erythema Day 6	Erythema Day 7	Erythema Day 8	Erythema Day 9	Erythema Day 10	Erythema Day 11
Male, V H-26138												
501	0	0	0	0	0	0	0	0	0	0	0	0
502	0	0	0	0	0	0	0	0	0	0	0	0
503	0	0	0	0	0	0	0	0	0	0	0	0
504	0	0	0	0	0	0	0	0	0	0	0	0
505	0	0	0	0	0	0	0	0	0	0	0	0
506	0	0	0	0	0	0	0	0	0	0	0	0
507	0	0	0	0	0	0	0	0	0	0	0	0
508	0	0	0	0	0	0	0	0	0	0	0	0
509	0	0	0	0	0	0	0	0	0	0	0	0
510	0	0	0	0	0	0	0	0	0	0	0	0

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 12	Erythema Day 13	Erythema Day 14	Erythema Day 15	Erythema Day 16	Erythema Day 17	Erythema Day 18	Erythema Day 19	Erythema Day 20	Erythema Day 21	Erythema Day 22	Erythema Day 23
Male, V H-26138												
501	0	0	0	0	0	C	0	0	0	0	0	0
502	0	0	0	0	0	C	0	0	0	0	0	0
503	0	0	0	0	0	C	0	0	0	0	0	0
504	0	0	0	0	0	C	0	0	0	0	0	0
505	0	0	0	0	0	C	0	0	0	0	0	0
506	0	0	0	0	0	C	0	0	0	0	0	0
507	0	0	0	0	0	C	0	0	0	0	0	0
508	0	0	0	0	0	C	0	0	0	0	0	0
509	0	0	0	0	0	C	0	0	0	0	0	0
510	0	0	0	0	0	C	0	0	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 24	Erythema Day 25	Erythema Day 26	Erythema Day 27
Male, V H-26138				
501	0	0	0	0
502	0	0	0	0
503	0	0	0	0
504	0	0	0	0
505	0	0	0	0
506	0	0	0	0
507	0	0	0	0
508	0	0	0	0
509	0	0	0	0
510	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 0	Erythema Day 1	Erythema Day 2	Erythema Day 3	Erythema Day 4	Erythema Day 5	Erythema Day 6	Erythema Day 7	Erythema Day 8	Erythema Day 9	Erythema Day 10	Erythema Day 11
Male, VII H-26139												
701	0	0	0	0	0	0	0	0	0	0	0	0
702	0	0	0	0	0	0	0	0	0	0	0	0
703	0	0	0	0	0	0	0	0	0	0	0	0
704	0	0	0	0	0	0	0	0	0	0	0	0
705	0	0	0	0	0	0	0	0	0	0	0	0
706	0	0	0	0	0	0	0	0	0	0	0	0
707	0	0	0	0	0	0	0	0	0	0	0	0
708	0	0	0	0	0	0	0	0	0	0	0	0
709	0	0	0	0	0	0	0	0	0	0	0	0
710	0	0	0	0	0	0	0	0	0	0	0	0

Study: 14992 1012

Print Date: 15-Jan-2004
Print Time: 12:07:19

Individual Edema and Erythema Scores

	Erythema Day 12	Erythema Day 13	Erythema Day 14	Erythema Day 15	Erythema Day 16	Erythema Day 17	Erythema Day 18	Erythema Day 19	Erythema Day 20	Erythema Day 21	Erythema Day 22	Erythema Day 23
Male, VII H-26139												
701	0	0	0	0	0	0	0	0	0	0	0	0
702	0	0	0	0	0	0	0	0	0	0	0	0
703	0	0	0	0	0	0	0	0	0	0	0	0
704	0	0	0	0	0	0	0	0	0	0	0	0
705	0	0	0	0	0	0	0	0	0	0	0	0
706	0	0	0	0	0	0	0	0	0	0	0	0
707	0	0	0	0	0	0	0	0	0	0	0	0
708	0	0	0	0	0	0	0	0	0	0	0	0
709	0	0	0	0	0	0	0	0	0	0	0	0
710	0	0	0	0	0	0	0	0	0	0	0	0

Study: 14992 1012

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Individual Edema and Erythema Scores

	Erythema Day 24	Erythema Day 25	Erythema Day 26	Erythema Day 27
Male, VII H-26139				
701	0	0	0	0
702	0	0	0	0
703	0	0	0	0
704	0	0	0	0
705	0	0	0	0
706	0	0	0	0
707	0	0	0	0
708	0	0	0	0
709	0	0	0	0
710	0	0	0	0

APPENDIX F

[redacted] Analytical Report

STUDY TITLE

H-26137, H26138, and H-26139: Repeated-Dose Dermal Toxicity 28-Day Study in Male Rats

DATA REQUIREMENTS

Analytical Method Requirements

STUDY DIRECTOR

Carol Finlay

ANALYTICAL PHASE COMPLETED ON

March 10, 2004

PERFORMING LABORATORY

Exygen Research
3058 Research Drive
State College, PA 16801
Phone: 814-272-1039

STUDY SPONSOR

E. I. du Pont de Nemours and Company (DuPont)
P.O. Box 50
Newark, DE 19714-0050 USA

PROJECT

Exygen Study Numbe[redacted]
Sponsor Study No.: DuPont-13882

Total Pages: 63

Exygen Study No [redacted]

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number [redacted] entitled "H-26137, H26138, and H-26139: Repeated-Dose Dermal Toxicity 28-Day Study in Male Rats," was conducted for DuPont in compliance with US EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with OECD Principles of Good Laboratory Practice (as revised in 1997), published in ENV/MS/CHEM(98)17, except for the item documented below. This item does not impact the validity of the study.

1. There was no in-lab audit for this study [redacted]. However, an in-lab audit was conducted for study [redacted] the same procedures performed by the same personnel were observed for the same client within the last six months. There were no technical problems observed. The in-lab audit for [redacted] will serve as a process-based inspection for this study.

Emily R. Decker
Principal Investigator
Exygen Research

3/10/04
Date

Carol Finlay
Study Director
DuPont

16-Mar-2004
Date

S. Mark Kennedy
Sponsor Representative
DuPont

16-MAR-2004
Date

Exygen Study No. [redacted]

QUALITY ASSURANCE STATEMENT

Exygen Research's Quality Assurance Unit reviewed Exygen Study Number [redacted], the analytical phase of the study entitled, "H-26137, H26138, and H-26139: Repeated-Dose Dermal Toxicity 28-Day Study in Male Rats". All phases were reviewed for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

Phase	Date Inspected	Date Reported to Principal Investigator	Date Reported to Exygen Management	Date Reported to Study Director and Sponsor Management
*1. Standard Preparation, Fortification, and Extraction	01/13/04	01/15/04	01/19/04	02/23/04
2. Raw Data & Draft Analytical Report Review	01/23,26/04	01/28/04	01/29/04	02/23/04
3. Final Analytical Report Review	02/23/04	02/23/04	02/23/04	02/23/04

Miwa Nabetani 3/10/04

Miwa Nabetani
Quality Assurance Auditor

Date

* Exygen QA observed an in-lab procedure for study [redacted] on the above date. This procedure was also used in the current study and was performed by the same personnel for the same client within a six-month period of the original lab inspection. Since no technical problems were observed during the original in-lab inspection, a process-based inspection for this procedure was issued for this study ([redacted]).

Exygen Study No.: [redacted]

CERTIFICATION OF AUTHENTICITY

This report, for Exygen Study Number [redacted] is a true and complete representation of the raw data for the study.

Submitted by: Exygen Research
3058 Research Drive
State College, PA 16801
(814) 272-1039

Principal Investigator, Exygen:

Emily R. Decker — Date: 3/10/04
Scientist/Principal Investigator
Exygen Research

Exygen Research Facility Management:

John M. Flaherty — Date: 3/10/04
Vice President
Exygen Research

Study Director, DuPont:

Carol Finlay — Date: 16-Mar-2004
DuPont

Sponsor Representative, DuPont:

S. Mark Kennedy — Date: 16-MAR-2004
DuPont

Exygen Study No.: [redacted]

EXYGEN STUDY IDENTIFICATION

H-26137, H26138, and H-26139: Repeated-Dose Dermal Toxicity 28-Day Study in Male Rats

SPONSOR STUDY NUMBER:	DuPont-13882
EXYGEN STUDY NUMBER:	[redacted]
~~TYPE OF ANALYTICAL PHASE~~:	~~Residue~~
SAMPLE MATRIX:	Rat Plasma
TEST SUBSTANCE:	[redacted]
SPONSOR:	E. I. du Pont de Nemours and Company P.O. Box 50 Newark, DE 19714-0050 Phone: (302) 366-5255
STUDY DIRECTOR:	Carol Finlay DuPont
PERFORMING LABORATORY:	Exygen Research 3058 Research Drive State College, PA 16801
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 11/18/03 Analytical Start Date: 01/12/04 Analytical Termination Date: 01/13/04 Analytical Report Completion Date: 03/10/04

Exygen Study No.: [redacted]

PROJECT PERSONNEL

The Study Director for this project at DuPont was Carol Finlay. The following personnel from Exygen Research were associated with various phases of the study:

Name	Title
Paul Connolly	Technical Lead-LC/MS
Emily Decker	Scientist
Karen Risha	Scientist
Mark Neeley	Scientist
Shawn Robb	Sample Custodian

Exygen Study No.: [redacted]

TABLE OF CONTENTS

LIST OF TABLES

Exygen Study No.: [redacted]

LIST OF FIGURES

LIST OF APPENDICES

Exygen Study No. [redacted]

1.0 SUMMARY

Exygen Research (Exygen) analyzed samples of rat plasma for residues of [redacted] in according to protocol DuPont-13882 (**Appendix A**) using the analytical method [redacted] sion 2 (**Appendix B**).

The limit of quantitation for this method is 10 ppb, which was established at Exygen in the method validation study 008-277.

Residues of [redacted] n the rat plasma samples ranged from < 10.0 ppb to 230 ppb.

The average recovery ± standard deviation fo [redacted] fortified rat plasma samples was 89% ± 11.1%.

2.0 OBJECTIVE

The objective of this analytical phase was to analyze rat plasma samples received at Exygen using the method [redacted] entitled "Method of Analysis for the Determination of P [redacted] Serum by LC/MS/MS".

3.0 INTRODUCTION

This report details the results of the analysis of rat plasma samples for PFOA using the analytical method [redacted]

The study was initiated on November 18, 2003, when the study director signed protocol number DuPont-13882. The analytical start date was January 12, 2004, and the analytical termination date was January 13, 2004.

4.0 TEST SYSTEM

The control rat plasma used for control samples and laboratory control fortified samples was received from DuPont on 10/01/03. Forty rat plasma samples were received at Exygen on January 06, 2004. All samples were logged in by Exygen personnel upon receipt and placed in frozen storage (≤ -20°C).

Sample login and chain of custody information can be found in the raw data package associated with this study. Storage records will be kept at Exygen Research and a true copy of the storage records will be furnished upon request.

Exygen Study No.: [redacted]

5.0 REFERENCE MATERIAL

The analytical standard [redacted] was received at Exygen on March 05, 2003 from Critical Path Services. The control article (internal standard) n-perfluorooctanoic acid (1,2 di-^{13}C) was received at Exygen on February 20, 2003 from DuPont.

The available information for the reference material is listed below. The analytical standard was stored in a refrigerator and the control article was stored at room temperature.

Compound	Exygen Inventory No.	Lot No.	Purity (%)	Expiration Date
[redacted]			97	03/05/04
			96.35	01/31/05

The structure of [redacted] and the internal standard are given below.

[redacted]
Chemical Name = [redacted]
Molecular weight = [redacted]

[redacted]

[redacted]
Chemical Name = [redacted]
Molecular weight = [redacted]

[redacted]

Exygen Study No.: [redacted]

6.0 DESCRIPTION OF ANALYTICAL METHOD

6.1 Extraction Procedure

1. Measure 0.1 mL of sample into 1 mL eppendorf centrifuge tubes (fortify with analyte as needed, close lid, and vortex ~ 10 seconds).
2. Add enough acetonitrile containing internal standard at 0.005 µg/mL (accounting for fortification volume) to make extraction volume 500 µL and vortex for ~ 10 seconds.
3. Centrifuge for ~ 10 minutes at ~ 14,000 rpm.
4. Analyze samples using electrospray LC/MS/MS.

6.2 Preparation of Standards and Fortification Solutions

The stock standard solution of [redacted] was prepared on September 17, 2003, at a concentration of 100 µg/mL by dissolving approximately 10 mg of the standard (corrected for purity) in methanol. The stock standard solution of ^{13}C[redacted] was prepared on September 17, 2003, at a concentration of 100 µg/mL by dissolving approximately 10 mg of the standard (corrected for purity) in methanol. A fortification solution of [redacted] at 10 µg/mL was prepared by taking 10 mL of the stock standard solution and bringing the volume up to 100 in acetonitrile. A 1.0 µg/mL fortification solution of ^{13}C[redacted] was prepared by taking 1.0 mL of the stock and bringing the volume up to 100 mL with acetonitrile.

A set of standards containing [redacted] with internal standards was prepared by dilution of the 10.0 µg/mL [redacted] solution and the 1.0 µg/mL ^{13}C[redacted] solution in the following manner. These solutions were used to fortify the samples and the extracted calibration standards.

Initial Conc. (µg/mL)[1]	Volume (mL)	Diluted to (mL)	Final Conc. (µg/mL)[1]
10.0	10.0	100[2]	1.0
10.0	1.0	100[2]	0.1
10.0	0.1	100[2]	0.01

[1] of [redacted] only
[2] 0.5 mL of the 1.0 µg/mL ^{13}C-[redacted] solution was added prior to making final volume to give a concentration of 0.005 µg/mL of ^{13}C[redacted] in each solution.

The calibration standards are processed through the extraction procedure, identical to the samples. The extracted standards are assigned a two-week expiration date from the date of extraction based on refrigerator stability data obtained during the method validation study (Exygen study: [redacted]). The fortification of the standards before extraction is done according to the following table:

Exygen Study No. [redacted]

Conc. Of [redacted] Fortification Solution (µg/mL)	Fortification Volume (µL)	Volume of Control Sample (mL)	Conc. of Extracted Calibration Standard (ppb)
0.01	100	0.1	10
0.01	200	0.1	20
0.1	50	0.1	50
0.1	100	0.1	100
0.1	200	0.1	200

The extraction solution was prepared by taking 0.5 mL of the 1.0 µg/mL ^{13}C[redacted] solution and bringing the volume up to 100 mL with acetonitrile. The dilution solution was prepared by taking 0.5 mL of the 1.0 µg/mL ^{13}C[redacted] solution and bringing the volume up to 100 mL with 50:50 methanol:water.

The stock standard solution and all fortification/calibration standard solutions were stored in a refrigerator (4° ± 2°C) when not in use. Documentation of standard preparation can be found in the raw data associated with this report.

6.3 Chromatography

Quantification of [redacted] was accomplished by analysis using electrospray LC-MS/MS. The retention time of [redacted] was ~ 2.0 min., with no significant interfering peaks (< 20% of the LOQ standard) in the control samples corresponding to the analyte retention times.

6.4 Instrument Sensitivity

The smallest amount of [redacted] injected during the chromatographic run was equivalent to 10 ppb.

6.5 Description of Instrument and Operating Conditions

A PE Sciex LC-MS/MS coupled to a Hewlett Packard HPLC system was used. Data acquisition and processing were performed using Analyst 1.2 software.
Detailed operating conditions are listed below:

Mass Spec:	PE SCIEX API 4000 Biomolecular Mass Analyzer
Interface:	SCIEX Turbo Ion Spray Liquid Introduction Interface Turbo Ion Spray temperature = 350° C with N2 at ~7 L/min
Computer:	Dell Optiplex GX110
Software:	PE Sciex Analyst: Version 1.2
HPLC:	Hewlett Packard (HP) Series 1100

HP Quat Pump
HP Vacuum Degasser
HP Autoinjector
HP Column Compartment

Filter Cartridge(s): 2 Keystone Hypercarbs in tandem, exiting pump
HPLC Column: Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temp.: 30° C
Mobile Phase (A): 2 mM Ammonium Acetate in water
Mobile Phase (B): Methanol

Time	%-A	%-B	Flow (mL/min)
0	40	60	0.3
3.0	40	60	0.3
3.5	0	100	0.3
3.7	0	100	0.5
7.0	0	100	0.5
7.5	40	60	0.5
9.0	40	60	0.5
9.5	40	60	0.3
12.0	40	60	0.3

Injection Vol.: 5 µL

Ions monitored:

Analyte	Mode	Transition Monitored
[redacted]	Negative	413 → 369
[redacted]	Negative	415 → 370

6.6 Quantitation and Example Calculation

Five microliters of sample or calibration standard were injected into the LC-MS/MS. The peak area was measured and the standard curve was generated by linear regression using 1/x weighting of the ratio analyte peak area/internal standard peak area versus the ratio of the concentration of analyte/concentration of internal standard using Analyst 1.2 (or equivalent) software system. The residue concentration for [redacted] was determined from the following equations:

Equation 1 was used to calculate the amount of analyte found (in ppb or ng/mL, based on ratio of analyte peak area to internal standard peak area) using the standard curve (linear regression parameters) generated by the Analyst software program.

Equation 1:

$$\text{Analyte found (ppb)} = \frac{((\text{Analyte Peak area/IS peak area}) - \text{intercept})) \times \text{IS conc. (ng/mL)} \times \text{DF}}{\text{slope}}$$

Exygen Study No.: [redacted]

For samples fortified with known amounts of analyte prior to extraction, Equation 2 was used to calculate the percent recovery.

Equation 2:
Recovery (%) =

$$\frac{\text{Analyte found (ppb)} - \text{avg. Analyte found in control (ppb)}}{\text{Amount Analyte added (ppb)}} \times 100$$

For plasma samples fortified with known amounts of analyte prior to extraction, Equation 3 was used to calculate the percent recovery.

Equation 3:
Recovery (%) =

$$\frac{\text{Analyte found (ppb)} - \text{avg. Analyte found in sample (ppb)}}{\text{Amount Analyte added (ppb)}} \times 100$$

An example of a calculation using an actual sample follows: Rat plasma sample Exygen [redacted], fortified at 10 ppb with [redacted]
Where:

analyte peak area	=	170017
IS peak area	=	413671
IS conc. (ng/mL)	=	4.167 ng/mL
intercept	=	0.0146455
slope	=	0.1712
dilution factor (DF)	=	1
ppb added (fort level)	=	10
amt found in control	=	ND

From equation 1:

$$\text{Analyte found (ppb)} = \frac{[(170017/413671) - 0.0146455] \times 4.167 \times 1}{0.1712}$$

$$= 9.65 \text{ ppb}$$

From equation 2:

$$\% \text{ Recovery} = \frac{(9.65 \text{ ppb} - 0)}{10 \text{ ppb}} \times 100$$

$$= 97\%$$

Note: This example calculation was done using rounded numbers, and therefore may be slightly different from the values shown in the RAW DATA.

Exygen Study No. [redacted]

7.0 EXPERIMENTAL DESIGN

Each analytical set was extracted according to method [redacted] and consisted of a reagent blank, one matrix control, two laboratory matrix controls fortified at known concentrations, one field sample fortified at a known concentration, 20 plasma samples, and one field sample extracted in duplicate.

8.0 RESULTS

The [redacted] found in the control rat plasma samples are listed in **Table I.**

Individual recoveries for [redacted] in the laboratory fortified rat plasma samples are detailed in **Table II.** The average percent recoveries ± standard deviation for [redacted] in the rat plasma samples was 89% ± 11.1%.

Individual results for the [redacted] in the rat plasma samples are given in **Table III.** Residues of [redacted] in the rat plasma samples ranged from < 10.0 ppb to 230 ppb.

A typical calibration curve for [redacted] and representative chromatograms are given in **Figures 1-5.**

9.0 CIRCUMSTANCES THAT MAY HAVE AFFECTED THE QUALITY OR INTEGRITY OF THE DATA

There are no known circumstances that have affected the quality or integrity of the data.

10.0 CONCLUSIONS

The rat plasma samples were successfully analyzed according to method [redacted]

11.0 RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Exygen Research will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Exygen Research's archives for the period of time specified in 40 CFR Part 792. Retained samples of reference substances are archived by the sponsor.

Exygen Study No. [redacted]

TABLES

Exygen Study No [redacted]

Table I. Summary of [redacted] (ppb) in Rat Plasma Control Samples

Sponsor ID	[redacted] (ng/mL)
670127 Control A 01/12/04	ND
670127 Control A2 01/12/04	ND

ND = Not Detected (less than 10.0 ppb)

Exygen Study No. [redacted]

Table II. Summary of Recoveries ([redacted]%) in Rat Plasma Samples

Sponsor ID	[redacted] (ppb)	Amount Fortified (ppb)	Recovery (%)
[redacted] S A) 01/12/04	9.65	10.0	96
[redacted]) 01/12/04	1030	1000	103
[redacted]) 01/12/04	697	1000	70
[redacted]) 01/12/04	8.61	10.0	86
[redacted]2) 01/12/04	896	1000	90
[redacted]) 01/12/04	1030	1000	91
		AVERAGE:	89
		STD DEV:	11.1
		% RSD:	12.5

LCS = Laboratory Control Spike

Exygen Study No. [redacted]

Table III. Summary of Residue Found for [redacted] (ppb) in Rat Plasma Samples

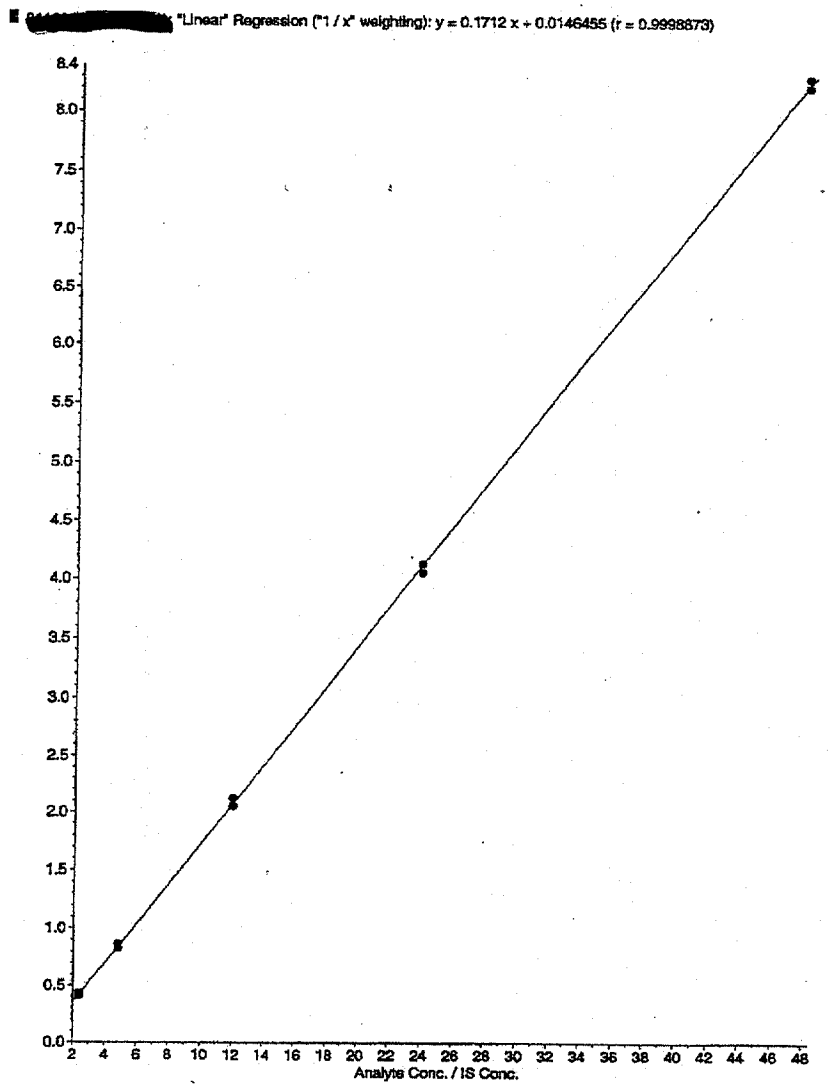
Sponsor ID	PFOA (ppb)
101	< 10.0
101 Dup	< 10.0
102	< 10.0
103	< 10.0
104	< 10.0
105	< 10.0
106	< 10.0
107	< 10.0
108	< 10.0
109	< 10.0
110	< 10.0
301	25.2
302	36.7
303	36.0
304	39.4
305	34.1
306	34.6
307	40.9
308	37.3
309	29.2
310	43.6
501	114
501 Dup	116
502	79.9
503	72.8
504	103
505	75.2
506	102
507	158
508	70.2
509	112
510	95.3
701	196
702	73.9
703	98.6
704	229
705	144
706	149
707	230
708	170
709	115
710	187

Exygen Study No. [redacted]

FIGURES

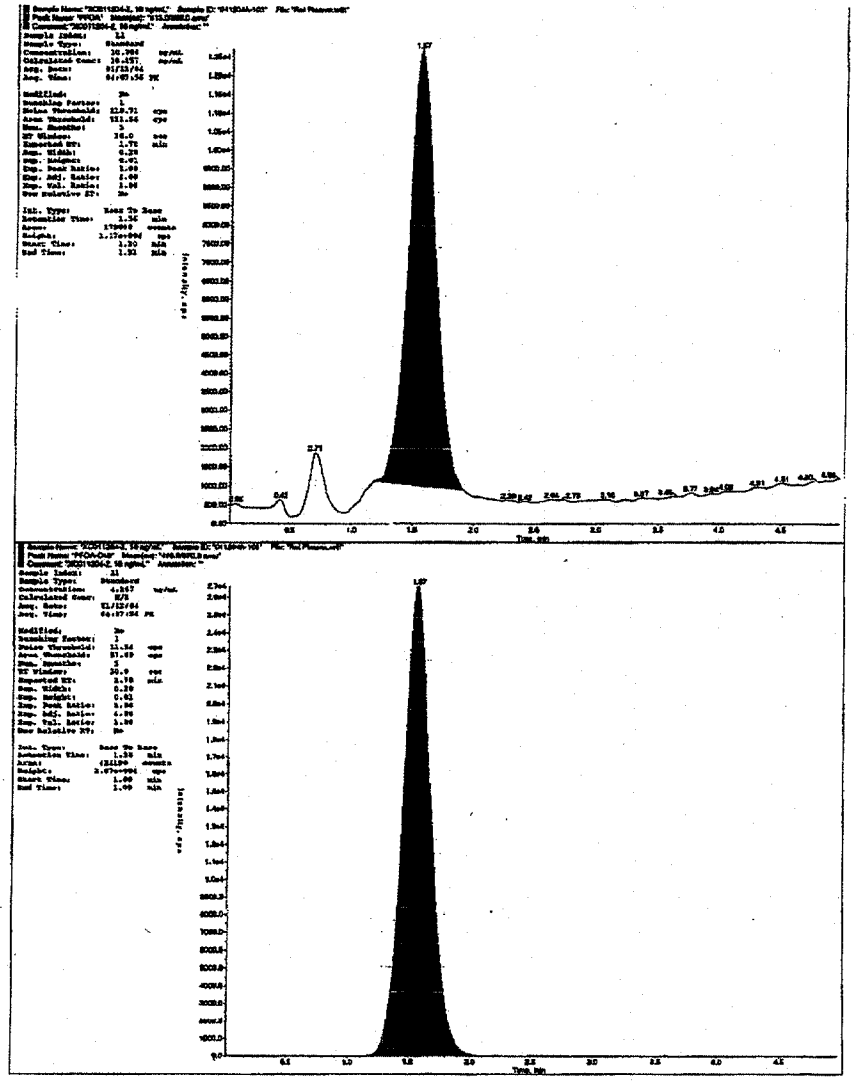
Exygen Study No. [redacted]

Figure 1. Typical Calibration Curve for [redacted]



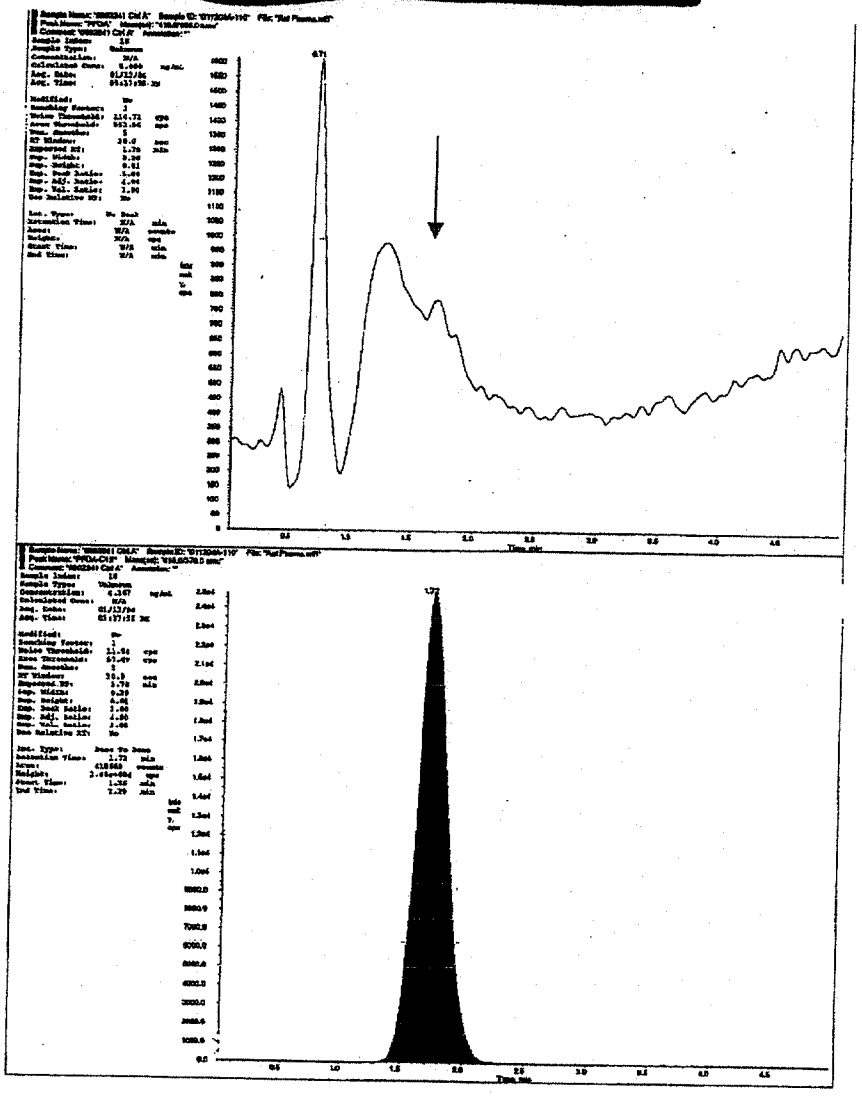
Exygen Study No. [redacted]

Figure 2. Chromatogram Representing a Calibration Standard for [redacted] at 10 ppb with ~ 5 ng/mL of ^{13}C[redacted]



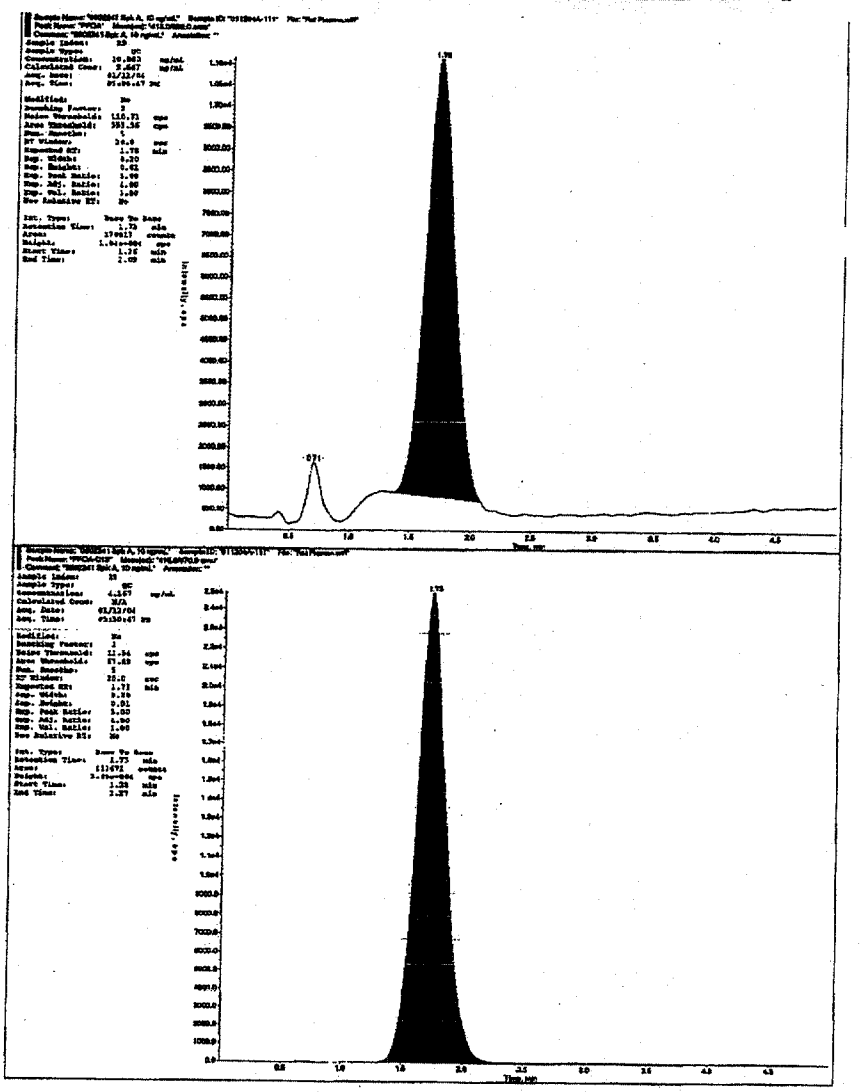
Exygen Study No. [redacted]

Figure 3. Chromatogram Representing Control Rat Plasma for [redacted] with ~ 5 ng/mL of ^{13}C[redacted] (Exygen ID: [redacted])



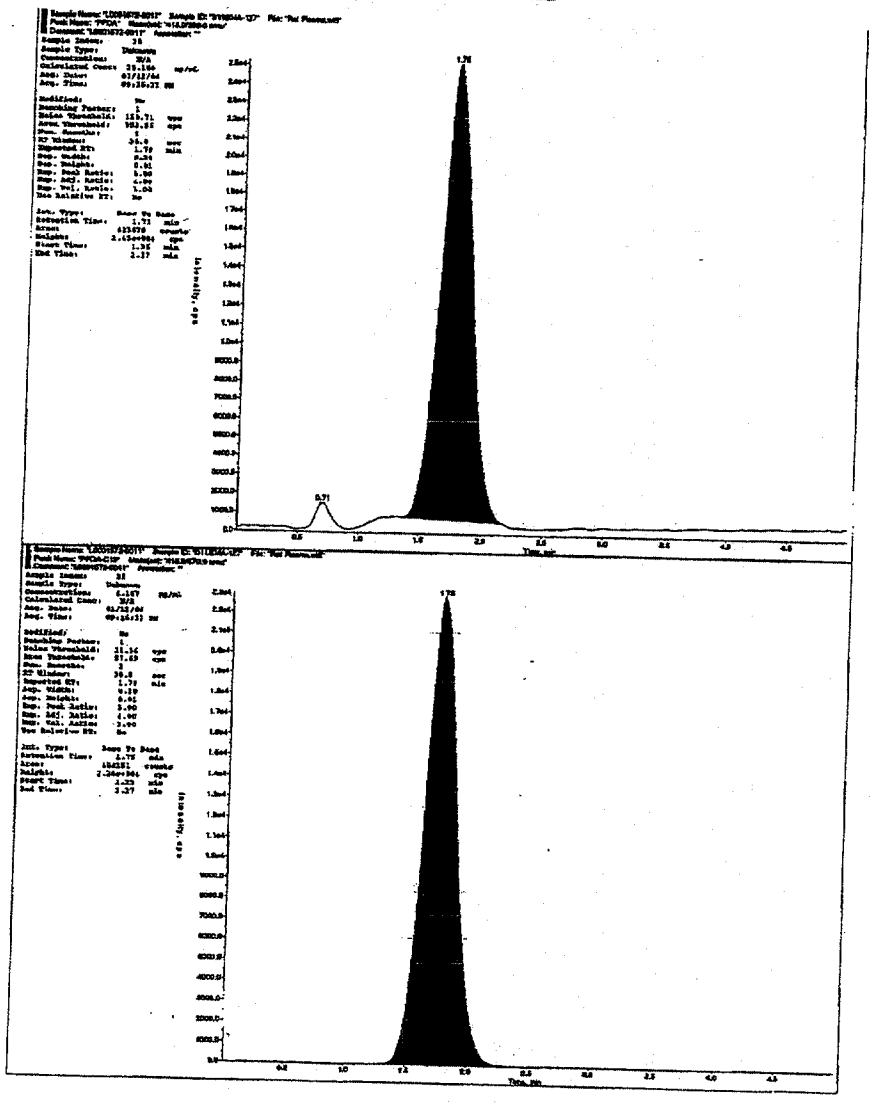
Exygen Study No.: [redacted]

Figure 4. Chromatogram Representing Control Rat Plasma Fortified at 10 ppb with [redacted] and ~ 5 ng/mL of ^{13}C-[redacted]



Exygen Study No.: [redacted]

Figure 5. Chromatogram Representing Rat Plasma Sample for [redacted] with ~ 5 ng/mL of [redacted]



Exygen Study No.: [redacted]

APPENDIX A

Study Protocol
DuPont-13882
(Exygen Study No. [redacted]
and
Amendment and Deviation

Exygen Study No. [redacted]

DuPont-13882

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats

Work Request Numbe[redacted]

Service Code[redacted]

Protocol

Haskell Animal Welfare Committee Number: [redacted]

Exygen Study No. [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

TABLE OF CONTENTS

Exygen Study No.: [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats
DuPont-13882

INTRODUCTION

The materials to be tested, H-26137, H-26138, and H-26139, are fabrics containing varying amounts of melt additive. The results of this study will be compared to the results of a previously conducted study with the neat melt additive.[1]

OBJECTIVE

The objective of this study is to evaluate the potential toxicity of H-26137, H-26138, and H-26139 when administered dermally to male rats for 28 days. The dermal route of administration was selected because it is a potential route of human exposure.

SPONSOR AND TEST FACILITY

This study is sponsored by 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 Work Authorization Form.

The study will be conducted at Haskell Laboratory for Health and Environmental Sciences, E.I. du Pont de Nemours and Company, Newark, Delaware.

REGULATORY COMPLIANCE

This study will be 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.[2,3] The study design is based on U.S. Environmental Protection Agency (EPA) Office of Prevention, Pesticides, and Toxic Substances (OPPTS),[4] Organization for Economic Cooperation and Development (OECD),[5] test guidelines. Areas of noncompliance will be documented in the final report.

Exygen Study No.: [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats
DuPont-13882

STUDY DESIGN

The study design is as follows:

Group	Number/Group	Haskell Laboratory Number
I	10	H-26136 (Control)
III	10	H-26137
V	10	H-26138
VII	10	H-26139

Study Parameters	Frequency
Body Weight	Test day 0 and weekly thereafter
Food Consumption	Test day 0 and weekly thereafter
Clinical Observations	Daily
Observations for dermal effects	Daily
Mortality/Moribundity Checks	Twice daily
Clinical Pathology	Week 4
PFOA blood analysis	Week 4
Necropsy	Week 4

MATERIALS AND METHODS

A. Materials

The materials (fabrics) were supplied by the sponsor and assigned the unique Haskell Laboratory Numbers H-26136 (for the control) and H-26137, H-26138, and H-26139 (for the test materials).

B. Test Species

Male Crl:CD®(SD)IGS BR rats will be obtained from Charles River Laboratories, Inc. The location of the supplier (city/state) will be documented in the study records and final report. The Crl:CD®(SD)IGS BR rat has been selected on the bases of extensive experience with this strain at Haskell Laboratory and its suitability with respect to hardiness, longevity, sensitivity, and low incidence of spontaneous diseases.

C. Animal Husbandry

All rats will be housed in stainless steel, wire-mesh cages suspended above cage boards. Animal rooms will be maintained at a temperature of 22 ± 3°C and a relative humidity of 50 ± 20%.

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H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

Animal rooms will be artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle.

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

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. Data are maintained separately from study records and may be included in the final report at the discretion of the study director.

D. Pretest Period

Upon arrival at Haskell Laboratory, all rats will be housed one per cage in quarantine. The rats will be:

- quarantined for a minimum of 5 days.
- identified temporarily by cage identification.
- weighed at least 3 times during quarantine.
- observed with respect to weight gain and any gross signs of disease or injury.

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

Rats that are accidentally killed or removed from study during the pretest period will be discarded without necropsy. Rats that are found dead or sacrificed *in extremis* during the pretest period will be sent to Pathology and given a gross examination to check for the presence of disease. Dependent upon these findings, further diagnostic procedures may be employed at the discretion of the study director, a pathologist, or the laboratory animal veterinarian. The results will not be reported in the final report unless considered significant to the evaluation of the study.

Exygen Study No.: [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

E. Assignment to Groups

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

Each rat will be housed individually and assigned an animal number. Prior to assignment to groups, each rat will be temporarily identified by cage identification. After assignment to groups, the animal number will be tattooed on the tail of each rat and included on the cage label.

At study start (test day 0) the rats will be 8 weeks old. On test day 0, when possible, rats with body weights that are not within ± 20% of the mean will be removed from study and replaced with rats having body weights within that range (subject to the same selection criteria as the original rats).

Rats that have not been assigned to a test group or which have been removed from study on test day 0, for out-of-range body weight, will be released for other laboratory purposes, or be sacrificed by carbon dioxide asphyxiation and discarded without pathological evaluation, at the discretion of the study director.

F. Administration of Test Substance

Approximately 24 hours prior to the first treatment, the fur of each rat will be closely shaved to expose the skin from the back and trunk. The fabric will be slightly moistened with deionized water, wrapped around the animal in a single layer, and secured. The approximate area of skin covered with each fabric will be documented in the study records. The rats will be fitted with plastic collars during the exposure period to prevent oral exposure to the wrapping.

The rats will be treated for 28 consecutive days. The exposure period will be approximately 6 hours each day. After the exposure period, the fabrics and collars will be removed and the shaved area will be washed with warm tap water. The shaved area of each rat will then gently be patted dry and the rat returned to its cage.

The animals will be reshaved during the study as needed. The entire area that was originally shaved will be reshaved. The animals will be reshaved only after an evaluation. On the day of necropsy, the fur will be shaved from an untreated area and outlined.

G. Body Weights

The rats will be weighed at weekly intervals during the study.

Exygen Study No.: [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

H. Food Consumption and Food Efficiency

The amount of food consumed by each rat will be determined weekly by weighing each feeder at the beginning and end of the week and subtracting the final weight and the amount of spillage from the feeder during the week from the initial weight. From these measurements, mean daily food consumption over the week will be determined. From the food consumption and body weight data, the mean daily food efficiency will be calculated.

I. Clinical Observations and Mortality

The rats will be observed for clinical signs and dermal effects after removal of the test fabric. The rats will also be observed for clinical signs at each weighing. The Draize Scale will be used to score skin irritation. Rats will be checked twice daily for mortality and for signs of illness, injury, or abnormal behavior.

J. Perfluorooctanoic Acid Level Evaluation

Blood (approximately 2 mL) will be collected from the vena cava of all rats at necropsy into a tube containing EDTA. Plasma will be prepared and stored frozen at -80°C to -20°C until analyzed for concentration of [redacted]. Plasma will be evaluated from all rats. Plasma samples will be extracted by organic solvent protein precipitation and then analyzed for [redacted] by an appropriate method at Exygen Research (3058 Research Drive, State College, PA 16801).

K. Clinical Pathology Evaluation

A clinical pathology evaluation will be conducted on all rats the day after the last dose. The day before collection of blood samples for the clinical pathology evaluation, the animals will be placed in metabolism cages. These animals will be fasted overnight (approximately 16 hours) and urine will be collected from each animal. Blood samples for clinical chemistry measurements will be collected from the orbital sinus of each animal while the animal is under light carbon dioxide anesthesia. Blood samples for PFOA will be collected at sacrifice from the abdominal *vena cava* of each animal while the animal is under carbon dioxide anesthesia. Additional blood collected from the *vena cava* will be placed in a serum tube, processed to serum, and frozen at -80°C to -20°C.

At the discretion of the study director or clinical pathologist, additional samples for selected clinical pathology tests will be collected from animals showing clinical evidence of toxicity or sacrificed *in extremis*.

Exygen Study No. [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

1. Clinical Chemistry

The following serum chemistry parameters will be determined:

cholesterol
triglycerides

2. Urinalysis

The following urinalysis parameter will be determined:

fluoride

L. Anatomical Pathology

1. Pretest

See Materials and Methods, Section D. Pretest Period.

2. Dosing Phase

All rats found dead, accidentally killed, sacrificed *in extremis*, or sacrificed by design will undergo a gross evaluation. All rats removed from study (except for out-of-range body weight on test day 0) will be sent to Pathology for gross evaluation and collection of tissues. Rats will be euthanatized by carbon dioxide anesthesia and exsanguination. Rats sacrificed by design will be fasted after 3 p.m. on the afternoon before their scheduled sacrifice. A final sacrifice will be performed on surviving rats following the final clinical pathology evaluation. The order of sacrifice for scheduled deaths will be random among all treatment groups.

The following tissues will be collected from rats which are found dead or accidentally killed (tissue integrity permitting), sacrificed *in extremis*, removed from study (except out-of-range body weight on test day 0), or sacrificed by design:

liver [a]
kidneys [a]
thyroid gland (after fixation) [a]
skin (treated and untreated)
gross observations [b]

a Organs to be weighed
b Gross observations made at necropsy for which histopathology is not appropriate (e.g., fluid, ruffled fur, and missing anatomic parts) will generally not be collected.

All tissues will be placed in the appropriate fixative.

Exygen Study No. [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

Rats sacrificed by design will have the following organs weighed: liver, kidneys, and thyroid. Relative organ weights (percent of final body weight) will be calculated. Final body weights determined just prior to necropsy will be used in the assessment of organ weight changes. Organs from rats found dead, sacrificed *in extremis*, or accidentally killed may be weighed at the discretion of the pathologist or study director.

Tissues collected from rats sacrificed by design, and from rats that are found dead or accidentally killed (tissue integrity permitting), or are sacrificed *in extremis*, may be further processed to slides, stained with hematoxylin and eosin, and examined microscopically at the discretion of the study director or pathologist. Selected gross observations for which a microscopic diagnosis would not be additive (e.g., osteoarthritis, pododermatitis, chronic tail dermatitis, calculus, and deformities of the teeth, toe, tail, or ear pinna) will be saved, but will generally not be processed for microscopic evaluation. Tissues from rats removed from study (for other than out-of-range body weight on test day 0), will not be processed for microscopic evaluation unless considered necessary by the study director or pathologist.

Additional procedures to identify and/or clarify histologic features of lesions may be performed at the discretion of the pathologist and will be documented in the final report.

Exygen Study No. [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
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STATISTICAL ANALYSES

Significance will be judged at $p < 0.05$.

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight Body Weight Gain Food Consumption Food Efficiency Organ Weight	Test for lack of trend[6]	Sequential application[7] of the Jonckheere-Terpstra trend test[6]	Preliminary tests for pairwise comparison
	OR[a]		
	Levene's test for homogeneity[9] and Shapiro-Wilk test[10] for normality[b]	One-way analysis of variance[11] followed with Dunnett's test[12]	Kruskal-Wallis test[13] followed with Dunn's test[14]
Clinical Pathology[c]	Levene's test for homogeneity[9] and Shapiro-Wilk test[10] for normality[b]	One-way analysis of variance[11] followed with Dunnett's test[12]	Kruskal-Wallis test[13] followed with Dunn's test[14]
Survival Incidence of Clinical Observations Incidence of Dermal Effects	None	Cochran-Armitage test for trend[11][d]	

a Pairwise comparisons and associated preliminary tests are only conducted if the test for lack of trend is significant.
b If the Shapiro-Wilk test is not significant but Levene's test is significant, a robust version of Dunnett's test will be used.
c When an individual observation is recorded as being less than a certain value, calculations are performed on half the recorded value. For example, if bilirubin is reported as <0.1, 0.05 is used for any calculations performed with that bilirubin data.
d If the incidence is not significant, but a significant lack of fit occurs, then Fisher's Exact test[15] with a Bonferroni correction is used.

Other methods will be used, if appropriate, at the time of analysis. The statistical methods used will be described in the final report.

SAFETY AND HOUSEKEEPING

Good housekeeping procedures will be practiced to avoid contamination of the test substance and to avoid potential health hazards. To avoid skin contact, gloves will be worn when handling the test substance. Animal carcasses and feces will be incinerated.

Exygen Study No. [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

RECORDS AND SAMPLE STORAGE

All data and records for analytical characterizations conducted by the sponsor will be archived by the sponsor. Laboratory-specific or site-specific raw data such as personnel files, instrument, equipment, refrigerator and/or freezer raw data will be retained at the facility where the work was done.

A sample of the test substance will be collected for archive purposes and retained at Haskell Laboratory, Newark, Delaware. 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.

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

REFERENCES

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Exygen Study No. [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats DuPont-13882

PROTOCOL APPENDIX

Study Function	Study Personnel
Study Director	Carol Finlay Staff Toxicologist
Clinical Pathology Evaluation	Nancy E. Everds Principal Research Clinical Pathologist and Manager
Anatomic Pathology Evaluation	Greg P. Sykes Veterinary Pathologist

Study Dates	
Initiation of Test Substance Administration	November 24, 2003
Clinical Pathology Evaluations	December 22, 2003
Anatomic Pathology Evaluation	December 22, 2003
Scheduled Sacrifice	December 22, 2003

Exygen Study No.: [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats
DuPont-13882

SIGNATURE

Approved by: _____ 18-Nov-2003
Carol Finley
Study Director
Date

cc: Haskell

Exygen Study No. [redacted]

H-26137, H-26138, and H-26139: Repeated-Dose Dermal Toxicity
28-Day Study in Male Rats
DuPont-13882

Work Request Numbe[redacted]

Service Code [redacted]

Protocol Amendment No. 1

Page 8, 2. Urinalysis

Add: "Urine volume" to list of urinalysis parameters to be determined.

Reason: Urine volume is needed to calculate the amount of fluoride in the urine.

Approved by: Carol Finlay 12/29/03
Carol Finlay Date

cc: Haskell

Exygen Study No. [redacted]

Exygen RESEARCH
Precise Research. Proven Results.

Page 1 of 1

PROTOCOL DEVIATION
Deviation Number: 1
Date of Occurrence: 01/13/04

Exygen Study Number [redacted] Protocol Number: DuPont-13882

DESCRIPTION OF DEVIATION
1. Section J. Analytical Method Section 4.5.4.e--accepted recovery of 70% for Exygen sample ID [redacted] at 1000 ppb.

ACTIONS TAKEN
for example - deviation issued, SOP revision, etc.
1. Protocol Deviation issued.

Recorded By: [signature] Date: 1/26/04

IMPACT ON STUDY
1. No negative impact because the LCS at the LLOQ and 1000 ppb were within the acceptable range for this analytical set.

[signature] Principal Investigator Signature — Date: 2/19/04
[signature] Study Director Signature — Date: 17-Feb-2004
[signature] Management Signature (Sponsor) — Date: 17-FEB-2004
Date
Exygen QAU Review [signature] 1/29/04

U:\Forms\PROTOCOL_DEVIATION.doc 9/25/2002/6

3058 Research Drive
State College, PA 16801, USA
T: 800.281.3219
F: 814.272.1019
exygen.com

APPENDIX B

Analytical Method [redacted] "Method of Analysis for the Determination of [redacted] in Serum by LC/MS/MS"

Exygen Study No.: [redacted]

Exygen RESEARCH
Precise Research, Proven Results.

TITLE

Method of Analysis for the Determination of [redacted] in Serum by LC/MS/MS - Revision 2

AUTHOR

Emily Decker

DATE ISSUED

January 8, 2004

SPONSOR

DuPont
Haskell Laboratory for Health & Environmental Sciences
P.O. Box 50
Newark, DB 19714-0050 USA
DuPont Study Number: DuPont-13090

PERFORMING LABORATORY

Exygen Research
3058 Research Drive
State College, PA 16801

METHOD NUMBER

[redacted]

TOTAL NUMBER OF PAGES

20

3058 Research Drive
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exygen.com

Exygen Study No.: [redacted]

MANAGEMENT APPROVAL

Emily R. Decker 1/8/04
Emily R. Decker Date
Scientist
Exygen Research

John M Flaherty 1/8/04
John Flaherty Date
Vice President
Exygen Research

S. Mark Kennedy 9-JAN-2004
S. Mark Kennedy Date
Sponsor Representative
DuPont

Exygen Study No. [redacted]

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Exygen Study No [redacted]

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LIST OF FIGURES

Exygen Study No. [redacted]

1. SUMMARY

This report details a method of analysis for [redacted] in serum.

[redacted] is extracted from serum by protein precipitation in acetonitrile. Quantification of [redacted] is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using selected reaction monitoring (SRM). The chemical formula of PFOA is given in section 2 of this method.

The lower limit of quantitation (LLOQ) for this method is 10 ppb for [redacted] in serum.

Quantification is performed using extracted calibration standards containing an internal standard.

This method was developed using monkey serum. The overall percent recovery ± standard deviation for [redacted] in monkey serum at 10, 100, and 1000 ppb was 94% ± 6.9% (Table I).

A representative calibration curve for [redacted] monkey serum is shown in Figure 1. Representative chromatograms for [redacted] monkey serum are shown in Figures 2 to 4.

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2. EXPERIMENTAL COMPOUNDS

The structure of [redacted] and the internal standard are given below.

[redacted]

Chemical Name = [redacted]
Molecular weight = [redacted]

[redacted]

[redacted]

Chemical Name = [redacted]
Molecular weight = [redacted]

[redacted]

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3. CHEMICALS AND SUPPLIES

3.1. CHEMICALS

Chemical	Grade	Source	Catalog No.
Methanol (MeOH)	HPLC	EM Science	JT9093-2
Acetonitrile (ACN)	HPLC	EM Science	AX0145-1
Ammonium Acetate	Reagent	Sigma-Aldrich	A-7330
OmniSolv Water	HPLC	EM Science	WX0004-1

3.2. STANDARDS

Standard	Lot Number	Purity (%)	Source
[redacted]			Oakwood Products DuPont

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3.3. Equipment and Supplies

Equipment	Supplier
Balance, analytical (display at least 0.0001 g)	Mettler
Centrifuge	Eppendorf
2 mL eppendorf centrifuge tubes	VWR
Disposable micropipets (50-100uL, 100-200uL)	Drummond (VWR)
Class A pipets and volumetric flasks	various suppliers
Hypercarb drop-in guard column (4 mm) (part # 844017-400)	Keystone
Stand-alone drop-in guard cartridge holder	Keystone
125-mL LDPE narrow-mouth bottles	Nalgene
2 mL clear HPLC vial kit (cat # 5181-3400)	HP
Standard lab equipment (graduated cylinders, disposable tubes etc.)	various suppliers
LC/MS/MS and HPLC systems	As described in section 4.5.

Note: Equivalent materials may be substituted for those specified in this method if they can be shown to produce satisfactory results.

Notes:

1. In order to avoid contamination, the use of disposable labware is highly recommended (tubes, pipets, etc.).
2. PTFE or PTFE lined containers or equipment, including PTFE-lined HPLC vials for the HPLC autosampler must not be used.
3. It is necessary to check the solvents (methanol) for the presence of contaminants by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
4. Use disposable micropipets or pipets to aliquot standard solutions to make calibration standards and sample fortifications.
5. The hypercarb cartridges should be changed when the system contaminants (elevated baseline) move to within 1 minute from the elution of PFOA.

3.4. Solutions

(1) 2 mM ammonium acetate solution is prepared by weighing 0.15 g of ammonium acetate and dissolving in 1 L of OmniSolv Water.

Note: The aforementioned example is provided for guidance, alternative volumes may be prepared as long as the ratios of the solvent to solute are maintained.

3.5. Preparation of Standards and Fortification Solutions

Analytical standards are used for three purposes:

1. Calibration Standards – These standards are prepared in control monkey serum and are used to calibrate the response of the detector used in the analysis.
2. Laboratory Control Spikes – These fortifications are usually prepared at concentrations corresponding to the LLOQ and 10x LLOQ and are used to determine analytical recovery. Laboratory control spikes are prepared in control monkey serum.
3. Matrix Spikes – These fortifications are prepared by spiking into the field samples at known concentrations. Matrix spikes are used to evaluate the effect of the sample matrix on analytical recovery.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained. Alternate concentrations may be prepared as long as the preparation and concentration are accurately recorded in the raw data. Note also that additional concentrations may be prepared if necessary.

3.5.1. Stock solution

Prepare individual stock solutions of ~ 100 µg/mL of [redacted] and ^{13}C-[redacted] by weighing out ~ 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 100-mL volumetric flask. Each stock solution (in 125-mL LDPE bottles) is to be stored in a refrigerator at 2°C to 6°C and is stable for a maximum period of 1 year from the date of preparation.

3.5.2. Fortification Solutions

a. Prepare a fortification standard of 10.0 µg/mL for PFOA by diluting 10.0 mL of the [redacted] solution described in 3.5.1 to 100 mL with acetonitrile in a volumetric flask.
b. Prepare a fortification standard of 1.0 µg/mL for ^{13}C[redacted] by diluting 1.0 mL of the ^{13}C[redacted] solution described in 3.5.1 to 100 mL with acetonitrile in a volumetric flask.
c. Prepare a fortification standard of 1.0 µg/mL for [redacted] containing 0.005 µg/mL of ^{13}C[redacted] by diluting 10.0 mL of the [redacted] solution described in 3.5.2.a and 0.5 mL of the ^{13}C[redacted] solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.
d. Prepare a fortification standard of 0.1 µg/mL for [redacted] containing 0.005 µg/mL of ^{13}C[redacted] by diluting 1.0 mL of the [redacted] solution described in 3.5.2.a and 0.5 mL of the ^{13}C-PFOA solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.
e. Prepare a fortification standard of 0.01 µg/mL for [redacted] containing 0.005 µg/mL of ^{13}[redacted] by diluting 0.1 mL of the [redacted] solution

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described in 3.5.2.a and 0.5 mL of the ^{13}C[redacted] solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.

Store all fortification standard solutions in a refrigerator (in 125-mL LDPE bottles) at 2°C to 6°C for a maximum period of 1 year from the date of preparation, after which time it is necessary to make new standards using the stock solution.

3.5.3. Calibration Standards

The calibration standards are processed through the extraction procedure, identical to the samples. The fortification of the standards before extraction is done according to the following table:

Conc. Of Mixed Fortification Solution (µg/mL)	Fortification Volume (µL)	Volume of Control Sample (mL)	Conc. of Extracted Calibration Standard (ppb)
0.01	100	0.1	10
0.01	200	0.1	20
0.1	50	0.1	50
0.1	100	0.1	100
0.1	200	0.1	200

4. METHOD

4.1. Flow Diagram

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram

Measure 0.1 mL of sample (using disposable micropipette)
(fortify samples when designated)
↓
Protein precipitation in acetonitrile
↓
LC/MS/MS analysis

4.2. Sample Processing

No sample processing is needed for serum samples. However, frozen samples must be allowed to completely thaw, un-aided, at room temperature. Samples

stored refrigerated should also be allowed to equilibrate to room temperature. All samples must be thoroughly mixed before being sampled for extraction.

4.3. Sample Preparation

a. Each batch of samples extracted (typically 30 or less) must include at least one reagent control (acetonitrile blank), one matrix control (method blank), and two matrix controls fortified at known concentrations to verify procedural recovery for the batch.
b. At least one sample per batch should be extracted in duplicate
c. At least one sample extracted should be separately fortified at a known concentration and carried through the procedure to verify recovery. Additional matrix spikes may be performed at the sponsor's request.

4.4. Extraction

1. Measure 0.1 mL of sample into 2 mL eppendorf centrifuge tubes (fortify with analyte as needed, close lid, and vortex ~ 10 seconds).
2. Add enough acetonitrile containing internal standard at 0.005 µg/mL (accounting for fortification volume) to make extraction volume 500 µL and vortex for ~ 10 seconds.
3. Centrifuge for ~ 10 minutes at ~ 14,000 rpm.
4. Analyze samples using electrospray LC/MS/MS.

4.5. Quantitation

4.5.1. LC/MS/MS System and Operating Conditions

Instrument:	PE SCIEX API 4000 Biomolecular Mass Analyzer SCIEX Turbo Ion Spray Liquid Introduction Interface
Computer:	Dell OptiPlex GX 110
Software:	PE Sciex Analyst 1.2
HPLC Equipment:	Hewlett Packard (HP) Series 1100 Quatpump G1311A Vacuum Degasser G1322A Autoinjector G1313A Column Compartment G1316A

Note: Two 4 × 10 mm hypercarb drop-in guard cartridges (Keystone, part # 844017-400) are attached in-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column: Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temperature: 30° C
Injection Volume: 5 µL
Mobile Phase (A): 2 mM Ammonium Acetate in OmniSolv Water
Mobile Phase (B): Methanol

Time	% A	% B	Flow (mL/min)
0.0	40	60	0.3
3	40	60	0.3
3.5	0	100	0.3
3.7	0	100	0.5
7	0	100	0.5
7.5	40	60	0.5
9	40	60	0.5
9.5	40	60	0.3
12	40	60	0.3

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance.

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time
[redacted]	Negative	413 → 369	~2.8 min.
	Negative	415 → 370	~2.8 min.

The retention times may vary, on a day-to-day basis, depending on the batch of mobile phase etc. Drift in retention times is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are interspersed throughout the analytical run.

Note: An alternative LC/MS/MS system may be used once demonstrated to be equivalent.

4.5.2. Tune File Parameters

The mass spectrometer is tuned for the analyte by infusing a ~ 1 µg/mL standard solution of [redacted] (at 10 µL/min, using an infusion pump) via a "T" into a stream of mobile phase containing 60% methanol and 40% 2mM ammonium acetate at 0.3 mL/min flow rate. The analyte is initially tuned for the parent ion and then tuned for the product ion. Once the instrument is tuned, the optimized parameters are saved as a tune file. This tune file is then used during routine analysis.

4.5.3. Calibration Procedures

a. Inject the same aliquot (5 µL) of each calibration standard into the LC/MS/MS.

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b. Use linear standard curves for quantitation. Linear standard curves are generated for each analyte by linear regression using 1/x weighting of the ratio analyte peak area/internal standard peak area versus the ratio of the concentration of analyte/concentration of internal standard using Analyst 1.2 (or equivalent) software system. Any calibration standard found to be a statistical outlier by using the appropriate outlier test (e.g. Huge Outlier Test), may be excluded from the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected and at least one calibration standard at the LLOQ must be retained.

c. The correlation coefficient (r) for calibration curves generated must be ≥0.9925 (r^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

4.5.4. Sample Analysis

a. Inject the same aliquot (5 µL) of each standard, sample, recovery, control, etc. into the LC/MS/MS system.

b. Standards corresponding to at least five or more concentration levels must be included in an analytical set.

c. An entire set of calibration standards should be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). As an alternative, an entire set of calibration standards may be included at the beginning and at the end of a sample set. In either case, calibration standards must be the first and last injection in a sample set.

d. The concentration of each sample/fortification/control is determined from the standard curve, based on the peak area of each analyte. The standard responses should bracket responses of the residue found in each sample set. If necessary, dilute the samples in 50:50 methanol:water to give a response within the standard curve range.

e. Fortification recoveries falling within 85 to 115% (80 to 120% for levels at the LLOQ) are considered acceptable.

f. Extracted samples must be stored refrigerated between 2°C to 6°C until analysis.

g. Samples in which no peaks are detected (i.e. signal : noise ratio < 3:1) at the corresponding analyte retention times will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention

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times but are less than the lowest concentration of the calibration standards (10 ppb where LLOQ = 10 ppb) will be reported as <10 ppb.

4.6. ACCEPTANCE CRITERIA

The following criteria must be met to ensure the presence of [redacted] and ^{13}C-PFOA:

1. The chromatograms must show the following peaks:
   [redacted] a daughter ion at 369 amu from a parent of 413 amu
   [redacted] daughter ion at 370 amu from a parent of 415 amu
2. Any analyte present in method blanks must be at least 5-fold lower than the LLOQ. Any analyte present in the reagent blank must be at least 5-fold lower than the LLOQ.
3. Recoveries of lab control spikes and matrix spikes must be between 85-115% (80-120% for levels at the LLOQ) of their known values. Any method fortification (lab control spike) falling outside the acceptable limits warrants re-extraction of the entire analytical set. Any matrix spike outside the acceptable range should be evaluated by the analyst to determine if re-extraction is warranted.
4. Any calibration standard found to be a statistical outlier by using an appropriate outlier test, may be excluded from the curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected and at least one calibration standard at the LLOQ must be retained.
5. The correlation coefficient (r) for calibration curves generated must be ≥0.9925 (r^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

4.7. PERFORMANCE CRITERIA

The following two criteria must be performed as a system suitability test, before the commencement of analysis when using an instrumentation set-up that has not been used for this method.

First Criterion:
Run a standard solution on LC/MS/MS corresponding to the estimated LLOQ and obtain a signal-to-noise ratio of at least 9:1, compared to a reagent blank. If this criterion cannot be met, optimize and change instrument operating parameters.

Second Criterion:
Run a set of standards of five or more concentration levels, spanning a range starting at or below the LLOQ up to the highest concentration level to be included in the analysis. Generate a calibration curve for each analyte and obtain a linear regression with a coefficient of determination (r^2) of at least 0.985. Once this criterion is met, samples may be analyzed with standards interspersed.

4.8. Time Required for Analysis

A set of 35 samples (1 reagent control, 1 matrix control, 1 laboratory spike, 2 laboratory control spikes, and 30 samples) can be taken through the extraction procedure in approximately 8 hours by one person. The LC/MS/MS analysis (standards and 35 samples) will take approximately 9 hours.

5. CALCULATIONS

a. Use Equation 1 to calculate the amount of analyte found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program.

Equation 1:

$$\text{Analyte found (ppb)} = \frac{(\text{Analyte Peak area/IS peak area}) - \text{intercept}) \times \text{IS conc. (ng/mL)}}{\text{slope}}$$

b. For samples fortified with known amounts of analyte prior to extraction, use Equation 2 to calculate the percent recovery.

Equation 2:

$$\text{Recovery (\%)} = \frac{\text{Analyte found (ppb)} - \text{avg. Analyte found in control (ppb)}}{\text{Amount Analyte added (ppb)}} \times 100\%$$

6. SAFETY

The analyst should read the material safety data sheets for all standards and reagents before performing this method. Use universal precautions when handling standards and reagents, including working in fume hoods and wearing laboratory coats, safety glasses, and gloves. Use blood-borne pathogen handling precautions when handling serum.

Exygen Study No.: [redacted]

Table I. Recovery of [redacted] from Fortifications In Monkey Serum

Summary of [redacted] Recoveries for 10 ppb Fortification in Monkey Serum

Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
[redacted]		05/30/03	05/30/03	10	95
		05/30/03	05/30/03	10	94
		05/30/03	05/30/03	10	98
				AVERAGE:	96
				STANDARD DEVIATION:	2.1
				RELATIVE STANDARD DEVIATION:	2.2

Summary of [redacted] Recoveries for 100 ppb Fortification in Monkey Serum

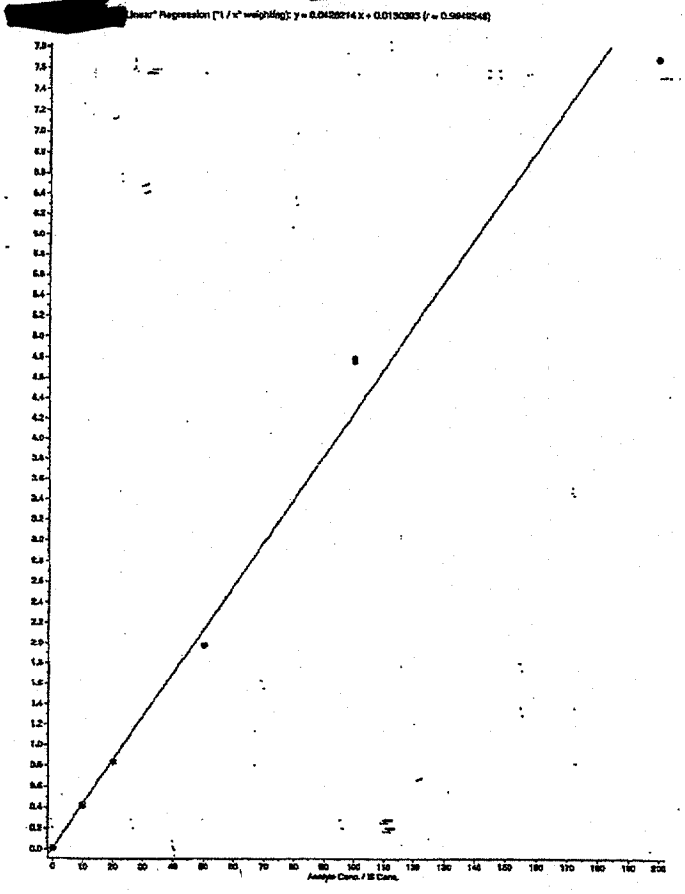
Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
[redacted]		05/30/03	05/30/03	100	92
		05/30/03	05/30/03	100	90
		05/30/03	05/30/03	100	93
				AVERAGE:	92
				STANDARD DEVIATION:	1.5
				RELATIVE STANDARD DEVIATION:	1.7

Summary of [redacted] Recoveries for 1000 ppb Fortification in Monkey Serum

Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
[redacted]		05/30/03	05/30/03	1000	107
		05/30/03	05/30/03	1000	95
		05/30/03	05/30/03	1000	81
				AVERAGE:	94
				STANDARD DEVIATION:	13.0
				RELATIVE STANDARD DEVIATION:	13.8
				OVERALL AVERAGE:	94
				OVERALL STANDARD DEVIATION:	6.9
				OVERALL RELATIVE STANDARD DEVIATION:	7.3

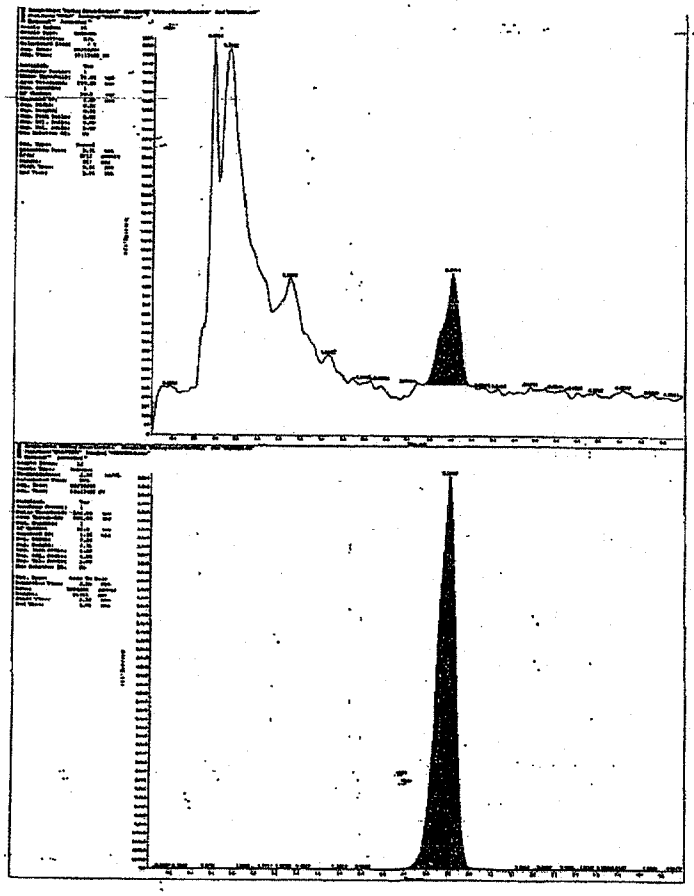
Exygen Study No.: [redacted]

Figure 1. Calibration curve for [redacted] Control Monkey Serum



Exygen Study No.: [redacted]

Figure 2. Representative Chromatogram of a Control Monkey Serum Sample for [redacted] with ~ 5 ng/mL of ^{13}C[redacted]



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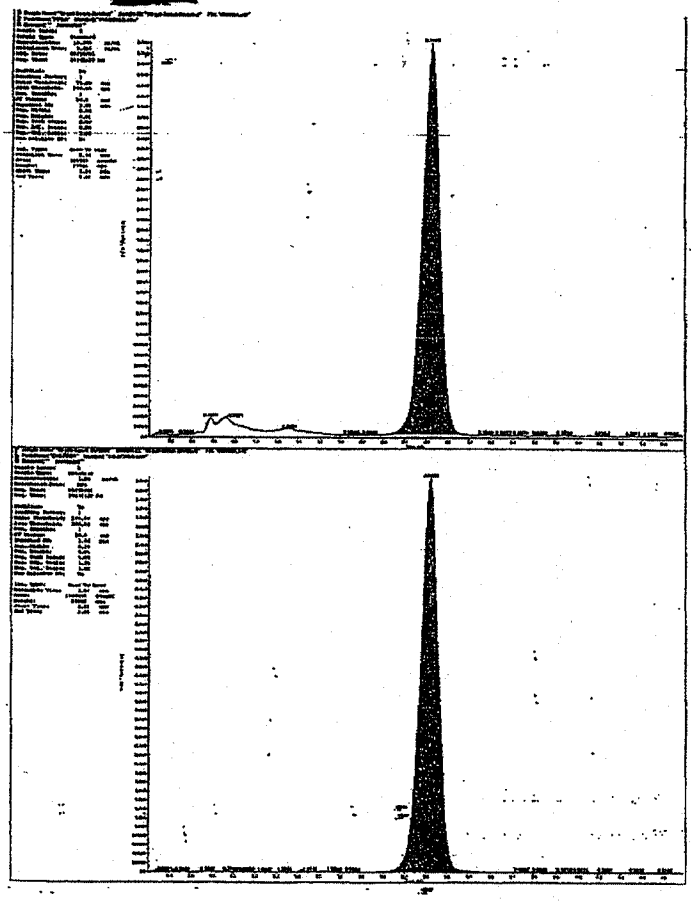
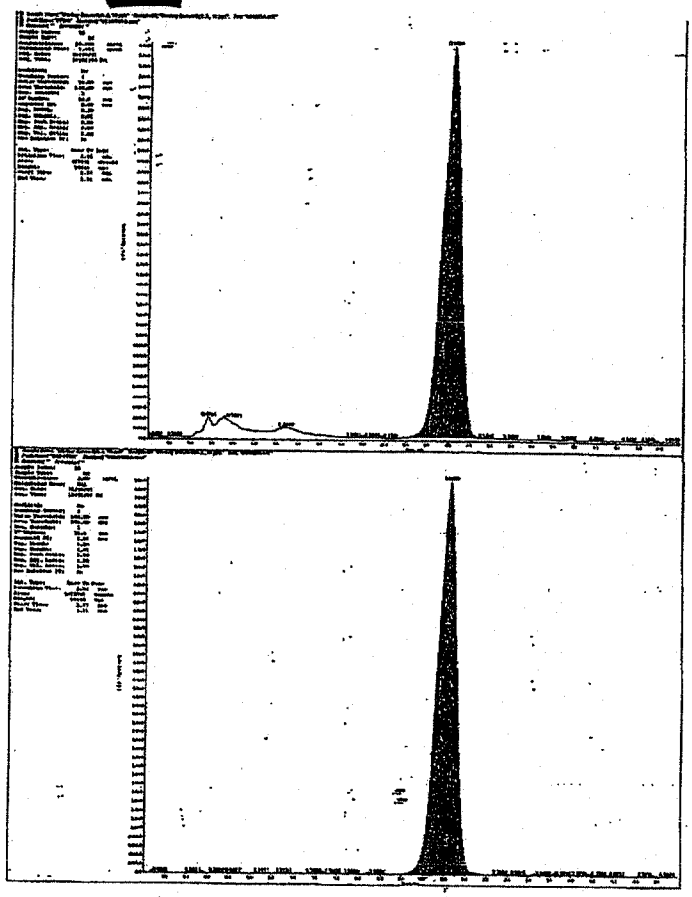
Exygen Study No.: [redacted]

Figure 3. Representative Chromatogram of a 10 ppb Standard for [redacted] Control Monkey Serum with ~ 5 ng/mL of ^{13}C-



Exygen Study No.: [redacted]

Figure 4. Representative Chromatogram of Control Monkey Serum Fortified at 10 ppb with [redacted] and with ~ 5 ng/mL of ^{13}C-[redacted]



APPENDIX G

INDIVIDUAL ANIMAL CLINICAL PATHOLOGY DATA

INDIVIDUAL ANIMAL CLINICAL PATHOLOGY DATA

EXPLANATORY NOTES

ABBREVIATIONS:

Individual Clinical Chemistry Values:
CHOL - cholesterol
TRIG - triglycerides

Individual Urinalysis Values:
UVOL - volume
UFLU - urine fluoride

NOTES:

When individual animal data are not reported, it may be due to one of the following reasons or other reasons, all of which are explained in the study records:

the sample was clotted (CLOT)
there was insufficient sample for testing (QNS)
a valid result could not be obtained (RNV)
the sample was not suitable for testing
the animal died prior to sample collection
no sample was available for testing (NSR)

Individual Animal Clinical Pathology Data

Male, Group I - H-26136 - Day 28

Animal	SHEM	SLIP	SICT	CHOL mg/dL	TRIG mg/dL
101	Trace	None	None	35	27
102	None	None	None	35	24
103	None	None	None	40	23
104	None	None	None	51	32
105	None	None	None	38	57
106	None	None	None	46	29
107	None	None	None	39	24
108	None	None	None	62	42
109	None	None	None	54	35
110	None	None	None	37	27

Male, Group III - H-26137 - Day 28

Animal	SHEM	SLIP	SICT	CHOL mg/dL	TRIG mg/dL
301	None	None	None	40	49
302	None	None	None	38	28
303	None	None	None	69	23
304	None	None	None	65	36
305	None	None	None	50	25
306	None	None	None	49	40
307	None	None	None	44	35
308	None	None	None	32	23
309	None	None	None	39	25
310	None	None	None	39	18

Individual Animal Clinical Pathology Data

Male, Group V - H-26138 - Day 28

Animal	SHEM	SLIP	SICT	CHOL mg/dL	TRIG mg/dL
501	None	None	None	50	21
502	None	None	None	21	15
503	None	None	None	44	43
504	None	None	None	28	27
505	None	None	None	41	54
506	None	None	None	32	27
507	None	None	None	31	20
508	None	None	None	41	21
509	None	None	None	29	23
510	None	None	None	35	28

Male, Group VII - H-26139 - Day 28

Animal	SHEM	SLIP	SICT	CHOL mg/dL	TRIG mg/dL
701	None	None	None	27	24
702	None	None	None	52	41
703	None	None	None	38	27
704	None	None	None	43	26
705	None	None	None	31	29
706	Trace	None	None	42	29
707	None	None	None	43	24
708	None	None	None	36	23
709	None	None	None	36	31
710	None	None	None	29	19

Individual Animal Clinical Pathology Data

Male, Group I - H-26136 - Day 28

Animal	UVOL mL	UFLU µg
101	13.0	11.8
102	5.0	9.5
103	13.8	12.5
104	10.6	8.4
105	4.0	9.7
106	17.0	13.6
107	8.2	7.7
108	4.0	6.6
109	9.0	14.1
110	4.0	7.6

Male, Group III - H-26137 - Day 28

Animal	UVOL mL	UFLU µg
301	6.6	10.2
302	6.8	9.7
303	17.0	13.6
304	7.6	10.4
305	8.2	9.8
306	16.6	12.6
307	25.0	15.0
308	7.0	9.3
309	11.6	12.3
310	23.6	11.6

Individual Animal Clinical Pathology Data

Male, Group V - H-26138 - Day 28

Animal	UVOL mL	UFLU µg
501	11.8	14.7
502	4.0	18.0
503	5.4	10.4
504	30.2	20.7
505	13.0	13.6
506	8.2	12.0
507	2.0	9.4
508	3.0	7.0
509	25.0	18.2
510	9.0	11.5

Male, Group VII - H-26139 - Day 28

Animal	UVOL mL	UFLU µg
701	4.6	11.8
702	9.0	21.6
703	7.4	11.6
704	8.4	13.6
705	6.8	13.4
706	7.0	10.0
707	8.0	12.5
708	4.8	6.1
709	8.2	15.9
710	14.6	15.5

APPENDIX H

Individual Animal Final Body and Organ Weights

Organ Weight Listing
Study: [redacted]

Individual Animal & Summary Stats Report
PLACES 2000 v1.400

Group : I H-26136 Treatment : I Sex :MALES

ANIMAL	FBW	KIDNEYS		LIVER		THYROID GLAND	
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	(Gms)	%FBW
101	283.30	2.694	0.9509	9.721	3.4313	0.017	0.0060
102	323.20	3.028	0.9369	11.295	3.4947	0.025	0.0077
103	342.40	3.328	0.9720	10.882	3.1782	0.020	0.0058
104	291.80	2.693	0.9229	9.582	3.2838	0.018	0.0062
105	343.10	2.934	0.8551	10.733	3.1282	0.021	0.0061
106	359.60	3.411	0.9486	12.121	3.3707	0.018	0.0050
107	309.80	2.895	0.9345	10.145	3.2747	0.015	0.0048
108	333.70	2.960	0.8870	11.533	3.4561	0.025	0.0075
109	348.50	3.253	0.9334	10.898	3.1271	0.023	0.0066
110	335.00	3.441	1.0272	11.884	3.5475	0.023	0.0069
Mean	327.04	3.064	0.9368	10.879	3.3292	0.021	0.0063
S.D.	24.920	0.279	0.0461	0.865	0.1536	0.003	0.0009

Group : III H-26137 Treatment : III Sex :MALES

ANIMAL	FBW	KIDNEYS		LIVER		THYROID GLAND	
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	(Gms)	%FBW
301	307.80	2.915	0.9470	11.221	3.6455	0.025	0.0081
302	331.50	2.928	0.8833	10.250	3.0920	0.018	0.0054
303	351.60	3.378	0.9608	12.466	3.5455	0.027	0.0077
304	316.90	2.858	0.9019	11.661	3.6797	0.017	0.0054
305	324.90	2.561	0.7882	10.152	3.1247	0.015	0.0046
306	353.30	3.507	0.9926	12.579	3.5604	0.024	0.0068
307	336.50	3.054	0.9076	11.324	3.3652	0.024	0.0071
308	306.70	2.939	0.9583	10.422	3.3981	0.016	0.0052

FBW - Final Body Weight

Organ Weight Listing
Study: [redacted]

Individual Animal & Summary Stats Report
PLACES 2000 v1.400

Group : III H-26137 Treatment : III Sex :MALES

ANIMAL	FBW	KIDNEYS		LIVER		THYROID GLAND	
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	(Gms)	%FBW
309	340.40	3.253	0.9556	10.238	3.0076	0.020	0.0059
310	318.00	2.975	0.9355	10.196	3.2063	0.021	0.0066
Mean	328.76	3.037	0.9231	11.051	3.3625	0.021	0.0063
S.D.	16.747	0.275	0.0575	0.946	0.2438	0.004	0.0012

Group : V H-26138 Treatment : V Sex :MALES

ANIMAL	FBW	KIDNEYS		LIVER		THYROID GLAND	
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	(Gms)	%FBW
501	286.00	2.901	1.0143	9.213	3.2213	0.016	0.0056
502	327.60	3.159	0.9643	10.632	3.2454	0.018	0.0055
503	334.00	2.825	0.8458	10.240	3.0659	0.020	0.0060
504	328.70	3.034	0.9230	10.799	3.2854	0.022	0.0067
505	321.80	3.032	0.9422	10.581	3.2881	0.018	0.0056
506	298.00	2.766	0.9282	9.292	3.1181	0.014	0.0047
507	309.50	3.258	1.0527	9.411	3.0407	0.019	0.0061
508	350.60	3.009	0.8582	10.268	2.9287	0.018	0.0051
509	316.40	2.841	0.8979	9.382	2.9652	0.018	0.0057
510	328.30	2.851	0.8684	10.207	3.1090	0.023	0.0070
Mean	320.09	2.968	0.9295	10.003	3.1268	0.019	0.0058
S.D.	18.562	0.159	0.0672	0.614	0.1295	0.003	0.0007

FBW - Final Body Weight

Organ Weight Listing
Study:1[redacted]

Individual Animal & Summary Stats Report
PLACES 2000 v1.400

Group : VII H-26139 Treatment : VII Sex :MALES

ANIMAL	FBW	KIDNEYS		LIVER		THYROID GLAND	
	(Gms)	(Gms)	%FBW	(Gms)	%FBW	(Gms)	%FBW
701	287.20	2.697	0.9391	9.428	3.2827	0.016	0.0056
702	368.80	3.466	0.9398	13.359	3.6223	0.013	0.0035
703	349.50	3.258	0.9322	11.525	3.2976	0.018	0.0052
704	313.00	2.792	0.8920	9.930	3.1725	0.022	0.0070
705	310.20	2.844	0.9168	10.251	3.3046	0.024	0.0077
706	360.00	3.154	0.8761	12.566	3.4906	0.020	0.0056
707	329.90	2.976	0.9021	10.651	3.2286	0.019	0.0058
708	322.60	3.041	0.9427	9.914	3.0732	0.017	0.0053
709	359.60	3.507	0.9753	13.845	3.8501	0.022	0.0061
710	310.30	2.720	0.8766	9.591	3.0909	0.021	0.0068
Mean	331.11	3.046	0.9193	11.106	3.3413	0.019	0.0058
S.D.	27.091	0.295	0.0323	1.624	0.2455	0.003	0.0012

*** Listing Complete ***

FBW - Final Body Weight

APPENDIX I

Individual Animal Gross Observations

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : I H-26136 Treatment: I Sex: Males

Animal Ref	Macroscopic Findings Only
101	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
102	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
103	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
104	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : I H-26136 Treatment: I Sex: Males

Animal Ref	Macroscopic Findings Only
105	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy
	No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
106	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy
	No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
107	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy
	No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
108	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy
	No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : I H-26136 Treatment: I Sex: Males

Animal Ref	Macroscopic Findings Only
109	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
110	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Date: 3-FEB-04

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : III H-26137 Treatment: III Sex: Males

Animal Ref	Macroscopic Findings Only
301	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
302	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
303	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
304	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : III H-26137 Treatment: III Sex: Males

Animal Ref	Macroscopic Findings Only
305	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
306	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
307	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
308	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy KIDNEYS : DILATATION, RIGHT, PELVIS. No Macroscopic Abnormality Observed : LIVER, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : III H-26137 Treatment: III Sex: Males

Animal Ref	Macroscopic Findings Only
309	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
310	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : V H-26138 Treatment: V Sex: Males

Animal Ref	Macroscopic Findings Only
501	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
502	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
503	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
504	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : V H-26138 Treatment: V Sex: Males

Animal Ref	Macroscopic Findings Only
505	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
506	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
507	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy KIDNEYS : CYST, BILATERAL, FEW, <2MM. No Macroscopic Abnormality Observed : LIVER, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
508	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Date: 3-FEB-04

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : V H-26138 Treatment: V Sex: Males

Animal Ref	Macroscopic Findings Only
509	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
510	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

10
Date: 3-FEB-04

Individual Animal Listing
Showing animal data for individual animals
STUDY :[redacted] (* APPROVED PROTOCOL *)
Dose Group : VII H-26139 Treatment: VII Sex: Males

Animal Ref	Macroscopic Findings Only
701	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
702	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy KIDNEYS : DILATATION, RIGHT, PELVIS. No Macroscopic Abnormality Observed : LIVER, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
703	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
704	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : VII H-26139 Treatment: VII Sex: Males

Animal Ref	Macroscopic Findings Only
705	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
706	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
707	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
708	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

Individual Animal Listing
Showing animal data for individual animals
STUDY : [redacted] (* APPROVED PROTOCOL *)
Dose Group : VII H-26139 Treatment: VII Sex: Males

Animal Ref	Macroscopic Findings Only
709	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED
710	Terminal Sacrifice Killed on Day : 28 Animal is signed off from necropsy No Macroscopic Abnormality Observed : LIVER, KIDNEYS, THYROID GLAND, SKIN, TREATED, SKIN, UNTREATED

*** Listing Complete ***