3M General Offices

3M Center
St. Paul, MN 55144-1000
612 733 1110

April 21, 2000

3M

Dr. Charles Auer
Room 7405
US EPA Headquarters
Ariel Rios Bldg.
1200 Pennsylvania Avenue, N. W.
Washington, D. C. 20460

FedEx
FedEx Tracking Number for Customer
8077 8211 8191

Dear Charlie:

Pursuant to our recent communications, 3M is enclosing additional information relating to perfluorooctanesulfonate (PFOS). We are providing this information to you on a voluntary basis as part of our continuing discussions with EPA regarding fluorochemistry. This information includes studies on PFOS relating to the following areas: (i) pharmacokinetics; (ii) teratology; and (iii) epidemiology and medical surveillance. A listing of the enclosed studies has been attached to this letter, and summaries also have been provided for each category of studies. Please note that incorrectly applied legends relating to legal privileges and proprietary protections have been removed from certain documents. Finally, you should be receiving under separate cover additional studies on PFOS relating to reproductive toxicology that 3M will also be submitting to the TSCA Section 8(e) docket.

3M plans to provide you with additional information on PFOS and other fluorochemicals in the near future. In this regard, we have been trying to contact you to address scope and procedural matters, and we hope to talk with you the week of April 24, 2000.

Please do not hesitate to call me or Dr. Larry Zobel, 3M's Medical Director, with any questions. I look forward to talking with you.

Very truly yours,

William A. Weppner

William A. Weppner, Ph.D
Director of Environmental, Health, Safety, and Regulatory Affairs
Specialty Materials Markets
3M
Bldg 236-1B-10
3M Center
St. Paul, MN 55144

Corporate Health Physics
Corporate Industrial Hygiene & Ergonomics
Corporate Occupational Medicine
Corporate Toxicology
3M Medical Department

3M Center, Building 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Attachments to April 21, 2000 letter to C. Auer from W. Weppner (Radiolabel Oral Absorption and Intravenous Pharmacokinetic Studies in Rats)

3M Technical

1. Synthesis and Characterization of FC-95-^{14}C, November 2, 1979.

2. Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95-^{14}C, December 28, 1979.

3. Absorption of FC-95-^{14}C in Rats after a Single Oral Dose, October 26, 1976

4. J. D. Johnson, et. al., Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [^{14}C]Perfluorooctanoate or Potassium [^{14}C]Perfluorooctanesulfonate, Fundamental and Applied Toxicology 4, pages 972-976 (1984).

Medicine, Health Physics
Industrial Hygiene, Toxicology
3M Medical Department

3M Center, Building 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
612 733 1110

3M

Attachments to April 21, 2000 letter to C. Auer from W. Weppner (Dermal Absorption and Intravenous Pharmacokinetics in Rabbits)

3M Technical Reports

1. Single-Dose Intravenous Pharmacokinetic Study of T-6246 in Rabbits. 3M Environmental Laboratory Study # AMDT-042095.1

2. Single-Dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits. 3M Environmental Laboratory Study # AMDT020895.1

3. Single-Dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits. 3M Environmental Laboratory Study #AMDT-112294.1

Corporate Health Physics
Corporate Industrial Hygiene & Ergonomics
Corporate Occupational Medicine
Corporate Toxicology
3M Medical Department

3M Center, Building 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Attachments to April 21, 2000 letter to C. Auer from W. Weppner (Teratology Studies in Rats and Rabbits)

1. Rat Teratology Study T-3351 Final Report Dec 19, 1983, Hazelton Laboratories America Project #154-160.

2. December 7, 1983 Letter to Mr. William C. McCormick, III from E. Marshall Johnson, Ph.D., reviewing draft of above.

3. Oral Teratology Study of FC-95 in Rats, Riker Laboratory, Experiment # 068TR0008, December, 1980.

4. November 12, 1982, Letter to Franklin D. Griffith, Ph.D. from E. Marshall Johnson, Ph.D., reviewing the above.

5. Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits, Study #418-012, January 11, 1999, Argus Laboratories.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

April 21, 2000

3M

Attachments To April 21, 2000 Letter to C. Auer from W. Weppner
(Medical Surveillance and Epidemiology)

Final Reports

1. An Epidemiologic Investigation of Clinical Chemistries, Hematology and Hormones in Relation to Serum Levels of Perfluorooctanesulfonate in Male Fluorochemical Production Employees. [Included is a 3M report of medical surveillance data. Also included is a published paper from this 3M report.]

2. Fluorochemical Exposure (Serum) Assessment of (3M) Decatur Chemical and Film Plant Employees. [Included are a 3M study protocol and report.]

3. Mortality Study of Employees at 3M Plant in Decatur, Alabama [Included is a University of Minnesota final report.]

4. Determination of Serum Fluorochemical Levels in Sumitomo 3M Employees [Included are a 3M study protocol and final report.]

5. Analysis of Selected Decatur Employee Serum for Sulfonic and Carboxylic Fluorochemicals [Included is a 3M technical report.]

6. Fluorochemical Control Study [Included is a 3M report of medical surveillance data.]

7. Working Memorandum on Data Quality Assessment [Included is a Battelle Laboratory memorandum regarding the mean and range of perfluorooctanesulfonate sera sample data collected by 3M from current and historical human populations.)

Study Protocols/Proposals of Ongoing Research

1. Identification of Fluorochemicals in Sera of Children in the United States. [Included is a 3M study protocol. Study is on-going. Final report due January 1, 2001]

2. Serum fluorochemical trends of out-of-county residents in CLUE I (1974) and Clue II (1989) epidemiologic investigations. [Included is a Johns Hopkins University study protocol. Study is on-going. Joint 3M/Johns Hopkins University final report is due January 1, 2001.]

3. Identification of Fluorochemicals in Human Tissue. [Included is 3M study protocol. Suty is on-going. Final report is due January 1, 2001.]

4. An Epidemiologic Analysis of the Inpatient and Outpatient Claims Event and Episode Experience of 3M-Decatur Employees, 1993-1998. [Included is a 3M study protocol. The study is on-going. Final report is due September 15, 2000.]

5. Determination of Serum Elimination Half-Lives of Ammonium Perfluorooctanoate, Perfluorooctane Sulfonic Acid, Perfluorohexane Sulfonic Acid and Total Organic Fluorine in Decatur Chemical Plant Retirees. [Included is a 3M study protocol. The study is on-going. Final report is due June 1, 2004. Interim draft reports will be issued.]

Synthesis and Characterization of FC-95-^{14}C

November 2, 1979

Conducted at:	Commercial Chemicals Division and Riker Laboratories, Inc. 3M St. Paul, Minnesota 55101
During:	April 1979 to July, 1979
Synthesis Conducted by:	F. E. Behr
Specific Activity and Radiochemical Purity Determination by:	J. D. Johnson and S. J. Gibson

Report by:

James D. Johnson 11/16/79

J. D. Johnson, M.S. Date
Senior Biochemical Pharmacologist
Riker Laboratories, Inc.

Fred E. Behr 11/16/79

F. E. Behr, Ph.D. Date
Senior Research Chemist
Commercial Chemicals Division

Specific Activity and Radiochemical Characterization Section Reviewed by:

R. E. Ober 11/19/79

R. E. Ober, Ph.D. Date
Manager, Drug Metabolism
Riker Laboratories, Inc.

Summary

The synthesis of a 20.0 g lot of FC-95-^{14}C (carbon-14 label α to sulfur atom) is described. The specific activity is 0.459 ± 0.008 μCi/mg. Thin-layer and column chromatography showed the FC-95-^{14}C to be at least 99% radiochemically pure. The FC-95-^{14}C was found suitable for metabolism studies.

Introduction

A series of experiments is planned to investigate the metabolism of FC-95 (a sulfonic acid salt) and the possibility of FC-807 being biotransformed to FC-95 and/or the alcohol (FM-3422). To facilitate these experiments, carbon-14 labeled FC-95 was synthesized and characterized. The synthetic pathway illustrated in Figure 1 is described.

A. Synthesis of FC-95-^{14}C

I. Fractional Distillation of $C_7F_{15}{}^{*}CF_2SO_2F$ (Step 1)

The crude cell drainings from cell run R-3256-B were filtered through glass wool to remove the cell tars. The fluorochemical product was washed twice with cold, dilute saturated $KHCO_3$ and twice with water; the product was dried over silica gel. A yield of 293.7g of base-washed cell drainings was obtained from cell run R-3256-B. GLC analysis showed 71.1% $C_8F_{17}SO_2F$. One hundred sixty grams of the base-washed cell drainings was purified by fractional distillation (see Table 1). Analysis for percent $C_8F_{17}SO_2F$ was done by gas chromatography (area percent). Fractions 11-16 were combined to give 98.3 g of product which was found to contain 98.15% $C_8F_{17}SO_2F$.

II. Preparation of FC-95-^{14}C ($KO_3S{*}CF_2C_7F_{15}$)

Water (31.1g) and potassium hydroxide pellets, (85% assay, 15.53g) were added to a 500 ml three-necked round-bottomed flask which had been equipped with a reflux condenser, thermometer, an air-driven mechanical stirrer, Tru-Bore® stirrer assembly and a small, pressure-equalized addition funnel. The flask contents were heated to 78-80°C. Fractional $C_7F_{15}{*}CF_2SO_2F$ (27.0g, 98.15% assay) was added dropwise through the addition funnel at a rate sufficient to maintain the temperature between 80-83°C. Gummy, white solids were formed during the addition. Upon completion of the addition of the radioactive perfluorooctane-sulfonyl fluoride, the mixture was heated at 85°C for 3 hours. At the end of the three hour reaction time, the gummy mass had broken into smaller solid pieces. Water (10.8 ml) was added. The pH of the solution was ∿ 13.5. The upper aqueous phase was removed (aspirator) and the solids were washed successively with water (54.0 ml) at 45-50°C. The solids were washed once more with water (17.8 ml), stirred at a high agitation rate, and the upper aqueous phase was discarded. Isopropanol (19.15g) and water (17.0g) were added to the solid product, and the mixture was heated at reflux temperature for one hour. All of the solids had dissolved after heating the mixture for one hour. The flask contents were poured into a glass evaporating dish. The flask was washed with isopropanol (24.7 ml)-water (17.0 ml). Concentration of the product was done on a steam bath. A final drying of the potassium perfluorooctane sulfonate was done at 60°C for 3 hours in a vacuum desiccator. The dry powder was triturated once with Freon 113 to remove small traces of silicon oil impurity used as stirrer lubricant. The dried product (27.8g) was assigned the following L number: FC-95-^{14}C, 20.0g, L-4544.

FC-95 normally is produced from one-plate distilled perfluorooctane-sulfonyl fluoride (assay 66-67%). The high assay $C_7F_{15}{*}CF_2SO_2F$ (98.15%) will also be used to prepare FC-807-^{14}C.

B. Specific Activity Determination of FC-95-^{14}C

Three standard solutions of FC-95-^{14}C[a] (L-4544, see Synthesis Section) were prepared by weighing[b] I, 25.00 mg; II, 26.84 mg; and, III, 26.79 mg into three 25 ml class A volumetric flasks. Solution I was prepared with methanol and solutions II and III were prepared with a 1:1 mixture (volume:volume) of water and methanol. The volumes were adjusted to 25 ml with the appropriate solvent and mixed by inverting by hand. Calibrated micropipettors[c] were used to aliquot six 10 µl and six 50 µl aliquots of each solution directly into scintillation counting vials. To three of the vials containing 10 µl aliquots and to three containing 50 µl aliquots from each primary solution, 1 ml of water and 15 ml of Aquasol®[d] were added. The remaining vials were prepared with 2.5 ml of methanol and 7.5 ml of MTSS[e]. The samples were cooled to refrigerator temperature and allowed to equilibrate in the dark before they were counted two times at 5 minutes each with a Packard Model 3385 Liquid Scintillation Spectrometer. The counting efficiency was determined for each sample by the internal standard method. The averaged data were reduced to dpm and the µCi/mg was calculated for each vial. The data are shown in Table 2. The mean µCi/mg found for each of the weighings was within 2% of each of the other means. The means between Aquasol®and MTSS were within 1.2% and the means between 10 µl and 50µl were within 1.3%. The overall average specific activity based on the 36 replicates (12 from each primary weighing) was 0.459 ± 0.0080 µCi/mg.

C. Radiochemical Analysis of FC-95-^{14}C

I. Thin-Layer Chromatography Systems and Carbon-14 Analysis

The analysis of radiochemical purity was carried out with a variety of thin-layer chromatography systems using either SGF 250 micron pre-scored Uniplates[f] or pre-adsorbent SGF 250 micron pre-scored Uniplates[f]. Plates were routinely developed in 10"x12"x4" thin-layer tanks[g] fitted with glass lids and lined with saturation pads[g]. Plates were allowed to develop 15 cm and were scraped laterally in 0.5 cm wide segments. The scraping was accomplished with a custom-made template and sharpened stainless steel spatula ground to exactly 0.5 cm width.

---

[a] Riker Isotope Number 442.
[b] Weighings were accomplished on a 5-place Mettler H64 electronic balance which had been recently calibrated by 3M Metrology.
[c] L/I Micropipettor, Lab Industries, Berkeley, California.
[d] New England Nuclear, Boston, Massachusetts.
[e] Modified TSS:25.2 g PPO, 1.01g Dimethyl POPOP and 3.8 liters toluene.
[f] Analtech, 75 Blue Hen Drive, Newark, Delaware.
[g] Supelco Inc., Bellefonte, Pennsylvania.

The scraped silica gel segments were collected in scintillation vials containing 2.5 ml of methanol. To the methanol, 7.5 ml of MTSS was added. The samples were counted and the counts per minute (cpm) were corrected for background using a suitable blank (usually two 0.5 cm segments scraped from below the origin on the plate being assayed). The cpm were not corrected for efficiency. The carbon-14 content of each segment was expressed as percent of total carbon-14 on the plate:

$$\frac{\text{cpm on segment}}{\text{sum of cpm on plate}} \times 100 = \%\ \text{carbon-14 content in segment.}$$

The percent carbon-14 content of each segment was plotted versus segment number. This provided a thin-layer radiochromatogram showing the radioactivity peaks corresponding to separated components in the material applied to the plate.

II. Solutions Used for Radiochemical Purity Analysis

Solution 1: The FC-95-^{14}C made from 25.00 mg of FC-95-^{14}C in 25.0 ml methanol described in the section on Specific Activity Determination, this report, was designated as Solution 1.

Solution 2: 28.12 mg of FC-95-^{14}C, L-4544, (Riker Isotope Inventory Number 442) was placed into a 10 ml volumetric flask and the volume adjusted to 10 ml with methanol. The solution was inverted several times to ensure mixing.

III. Column Chromatography of FC-95-^{14}C

A silicic acid (Unisil[a]) column was prepared by pouring a chloroform slurry of silicic acid into a 14mm I.D. glass column[b] to a height of 21 cm. A 1.0 ml aliquot of Solution 2 was applied to the top of the column. Three successive 200 ml fractions were collected after applying 200 ml of $CHCl_3$, Fraction 1; 200 ml of chloroform-methanol 1:1 (volume: volume), Fraction 2; and 200 ml of methanol, Fraction 3. Each of the 200 ml fractions was evaporated with a rotating evaporator, transferred to a 12 ml centrifuge tube, evaporated to near dryness with a stream of nitrogen, and reconstituted with 0.20 ml of chloroform-methanol 1:1 (volume:volume). Fifty microliters was applied to thin-layer plates and analyzed as described previously.

IV. Results and Discussion

Since the chemical identity of the FC-95-^{14}C is well established by synthesis (see synthesis of FC-95-^{14}C, this report), unlabeled FC-95 was not co-chromatographed with the labeled FC-95-^{14}C. The results of the radiochemical purity analysis of FC-95-^{14}C by thin-layer chromatography are shown in Table 3 and in Figures 2-5. These analyses did not discern any impurities in FC-95-^{14}C. Thin-layer analysis of three column fractions from a silicic acid column showed 99.1% of the radioactivity recovered in Fraction 2 (1:1 chloroform-methanol) (see Figure 6). Fraction 3 (methanol) contained 0.8 per cent of the

---

[a] Unisil, Activated Silicic Acid, 100-200 mesh. Clarkson Chemical Company, Inc., Williamsport, Pennsylvania.

[b] Column was fitted with a sintered-glass base and stopcock.

recovered radioactivity (see Figure 7). The impurity contained in Fraction 3 has a different R_f (0.40) from that of FC-95 (0.27) in the same solvent systems. The impurity was not identified.

Overall, the radiochemical purity of FC-95-^{14}C is at least 99%. The FC-95-^{14}C is suitable for metabolism studies.

Acknowledgement

The authors gratefully acknowledge the gas chromatographic analysis of the carbon-14 labeled $C_7F_{15}CF_2SO_2F$ fractions done by Mr. Todd Mathisen of Commercial Chemicals Division and the assistance of John C. Hansen and Larry Headrick during the electrochemical fluorination of labeled $C_7F_{15}CF_2SO_2F$.

List of Tables and Figures

Table 1: Data from Fractional Distillation of Base-washed $C_7F_{15}{}^*CF_2\ SO_2F$.
NB 50942 p. 34,35.

Table 2: Specific Activity Determination of FC-95-^{14}C.
NB 51312 p. 33.

Table 3: Thin-Layer Chromatography Systems for FC-95-^{14}C.
NB 51807 p. 21.

Figure 1. Pathway for Synthesis of FC-95-^{14}C.
NB 50942 p. 36.

Figure 2: Thin-Layer Radiochromatogram Plate 1.
NB 51807 p. 4.

Figure 3: Thin-Layer Radiochromatogram Plate 2.
NB 51807 p. 4.

Figure 4: Thin-Layer Radiochromatogram Plate 3.
NB 51807 p. 5.

Figure 5: Thin-Layer Radiochromatogram Plate 4.
NB 51807 p. 5.

Figure 6: Thin-Layer Radiochromatogram of FC-95-^{14}C Silicic Acid Column Eluent, Fraction 2 (Chloroform-Methanol).
NB 51807 p. 18.

Figure 7: Thin-Layer Radiochromatogram of FC-95-^{14}C Silicic Acid Column Eluent, Fraction 3 (Methanol)
NB 51807 p. 18.

Table 1

Data from Fractional Distillation of Base-Washed $C_7F_{15}\overset{*}{C}F_2SO_2F$

Fraction	bP °C	Yield (g)	Area %[a]				
			LB'S[b]	C_8F_{18}	$C_6F_{13}SO_2F$ $C_7F_{15}SO_2F$	$C_8F_{17}SO_2F$	HB'S[c]
1	< 40	4.3	99.3				
2	< 80	10.8	95.3	2.30	0.12	2.20	---
3	80-83	8.7	80.99	8.82	0.95	7.86	---
4	83-92	4.1	73.55	12.46	1.55	13.36	---
5	92-94	5.7	63.63	15.39	2.17	18.94	---
6	94-97	4.3	50.23	18.54	3.09	27.52	---
7	97-104	1.8	41.92	21.18	3.80	32.42	---
8	104-108	2.5	22.02	18.90	5.27	53.51	---
9	108-118	1.8	12.16	15.03	5.82	66.66	---
10	118-123	2.7	6.17	11.12	5.84	76.78	---
11	126-128	9.4	7.23	5.44	5.33	87.53	---
12	128-130	7.1	0.49	1.93	4.39	95.66	---
13	131- 33	9.4	---	---	0.43	96.64	0.46
14	138- 40	31.2	---	---	1.52	97.92	0.49
15	> 140	16.0	---	---	0.22	99.55	0.09
16	---	25.2	---	---	0.058	93.22	---
Bottoms	---	---	---	---	---	5.13	64.0
Blend FR 11-16						98.15	

[a] Area percent was determined by gas chromatographic analysis.

[b] Low boiling non-functional fluorocarbons below C_8F_{18}.

[c] High-boiling, unidentified fluorocarbons boiling above $C_8F_{17}SO_2F$.

Table 2

Specific Activity Determination of FC-95-^{14}C

Comparison of Primary Solutions

	Solution I (25.00 mg/25.0 ml) µCi/mg	Solution II (26.84 mg/25.0 ml) µCi/mg	Solution III (26.79 mg/25.0 ml) µCi/mg
	0.4535	0.4610	0.4660
	0.4558	0.4530	0.4647
	0.4582	0.4535	0.4598
	0.4658	0.4652	0.4532
	0.4816	0.4607	0.4554
	0.4736	0.4611	0.4644
	0.4521	0.4565	0.4425
	0.4637	0.4422	0.4576
	0.4589	0.4486	0.4567
	0.4637	0.4540	0.4625
	0.4653	0.4576	0.4490
	0.4685	0.4518	0.4513
$\bar{X} \pm$ S.D.	0.4634 ± 0.0085	0.4554 ± 0.0063	0.4569 ± 0.0071

Comparison of Scintillation Solvents

	MTSS µCi/mg	Aquasol® µCi/mg
	0.4658	0.4535
	0.4816	0.4558
	0.4736	0.4582
	0.4637	0.4521
	0.4653	0.4637
	0.4685	0.4589
	0.4652	0.4610
	0.4607	0.4530
	0.4611	0.4535
	0.4540	0.4565
	0.4576	0.4422
	0.4518	0.4486
	0.4532	0.4660
	0.4554	0.4647
	0.4644	0.4598
	0.4625	0.4425
	0.4490	0.4576
	0.4513	0.4567
$\bar{X} \pm$ S.D.	0.4614 ± 0.0084	0.4558 ± 0.0067

Comparison of 10 µl and 50 µl Aliquots

	10 µl µCi/mg	50 µl µCi/mg
	0.4535	0.4521
	0.4558	0.4637
	0.4582	0.4589
	0.4658	0.4637
	0.4816	0.4653
	0.4736	0.4653
	0.4610	0.4685
	0.4530	0.4565
	0.4535	0.4422
	0.4652	0.4486
	0.4607	0.4576
	0.4611	0.4518
	0.4660	0.4425
	0.4647	0.4576
	0.4598	0.4567
	0.4532	0.4625
	0.4554	0.4490
	0.4644	0.4513
$\bar{X} \pm$ S.D.	0.4615 ± 0.0076	0.4557 ± 0.0075

Table 3

Thin-Layer Chromatography Systems for FC-95-^{14}C

Plate No.	Type of Plate	Solvent System[a]	R_f[b] of FC-95-^{14}C
1	Uniplate	50 chloroform 50 acetone	0.17
2	Uniplate	100 chloroform 100 methanol 2 acetic acid[c]	0.70
3	Uniplate	100 butanol 10 water 10 acetic acid[c]	0.67
4	Uniplate	150 chloroform 50 methanol 5 ammonium hydroxide[c]	0.30
5	Pre-adsorbent Uniplate	100 butanol 10 water 10 acetic acid[c]	0.67

[a] Solvents were prepared volume:volume; a 100 ml aliquot of solvent mixture was added to chromatography tank.

[b] R_f is of only radioactive peak on plate.

[c] Acetic acid and ammonium hydroxide were concentrated.

Figure 1

Pathway for Synthesis of FC-95-^{14}C

$$C_7H_{15}\overset{*}{C}H_2SO_2F^{a} \xrightarrow[HF]{ECF} C_7F_{15}\overset{*}{C}F_2SO_2F$$

R-3256-B

$$C_7F_{15}\overset{*}{C}F_2SO_2F \text{ (Assay 71.1\%)} \xrightarrow[\text{Distillation}]{\text{Fractional}} C_7F_{15}\overset{*}{C}F_2SO_2F \text{ (Assay 98.15\%)}$$

I

$$I + 2KOH \xrightarrow[80°C]{H_2O} KO_3S\overset{*}{C}F_2C_7F_{15}$$

FC-95-^{14}C[b]

* Denotes position of Carbon-14.

[a] $C_7H_{15}\overset{*}{C}H_2SO_2F$ was prepared by Pathfinder Laboratories.

[b] FC-95-^{14}C, potassium perfluorooctanesulfonate.

Figure 2

Thin-layer Radiochromatogram of
FC-95-^{14}C, Plate No. 1

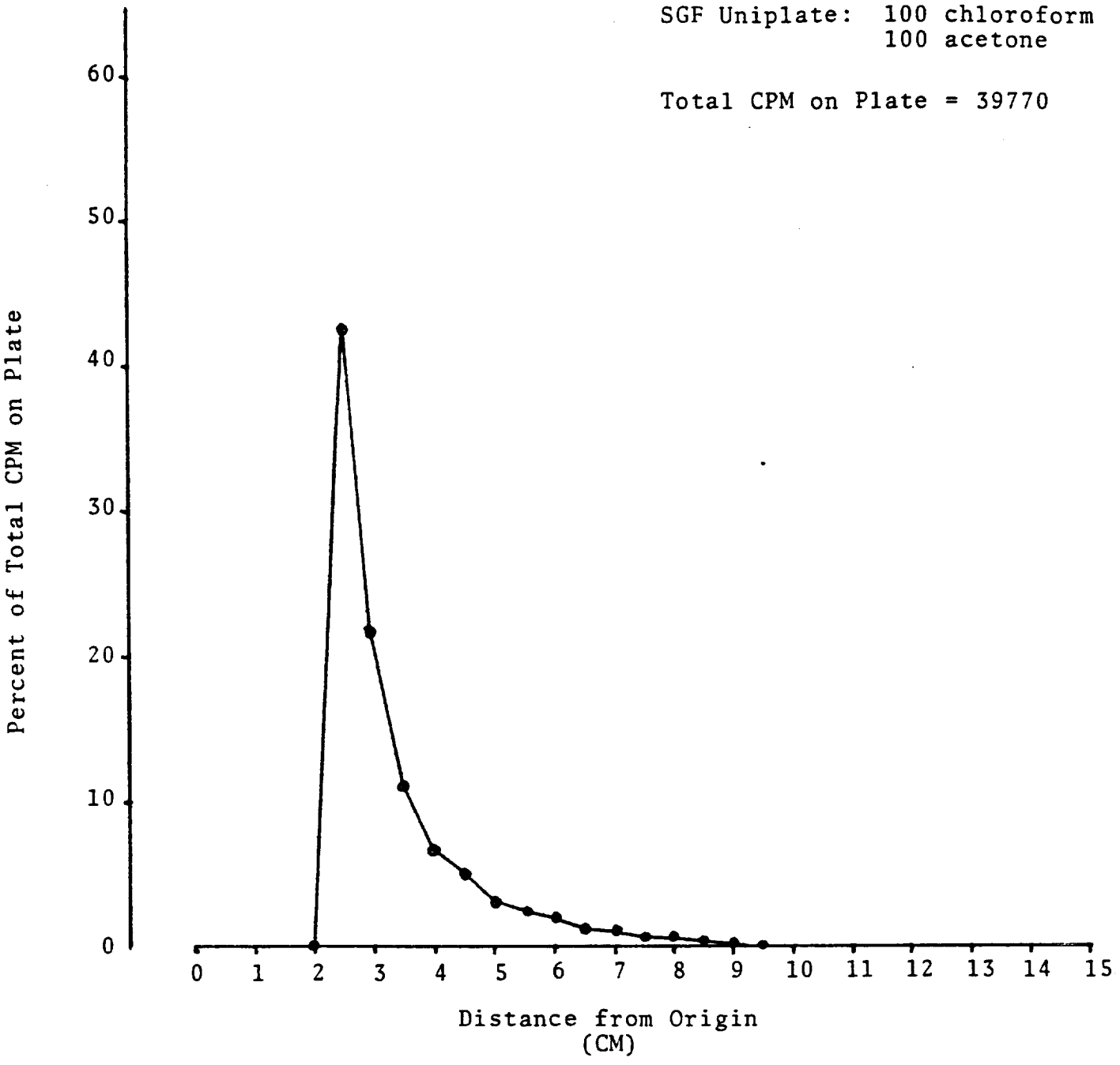


Figure 3

Thin-layer Radiochromatogram of
FC-95-^{14}C, Plate No. 2

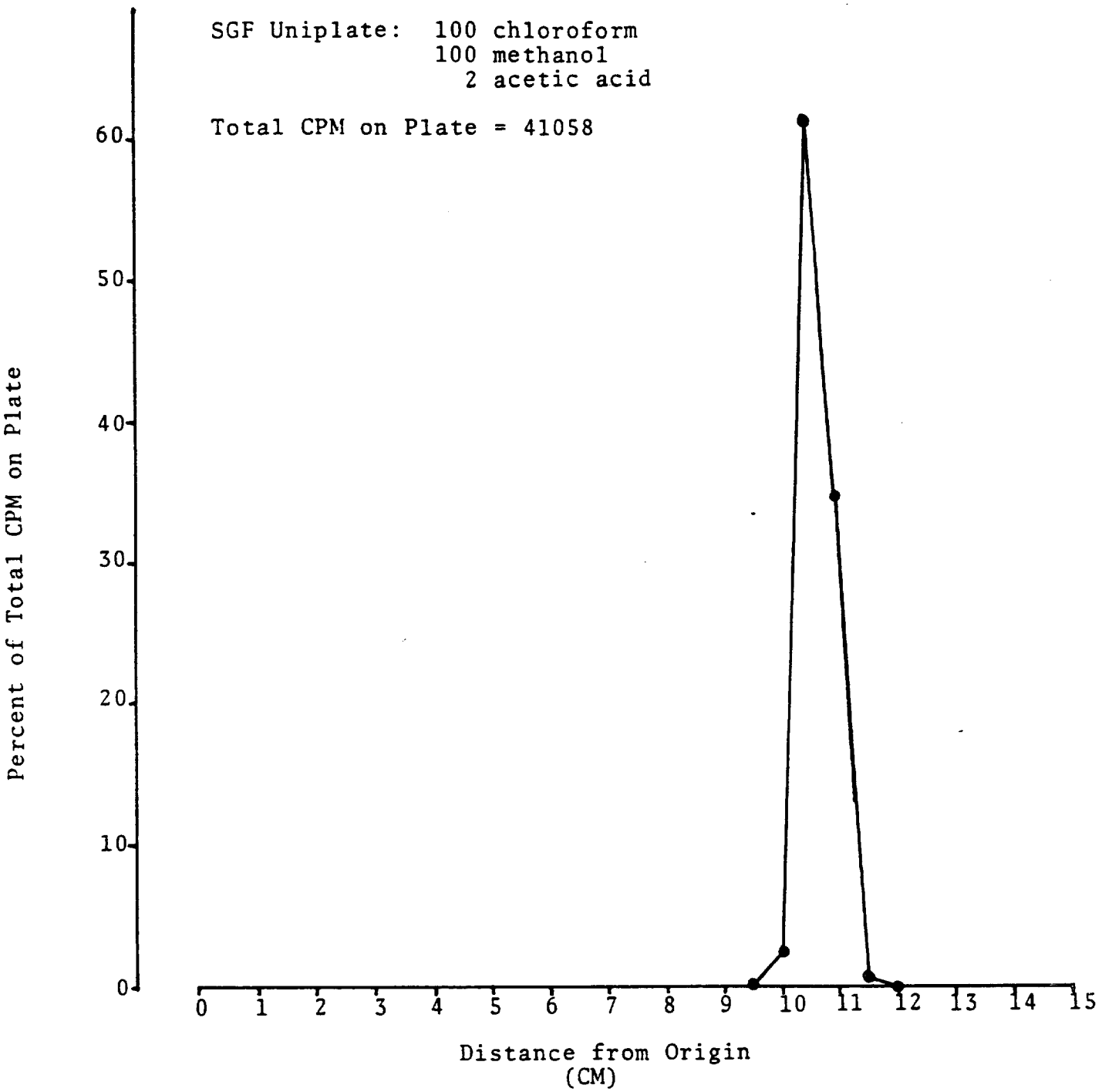


Figure 4

Thin-layer Radiochromatogram of
FC-95-^{14}C, Plate No. 3

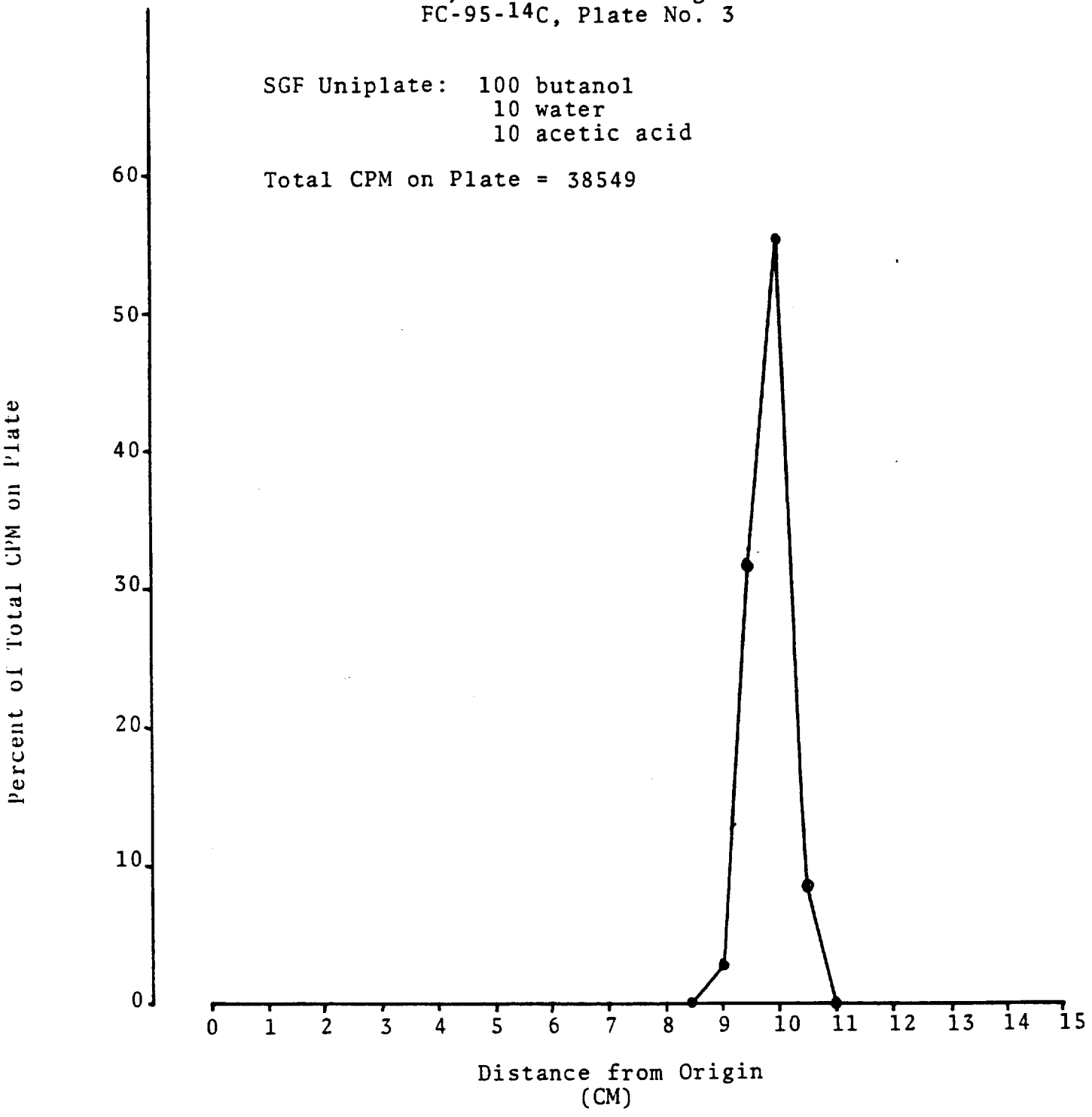


Figure 5

Thin-layer Radiochromatogram of
FC-95-^{14}C, Plate No. 4

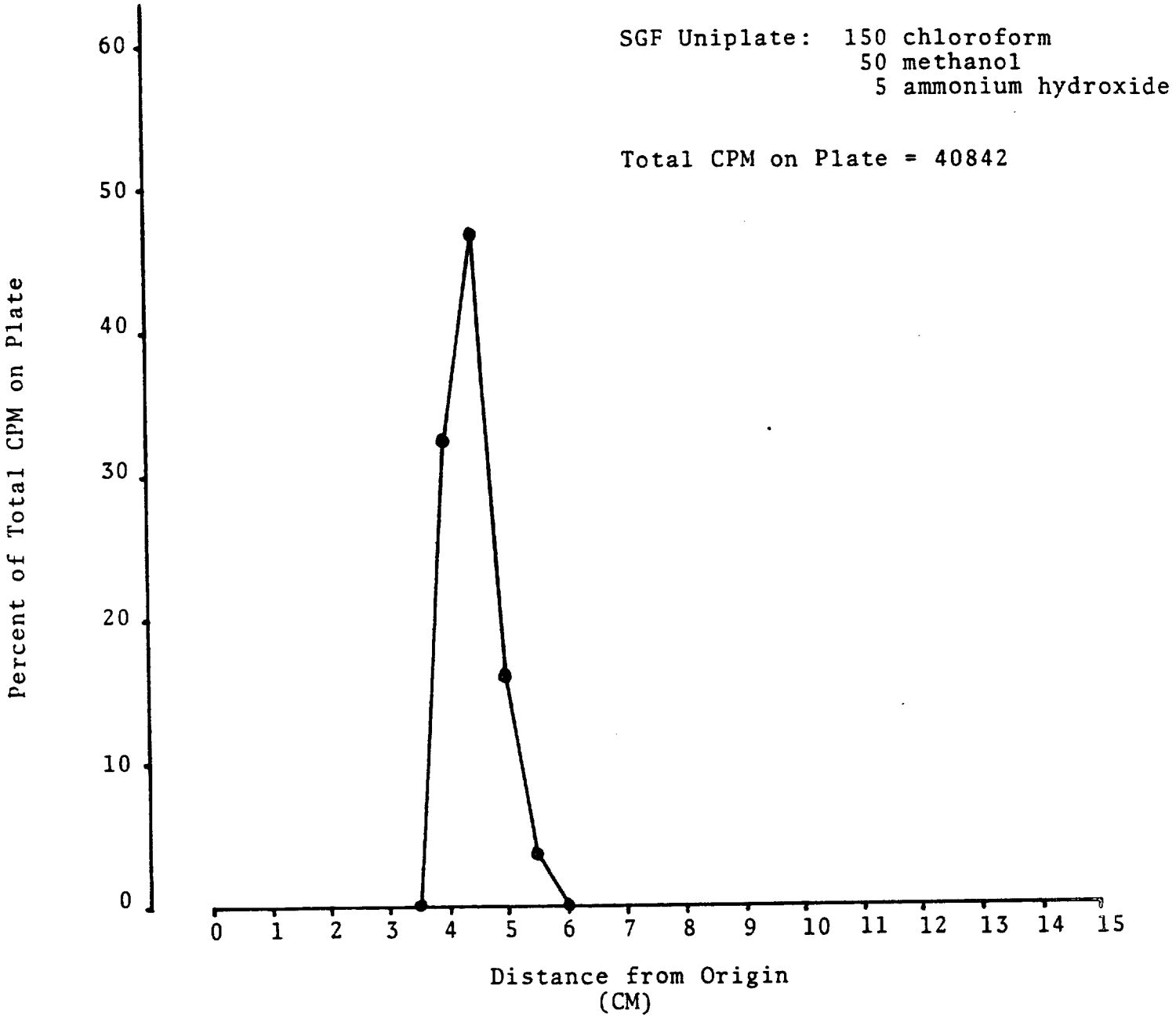


Figure 6

Thin-layer Radiochromatogram of
FC-95-^{14}C Silicic Acid Column Eluent, Fraction 2
(Chloroform-Methanol)

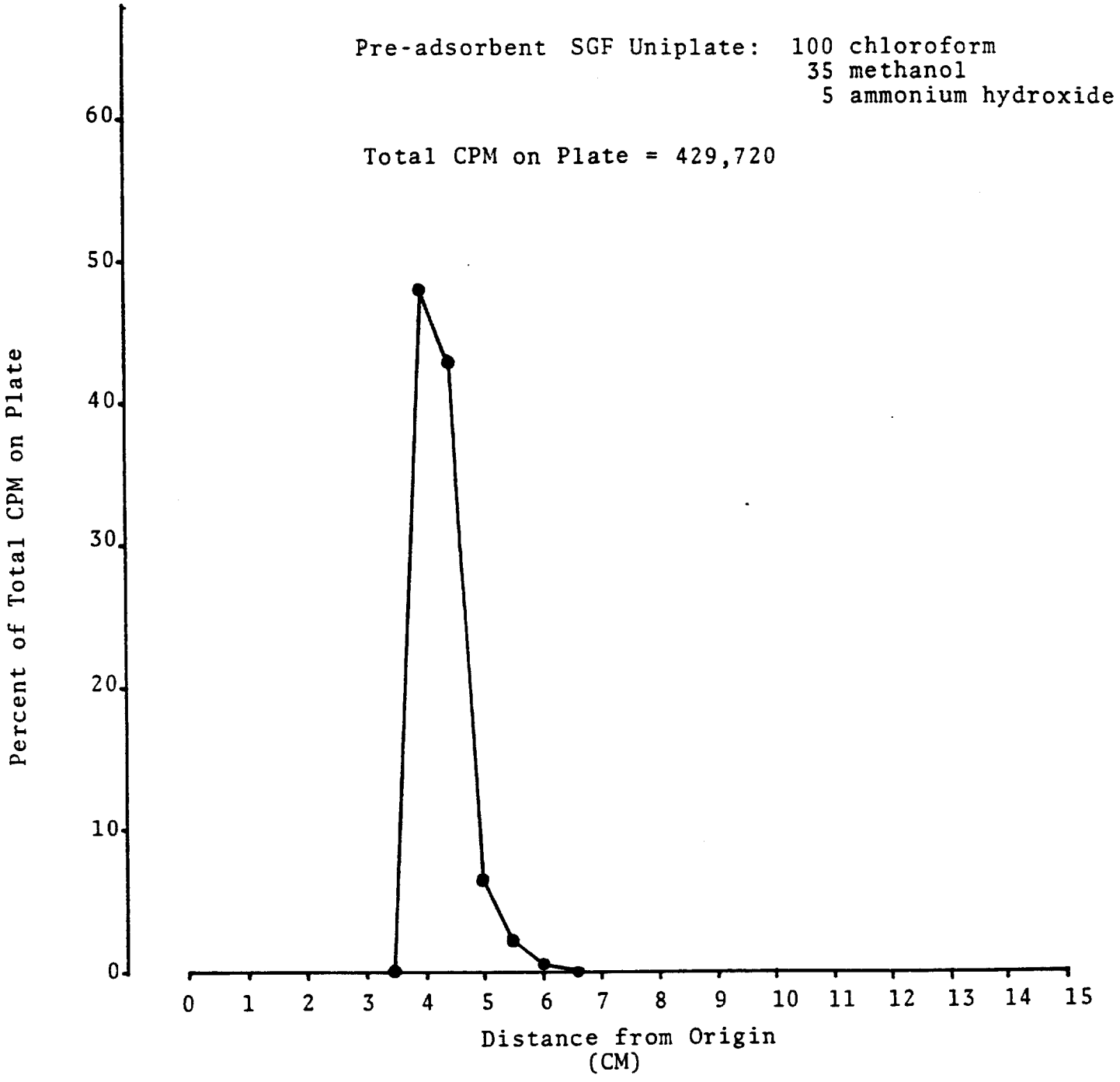
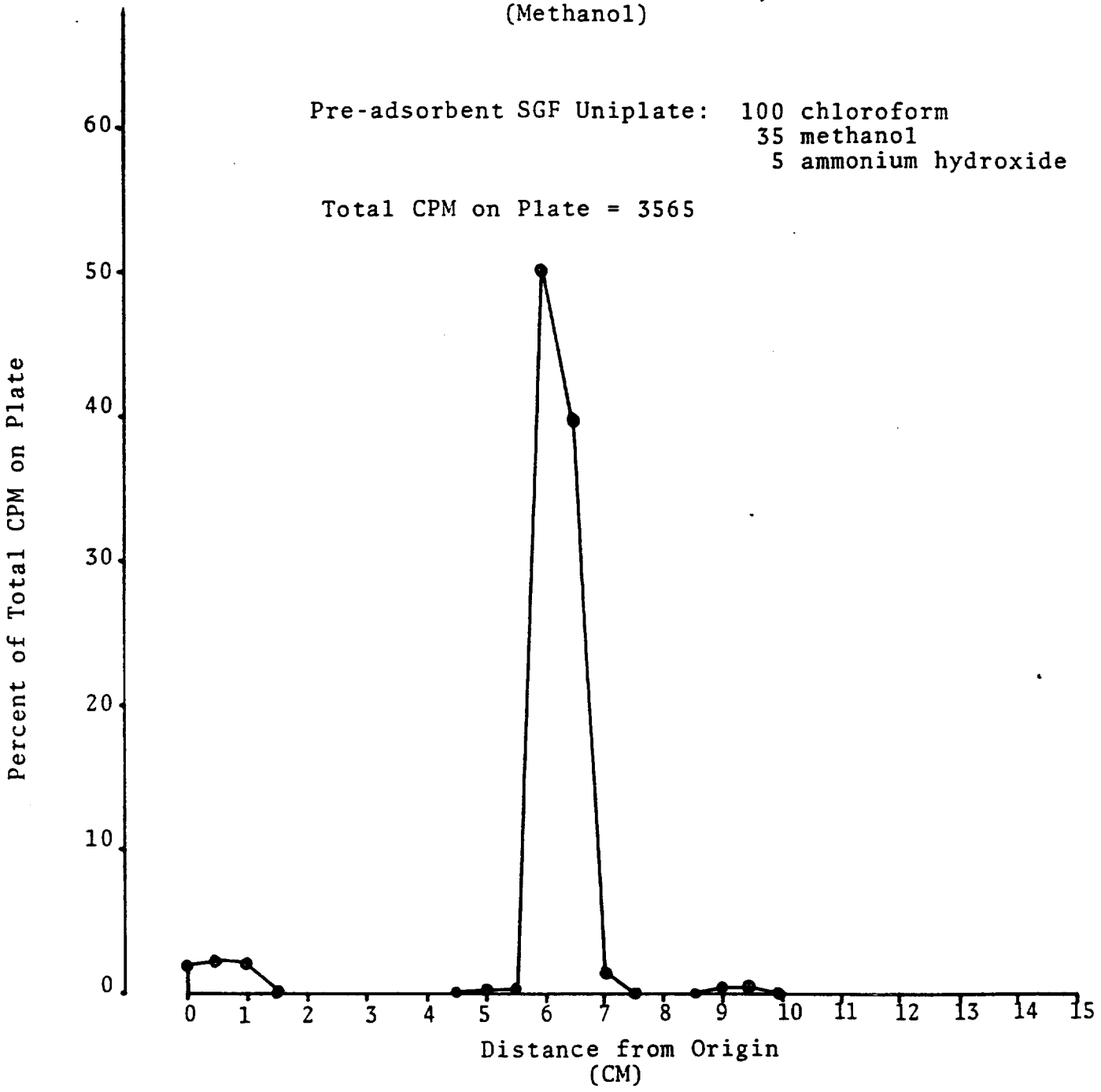


Figure 7

Thin-layer Radiochromatogram of
FC-95-^{14}C Silicic Acid Column Eluent, Fraction 3
(Methanol)



File

RECEIVED
JAN 30 1980
3M
TOXICOLOGY

Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95-^{14}C

December 28, 1979

Conducted at: Riker Laboratories, Inc.
Subsidiary of 3M
St. Paul, Minnesota 55101

During: June to October, 1979

Conducted by: S. J. Gibson and J. D. Johnson

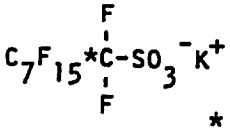
Report by: James D. Johnson 1/16/80
James D. Johnson, M.S. Date
Senior Biochemical Pharmacologist

Reviewed by: Robert E. Ober 1/16/80
Robert E. Ober, Ph.D. Date
Manager, Drug Metabolism

Summary

By 89 days after a single iv dose of FC-95-^{14}C (mean dose, 4.2 mg/kg) six male rats excreted a mean of 30.2% of the total carbon-14 via urine. Mean cumulative fecal excretion was 12.6%. At 89 days, mean tissue concentration of total carbon-14 expressed as µg FC-95-^{14}C equivalents/g were: liver, 20.6; plasma, 2.2; kidney, 1.1; lung, 1.1; spleen, 0.5; and bone marrow, 0.5. Lower concentrations ($\leq$ 0.5) were measured in adrenals, skin, testes, muscle, fat, and eye. No radioactivity (< 0.05) was detected in brain. The carbon-14 in liver and plasma represents 25 and 3 percent of the dose, respectively.

Introduction



* Denotes position of carbon-14

FC-95 is the potassium salt of a perfluorinated sulfonic acid. A series of experiments has been planned to investigate possible means of increasing the rate of elimination of FC-95 from the body. FC-95 absorption, elimination from plasma, and excretion data were necessary to form a basis for efficient design of these experiments. This FC-95-^{14}C intravenous experiment (FC-Experiment 3) which was designed to provide data on the route and extent of total carbon-14 excretion was paired with an FC-95-^{14}C oral dosing experiment which was designed to provide data on total carbon-14 absorption and elimination from plasma. (2) In addition, these FC-95 absorption, plasma elimination, and excretion data will be used to interpret and guide other experiments on the possible biotransformation of FC-807 (3) to FC-95.

Methods

Radiolabeled FC-95-^{14}C

The carbon-14 label is at the position α to the sulfur atom (see above structure). The specific activity of this lot of FC-95-^{14}C (Riker Isotope Inventory Number 442) is 0.459 µCi/mg. The FC-95-^{14}C was found to be suitable for metabolism studies; details of the synthesis, specific activity determination, and radiochemical purity determination have been reported. (1)

Animals

Six male Charles River[a] CD rats, eight weeks old, were conditioned to individual metal metabolism cages for 24 hours prior to dosing. The body weights ranged from 262 to 303 g, mean 288 g. The rats were allowed free access to Purina[b] Ground Chow and water before and after dosing.

Dosing

Each rat was weighed, anesthetized with diethyl ether, and then given a single iv dose (via tail vein) of FC-95-^{14}C. The dose was 2.0 ml of a 0.9% NaCl solution containing 1.21 mg FC-95-^{14}C/2.0 ml. The average dose was 4.2 mg/kg. The dose was delivered with a 3.0 cc disposable plastic syringe (Monoject®[c]) fitted with a 26 gauge 1/2" needle. The

---

[a] Charles River Breeding Laboratories, Wilmington, Mass.

[b] Purina Lab Chow, Ralston Purina Company, St. Louis, Missouri.

[c] Sherwood Medical Industries, Inc., Deland, Florida 32720.

dosing solution was prepared by adding ≈ 200 mg of FC-95-^{14}C to 0.9% NaCl, shaking for one-half hour at moderate speed in a mechanical shaker, and centrifuging. The supernatant was removed and used for dosing solution. The carbon-14 content of the dosing solution was determined by direct counting (see Appendix 1).

Sample Collection

Urine and feces were collected at intervals (see Tables 1 and 2) for each of the six rats for 89 days. At 89 days post dose, the rats were anesthetized with diethyl ether; blood was drawn from the descending aorta and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. The rats were sacrificed by exsanguination; and spleen, liver, brain, testes, adrenals, kidneys, lungs, and eyes were collected as whole organs. Bone marrow was obtained from the four major bones of the rear legs by splitting the bone and collecting the marrow on pieces of a tared combustion pad[a]. Samples of skin, thigh muscles, subcutaneous and abdominal fat, and the total remaining carcass were collected.

Sample Analysis for Carbon-14

Feces and major organs were prepared for carbon-14 analysis by homogenizing and aliquoting a sample of the homogenate into combustion cone[a]. Homogenizing was done in Waring blenders by adding nine parts of water (w/w) to one part of biological material. The homogenates were weighed into combustion cones in triplicate on a top-loading balance by taring the cone and adding 1.0 g of the homogenate. Care was taken to mix the homogenate between samplings. Urine, red blood cells, and plasma were measured into combustion cones by weight. Whole eyes, adrenals, and samples of bone marrow, skin and fat were also weighed in combustion cones. Care was taken to weigh these samples promptly (1-2 minutes) after removal in order to avoid loss of weight by drying. Homogenates, tissue samples weighed directly, and weighed samples of plasma, red blood cells, and urine were combusted with a Packard Model 306 Oxidizer. Recovery of carbon-14 from biological samples was determined by combusting suitable blank homogenates (feces, liver, muscle, and spleen) and blank rat plasma spiked with dilutions of FC-95-^{14}C dosing solution at the beginning, middle, and end of the experimental sample set (see Appendix 2). Urine collections were sampled before freezing and were counted directly; triplicate 1.0 ml aliquots of each sample were pipetted directly into scintillation vials and 15 ml Aquasol® was added. All samples were cooled to refrigerator temperature in the dark before counting. All radiometric analyses were done using a Packard Model 3385 Tri-Carb Liquid Scintillation Spectrometer.

Counting efficiency for each sample was determined by use of the AES (Automatic External Standardization) ratio method. To calibrate the external standard, internal standard was added to selected samples from the group of samples (three with low AES ratios and three with high ratios), and these samples were recounted along with a sealed standard. Data were collected on punch tape and processed by the CDC 1700 Computer System in the 3M Central Research Data Processing Laboratory. Data reduction to dpm was accomplished with the Biological Automatic External Standardization computer program.

---

[a] Packard Instrument Company, Inc., 2200 Warrenville Road, Downers Grove, IL.

Results and Discussion

The results of the fecal and urinary analyses are shown in Tables 1 and 2 and in Figures 1 and 2. Tables 1 and 2 list the total carbon-14 expressed as percent of dose. The data are expressed as cumulative percent of dose excreted versus time in Figures 1 and 2. As illustrated in Figures 1 and 2, excretion of total carbon-14 is slow. By 89 days after dosing, total excretion (urine + feces) is only 43 percent of the dose. Radioactive content in feces was too low to measure after 64 days; total carbon-14 excreted is thus the sum of fecal excretion (0 to 64 days) and urinary excretion (0 to 89 days). The mean ratio of cumulative excretion of total carbon-14 in urine (30.2%) to that in feces (12.6%) was 2.4 ± 0.2. This is in contrast to the ratio of cumulative excretion of total carbon-14 in urine to that in feces ($\leq$ 0.1) at 124 days post dose reported after a single iv dose of FC-807-^{14}C. (3) This indicates that FC-807-^{14}C is not likely extensively biotransformed to FC-95-^{14}C. Since 50-60 percent of the FC-95-^{14}C dose was not excreted by either feces or urine at 89 days post dose, selected tissues were analyzed for carbon-14 content to identify those tissues in which total carbon-14 might be retained.

The results of the tissue analyses are shown in Table 3. Mean tissue carbon-14 concentrations above one µg FC-95-^{14}C equivalents/g were as follows: liver, 20.6; plasma, 2.2; kidney, 1.1; and lung, 1.1. Other tissues such as muscle, skin, bone marrow, and spleen had concentrations ranging from 0.2 to 0.6 µg/g. There was a difference in carbon-14 content of subcutaneous fat (0.2 µg/g) and abdominal fat ($\leq$ 0.08µg/g). Very little total carbon-14 was found in whole eye (0.16 µg/g) and no detectable total carbon-14 was found in brain. There is a striking difference in the pattern of total carbon-14 retention when liver, bone marrow, and spleen after iv dosing with FC-95-^{14}C is compared to the pattern of retention found for these tissues after dosing with FC-807-^{14}C. (3) The mean carbon-14 content for FC-807-^{14}C dosed rats normalized to liver content are: liver, 1.0; spleen, 8.9; and bone marrow, 2.4. For FC-95-^{14}C dosed rats, the mean ratios are liver, 1.0; spleen, 0.02; and bone marrow, 0.02. Since the spleen/liver ratio following FC-95-^{14}C is 8.9 and bone marrow/liver is 2.4, this data supports the conclusion that FC-807 is not extensively or rapidly biotransformed to FC-95.

The total carbon-14 contents expressed as percent of dose for selected whole organs and plasma and red blood cells are listed in Table 4. Only liver and plasma contain a substantial percentage of the dose at 89 days post dose. The low levels of radioactivity found for kidney, lung, testes, and spleen, are (in view of the relatively high carbon-14 content of plasma and red blood cells) due in part to the blood still contained in these organs when homogenized and analyzed.

References

(1) Johnson JD, Behr FE: Synthesis and Characterization of FC-95-^{14}C (Report), November 2, 1979.

(2) Johnson JD: Absorption of FC-95-^{14}C in Rats After a Single Oral Dose (Report) October 26, 1979.

(3) Johnson JD: Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats After a Single IV Dose of FC-807-^{14}C (Report), September 28, 1979.

(4) Altman PL, Dittmer DS: Blood and Other Body Fluids. Bethesda, Maryland, Federation of American Societies for Experimental Biology, 1971, p. 5.

List of Tables and Figures

Table 1: Excretion of Total Carbon-14 in Urine in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg). NB 52584 p. 17.

Table 2: Excretion of Total Carbon-14 in Feces in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg). NB 52584 p. 14.

Table 3: Tissue Distribution of Total Carbon-14 in Rats at 89 Days after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg). NB 52584 p. 20.

Table 4: Percent of Dose Present in Tissues at 89 Days after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg). NB 52584 p. 21.

Figure 1: Cumulative Excretion of Total Carbon-14 in Urine after a Single IV Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg) Mean of 6 Rats. NB 52584 p. 18.

Figure 2: Cumulative Excretion of Total Carbon-14 in Feces after a Single IV Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg) Mean of 6 Rats. NB 52584 p. 14.

Appendix, Table 1: Determination of Carbon-14 Content of Dosing Solution. NB 51312 p. 35.

Appendix, Table 2: Recovery of Total Carbon-14 from Blank Fecal Homogenate Samples Spiked with FC-95-^{14}C. NB 52584 p. 16.

Appendix, Table 3: Comparison of Carbon-14 Analysis of Urine Samples by Oxidation Versus Direct Counting in Aquasol®. NB 52584 p. 9.

Table 1

Excretion of Total Carbon-14 in Urine in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)

Collection Period (days)	Rat Number 1	2	3	4	5	6	Mean
0-0.5	1.09[a]	0.69	1.03	0.77	1.03	0.86	0.91
0.5-1	0.70	0.90	0.72	0.60	0.77	0.94	0.77
1-2	1.56	1.11	1.47	0.78	1.08	1.27	1.21
2-3	1.31	1.05	1.14	0.68	0.91	1.11	1.03
3-4	1.16	0.87	1.06	0.68	0.93	0.85	0.93
4-5	1.09	0.80	0.81	0.60	0.80	0.90	0.83
5-6	0.86	0.72	0.67	0.49	0.76	0.77	0.71
6-7	1.07	0.71	0.75	0.52	0.74	0.75	0.76
7-8	0.90	0.69	0.85	0.54	0.76	0.74	0.75
8-9	0.86	0.57	0.70	0.60	0.68	0.67	0.68
9-10	0.82	0.66	0.79	0.59	0.48	0.74	0.68
10-11	0.60	0.36	0.65	0.64	0.63	0.64	0.59
11-12	0.62	0.52	0.54	0.69	0.55	0.57	0.58
12-13	0.56	0.53	0.63	0.60	0.60	0.59	0.59
13-14	0.55	0.53	0.51	0.58	0.57	0.55	0.55
14-15	0.65	0.51	0.46	0.57	0.55	0.51	0.54
15-16	0.54	0.48	0.56	0.45	0.58	0.46	0.51
16-17	0.57	0.43	0.40	0.45	0.48	0.55	0.48
17-18	0.53	0.40	0.34	0.35	0.49	0.44	0.43
18-19	0.41	0.39	0.28	0.42	0.38	0.46	0.39
19-21	0.93	0.81	0.79	0.77	0.84	0.92	0.84
21-23	0.91	0.75	0.69	0.77	0.66	0.88	0.78
23-25	0.63	0.59	0.59	0.66	0.70	0.77	0.66
25-27	0.76	0.67	0.64	0.66	0.68	0.64	0.68
27-29	0.73	0.66	0.73	0.68	0.66	0.64	0.68
29-32	1.04	0.88	0.69	0.89	0.82	0.86	0.86
32-36	1.06	1.05	1.16	1.21	1.14	0.66	1.05
36-40	0.97	1.08	0.97	1.01	1.01	0.91	0.99
40-43	0.59	0.58	0.88	0.82	0.79	0.83	0.75
43-47	0.59	0.78	1.00	0.94	0.96	1.25	0.92
47-50	0.70	0.52	0.66	0.70	0.69	0.79	0.68
50-54	0.52	0.77	0.82	0.83	0.75	0.98	0.78
54-57	0.63	0.56	0.62	0.63	0.58	0.61	0.61
57-61	0.56	0.82	0.74	0.89	0.81	0.94	0.79
61-69	1.38	1.33	1.41	1.64	1.66	1.59	1.50
69-78	1.51	1.77	1.49	1.60	1.64	1.81	1.64
78-89	1.59	2.03	1.97	2.06	2.45	2.39	2.08
TOTAL	31.6	28.6	30.2	28.4	30.6	31.8	30.2

[a] Data are expressed as percent of dose excreted during collection period.

Table 2

Excretion of Total Carbon-14 in Feces in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)

Collection Period (days)	Rat Number 1	2	3	4	5	6	Mean
0-0.5	0.043[a]	-	0.069	0.004	0.178	-	0.049
0.5-1	0.711	0.780	0.919	0.988	0.777	0.877	0.842
1-2	0.955	0.770	0.865	0.465	0.682	1.032	0.795
2-3	0.761	0.572	0.726	0.466	0.652	0.719	0.649
3-4	0.736	0.604	0.599	0.667	0.639	0.689	0.656
4-5	0.764	0.630	0.606	0.395	0.538	0.529	0.577
5-6	0.636	0.595	0.352	0.397	0.502	0.578	0.510
6-7	0.560	0.464	0.911	0.434	0.522	0.635	0.588
7-8	0.539	0.423	0.832	0.306	0.343	0.451	0.482
8-9	0.441	0.336	0.597	0.337	0.411	0.401	0.421
9-10	0.478	0.400	0.342	0.299	0.371	0.433	0.387
10-11	0.524	0.407	0.327	0.295	0.294	0.372	0.370
11-12	0.311	0.278	0.240	0.304	0.269	0.374	0.296
12-13	0.319	0.279	0.301	0.363	0.300	0.296	0.310
13-14	0.351	0.255	0.280	0.299	0.222	0.278	0.281
14-15	0.328	0.233	0.265	0.309	0.247	0.275	0.276
15-16	0.291	0.276	0.276	0.252	0.252	0.283	0.272
16-17	0.246	0.174	0.174	0.176	0.190	0.164	0.187
17-18	0.228	0.130	0.171	0.156	0.117	0.178	0.163
18-19	0.124	0.105	0.170	0.111	0.110	0.153	0.129
19-21	0.378	0.274	0.295	0.294	0.325	0.301	0.311
21-23	0.357	0.258	0.280	0.303	0.276	0.340	0.302
23-25	0.088	0.349	0.294	0.255	0.280	0.308	0.262
25-27	0.288	0.176	0.138	0.204	0.218	0.226	0.208
27-29	0.266	0.140	0.197	0.181	0.149	0.280	0.202
29-32	0.270	0.114	0.220	0.260	0.239	0.237	0.223
32-36	0.570	0.366	0.490	0.646	0.587	0.499	0.526
36-50	1.397	1.074	1.562	1.768	1.352	2.028	1.530
50-64	1.086	0.663	0.957	1.125	0.452	0.712	0.833
TOTAL	14.0	11.1	13.5	12.1	11.5	13.6	12.6

[a] Data are expressed as percent of dose excreted during collection period.

Table 3

Tissue Distribution of Total Carbon-14
in Rats at 89 Days after a Single Intravenous
Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)

	Rat Number						
	1	2	3	4	5	6	Mean
Tissue[a]	µg/g[b]	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Liver	17.17	21.46	21.22	22.25	18.99	22.27	20.56
Plasma	2.37	2.17	2.37	2.27	2.05	2.05	2.21
Kidney	1.05	0.89	1.10	1.20	1.10	1.22	1.09
Lung	0.98	0.90	1.03	1.31	1.10	1.03	1.06
Spleen	0.48	0.50	0.51	0.57	0.48	0.49	0.51
Bone Marrow	0.41	0.43	0.39	0.79	0.35	0.38	0.46
Red Blood Cells	0.43	0.59	0.58	0.37	0.37	0.34	0.45
Adrenals	0.44	0.42	0.49	0.32	0.35	0.41	0.41
Testes	0.33	0.33	0.35	0.41	0.35	0.37	0.36
Skin	0.38	0.38	0.35	0.32	0.34	0.32	0.35
Muscle	0.41	0.31	0.31	0.29	0.26	0.18	0.29
Subcutaneous Fat	0.15	0.28	0.19	0.27	0.13	0.15	0.20
Eye	0.18	0.15	0.16	0.18	0.13	0.16	0.16

[a] Brain and abdominal fat were also assayed but level of carbon-14 was too low to quantitate ($\leq$ 0.08 µg/g) in abdominal fat, and too low to detect in brain (< 0.05 µg/g).

[b] Total carbon-14 concentration is expressed as µg equivalents of FC-95-^{14}C/g.

Table 4

Percent of Dose Present in Tissues
at 89 Days after a Single Intravenous
Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)

Tissue	Rat Number						Mean
	1	2	3	4	5	6	
	%	%	%	%	%	%	%
Liver[a]	20.16	27.11	25.39	29.06	24.96	24.58	25.21
Plasma[b]	2.81	2.82	2.84	3.00	2.75	2.64	2.81
Red Blood Cells[c]	0.42	0.64	0.58	0.41	0.41	0.37	0.47
Kidney[a]	0.27	0.22	0.24	0.30	0.27	0.30	0.27
Lung[a]	0.15	0.11	0.12	0.17	0.13	0.11	0.13
Testes[a]	0.10	0.09	0.11	0.12	0.10	0.10	0.10
Spleen[a]	0.02	0.02	0.02	0.03	0.02	0.02	0.02

[a] Data is expressed as percent of dose present in whole organ.

[b] Data is estimate of percent of dose present in plasma.
Plasma volume = 31.3 ml/kg body weight (4).

[c] Data is estimate of percent of dose present in red blood cells.
Red blood cells = 26.3 ml/kg body weight (4).

Figure 1

Cumulative Excretion of Total Carbon-14 in Urine
after a Single IV Dose of FC-95-^{14}C
(Mean Dose, 4.2 mg/kg)
Mean of 6 Rats

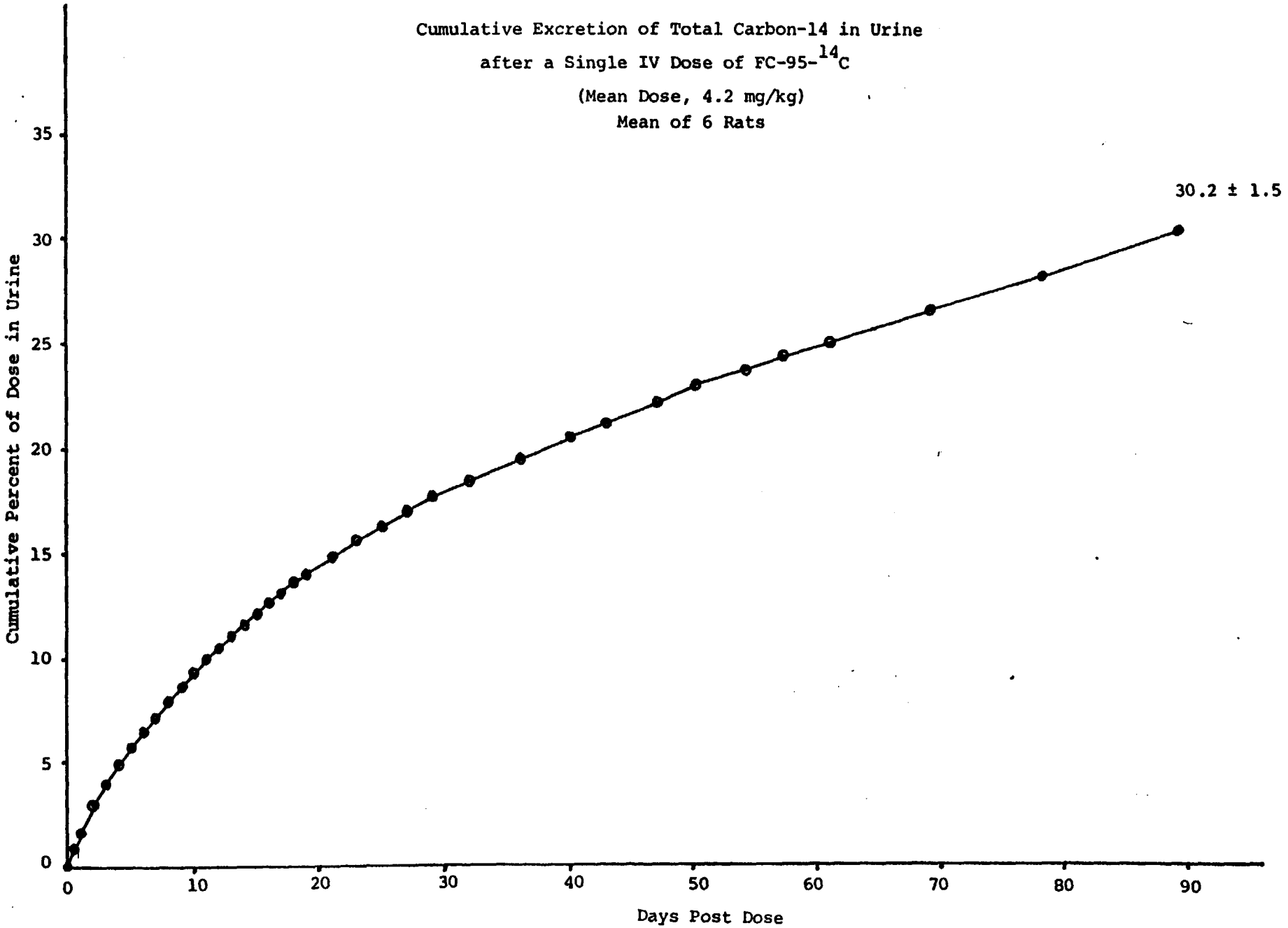
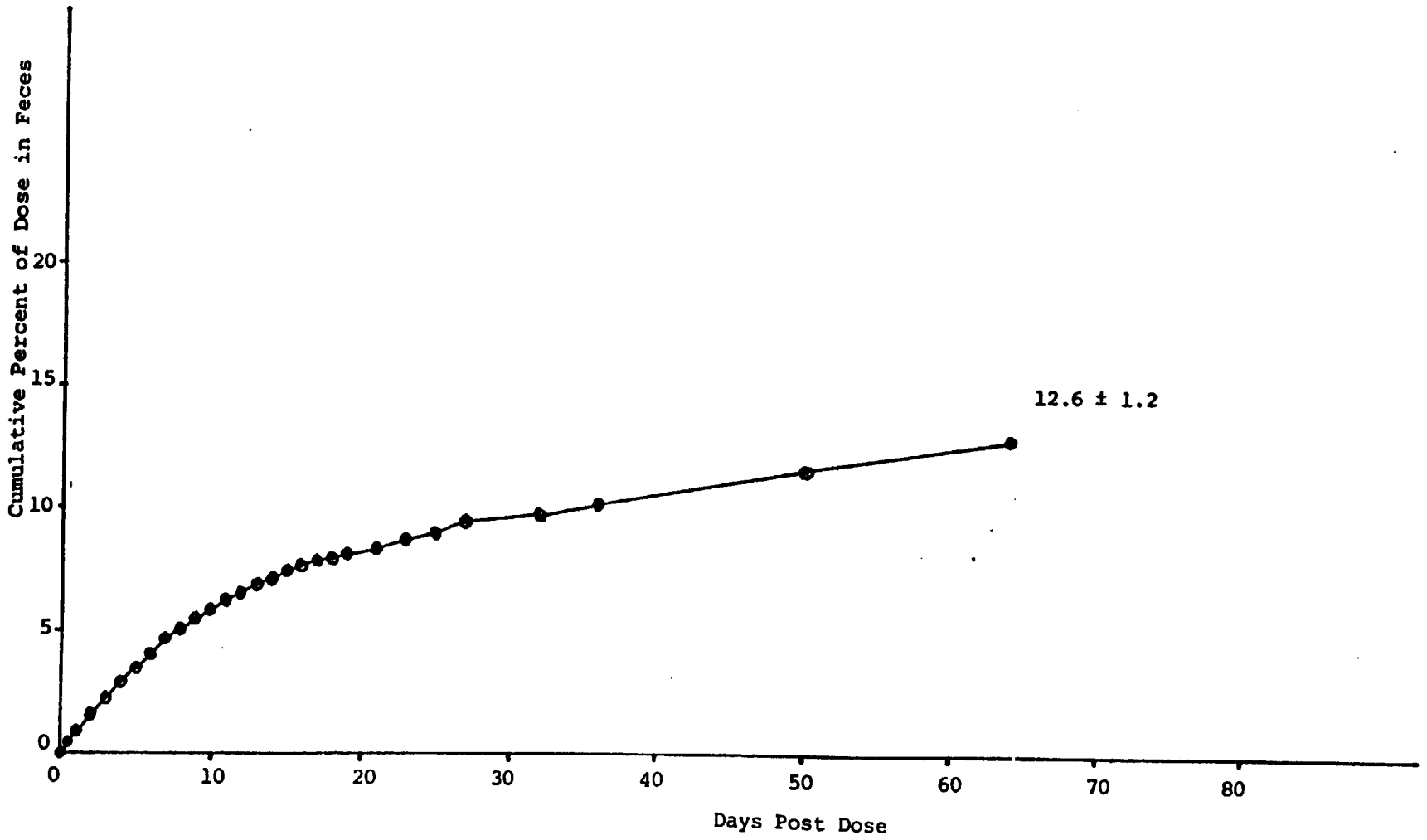


Figure 2

Cumulative Excretion of Total Carbon-14 in Feces
after a Single IV Dose of FC-95-^{14}C
(Mean Dose, 4.2 mg/kg)
Mean of 6 Rats



Appendix 1

Determination of Carbon-14 Content of Dosing Solution

Just prior to dosing of rats, the FC-95-^{14}C dosing solution was sampled with calibrated micropipettors[a] directly into counting vials. Six 10 µl and six 50 µl aliquots were pipetted, 1 ml of water and 15 ml of Aquasol® were added[b], corrections were made for background and counting efficiency and, using the specific activity of FC-95-^{14}C already determined[c], the µCi/2.0 ml was calculated. The data are shown in Appendix Table 1. The dose administered each rat is 2.0 ml of solution containing 0.553 µCi of FC-95-^{14}C which is 1.21 mg of FC-95-^{14}C. This is a mean dose of 4.2 mg/kg.

---

[a] L/I Micropipettor, Lab Industries, Berkeley, California.

[b] Aquasol® was found to be a suitable solvent-scintillent for FC-95-^{14}C during specific activity determination (1).

[c] Specific activity = 0.459 µCi/mg. Specific activity determination has been reported (1).

Appendix

Table 1

Carbon-14 Content of Dosing Solution

10 µl Aliquot µCi/2.0 ml	50 µl Aliquot µCi/2.0 ml
0.5602	0.5624
0.5554	0.5532
0.5614	0.5290
0.5628	0.5390
0.5612	0.5382
0.5554	0.5534

Overall average = 0.5526

$\frac{0.553}{0.4586\ \mu Ci/mg}$ = 1.21 mg FC-95-^{14}C/2.0 ml

Appendix 2

Recovery of Total Carbon-14 from Blank Biological Samples Spiked with FC-95-^{14}C

The good recovery of total carbon-14 from combusted FC-807-^{14}C in biological samples has been reported. (3) Since the carbon label is α to the sulfur atom in both FC-95-^{14}C and the components of FC-807-^{14}C, it is reasonable to expect that good recovery of total carbon-14 from FC-95-^{14}C and/or its metabolites in biological samples can be attained. For each set of samples combusted, four replicates of 10 µl, 50 µl, and 100 µl of diluted FC-95-^{14}C dosing solution were aliquoted with calibrated micropipettors directly into scintillation vials. At the same time using the same solution and pipettes, either three or four replicates of 10 µl, 50 µl and 100 µl were aliquoted directly into combustion cones which already contained blank biological material (fecal, liver, muscle, or spleen homogenates). The combustion cones were dried and then pelletized with 5 cm ashless filter paper. Blank filter paper pellets were combusted and the solvents collected in the vials to which the FC-95-^{14}C had been added directly. One each of the 10 µl, 50 µl, and 100 µl FC-95-^{14}C spiked pellets were routinely combusted at the beginning, middle, and end of each set of biological samples. After correction for background and counting efficiency, percent recovery was calculated by comparing mean results from direct addition and combustion. The mean recovery data for three sets of fecal samples that were analyzed on different days for total carbon-14 (FC-95-^{14}C) are shown in Appendix Table 2. The mean recovery is 98.0 ± 1.5%. Since each entry in the table is the mean of four determinations, this mean recovery is based on 36 determinations. Recovery of total carbon-14 from spiked tissue homogenates (not shown) was also 98%. Thus, good recovery of total carbon-14 from FC-95-^{14}C spiked biological material is shown.

Appendix

Table 2

Recovery of Total Carbon-14 from Blank Fecal Homogenate Samples Spiked with FC-95-^{14}C

10 µl[a]	50 µl	100 µl
97.4[b,c]	99.5	97.7
100.6	97.0	98.1
97.9	95.4	98.1
98.6	97.3	98.0

Overall average = 98.0 ± 1.5% (mean ± S.D.)

[a] Amount of FC-95-^{14}C reference solution added.

[b] Data are expressed as % recovery $\frac{\text{dpm from combustion}}{\text{dpm from direct addition}} \times 100)$

[c] Each entry is the mean of 4 combustion recovery determinations.

Appendix 3

Comparison of Carbon-14 Analysis of Urine Samples by Oxidation Versus Direct Counting in Aquasol®

The urine carbon-14 excretion data reported in this report are based on direct counting in Aquasol®. However, there is a concern that an insoluble metabolite or complex might exist in the urine so that not all of the radioactivity in the urine is counted. To investigate this possibility, concentration data from combustion were compared to concentration data obtained from direct counting of the same urine samples. The data are shown in Appendix Table 3. The mean ratio of the results from the comparison is 0.97. Thus, direct counting in Aquasol® is a valid method to assay total carbon-14 in the urine samples.

Appendix

Table 3

Comparison of Carbon-14 Analysis of Urine Samples by Oxidation versus Direct Counting in Aquasol®

Sample Number	Direct Counting (µg/ml)[a]	Oxidized (µg/g)[b]	Ratio Oxidized/ Direct Counting
1	0.98	0.95	0.97
2	1.04	1.01	0.97
3	0.76	0.75	0.99
4	0.66	0.62	0.94
5	0.86	0.72	0.84
6	0.77	0.84	1.09
		Mean	0.97

[a] Data expressed as µg FC-95-^{14}C equivalents/ml.

[b] Data expressed as µg FC-95-^{14}C equivalents/g.

Distribution List

M.T. Case	218-2
S.F. Chang	218-2
G.J. Conard	218-2
H.E. Freier	201-1S
~~F.D. Griffith~~	~~220-2E~~
L.I. Harrison	218-2
T.L. Kerley	218-1
J.D. LaZerte	236-1
L.J. Magill	223-6S
R.A. Nelson	218-1
R.E. Ober	218-2
J.A. Pendergrass	220-2E
R.A. Prokop	236-3B
A.L. Rosenthal	230-3
F.A. Ubel	220-2E
P. Venkateswarlu	236-3A
DMG Central File	218-2
Tech Communications	201-2CN
Central Files	218-1

Absorption of FC-95-^{14}C in Rats After a Single Oral Dose

October 26, 1979

Conducted at: Riker Laboratories, Inc.
Subsidiary of 3M
St. Paul, Minnesota 55101

During: June to July, 1979

Conducted by: S. J. Gibson and J. D. Johnson

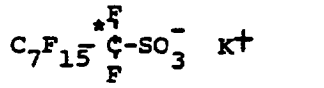
Report by: James D. Johnson 11/1/79
James D. Johnson, MS — Date
Senior Biochemical Pharmacologist

Reviewed by: Robert E. Ober 11/2/79
Robert E. Ober, Ph.D. — Date
Manager, Drug Metabolism

Summary

After a single oral dose of FC-95-^{14}C (mean dose, 4.2 mg/kg) in solution to groups of three male rats, at least 95% of the total carbon-14 is systemically absorbed at 24 hours. The half-life for elimination of total carbon-14 from plasma is 7.5 days.

Introduction



FC-95 *Denotes Position of Carbon-14

FC-95 is the potassium salt of a perfluorinated sulfonic acid. A series of experiments has been planned to investigate possible means of increasing the rate of elimination of FC-95 from the body. FC-95 absorption, elimination from plasma, and excretion data were necessary to form a basis for efficient design of these experiments. This FC-95-^{14}C oral dosing experiment (FC-Experiment 4) which was designed to provide total carbon-14 absorption and plasma elimination data was paired with an FC-95-^{14}C iv dosing experiment (FC-Experiment 3) which was designed to provide data on the route and extent of total carbon-14 excretion. In addition, these FC-95 absorption, plasma elimination, and excretion data will be used to interpret and guide other experiments on the possible biotransformation of FC-807 to FC-95.

Methods

Radiolabeled FC-95-^{14}C

The carbon-14 label is at the position α to the sulfur atom. (See above structure). The specific activity of this lot of FC-95-^{14}C (Riker Isotope Inventory Number 442) is 0.459 μCi/mg. The FC-95-^{14}C was found to be suitable for metabolism studies; details of the specific activity determination, chemical characterization, and radiochemical purity determination will be reported separately.

Animals

Male Charles River[a] CD rats, eight weeks old, were conditioned to individual metal metabolism cages for 24 hours prior to dosing. The body weights ranged from 243 to 315g, mean 285g. The rats were allowed free access to Purina[b] Ground Chow and water before and after dosing.

Dosing

Each non-fasted rat was weighed immediately before being given a single oral dose of FC-95-^{14}C. The dose was 2.0 ml of a 0.9% NaCl solution containing 1.21 mg FC-95-^{14}C/2.0 ml. The average dose was 4.2 mg/kg. The dose was delivered with a 2.0 cc glass syringe (Trylon®[c]) fitted with a stainless steel intubation tube. The dosing solution was prepared by adding ∿ 200 mg of FC-95-^{14}C to 0.9% NaCl, shaking for one half hour at moderate speed in a mechanical shaker, and centrifuging. The supernatant was removed and used for dosing solution. The carbon-14 content of the dosing solution was determined by direct counting (see Appendix 1).

---

[a] Charles River Breeding Laboratories, Wilmington, Mass.

[b] Purina Lab Chow, Ralston Purina Company, St. Louis, Missouri.

[c] Aloe Medical, Division of Brunswick, St. Louis 3, Missouri.

Sample Analysis for Carbon-14

Homogenizing of feces and tissue was done in Waring blenders by adding nine parts of water (w/w) to one part of biological material. The homogenates were weighed into combustion cones[a] in triplicate on a top-loading balance by taring the cone and adding 1.0g of the homogenate. Care was taken to mix the homogenate between samplings. Urine, red blood cells, and plasma samples (from the 24 and 48 hour post dose groups) were also measured into combustion cones by weight. The concentration of carbon-14 in plasma for the 3 rats in the 24 and 48 hour groups was determined by both combustion and direct counting. Plasma samples (including the 24 and 48 hour post dose groups) were counted directly by transferring 1.0 ml of plasma and 15 ml of Aquasol®[b] to a scintillation vial and counting in a liquid scintillation spectrometer. Homogenate, plasma, urine and red blood cell samples from the 24 and 48 hour post dose group were combusted with a Packard Model 306 Oxidizer. Combustion recovery of carbon-14 from biological samples was determined by combusting blank fecal homogenates and blank red blood cells spiked with dilutions of FC-95-^{14}C dosing solution at the beginning, middle, and end of the analysis of the experimental sample set (see Appendix 2).

All radiometric measurements were done using a Packard Model 3385 Tri-Carb Liquid Scintillation Spectrometer. For plasma samples counted directly, the efficiency for each sample was determined by adding internal standard to each sample and recounting, correcting for background from appropriate blanks, and comparing the results to a sealed standard of known activity. After correcting for background with appropriate blanks and for efficiency, the carbon-14 content of each sample was calculated. For oxidized samples, counting efficiency for each sample was determined by use of the AES (Automatic External Standardization) ratio method. To calibrate the external standard, internal standard was added to selected samples from the group of samples (three with low AES ratios and three with high ratios) and these samples were recounted along with a sealed standard. Data were collected on punch tape and processed by the CDC 1700 Computer System in the 3M Central Research Data Processing Laboratory. Data reduction to dpm was accomplished with the Biological Automatic External Standardization computer program.

Sample Collection

Groups of three rats were sacrificed by exsanguination at 1, 2, 6, 12, 24, 48, 96, and 144 hours post dose. Rats were anesthetized with diethyl ether and blood was drawn from the descending aorta of each rat and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. In addition to plasma and red blood cells, total urine, total feces, spleen, digestive tract plus contents (esophagus, stomach, small intestine, large intestine, and colon), and remainder of carcass were saved from each of the three rats in the 24 and 48 hours post dose groups for carbon-14 analysis.

---

a Packard Instrument Company, Inc., 2200 Warrenville Rd., Downers Grove, Illinois.

b New England Nuclear, Boston, Mass.

Results and Discussion

The results of the urine, feces, digestive tract plus contents, and carcass analyses are shown in Table 1. The digestive tract and contents contained on the average, 3.45% of the dose. The mean fecal excretion is 1.55% of the dose at 24 hours and 3.24% at 48 hours. At 24 hours, the mean sum of total carbon-14 in feces and digestive tract plus contents is 5% of the dose. Some of this 5% likely represents systemically absorbed carbon-14 present either in the digestive tract tissues or in the digestive tract contents as a result of excretion. The data from the 48 hour post dose group of rats are consistent with the 24 hour post dose data. Thus, at least 95% of the FC-95-^{14}C dose was absorbed from solution after administration to non-fasted rats. The major portion of the radioactivity recovered was found in the carcass. The carcass data are not as reliable as the other tissue data since large volume homogenates were necessary and homogeneity of sample aliquots was difficult to assure. There is some excretion of total carbon-14 in urine (1-2%/day); this is in contrast to the much slower rate of urinary excretion of total carbon-14 after an iv dose of FC-807-^{14}C (0.067%/day) (1). The spleens from the 24 hour and 48 hour post dose rats were analyzed for total carbon-14 content, and the percent of the dose in the whole organ was ∿ 0.2%. This is much lower than the 6% of the dose in spleen observed at 124 days post iv dose for FC-807-^{14}C (1).

The concentrations of total carbon-14 in red blood cells and plasma were compared (Table 2). The mean ratio of red blood cell to plasma concentration at 24 and 48 hours is 0.25 and 0.39, respectively. Thus, at 24 and 48 hours after a single oral dose of FC-95-^{14}C, there is no selective retention of carbon-14 in red blood cells.

The results from analysis of plasma samples from groups of three rats at 1, 2, 6, 12, 24, 48, 96, and 144 hours after a single oral dose of FC-95-^{14}C are shown in Table 3. The log of mean concentration versus time for these data is plotted in Figure 1. The least squares line through the individual points from 24 to 144 hours for these data fits the equation:

$C_p = 15.65e^{-0.00387t}$; C_p is plasma concentration. The half-life of elimination from plasma is 179 hours (7.5 days). Thus, elimination from plasma of total carbon-14 after a single oral dose of FC-95-^{14}C is slow.

References

1. Johnson, JD: Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single IV Dose of FC-807-^{14}C (Report), September 29, 1979.

2. Johnson, JD: Absorption of FC-807-^{14}C in Rats after a Single Oral Dose (Report), July 10, 1979.

List of Tables and Figures

Table 1: Percent Recovery of Total Carbon-14 after a Single Oral Dose of FC-95-^{14}C to Rats (Mean Dose, 4.2 mg/kg) at 24 and 48 Hours Post Dose.
NB 52584 p. 7.

Table 2: Comparison of Total Carbon-14 Content of Red Blood Cells to Plasma after a Single Oral Dose of FC-95-^{14}C to Rats (Mean Dose, 4.2 mg/kg) at 24 and 48 Hours Post Dose.
NB 52584 p. 7.

Table 3: Concentration of Carbon-14 in Rat Plasma after a Single Oral Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg) at 1, 2, 6, 12, 24, 48, 96, and 144 Hours Post Dose.
NB 52584 p. 10.

Figure 1: Mean Concentration of Carbon-14 in Rat Plasma after a Single Oral Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg).
NB 52584 p. 10.

Appendix, Table 1: Determination of Carbon-14 Content of Dosing Solution.
NB 51312 p. 35.

Appendix, Table 2A: Recovery of Total Carbon-14 from Blank Fecal Homogenate Samples Spiked with FC-95-^{14}C.
NB 52584 p. 12.

Appendix, Table 2B: Recovery of Total Carbon-14 from Blank Fecal Homogenate and Red Blood Cell Samples Spiked with FC-95-^{14}C.
NB 52584 p. 8.

Appendix, Table 3: Comparison of Oxidation with Direct Counting of Plasma Samples from Rats at 24 and 48 Hours after a Single Oral Dose of FC-95-^{14}C.
NB 52584 p. 11.

Table 1

Percent Recovery of Total Carbon-14 after a Single Oral Dose of FC-95-^{14}C to Rats (Mean Dose, 4.2 mg/kg) at 24 and 48 Hours Post Dose

Rat Number	Time Post Dose (Hours)	Percent of Dose			
		Carcass	Digestive Tract plus Contents	Feces	Urine
1	24	77.34[a]	3.27	1.68	1.67
2	24	77.06	3.45	1.72	1.94
3	24	82.62	4.02	1.24	1.09
Mean		79.01	3.58	1.55	1.57
4	48	103.61	3.55	3.25	1.91
5	48	86.21	3.17	3.10	2.78
6	48	92.63	3.24	3.37	2.88
Mean		94.15	3.32	3.24	2.52

[a] Data were adjusted for combustion recovery (see Appendix 2).

Table 2

Comparison of Total Carbon-14 Content of Red Blood Cells and Plasma after a Single Oral Dose of FC-95-^{14}C to Rats (Mean Dose, 4.2 mg/kg) at 24 and 48 Hours Post Dose

Rat Number	Time Post Dose (Hours)	Red Blood Cells	Plasma	Ratio Red Blood Cells/Plasma
1	24	3.63[a,b]	16.34[b]	0.22
2	24	4.15	14.62	0.28
3	24	3.18	13.40	0.24
Mean				0.25
4	48	2.80	14.27	0.20
5	48	7.85	14.25	0.55
6	48	4.94	11.76	0.42
Mean				0.39

[a] Data are expressed as µg equivalents of FC-95-^{14}C/g.

[b] The data were obtained by combustion and were adjusted for recovery (see Appendix 2).

Table 3

Concentration of Carbon-14 in Rat Plasma
after a Single Oral Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)
at 1, 2, 6, 12, 24, 48, 96, and 144 Hours Post Dose

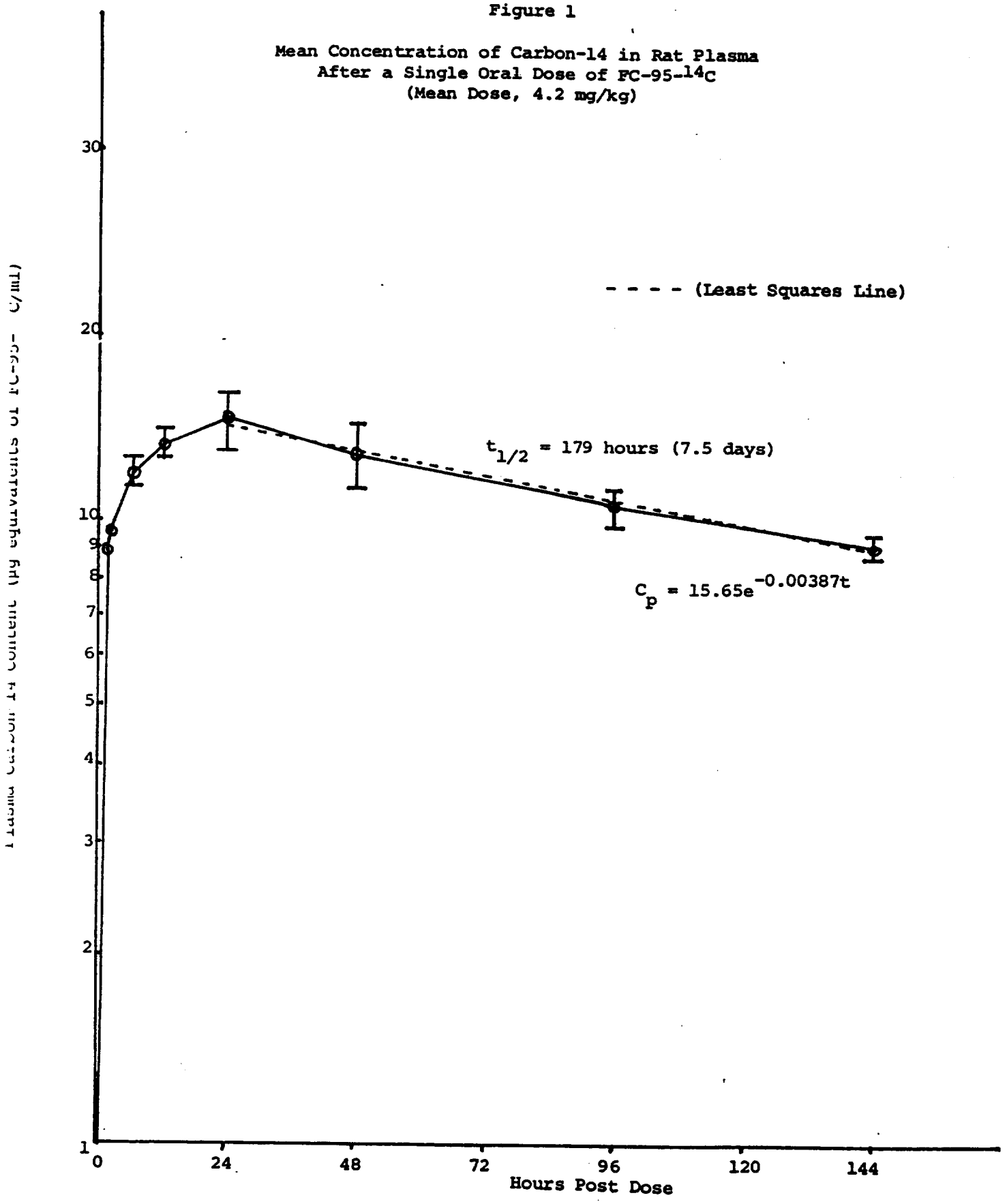
Hours Post Dose	Rat[a] A	B	C	Average ± SD
1	11.35[b]	7.47	7.62	8.81 ± 2.20
2	9.84	12.88	6.12	9.61 ± 3.39
6	11.74	11.59	12.60	11.98 ± 0.55
12	13.20	14.14	12.61	13.32 ± 0.77
24	16.35	14.43	13.02	14.60 ± 1.60
48	13.64	13.98	11.06	12.89 ± 1.60
96	11.05	9.67	11.00	10.57 ± 0.78
144	8.71	9.53	9.09	9.11 ± 0.41

[a] Three rats were dosed and sacrificed for each time interval.

[b] Data were obtained by direct counting; data are expressed as µg equivalents of FC-95-^{14}C/ml of plasma.

Figure 1

Mean Concentration of Carbon-14 in Rat Plasma
After a Single Oral Dose of FC-95-^{14}C
(Mean Dose, 4.2 mg/kg)



Appendix 1

Determination of Carbon-14 Content of Dosing Solution

Just prior to dosing of rats, the FC-95-^{14}C dosing solution was sampled with calibrated micropipettors[a] directly into counting vials; six 10 µl and six 50 µl aliquots were pipetted. One ml of water and 15 ml of Aquasol® were added[b], corrections were made for background and counting efficiency and, using the specific activity of FC-95-^{14}C already determined[c], the µCi/2.0 ml was calculated. The data are shown in Appendix Table 1. The dose administered each rat was 2.0 ml of solution containing 0.553 µCi of FC-95-^{14}C which is 1.21 mg of FC-95-^{14}C. This is a mean dose of 4.2 mg/kg.

---

[a] L/I Micropipettor, Lab Industries, Berkeley, California.

[b] Aquasol® was found to be a suitable solvent-scintillent for FC-95-^{14}C during specific activity determination (to be reported separately).

[c] Specific activity = 0.459 µCi/mg. (Specific activity determination to be reported separately).

Appendix

Table 1

Carbon-14 Content of Dosing Solution

10 µl Aliquot µCi/2.0 ml	50 µl Aliquot µCi/2.0 ml
0.5602	0.5624
0.5554	0.5532
0.5614	0.5290
0.5628	0.5390
0.5612	0.5382
0.5554	0.5534

Overall average = 0.5526

$$\frac{0.553}{0.4586\ \mu Ci/mg} = 1.21\ \mu Ci\ FC\text{-}95\text{-}^{14}C/2.0\ ml$$

Appendix 2

Recovery of Total Carbon-14 from Blank Biological Samples Spiked with FC-95-^{14}C

The good recovery of total carbon-14 from combusted FC-807-^{14}C in biological samples has been reported (2). Since the carbon label is α to the sulfur atom in both FC-95-^{14}C and the components of FC-807-^{14}C, it is reasonable to expect that good recovery of total carbon-14 from FC-95-^{14}C and/or its metabolites in biological samples can be attained. For each set of samples combusted, four replicates of 10 µl, 50 µl, and 100 µl of diluted FC-95-^{14}C dosing solution were aliquoted with calibrated micropipettors directly into scintillation vials. At the same time using the same solution and pipettes, either three or four replicates of 10 µl, 50 µl or 100 µl were aliquoted directly into combustion cones which already contained blank biological material (fecal homogenates, plasma, or red blood cells). The combustion cones were dried and then pelletized with 5 cm ashless filter paper. Blank filter paper pellets were combusted and the solvents collected in the vials to which the FC-95-^{14}C had been added directly. One each of the 10 µl, 50 µl, and 100 µl FC-95-^{14}C spiked pellets were routinely combusted at the beginning, middle, and end of each set of biological samples. After correction for background and counting efficiency, percent recovery was calculated by comparing mean results from direct addition and combustion. The mean recovery data for three sets of fecal samples that were analyzed on different days for total carbon-14 (FC-95-^{14}C) for FC-Experiments 3(to be reported later) are shown in Appendix Table 2A. The mean recovery is 98.0 ± 1.5%. Since each entry in the table is the mean of four determinations, this mean recovery is based on 36 determinations. Thus, good recovery of total carbon-14 from FC-95-^{14}C spiked biological material is shown. The mean recovery for the set of samples in the report (FC-Experiment 4) which is based on triplicate 10 µl, 50 µl, and 100 µl FC-95-^{14}C spiked blank fecal homogenates and red blood cells was 93.6%. (See Appendix Table 2B). This slightly lower recovery was due to a minor malfunction of the Packard Oxidizer and was fairly uniform throughout the set of samples. Thus, the total carbon-14

data obtained from combustion in this report were adjusted for recovery.

There was no apparent differences in recovery between spiked blank red blood cells and spiked fecal homogenates.

Appendix

Table 2A

Recovery of Total Carbon-14 from Blank Fecal Homogenate Samples Spiked with FC-95-^{14}C

10 µl[a]	50 µl	100 µl
97.4[b,c]	99.5	97.7
100.6	97.0	98.1
97.9	95.4	98.1
98.6	97.3	98.0

Overall average = 98.0 ± 1.5%

[a] Amount of FC-95-^{14}C reference solution added.

[b] Data are expressed as % recovery $\left(\frac{\text{dpm from combustion}}{\text{dpm from direct addition}} \times 100\right)$

[c] Each entry is the mean of 4 combustion recovery determinations.

Appendix

Table 2B

Recovery of Total Carbon-14 from Fecal Homogenate and Red Blood Cell Samples Spiked with FC-95-^{14}C

DIRECT ADDITION

10 µl[a]	50 µl	100 µl
756[b]	3868	7403
756	3688	7404
727	3719	---[c]
723	3671	7394
741 ± 18	3737 ± 90	7400 ± 6

SPIKED AND OXIDIZED

	10 µl		50 µl		100 µl	
	Fecal Homogenates	Red Blood Cells	Fecal Homogenates	Red Blood Cells	Fecal Homogenates	Red Blood Cells
	619	671	3581	3583	7408	7093
	613	---[d]	3775	3583	7104	7098
	677	711	3484	3347	7105	7081
$\bar{X}$	636	691	3613	3504	7106	7091

$\frac{636}{741} \times 100 = 85.8\%$ $\frac{3613}{3737} \times 100 = 96.7\%$ $\frac{7106}{7400} \times 100 = 96.0\%$

$\frac{691}{741} \times 100 = 93.3\%$ $\frac{3504}{3737} \times 100 = 93.8\%$ $\frac{7091}{7400} \times 100 = 95.8\%$

Overall average = 93.6%

[a] Amount of FC-95-^{14}C spiking solution added.

[b] Data are expressed as dpm.

[c] Sample was not used; cracked vial.

[d] Spiking error; sample was not used.

Appendix 3

Comparison of Oxidation with Direct Counting of Plasma Samples from Rats at 24 and 48 Hours after a Single Oral Dose of FC-95-^{14}C

Concentration of Carbon-14 in plasma for the three rats in both the 24 and 48 hour post dose groups was determined by both oxidation and direct counting. Samples were counted directly by transferring 1.0 ml of plasma and 15 ml of Aquasol® to scintillation vials and counting in the liquid scintillation counter. Corrections for efficiency and background were made for each sample. For determination by oxidation, plasma samples were measured by weight into combustion cones and carbon-14 was determined by combusting and counting. The data from the plasma oxidation - direct counting comparison are shown in Appendix Table 3. The mean ratio of oxidized to direct counting is 0.97± 0.02. Thus, the plasma level data obtained via either of these two methods will be essentially the same. The direct counting method, being much more convenient, was chosen to analyze the plasma, and the data quoted for the plasma concentration versus time (Figure 1) is from direct counting. Though a direct measurement of absolute recovery from plasma was not done, these results indicate that recovery of total carbon-14 from plasma spiked with FC-95-^{14}C would be similar to that found for recovery from fecal homogenates (98%, Appendix 2).

Appendix

Table 3

Comparison of Oxidation with Direct Counting of Plasma Samples from Rats at 24 and 48 Hours after a Single Oral Dose of FC-95-^{14}C

Rat	Direct Counting (µg/ml)[a]	Oxidation (µg/g)[a]	Ratio Oxidation/ Direct Counting
1 (24 hours)	16.35	15.30	0.94
2 (24 hours)	14.43	13.99	0.97
3 (24 hours)	13.02	12.55	0.96
1 (48 hours)	13.64	13.36	0.98
2 (48 hours)	13.98	13.33	0.95
3 (48 hours)	11.06	11.01	1.00
			X ± SD 0.97 ± 0.02

[a] Data are expressed as µg equivalents of FC-95-^{14}C.

FUNDAMENTAL AND APPLIED TOXICOLOGY 4, 972–976 (1984)

Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [^{14}C]Perfluorooctanoate or Potassium [^{14}C]Perfluorooctanesulfonate

JAMES D. JOHNSON,[1] SHEILA J. GIBSON, AND ROBERT E. OBER

Riker Laboratories, Inc., 3M Center, Building 270-3S-05, St. Paul, Minnesota 55144

Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [^{14}C]Perfluorooctanoate or Potassium [^{14}C]Perfluorooctanesulfonate. JOHNSON, J. D., GIBSON, S. J., AND OBER, R. E. (1984). *Fundam. Appl. Toxicol.* 4, 972–976. After a single intravenous dose of ammonium [^{14}C]perfluorooctanoate ([^{14}C]PFO, 13.3 mg/kg) or of potassium [^{14}C]perfluorooctanesulfonate ([^{14}C]PFOS, 3.4 mg/kg) to rats, cholestyramine fed daily as a 4% mixture in feed was shown to increase the total carbon-14 eliminated via feces and to decrease liver concentration of carbon-14. Rats were fed cholestyramine in feed for 14 days after administration of [^{14}C]PFO and for 21 days after administration of [^{14}C]PFOS. Control rats were administered radiolabeled fluorochemical but were not treated with cholestyramine. Cholestyramine treatment increased mean cumulative carbon-14 elimination in feces by 9.8-fold for rats administered [^{14}C]PFO and by 9.5-fold for rats administered [^{14}C]PFOS. After [^{14}C]PFO, a mean of 4% of the dose of carbon-14 was in liver of cholestyramine-treated rats at 14 days versus 7.6% in control rats; after [^{14}C]PFOS, 11.3% of the dose was in liver at 21 days versus 40.3% in control rats. After administration of either radiolabeled compound, plasma and red blood cell carbon-14 concentrations, which were relatively lower than liver concentrations, were also significantly reduced by cholestyramine treatment. © 1984 Society of Toxicology.

The anion of perfluorooctanoic acid has been found in the serum of manufacturing plant workers (Ubel *et al.*, 1980). Toxicity studies in animals have been reported (Griffith and Long, 1980). In view of the slow elimination and the presence of the compound in plant workers' serum, a means of enhancing elimination was sought. In addition, since both ammonium perfluorooctanoate and potassium perfluorooctanesulfonate are commercial products and intermediates of products, the possibility of affecting the elimination of perfluorooctanesulfonate was also of interest. Cholestyramine, an agent approved for use in humans to lower cholesterol blood levels, is an anion exchange resin which has been used to promote fecal excretion of the pesticide chlordecone (Kepone) in rats (Cohn *et al.*, 1978). Since both perfluorooctanoic acid and perfluorooctanesulfonic acid are prepared as salts and exist as anions at physiologic pH, they would be expected to complex with cholestyramine in vivo. Although Pastoor *et al.* (1983) investigated cholestyramine in mice and rats and found no enhanced elimination of carbon-14 with a single 1000-mg/kg dose of cholestyramine given at 24 hr after a 10-mg/kg oral dose of ammonium [^{14}C]perfluorooctanoate, the usual application of cholestyramine administered to enhance elimination of xenobiotics is with repeated doses. In the study reported here, we investigated the effect of cholestyramine administered in feed for several days on the rate of fecal elimination of carbon-14 after either a subtoxic intravenous dose of ammonium [^{14}C]perfluorooctanoate ([^{14}C]PFO) or potassium [^{14}C]perfluorooctanesulfonate ([^{14}C]PFOS).

[1] To whom all correspondence should be addressed.

0272-0590/84 $3.00
Copyright © 1984 by the Society of Toxicology.
All rights of reproduction in any form reserved.

Notice: This material may be protected by copyright law (Title 17 U.S. Code).

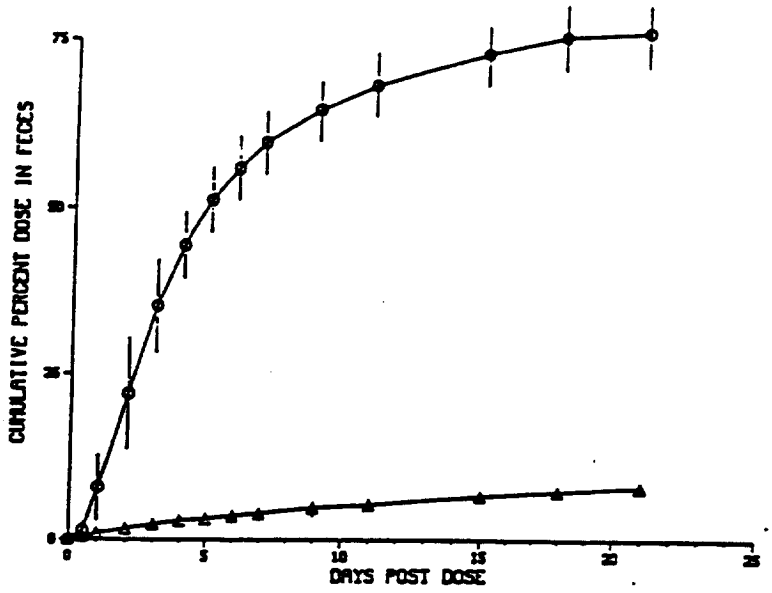


FIG. 1. Cumulative excretion of total carbon-14 in feces after a single intravenous dose of potassium [^{14}C]perfluorooctanesulfonate ([^{14}C]PFOS). Mean dose of [^{14}C]PFOS: cholestyramine treated, 3.4 mg/kg (O); control 3.5 mg/kg (Δ). Total cumulative percentage excreted in feces: cholestyramine treated, 75.8 ± 5.0, control, 8.0 ± 0.8.

sents 4 and 8% of the dose for cholestyramine-treated and control rats, respectively. The mean carbon-14 concentration of plasma and red blood cells with cholestyramine treatment is significantly less than the mean concentration in controls.

DISCUSSION

The carbon-14 present in rat liver after administration of [^{14}C]PFOS or [^{14}C]PFO is probably in the form of anions. (The pK_a of these compounds is <1.0.) Thorough inves-

TABLE 1

EFFECT OF CHOLESTYRAMINE TREATMENT ON CONCENTRATION OF CARBON-14 IN RAT LIVER, PLASMA, AND RED BLOOD CELLS AFTER A SINGLE INTRAVENOUS DOSE OF POTASSIUM [^{14}C]PERFLUOROOCTANESULFONATE[a] ([^{14}C]PFOS) OR AMMONIUM [^{14}C]-PERFLUOROOCTANOATE[b] ([^{14}C]PFO)

Treatment group	Carbon-14 concentration[c]		
	Liver	Plasma	Red blood cells
[^{14}C]PFOS			
Cholestyramine-treated (21 days)	9.4 ± 1.6[d]	0.9 ± 0.1[d]	0.3 ± 0.1[d]
Controls	35.6 ± 5.6	6.9 ± 0.6	1.8 ± 0.4
[^{14}C]PFO			
Cholestyramine-treated (14 days)	12.1 ± 2.1[d]	5.1 ± 1.7[d]	1.8 ± 0.7[d]
Controls	22.3 ± 6.2	14.7 ± 6.8	4.2 ± 2.4

[a] Each rat received 1.13 mg [^{14}C]PFOS; mean dose: cholestyramine-treated rats, 3.4 mg/kg; control rats, 3.5 mg/kg.

[b] Each rat received 4.2 mg [^{14}C]PFO; mean dose: cholestyramine-treated rats, 13.3 mg/kg; control rats .5 mg/kg.

[c] Concentration is expressed as μg eq of radiolabeled compound/g of tissue or ml of fluid.

[d] Significantly different from control values ($p < 0.05$).

remaining in the liver. This difference is most likely due to small additive losses in collection of the many urine and feces samples as well as the small but cumulative error in not correcting for combustion recovery in the many samples (>95%). Since the experimental conditions for cholestyramine-treated and control rats were identical with respect to the dosing, sample collection, and analyses, the less than 100% recovery of carbon-14 (urine, feces, and liver) was judged to not affect the results or conclusion.

Overall, the data support possible utility of cholestyramine in promoting the excretion of perfluorooctanoate and perfluorooctanesulfonate in humans.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the synthesis of ammonium [^{14}C]perfluorooctanoate and potassium [^{14}C]-perfluorooctanesulfonate by F. E. Behr of Commercial Chemicals Division, 3M, and the typing of this manuscript by K. F. Gibson and J. E. Warn of Riker Laboratories.

REFERENCES

BOYLAN, J. J., EGLE, J. L., AND GUZELIAN, P. S. (1978). Cholestyramine: Use as a new therapeutic approach for chlordecone (Kepone®) poisoning. *Science (Washington, D.C.)* 199, 893–895.

COHN, W. J., BOYLAN, J. J., LANKE, R. V., FARISS, M. W., HOWELL, J. R., AND GUZELIAN, P. S. (1978). Treatment of chlordecone (Kepone®) toxicity with cholestyramine. *N. Engl. J. Med.* 298, 243–278.

GRIFFITH, F. D., AND LONG, J. E. (1980). Animal toxicity studies with ammonium perfluorooctanoate. *J. Amer. Ind. Hyg. Assoc.* 41, 576–583.

PASTOOR, T. P., KINZLER, K. W., AND KENNEDY, G. L. JR. (1983). The effect of cholestyramine on elimination of ammonium perfluorooctanoate from the blood of rats and mice. *Toxicologist* 3, 82.

UBEL, F. A., SORENSON, S. D., AND ROACH, D. E. (1980). Health status of plant workers exposed to fluorochemicals—A preliminary report. *J. Amer. Ind. Hyg. Assoc.* 41, 584–589.

3M Environmental Laboratory

Final Report- Analytical Study

Single-dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits

In-Vivo Study Reference Number: HWI#6329-130

Study Number: AMDT-020895.1
Test Substance: FC-95 (T-6049)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology & Services
935 Bush Avenue
St. Paul, MN 55106

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/20/95
Completion Date

1.0 SUMMARY

Samples of rabbit liver at 28 days post dermal administration of 0, 0.003, 0.06, and 0.30 mg/kg FC-95 (T-6049) were analyzed by combustion for total organic fluorine. There was no detectable level of total organic in any of the treated groups above that observed in control rabbit liver. Since in a pharmacokinetic study (HWI#6329-159), it was established that perfluorooctanesulfonate is persistent in rabbit liver with a biological half-life in excess of one month and that perfluorooctanesulfonate will serve as a good marker for absorption of FC-95, it is concluded that detectable dermal absorption does not occur at these dose levels. These dose levels are very low and possibly too low to make an assessment of dermal absorption.

2.0 INTRODUCTION

FC-95 is the potassium salt of perfluorooctanesulfonic acid. In a previous pharmacokinetic study (HWI#6329-159), it was shown that the biological half-life is >1 month. It is persistent in serum and liver after an intravenous dose. From previous studies in rats, the molecule is not known to biotransform or conjugate. Thus, analysis by combustion with subsequent measurement of fluoride will estimate the amount of perfluorooctanesulfonate present. At later times, the concentration in liver is higher than the amount in serum on the average. For use as a marker to assess dermal absorption, the level of total organic fluorine in liver is therefore a more sensitive test at 28 days post dose.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company.

3.3. Purity and Strength of Reference Substance: Responsibility of Sponsor

3.4 Stability of Reference Substance: To be determined by Sponsor

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples, the storage is -20±10^0 C.

3.6 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

4.0 EXPERIMENTAL - Overview

Samples of tissue and serum were available from HWI#6329-130 for analysis for total organic fluorine. The samples were combusted and analyzed for total organic fluorine. The data were assessed for extent of dermal absorption of FC-95.

5.0 EXPERIMENTAL - Methods

5.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

5.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

5.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

5.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

6.0 DATA ANALYSIS

The data from combustion analysis at 28 days post dermal administration of FC-95 (T-6049) are attached. The dosing material T-6049 is a 0.06% solution of FC-95. Doses in this analysis are expressed as the FC-95 equivalents. The liver was combusted and subsequently analyzed for total organic fluoride. There is no evidence of dermal absorption at any dose level for the groups of rabbits in the control, 0.003 mg/kg, 0.06 mg/kg, and 0.30 mg/kg groups. In all cases the amount of organic fluorine was below the practical quantitation levels and apparently below the detection limits.

Electrospray mass spectrometry did detect perfluorooctanesulfonate anion, but again the results are nebulous and below the practical quantitation limits.
There does not appear to be dermal absorption of FC-95 at these dose levels.

6.1 Circumstances That May Have Affected the Quality of the Data: No problems with this analysis are recognized that could affect the conclusion. However, the doses administrated are so low that even if all the dose had been absorbed it would be difficult to quantitate. In the higher dose group, 2 kg rabbits would receive only 600 ug total. For dermal dosing with analysis at 28 days and a half life on the order of a month, there would be less than 300 ug present in liver even if all the absorbed perfluorooctanesulfonate had gone to the liver.

7.0 CONCLUSION

FC-95 is not dermally absorbed at 0.30 mg/kg dose. This is a very low dose and possibly too low to make an assessment of dermal absorption.

8.0 MAINTENANCE OF RAW DATA AND RECORDS

8.1 Raw Data and Data: Raw data, approved protocol, approved final report, appropriate specimens, and electronic data will be maintained in the AMDT archives.

9.0 APPENDICES

9.1 Protocol and Amendments

9.1.1 Protocol and Final Report: HWI#6329-130 "Single Dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits (Protocol type TP-3016.AB for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-020895.1 including methods.

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-020895.1

9.10 Instrument Settings: See methods

9.11 Data: See attached:

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

9.1.1 Protocol and Final Report: HWI#6329-130 "Single Dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707-7545

a CORNING Company

Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

JUN 29 1995
3M
TOXICOLOGY

FINAL REPORT

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

June 27, 1995

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-130

Phone 608-241-4471 Fax 608-241-7227
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD MADISON, WI 53704

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
12/21/94	12/21/94	Protocol Review	12/22/94	01/10/95
01/10/95	01/10/95	Animal Observation	01/10/95	02/10/95
01/24/95	01/24/95	Protocol Amendment	01/24/95	02/10/95
04/03/95	04/13/95	Data/Report Review	04/14/95	05/10/95
04/03/95	04/13/95	Data Review	04/14/95	05/10/95
06/27/95	06/27/95	Report Rereview	06/27/95	07/10/95

Randy Lester
Representative, Quality Assurance Unit

6·27·95
Date

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits

Test Material	T-6049
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Study Initiation Date	December 30, 1994
Experimental (In-life) Start Date	January 7, 1995
In-life End Date	February 10, 1995
Experimental Termination Date	June 27, 1995
Study Completion Date	June 27, 1995

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Francis (Bud) W. McDonald
Study Coordinator

Patricia Padgham
In-life Supervisor

Rose M. Bridge
Report Supervisor

Quality Assurance

Sherry R. W. Petsel
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Thomas E. Palmer, PhD
Anatomical Pathologist

Jack Serfort/
Deborah L. Pirkel
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

	Page
Quality Assurance Statement	2
Study Identification	3
Key Personnel	4
Summary	6
Objective	8
Regulatory Compliance	8
Test and Control Materials	8
Test System	9
Procedures	10
Results	13
Discussion	14
Signature	14
Reference	14
Pathology Report	15
Table	
1 Individual and Mean Body Weights (g)	16
2 Individual Clinical Signs	17
3 Individual Dermal Irritation Scores	19
4 Individual Pathology Comments	23
5 Individual Animal Tissue Weights and Bile Volumes	25
Appendix A	27
Protocol Deviations	28
Protocol TP3016.AB	29
Protocol Amendment No. 1	41

SUMMARY

This study was done to assess the systemic absorption/toxicity and relative skin irritancy of T-6049 when applied to the skin of rabbits.

The study was conducted using three male and three female acclimated rabbits of the Hra:(NZW)SPF strain for each treatment group.

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Number of Animals Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6049	5	3	3*
3	T-6049	100	3	3*
4	T-6049	500	3	3

a Administered at a dose volume of 2.0 mL/kg.
* One animal sacrificed on Day 2 due to possible broken back and replaced with another female animal.

The back of each rabbit was clipped free of hair and a single dose of the respective material at the indicated dose level was administered to the skin of the rabbits. The treatment sites remained intact. The area of application was covered with a gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing for a 24-hour exposure period.

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks were conducted daily thereafter for 28 days. The only exceptions to this were on Days 4 and 6 when the afternoon mortality check was inadvertently not conducted for the two replacement animals. Body weights were determined on Day -4 for randomization purposes, before test or control material administration (Day 1), and at in-life termination (Day 29). The initial dermal irritation reading was made before test or control material administration (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8. Blood samples were collected from a marginal ear vein of the animals before in-life initiation (Day 1), approximately 24-hours postdose (Day 2), on Days 4, 9, 16, and 23. Replacement animals were bled on Days 1, 2, 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from each animal. All samples were centrifuged and separated into serum and cellular fractions. All animals were euthanized at termination of the in-life phase and necropsied. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected at necropsy

and weighed (volume only determined for bile). The blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen to the Sponsor after termination of the in-life phase.

Application of T-6049 did not result in any test material-related changes in body weight gain. All animals appeared clinically normal throughout the study with the exception of one Group 2 and one Group 3 female animals that were sacrificed on Day 2 due to injury (possible broken backs). These animals were replaced in the study and the replacement animals appeared normal throughout the study. No dermal irritation was observed at the dermal scoring intervals as a result of the application of distilled water or T-6049 at any of the dose levels. There were no test material-related lesions observed at necropsy.

OBJECTIVE

The objective of this study was to assess the systemic toxicity/absorption and relative skin irritancy of a test material when applied to the skin of rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test material mixture prepared for the Group 2 animals for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6049 and described as a clear, colorless liquid. The control material was distilled water and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). Analysis of the test material mixture prepared for the Group 2 animals for concentration, homogeneity/solubility, and stability was not conducted or requested by the Sponsor. The purity and stability of the control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test and control materials were stored at room temperature. A reserve sample of each test and control material was taken and will be retained in a freezer set to maintain a temperature of -20°C ±10° for 10 years in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any unused test material was returned to the Sponsor after completion of the in-life phase according to HWI SOP. Any remaining control material is retained for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were procured from HRP, Inc., Denver, Pennsylvania on December 28, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were placed into study groups using a stratified body weight randomization program. The randomization body weights were determined on Day -4. The weight variation of the animals for each group of each sex selected for the study did not exceed ±2 standard deviations of the mean weight, and the mean body weights for each group of each sex were not statistically different at the 5% probability level. Two female animals (Nos. F53398 and F53355) were replaced after they were treated due to possible broken backs. These animals were replaced with two other female animals (Nos. F52999 and F53351) which were treated in the same manner.

Study Design

Animals weighing from 2,018 to 2,625 g at initiation of treatment were placed into the following study groups:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Number of Animals Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6049	5	3	3*
3	T-6049	100	3	3*
4	T-6049	500	3	3

a Administered at a dose volume of 2.0 mL/kg.
* One animal sacrificed on Day 2 due to possible broken back and replaced with another female animal. This animal weighed 2,625 g at initiation.

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Preparation of Exposure Area

On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit was clipped free of hair. The clipped area made up approximately 20% of the total body surface area. The intact skin of the test sites was inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals were clipped as needed throughout the study to aid in visualizing the application sites.

Dose Administration

All animals received a single administration of the respective test or control material. The day of treatment was designated as Day 1.

Group 1. An individual dose (2.0 mL/kg) was calculated and measured based on each animal's body weight on the day of treatment. The control material (distilled water) was applied evenly to the test site at a rate of approximately 0.05 mL/cm^2.

Groups 2, 3, and 4. For the Group-2 animals (5 mg/kg), the test material (T-6049) was mixed with distilled water to a concentration of 500 mg/mL and applied at a dose volume of 0.01 mL/kg. The mixture was stored at room temperature until administered. The test material was administered undiluted to the test sites of the Groups 3 and 4 animals (100 or 500 mg/kg, respectively) using the average bulk density of 0.99 g/mL to determine the dose volume for each dose level (0.10 and 0.51 mL/kg, respectively). The area of exposure for the 5, 100, and 500 mg/kg dose levels was 4, 25 (16 for replacement animal), and 100 cm^2, respectively. The approximate rate of application ranged from 0.006 to 0.014 mL/cm^2. An individual dose of the respective test material or test material mixture was calculated for each animal based on its body weight on the day of treatment.

Each area of application was covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. Collars were used to restrain the animals during the 24-hour exposure period.

Approximately 24 hours after test or control material application, the restraining collars and bandages were removed and any residual test material was removed with tap water and disposable paper towels.

Reason for Route of Administration

The dermal route is a potential route of exposure in humans.

Observations of Animals

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks (morning and afternoon) were conducted daily thereafter for 28 days. The only exceptions to this were on Days 4 and 6 when the afternoon mortality check was inadvertently not conducted for the two replacement animals.

Body weights were determined for randomization purposes on Day -4, before test material administration (Day 1), and at in-life termination (Day 29).

The initial dermal irritation reading was made before test or control material administration according to the Draize[1] technique (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8.

Sample Collections

Blood samples (approximately 4 mL) were collected from a marginal ear vein of all animals before experimental initiation (Day 1). Subsequent collection of blood was conducted approximately 24-hours postdose (Day 2), and on Days 4, 9, 16, and 23. Replacement animals were bled on Days 1, 2, 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were centrifuged and separated into serum and cellular fractions. These samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the experimental phase (Day 29), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, exsanguinated, and necropsied in random order. The sites of test and control material application were washed with lukewarm tap water before the necropsy procedure. All animals were subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female in each group were collected. The tissue samples for all animals, with the exception of the animal sacrificed and necropsied on Day 2, were weighed (volume only determined for bile) and immediately placed on dry ice, then placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals were discarded.

Shipment of Blood, Bile, and Tissues

After experimental termination, the blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106), along with the collected corresponding weights or volumes. The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the tissue samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual and mean body weights are in Table 1. All animals exhibited body weight gains from Day 1 to Day 29.

Clinical Observations

Individual clinical signs are in Table 2. All animals appeared normal throughout the study with the following exceptions:

- One Group 2 female (No. F53398) treated with T-6049 at 5 mg/kg appeared to have injured its back at the time of the 24-hour blood collection. This animal was sacrificed, necropsied, and replaced with No. F52999. The replacement animal appeared normal throughout the study.

- One Group 3 female (No. F53355) treated with T-6049 at 100 mg/kg was found prior to completion of the exposure period with its leg caught in the cage floor, appeared to have injured its back, and was sacrificed. Since this animal did not receive its full test material exposure, the animal was discarded without a gross necropsy examination. The animal was replaced with No. F53351. The replacement animal appeared normal throughout the study.

Dermal Irritation

Individual dermal irritation scores are in Table 3. The control material produced no dermal irritation. No dermal irritation was observed in the animals treated with T-6049 at any of the dose levels.

Pathology

Individual animal pathology comments are presented in Table 4. Individual animal tissue weights and bile volumes are in Table 5. The treated skin in two animals dosed at 0 mg/kg, two animals dosed at 5 mg/kg, and one animal dosed at 500 mg/kg was diffusely red or had multiple red areas of variable size. These findings are not considered to be test material-related. There were no lesions observed in any of the remaining animals.

Page 15 contains a pathology report by the study pathologist.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6049 were evaluated in male and female albino rabbits when administered as a single dermal application. There were no test material-related changes in body weight gain or in-life clinical findings at any of the dose levels. No test material-related dermal irritation was observed during the study. There were no test material-related lesions observed at necropsy.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

6-27-95
Date

REFERENCE

1. Draize, J. H., "Acute Dermal Toxicity (Single Exposure)," In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity*, Association of Food and Drug Officials of the U.S., pp. 54-56 (1959).

PATHOLOGY REPORT

There were six rabbits (three males and three females) each from four dose levels of 0, 5, 100, and 500 mg/kg of body weight euthanized and necropsied at the termination of the study. One female (Animal No. F53398) dosed at 5 mg/kg of body weight was sacrificed on Day 2 because of an apparent broken back. This animal was replaced with another female (Animal No. F52999). The test material, dose level, day of death, and gross observations recorded for each animal are in the Individual Pathology Comments that follow this report.

At necropsy, the treated skin in some animals was diffusely red or had multiple red areas of variable size. This included two animals dosed at 0 mg/kg, two animals dosed at 5 mg/kg, and one animal dosed at 500 mg/kg. There were no visible lesions in any of the remaining animals. The liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from each of these animals, and both kidneys from one male and one female in each group were collected. The tissue samples were weighed (volume only determined for bile), frozen, and sent to the Sponsor. The tissues from the female dosed at 5 mg/kg and sacrificed on Day 2 were collected but were not weighed. After necropsy, the animals were discarded.

The animal sacrificed on Day 2 had multiple dark red areas of variable size within the skeletal muscle of the lumbar-sacral region of the spinal column. These findings correlated with the clinical observation of an apparent broken back.

Thomas E. Palmer, PhD
Pathologist

6-27-95
Date

(6329-130.slh)
03/22/95

Table 1
Individual and Mean Body Weights (g)

Male				Female			
Animal Number	Randomization Day -4	Day 1	Day 29	Animal Number	Randomization Day -4	Day 1	Day 29
Group 1 (Control) - Distilled Water (0 mg/kg)							
F53358	2,066	2,231	2,816	F53404	2,200	2,286	2,997
F53359	2,030	2,093	2,670	F53405	2,426	2,474	2,911
F53353	2,136	2,312	3,012	F53356	2,110	2,224	2,685
Mean	2,077	2,212	2,833		2,245	2,328	2,864
Group 2 - T-6049 (5 mg/kg)							
F53402	2,288	2,382	2,938	F53399	2,095	2,122	2,580
F53364	2,244	2,364	2,923	F53349	2,376	2,492	2,919
F53352	2,102	2,018	2,622	F53398[a]	2,331	2,419	-
				F52999	2,485	2,625	3,272
Mean	2,211	2,255	2,828		2,319	2,413	2,924
Group 3 - T-6049 (100 mg/kg)							
F53360	2,157	2,194	2,943	F53350	2,320	2,409	2,996
F53400	2,076	2,122	2,696	F53355[b]	2,280	2,297	-
F53346	2,115	2,211	2,694	F53397	2,145	2,245	2,706
				F53351	2,059	2,253	2,606
Mean	2,116	2,176	2,778		2,175	2,302	2,769
Group 4 - T-6049 (500 mg/kg)							
F53365	2,126	2,276	2,951	F53403	2,170	2,270	2,860
F53354	2,325	2,478	3,008	F53000	2,241	2,268	2,914
F53347	2,103	2,152	2,646	F53345	2,306	2,341	2,951
Mean	2,185	2,302	2,868		2,239	2,293	2,908

a Animal No. F53398 was originally selected by the randomization program for use in the study and was treated. This animal was sacrificed after completion of the exposure period due to a possible broken back and was replaced with No. F52999. The body weights for No. F53398 are not included in the group means.

b Animal No. F53355 was originally selected by the randomization program for use in the study and was treated. This animal was sacrificed before completion of the exposure period due to a possible broken back and was replaced with No. F53351. The body weights for No. F53355 are not included in the group means.

\- Not applicable.

Table 2
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2	Day 3 through 29
		Group 1 (Control) - Distilled Water (0 mg/kg)			
Male	F53358	Appeared normal	✓	✓	✓
	F53359	Appeared normal	✓	✓	✓
	F53353	Appeared normal	✓	✓	✓
Female	F53404	Appeared normal	✓	✓	✓
	F53405	Appeared normal	✓	✓	✓
	F53356	Appeared normal	✓	✓	✓
		Group 2 - T-6049 (5 mg/kg)			
Male	F53402	Appeared normal	✓	✓	✓
	F53364	Appeared normal	✓	✓	✓
	F53352	Appeared normal	✓	✓	✓
Female	F53399	Appeared normal	✓	✓	✓
	F53349	Appeared normal	✓	✓	✓
	F53398	Appeared normal	✓	-	
		Possible broken back (at time of 24-hour bleeding interval)	-	✓	
		Moribund sacrifice	-	✓	
	F52999[a]	Appeared normal	✓	✓	✓

✓ Condition existed.
- Condition not evident.
a Animal No. F52999 replaced Animal No. F53398.

Table 2 (Continued)
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2	Day 3 through 29
		Group 3 - T-6049 (100 mg/kg)			
Male	F53360	Appeared normal	✓	✓	✓
	F53400	Appeared normal	✓	✓	✓
	F53346	Appeared normal	✓	✓	✓
Female	F53350	Appeared normal	✓	✓	✓
	F53355	Appeared normal	✓	-	
		Leg caught in cage floor (possible broken back)	-	✓	
		Moribund sacrifice	-	✓	
	F53397	Appeared normal	✓	✓	✓
	F53351[a]	Appeared normal	✓	✓	✓
		Group 4 - T-6049 (500 mg/kg)			
Male	F53365	Appeared normal	✓	✓	✓
	F53354	Appeared normal	✓	✓	✓
	F53347	Appeared normal	✓	✓	✓
Female	F53403	Appeared normal	✓	✓	✓
	F53000	Appeared normal	✓	✓	✓
	F53345	Appeared normal	✓	✓	✓

✓ Condition existed.
- Condition not evident.
a Animal No. F53351 replaced Animal No. F53355.

Table 3
Individual Dermal Irritation Scores

Group 1 (Control) - Distilled Water (0 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53358				Animal No. F53404			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53359				Animal No. F53405			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53353				Animal No. F53356			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 2 - T-6049 (5 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53402				Animal No. F53399			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53364				Animal No. F53349			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53352				Animal No. F53398[a]			
Erythema	0	0	0	0	0	0	-	-
Edema	0	0	0	0	0	0	-	-
Atonia	0	0	0	0	0	0	-	-
Desquamation	0	0	0	0	0	0	-	-
Coriaceousness	0	0	0	0	0	0	-	-
Fissuring	0	0	0	0	0	0	-	-
					Animal No. F52999[b]			
Erythema					0	0	0	0
Edema					0	0	0	0
Atonia					0	0	0	0
Desquamation					0	0	0	0
Coriaceousness					0	0	0	0
Fissuring					0	0	0	0

a Animal replaced with No. F52999.
b Replacement animal for No. F53398.
- Not applicable.

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 3 - T-6049 (100 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53360				Animal No. F53350			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53400				Animal No. F53355[a]			
Erythema	0	0	0	0	0	-	-	-
Edema	0	0	0	0	0	-	-	-
Atonia	0	0	0	0	0	-	-	-
Desquamation	0	0	0	0	0	-	-	-
Coriaceousness	0	0	0	0	0	-	-	-
Fissuring	0	0	0	0	0	-	-	-
	Animal No. F53346				Animal No. F53397			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
					Animal No. F53351[b]			
Erythema					0	0	0	0
Edema					0	0	0	0
Atonia					0	0	0	0
Desquamation					0	0	0	0
Coriaceousness					0	0	0	0
Fissuring					0	0	0	0

a Animal replaced with No. F53351.
b Replacement animal for No. F53355.
- Not applicable.

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 4 - T-6049 (500 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53365				Animal No. F53403			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53354				Animal No. F53000			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53347				Animal No. F53345			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 4
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
				Group 1 (Control) - Distilled Water (0 mg/kg)
F53358	M	-	29	No visible lesions.
F53359	M	-	29	No visible lesions.
F53353	M	-	29	The skin has multiple red areas within the dermal application site, up to 3 cm x 2 cm. Collected with routine section.
F53404	F	-	29	No visible lesions.
F53405	F	-	29	No visible lesions.
F53356	F	-	29	The treated skin area appears diffusely dark red. Collected with routine section.
				Group 2 - T-6049 (5 mg/kg)
F53402	M	-	29	No visible lesions.
F53364	M	-	29	No visible lesions.
F53352	M	-	29	No visible lesions.
F53399	F	-	29	The skin has diffusely dark red areas along the entire dorsal lumbar and thoracic region. Collected with routine section.
F53349	F	-	29	The skin has multiple red areas on the application site up to 1 cm in diameter. Collected with routine section.
F53398[a]	F	-	2	There are multiple dark red areas within the skeletal muscle of the lumbar sacral region surrounding the spinal column, up to 1.5 x 2.0 cm.
F52999[b]	F	-	29	No visible lesions.

- Not applicable.
a Animal replaced with No. F52999.
b Replacement animal for No. F53398.

Table 4 (Continued)
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
			Group 3 - T-6049 (100 mg/kg)	
F53360	M	-	29	No visible lesions.
F53400	M	-	29	No visible lesions.
F53346	M	-	29	No visible lesions.
F53350	F	-	29	No visible lesions.
F53397	F	-	29	No visible lesions.
F53351	F	-	29	No visible lesions.
			Group 4 - T-6049 (500 mg/kg)	
F53365	M	-	29	No visible lesions.
F53354	M	-	29	No visible lesions.
F53347	M	-	29	No visible lesions.
F53403	F	-	29	No visible lesions.
F53000	F	-	29	The skin has multiple red areas on the application site, up to 1 cm x 3 cm. Collected with routine section.
F53345	F	-	29	No visible lesions.

- Not applicable.

Table 5
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 1 (Control) - Distilled Water (0 mg/kg)			
Male	F53358	81.381	-	0.783	1.4
	F53359	73.857	-	0.761	0.8
	F53353	77.411	15.761	0.644	0.6
Female	F53404	70.277	12.585	0.620	1.8
	F53405	78.270	-	0.775	1.1
	F53356	74.687	-	0.915	0.5
		Group 2 - T-6049 (5 mg/kg)			
Male	F53402	78.731	14.611	0.468	2.3
	F53364	84.099	-	0.641	1.8
	F53352	67.613	-	0.706	1.8
Female	F53399	65.818	-	0.546	0.8
	F53349	77.596	14.211	0.489	0.9
	F53398[a]	-	-	-	-
	F52999[b]	87.510	-	0.660	0.5

- Not applicable.
a Moribund sacrifice on Day 2 (01/08/95) due to possible broken back. Tissues were collected, however, the corresponding weight or volume was not taken since this requirement became effective on 01/24/95. Animal replaced with No. F52999.
b Replacement animal for No. F53398.

Table 5 (Continued)
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 3 - T-6049 (100 mg/kg)			
Male	F53360	76.347	15.873	0.802	0.9
	F53400	74.958	-	0.395	1.6
	F53346	75.414	-	0.570	1.3
Female	F53350	74.050	15.120	0.784	1.4
	F53397	74.882	-	0.801	0.9
	F53351	80.686	-	0.279	0.5
		Group 4 - T-6049 (500 mg/kg)			
Male	F53365	82.598	-	0.441	1.8
	F53354	70.968	15.234	0.540	1.3
	F53347	83.696	-	0.716	2.0
Female	F53403	70.624	15.427	0.910	1.4
	F53000	79.617	-	0.714	0.8
	F53345	81.341	-	0.633	1.8

- Not applicable.

APPENDIX A

Protocol Deviations
Protocol TP3016.AB
Protocol Amendment No. 1

Protocol Deviations

Protocol	Actual Procedure
Page 7, 7. Experimental Design, C. Observation of Animals, (1) Clinical Observations, First Sentence. For clinical signs before test or control material administration and for clinical signs and mortality at approximately 1, 2.5, and 4 hours after test material administration (Day 1) and Daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality for at least 28 days.	On Day 4 and 6, the afternoon mortality check was inadvertently not conducted for the two replacement animals (Group 2, No. F52999, and Group 3, No. F53351).
Page 7, 7. Experimental Design, C. Observation of Animals, (3) Body Weights. For randomization, before test or control material application (Day 1), on Day 29, and at unscheduled death (when survival exceeds 1 day).	The body weight of the Group 2 animal that was sacrificed on Day 2 was inadvertently not documented.

These deviations are not considered to have had an adverse effect on the outcome of the study.

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707 7545

a CORNING Company

Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

PROTOCOL TP3016.AB

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of
T-6049 in Rabbits

Date:

December 30, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-130

Phone 608-241-4471 Fax 608 241-7227
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI 53704

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of
T-6049 in Rabbits

HWI No.	6329-130
Test Material	T-6049
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	January 7, 1995
Experimental Termination Date	February 4, 1995
Draft Report Date	March 18, 1995

1. Study
Single-Dose Dermal Absorption/Toxicity Study in Rabbits

2. Purpose
To assess the systemic absorption and toxicity and relative skin irritancy of a test material when applied to the skin of rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] 59 Nohsan No. 3850 (Japanese MAFF)
[ ] Notification No. 313 (Japanese MOHW)

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Material

A. Identification
T-6049

B. Physical Description
(To be documented in the raw data)

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

D. Storage
Room temperature

E. Reserve Samples
Reserve sample(s) of each batch/lot of test and control materials will be taken for this study.

The test and control material reserve samples will be stored at HWI in a freezer set to maintain a temperature of -20°C ±10°C for 10 years per HWI SOP. The Sponsor will be contacted after 10 years for disposition in accordance with the appropriate regulatory Good Laboratory Practices.

F. Retention
Any unused test material will be returned to the Sponsor after completion of the in-life phase of the study.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Distilled water

B. Physical Description
Clear, colorless liquid

C. Purity and Stability
The purity and stability of this manufactured material is considered to be adequate for the purposes of this study.

D. Storage Conditions
Room temperature

E. Reserve Samples
See Section 5. E. Reserve Samples

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.0 to 3.0 kg

(5) Number and Sex
12 males and 12 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The animals will be placed into study groups using a stratified body weight randomization program within nine days of study initiation.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Number of Animals Females
1 (Control)	Distilled water	0*	3	3
2	T-6049	5**	3	3
3	T-6049	100	3	3
4	T-6049	500	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered at a dose volume of .01 mL/kg

(2) Preparation of Exposure Area
On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit will be clipped free of hair. The shaved area will constitute approximately 20% of the total body surface area. The treatment sites (intact skin) will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals will be clipped as needed throughout the study.

(3) Dose Administration
All animals will receive a single administration of the respective test or control material. The day of treatment will be designated as Day 1. The dose for each animal in Group 2 will be diluted with distilled water and applied at a dose volume of .01 mL/kg. The respective dose for each animal in Groups 3 and 4 will be applied undiluted. All doses in Groups 1-4 will be based on the animal's body weight just before administration and will be spread onto the area of exposure in a thin and uniform a layer. The area of application (Groups 1-4) will be covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. The rabbits will be collared during the 24-hour application period.

(4) Reason for Route of Administration
The dermal route is a potential route of exposure in humans.

(5) Removal of Test Material
Approximately 24 hours after test or control material application the bandages and collars will be removed and the residual test material will be removed using water or an appropriate solvent, if necessary.

C. Observation of Animals

(1) Clinical Observations
For clinical signs before test or control material administration and for clinical signs and mortality at approximately 1, 2.5, and 4 hours after test material administration (Day 1) and daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality for at least 28 days. Observations may be extended when directed by the study director.

(2) Reading of Dermal Irritation
Before test or control material administration the initial dermal irritation reading will be made and recorded as the Day 1 reading (Attachment 1). Additional dermal irritation readings will be made approximately 30 minutes after bandage removal (Day 2) and on Study Days 4 and 8. Individual dermal irritation records will be maintained for each animal.

(3) Body Weights
For randomization, before test or control material application (Day 1), on Day 29, and at unscheduled death (when survival exceeds 1 day)

(4) Sample Collections

(a) Frequency
Before initiation (Day 1), approximately 24 hours post-dose (Day 2), Days 4, 9, 16, 23, and at experimental termination (Day 29)

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from the marginal ear vein of either ear on Days 1, 2, 4, 9, 16, and 23. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal sacrificed in a moribund condition or sacrificed at the time of necropsy (Day 29). The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain -20°C ±10°C. The separated serum and cellular fractions will be sent frozen on dry ice to the Sponsor after experimental termination.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson or alternate will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

D. Pathology

(1) Unscheduled Sacrifices and Deaths
Any animal dying during the study or sacrificed in a moribund condition will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. Tissues, as described in section D. Pathology, (3) Sample Collection, will be collected. After necropsy, the animals will be discarded.

(2) Scheduled Sacrifice
At termination of the experimental phase (Day 29), surviving animals will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, exsanguinated, and subjected to an abbreviated gross necropsy examination. The animals will be necropsied in random order and all abnormalities will be recorded.

(3) Sample Collection
The sites of test and control material application will be washed with lukewarm tap water prior to the necropsy procedure. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necopsied in each group will be collected and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals will be discarded.

The tissues (liver, bile, dermal application site, kidneys) will be sent frozen on dry ice to the Sponsor after experimental termination. The samples will be shipped to the person listed in Section 7.C.(4).(c). The Sponsor is responsible for the retention and disposition of the samples.

E. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Tabulation of mortality data by sex and dose level
Description of any toxic effects/dermal irritation
Tabulation of mean body weights by sex and dose level
Gross pathology findings/gross pathology report

9. Location of Raw Data, Records, and Final Report
Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Anatomical pathology records
Sample collection records
Shipping records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

Date: 1-5-95

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

Date: 12-30-94

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

Date: 12/30/94

(6329-130.protdsk2)

Attachment 1

Scoring Scale for Acute Dermal Reactions

Erythema

0 - None
1 - Slight
2 - Moderate
3 - Severe

Edema

0 - None
1 - Slight (barely perceptible to well defined by definite raising)
2 - Moderate (raised approximately 1 mm)
3 - Severe (raised more than 1 mm)

Atonia

0 - None
1 - Slight (slight impairment of elasticity)
2 - Moderate (slow return to normal)
3 - Marked (no elasticity)

Desquamation

0 - None
1 - Slight (slight scaling)
2 - Moderate (scales and flakes)
3 - Marked (pronounced flaking with denuded areas)

Coriaceousness

0 - None
1 - Slight (decrease in pliability)
2 - Moderate (leathery texture)
3 - Marked (tough and brittle)

Fissuring

0 - None
1 - Slight (definite cracks in epidermis)
2 - Moderate (cracks in dermis)
3 - Marked (cracks with bleeding)

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707-7545

a CORNING Company

PROTOCOL TP3016.AB

Single-Dose Dermal Absorption/Toxicity Study
of T-6049 in Rabbits

HWI 6329-130

Sponsor	Contractor
3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220	Hazleton Wisconsin, Inc 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	**Study Director**
John L. Butenhoff, PhD	Steven M. Glaza

Amendment No. 1

This amendment modifies the following portions of the protocol:

Effective January 10, 1995

1. Page 6, 7. Experimental Design; B. Dose Administration; (3) Dose Administration. Animal Nos. F53355 (Group 3 female) and F53398 (Group 2 female) were sacrificed on Day 2 due to injury (apparent broken backs). These animals will be replaced in this study. Replacement animals will be dosed, bled, and necropsied (includes tissue collection) at the same time as the animals in another 3M sponsored study (6329-137). Add the following as the second paragraph to this section:

   Due to the sacrifice on Day 2 of one Group 2 female (Animal No. F53398) and one Group 3 female (Animal No. F53355), replacement animals will be treated at the respective dose levels in the same manner as for the other animals in the study. The observations (clinical observations, reading of dermal irritation, and body weights) and the pathology of the animals (unscheduled sacrifices and deaths, scheduled sacrifice, and sample collection) will be conducted in the same manner as for the other animals in the study. The method of blood sample collection will be in the same manner as for the other animals in the study. The frequency of blood collection will be as follows:

   Before initiation (Day 1), approximately 24 hours post-dose (Day 2), Days 4, 8, 15, 22, and at experimental termination (Day 29).

Phone 608-241-4471 Fax 608-241-7203
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI 53704

Effective January 24, 1995

At the request of the Sponsor, the weights of tissues collected and the volume of bile collected will be documented in the raw data. These weights and volumes will be included with the sample shipment. Modify the following sections of the protocol to include these additions.

2. Page 9, 7. Experimental Design; D. Pathology; (3) Sample Collection. Modify the second sentence in the first and second paragraphs of this section with the following underlined additions:

> The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necropsied in each group will be collected, weighed (volume only determined for bile), and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C.
>
> The samples and their corresponding weights or volumes will be shipped to the person listed in Section 7.C.(4).(c).

3. Page 9, 8. Report. Add the following to this section:

> Individual animal tissue weights and bile volumes

PROTOCOL AMENDMENT APPROVAL

John L. Butenhoff — 2/9/95
John L. Butenhoff, PhD — Date
Sponsor's Representative
3M Toxicology Service Medical Department

Steven M. Glaza — 2-1-95
Steven M. Glaza — Date
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

Tracy Thiel — 2-02-95
Representative — Date
Quality Assurance Unit
Hazleton Wisconsin, Inc.

(6329-130.Am1.dsk2)

9.1.2 Analytical protocol AMDT-020895.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits

In-Vivo Study Reference Number: HWI#6329-130

Study Number: AMDT-020895.1
Test Substance: FC-95 (T-6049)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Initiation Date: July 25, 1995
Proposed Completion Date: August 25, 1995

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochenmicals Using Electrospray Mass Spectrometry

Author: James D. Johnson

Approved By:

James D. Johnson 10/30/95
James D. Johnson — Date
Study Director

John L. Butenhoff 10/30/95
John Butenhoff, PhD — Date
Sponsor Representative

1.0 PURPOSE

The purpose of this study is to assess the dermal absorption of FC-95 (T-6049) in rabbits after a single dermal dose of FC-95.

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

2.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.
2.1.2 Analytical Reference Matrix: Bovine liver and bovine serum
2.1.3 Analytical Control Substance: None
2.1.4 Analytical Control Matrix: Bovine liver and bovine serum

2.2 Source of Materials: 3M ICP/PCP Division (2.1.1), grocery store (2.1.2, 2.1.4 liver), Sigma Chemical Company (2.1.2, 2.1.4 serum)

2.3 Number of Test and Control Samples: Tissues and fluids from 18 test animals and 6 control animals. One animal was replaced on day 2 (animal F53398 in the 5 mg/kg dose group replaced by animal F52999). Tissues and fluids include liver, kidney, serum, cellular fraction, dermal application site and bile. Analysis of these tissues will be at the discretion of the Study Director.

2.4 Identification of Test and Control Samples: The samples are identified using the HWI animal identification number which consists of a letter and five digit number, plus the tissue identity and day identity (serum).

2.5 Purity and Strength of Reference Substance: To be determined by Sponsor.

2.6 Stability of Reference Substance: To be determined by Sponsor.

2.7 Storage Conditions for Test Materials: Room temperature (2.1.1), -20 ± 10°C (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required studies longer than 28 days.

2.9 Safety Precautions: Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting the samples.

3.0 EXPERIMENTAL - Overview

Rabbits were dosed with T-6049 in a control group, 5 mg/kg, 100 mg/kg, and 500 mg/kg dermally. (See HWI#6329-130 for dosing, animal weights, tissue collection, etc.) Tissues and serum are available from these animals for analysis of total organic fluorine content. At the discretion of the Study Director samples will be analyzed to the extent necessary to provide information to assess the extent of dermal absorption.

4.0 EXPERIMENTAL - Methods

4.1 Liver and Serum screening methods: (attached)

4.1.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

4.1.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

4.1.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

4.1.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochenmicals Using Electrospray Mass Spectrometry

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-95 per tissue or fluid. Statistics used, at the discretion of the Study Director, may include serum averages and standard deviations of concentrations for different dose groups. If necessary, simple statistical tests such as Student's t test may be applied to determine statistical difference.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

6.1 Raw Data and Records: Raw data, approved protocol, appropriate specimens, approved final report, and electronic data will be maintained in the AMDT Archives.

7.0 REFERENCES

7.1 AMDT-S-10-0, Sample Tracking System

8.0 ATTACHMENTS

8.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

8.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

8.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

8.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochenmicals Using Electrospray Mass Spectrometry

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Liver

Method Identification Number: AMDT-M-1 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95 Date

Quality Assurance 10-4-95 Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological tissues, particularly liver.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 150 mg, depending on sample moisture content. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are not to be handled with bare hands. Fluoride may leach from the skin to the sample. Use forceps or probe to transfer tissues.
7.2 Samples of liver are cut from frozen liver and placed in a tared and labeled weigh boat. Use a clean scalpel and cutting board. The cutting board and scalpel should be cleaned with water, methanol, or methanol-water solution after each liver is cut.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit liver studies, use beef liver as the matrix. Cut a piece of frozen beef liver (100 - 150 mg) and weigh it in a labeled and tared weigh boat.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked liver curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef liver as the matrix. Prepare at least 3 samples of beef liver (100 - 150 mg) for blanks.
8.3.2.3 Put sample in Dohrmann boat. Combust each sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.

8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 Weigh out at least 15 matrix samples (5 standards with 3 replicates each) in tared and labeled weigh boats. For rabbit studies, weigh 100-150 mg beef liver samples. Record weights in study data. Store the matrix samples on dry ice or ice packs to keep them frozen until used.
8.3.3.2 Place weighed beef liver sample in Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef liver.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.
9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-SOLIDS program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control
10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved By:

Group Leader 10/3/95
Date

Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-1 Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 SCOPE: This method is for the calibration and operation of an Orion EA940 Expandable Ion Analyzer.

1.2 APPLICABLE COMPOUNDS: Fluoride.

1.3 APPLICABLE MATRICES: Liquid samples in an appropriate buffer solution. Preferred pH of 6.0.

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

4.1 Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.
4.2 Orion Model 900001 electrode filling solution (AgCl) or equivalent.
4.3 Orion 940907 100 ppm fluoride standard or equivalent.
4.4 Milli-Q™ water or equivalent.
4.5 Magnetic stir bars.
4.6 Lab tissues.
4.7 Sample collection vials.
4.8 Plastic 100 mL volumetric flasks.
4.9 Polystyrene pipettes.
4.10 Miscellaneous laboratory glassware.

5.0 EQUIPMENT

5.1 Orion Model EA940 Expandable Ion Analyzer or equivalent.
5.2 Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.
5.3 Magnetic Stir Plate.
5.4 IBM compatible 386 or 486 computer (only needed if using Orion 3E software).
5.5 Orion RS232 interface cable (only needed if using Orion 3E software).
5.6 Microsoft Excel 5.0 (only needed if using Orion 3E software).

6.0 INTERFERENCES

6.1 It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.
6.2 Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.
6.3 The rate the samples are stirred at should be consistent with the rate the standards were stirred.

6.4 Air bubbles trapped under electrode can give erroneous readings. Make sure no air is trapped under electrode.

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards
8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.
8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.
8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.
8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.
8.1.5 Shake the flasks to mix the solutions.
8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.
8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.
8.1.8 Invert and shake the flasks for the final mixing.
8.1.9 Record standards in Standards Log Book.

8.2 Calibration
8.2.1 If necessary, remove tape from electrode filling hole.
8.2.2 Invert probe to wet top seal.
8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.
8.2.4 Fill the electrode with filling solution.
8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.
8.2.6 Record the slope in the appropriate log book.
8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

8.3 Storage Conditions for Standards
8.3.1 Calibration standards are stored at room temperature.

9.0 PROCEDURES

9.1 Calibration and Measurement, Standard method:
9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.
9.1.2 Place a stir bar in the sample and place the sample on the stir plate.
9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.
9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.
9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

9.2 Calibration And Measurement, Using Orion 3E Software:

9.2.1 Calibration:
9.2.1.1 Follow steps 8.2.1 to 8.2.4.
9.2.1.2 Press Function Key #8 (F8).
9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.
9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.
9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.
9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.
9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

9.2.2 Data Spreadsheet:
9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.
9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

9.2.3 Fluoride Measurement:
9.2.3.1 Follow steps 9.2.1 through 9.2.4
9.2.3.2 Enter the name of the sample in the appropriate place on the screen.
9.2.3.3 Click on the NEW SAMPLE button
9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

10.3 Other Validation Parameters According to Reference 13.2, the range of detection is 0.02 ppm fluoride up to a saturated solution of fluoride.

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Orion Model EA940 Expandable Ion Analyzer Instruction Manual, Orion Research Incorporated, 1991.
13.2 Orion Model 960900 Solid State Combination Fluoride Electrode Instruction Manual, Orion Research Incorporated, 1991.

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 10-31-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 10-31-95

Quality Assurance — Date: 10-31-95

Software: MS Word, 6.0
Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

1.0 SCOPE

1.1 **Scope:** This method is for the extraction of fluorochemicals from rabbit livers. Ethyl acetate is used to extract fluorochemicals from the livers for analysis by electrospray mass spectroscopy.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

2.1 Fluorochemicals, rabbit livers, electrospray mass spectrometer, fluorinated compounds, extraction.

3.0 PRECAUTIONS

3.1 Use gloves when handling the rabbit livers, they may contain pathogens.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Syringe, capable of measuring 100 µL
4.1.2 Eppendorf type or disposable pipets
4.1.3 Gloves
4.1.4 Plastic grinding tubes
4.1.5 Plastic centrifuge tubes, 15 mL
4.1.6 Labels
4.1.7 Nitrogen
4.1.8 Timer
4.1.9 Filters, Titan nylon syringe filters, 0.2 µm.
4.1.10 Analytical pipets: glass volumetric pipets.
4.1.11 Disposable plastic 3 cc syringes.
4.1.12 Crimp cap autovials.

4.2 **Reagents**

4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.
4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
4.2.4 Ethyl Acetate
4.2.5 Methanol
4.2.6 Milli-Q water
4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

5.1 Ultra-Turrax T25 Grinder for grinding liver samples.
5.2 Vortex mixer
5.3 Centrifuge
5.4 Shaker
5.5 Analytical Evaporator

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 The rabbit livers are received frozen, and must be kept frozen until the extraction is performed.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.
8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.
8.1.3 Bring it up to volume with methanol, this is the stock standard.
8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

$$\frac{\text{actual mg perfluoroctane-sulphonic acid}}{0.1\ \text{L}} \times \frac{3\ \text{mL}}{250\ \text{mL}} = \text{actual concentration, ppm}$$

8.2 Prepare FC-95 Anion Standards

8.2.1 Prepare FC-95 standards for the standard curve.
8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.
8.2.3 Bring up to volume with dilute acetonitrile.
8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.
8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 Prepare Beef Liver Homogenate to Use for Standards

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.
8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

8.3.3 Spike seven of the 1 mL aliquots with the following amounts of working standards in step **9.12** of the procedure. One 1 mL aliquot serves as the blank.

Working Standard (Approximate Conc.)	uL	Approximate final concentration of FC-95 in liver
-	-	Blank
500 ppb	100	0.292 ppm
500 ppb	200	0.584 ppm
500 ppb	300	0.877 ppm
500 ppb	400	1.168 ppm
1 ppm	500	2.924 ppm
5 ppm	200	5.848 ppm
5 ppm	300	8.772 ppm

8.4 Calculate the actual value of the standards:

$$\frac{\text{uL of standard} \times \text{concentration (in ppm)}}{\text{171 mg liver}^{*} / \text{1 ml homogenate}} = \text{final concentration (ppm) of FC -95 in liver}$$

*Average weight of bovine liver in solution as determined by weighing 1 mL homogenates of 40 mg liver in 200 mL of Milli-Q water. The amount of FC-95 is reported as equivalents of FC-95 potassium salt.

8.5 Calibration

8.5.1 Extract the spiked beef liver homogenate following 9.13 to 9.23 of this method. Use these standards to establish your curve on the mass spectrometer.

8.5.2 Alternatively, a standard curve may be generated using ratios of responses of the perfluorooctansulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

8.6 Storage Conditions for Standards

8.6.1 New standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectrometer is performed.

8.7 Storage Conditions for Standards

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

9.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

9.2 Use a dissecting scalpel and cut off approximately 1 g of liver.

9.3 Weigh the sample directly into a tared plastic grinding tube.

9.4 Record the liver weight in the study note book.

9.5 Put a label on the vial with the study number, weight, rabbit ID, date and analyst initials.

9.6 Add 2.5 mLs water.
9.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, until the sample is a homogeneous solution with no large chunks.
9.8 Rinse the probe off into the sample with 2.5 mLs water using a pipet.
9.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
9.10 Cap the sample and vortex for 15 seconds.
9.11 Pipet 1 mL into a 15 mL centrifuge tube. Label the centrifuge tube with the identical information as the grinding tube. (See AMDT-M-4 Worksheet for documenting the remaining steps.)
9.12 Spike the beef liver homogenates with the appropriate amount of FC-95 standard as described in **8.3**.
9.13 Spike the samples and beef liver homogenates with 100 uL of internal standard.
9.14 Add 1 mL of the sodium carbonate/sodium bicarbonate buffer and 1 mL ammonium acetate.
9.15 Using an analytical pipet, add 5 mL ethyl acetate.
9.16 Cap the sample and vortex 20 to 30 seconds.
9.17 Put them in the shaker for 20 min.
9.18 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.19 Remove 4 mLs of the top organic layer to a fresh 15 mL centrifuge tube with a 5 mL graduated glass pipet. Transfer the label to the fresh tube.
9.20 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.21 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.22 Vortex 15 seconds.
9.23 Transfer the sample to a 3 mL syringe. Attach a 0.2 µm nylon mesh filter, and filter the sample into a fresh centrifuge tube or a autovial. Label the tube or vial with the study number and animal number.
9.24 Cap and hold for analysis by electrospray mass spectroscopy.
9.25 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 **Quality Control** - not applicable
10.2 **Precision and Accuracy**- not applicable
10.3 **Other Validation Parameters**- not applicable

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22 Routine Maintenance of Ultra-Turrax T-25

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W_____	FC-95 approx 1 ppm actual ppm #W_____	FC-95 approx. 5 ppm actual ppm #W_____	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
[1]		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

[1] study number where the original worksheet is located and place a copy.

Liver Extraction Process:		Date & Initials
Pipet 1 mL of Liver Solution		
Pipet 100 uL of 12 ppm Internal Standard	Std. #	
Vortex 15 sec.		
Pipet 1 mL of 250 ppm Ammonium Acetate	Std. #	
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer		
Pipet 5 mL of Ethyl Acetate		
Vortex 20-30 sec.		
Shake 20 min.		
Centrifuge 20-25 min.		
Remove a 4 mL aliquot of organic layer		
Blow down to near dryness (<0.25 mL) with N_2		
Add 1 mL of 1:1 Acetonitrile/H_2O	TN#	
Vortex 15 sec.		
Filter using a 3cc B-D syringe with a 0.2um SRI filter into a 1.5 mL autosample vial.		

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5 **Adoption Date:** 6-6-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 6/6/95

Quality Assurance — Date: 6/6/95

Software: MS Word, 6.0
Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

1.0 SCOPE

1.1 Scope: This method is for the analysis of extracts of rabbit liver or other tissues or fluids for fluorochemicals using the electrospray mass spectrometer. The analysis is performed by single ion monitoring of FC-95 anion, M/Z= 499, the internal standard M/Z = 427, and other appropriate masses.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers (samples), Beef Liver (standards), other tissues and fluids.

2.0 KEYWORDS

2.1 Fluorochemicals, fluorinated compounds, electrospray mass spectroscopy, mass spectrometer, rabbit livers.

3.0 PRECAUTIONS

3.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

3.2 Do not run the pump above it's capacity of 4000 psi. If pressure goes over 4000 psi stop and release pressure. The peak tubing may be plugged. Troubleshoot back to find the plug and replace the plugged tubing. See AMDT-EP-15

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

4.1.3 100 uL or 250 uL flat tip syringe for sample injection.

4.2 **Reagents**

4.2.1 Dilute acetonitrile mobile phase, dilute acetonitrile 1:1 with Milli-Q water.

4.2.2 Milli-Q water, all water used in this method should be Milli-Q water.

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 Keep the extracted samples in capped 15 mL centrifuge tubes or in capped autovials until ready for analysis.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Seven beef liver standards and one blank beef liver are prepared during the extraction procedure. (See AMDT-M-4, section 8.0)

8.2 Calibration

8.2.1 Run the seven beef liver standards twice, starting with the lowest standard to obtain the standard curve.

8.2.2 Typically one standard is run after each 5 to 7 samples. Choose a standard in the same range of concentration as the samples.

8.3 Storage Conditions for Standards

8.3.1 Fresh standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

9.1.1 Set software to "Operate on", Ion Mode ES^-.

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

9.1.4 Set the pump to "Run". Set the flow to 1000 uL/min. Observes droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

9.1.5 Check the fused silica at the end of the probe. Use an eye piece to check for chips. The tip should be flat with no jagged edges. If any chips are found cut off the tip of the silica with a column cutter and pull the silica through to the appropriate length.

9.1.6 Check your nitrogen supply. Turn on the nitrogen. There should be no nitrogen leaking around the tip of the probe. A fine mist should be coming out of the tip.

9.1.7 Carefully guide the probe into the opening. Insert it until it won't go any further. Connect the voltage cable to the probe.

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES^-, and the appropriate masses to 427 and 499.

9.1.9 If it is not in single ion mode go to "Option" and set SIR.

9.1.10 Start Acquisition. Assign a file name, MO-DAY-YR + letter. Record it in the log book.

9.1.11 Run the beef liver samples first, running each standard twice at the beginning of the run.. Run a QC check by running one standard after every 5 to 7 samples.

9.2 Manual Injection

9.2.1 Draw 150 uL of sample into a syringe. Inject the sample into the rheodyne injection port. Inject slowly. Record the sample ID in the log book.

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

9.2.4 Record the scan number for each sample in the logbook.

9.3 **Using the Autosampler**

9.3.1 Set up sample tray A, B, or C.

9.3.2 Record the samples and their positions in the instrument log book. Up to 17 vials may be in each run.

9.3.3 Set-up the sampler:

- 9.3.3.1 Push the sample button
- 9.3.3.2 Set sample loop size = 100 uL
- 9.3.3.3 Set inject/sample = 2
- 9.3.3.4 Set Cycle time = 0
- 9.3.3.5 Name the file: Livers
- 9.3.3.6 Identify the tray used
- 9.3.3.7 Add the samples to Queue by pressing "Enter"
- 9.3.3.8 Press "Run" to start

10.0 VALIDATION

10.1 **Quality Control**

10.1.1 Run a standard every 5 to 7 samples. If a significant change(± 50%) in peak height occurs stop the run. Only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 **Precision and Accuracy**

10.2.1 See Method Validation Report number AMDT-M-5.0.V1

10.3 **Other Validation Parameters**

10.4 Refer to Method Validation Report Number AMDT-M-5.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.2 Plot the standard curve, using the mean of the two values obtained for each standard.

11.2.1 Read peak heights or areas for the samples from the printout. Use linear regression to determine the sample concentrations.

11.2.2 Calculate the mg of FC-95 anion, or other fluorochemical in the total rabbit liver:

mg FC-95 anion in the total rabbit liver =

$$\frac{\text{mg FC-95 anion from std. curve}}{\text{gms of liver used for analysis}} \times \text{Total mass of liver, gms}$$

11.3 Make a results table and enter it in the study book.

11.4 Print a chromatogram for each sample, with the peaks labeled with the sample or standard ID. Write the study number on the printout, initial, date, and put it in the study folder. Staple all chromatograms together and number pages.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

Completed by: QAU Auditor Original to: Study Director Copies to: QAU Files

Study Title: **Single-dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits**

Study Number: AMDT-020895.1 Name of Auditor: Kari Rambo

This study has been inspected by the Quality Assurance Unit as indicated in the following table. The findings were reported to the study director and management.

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
10/13/95	10/19/95	Final Report	10/19/95	10/19/95

Kari Rambo 10-19-95

QAU Auditor Date

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:
Jim Johnson
Dave Christenson

Documentation and Reporting:
Jim Johnson
Rich Youngblom

Quality Assurance Unit:
Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

Summary of Combustion Data - Liver
AMDT-020895.1, HWI 6329-130
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total µg Fluoride in Whole Liver
Mean per Dose Group*

	µg	Std. Dev.
Control Group	22.4 ±	5.7
5.0 mg/kg dose (T6049) (0.003 mg/kg)**	17.6 ±	4.9
100 mg/kg dose (T6049) (0.06 mg/kg)**	15.8 ±	2.6
500 mg/kg dose (T6049) (0.30 mg/kg)**	22.3 ±	2.2

*Calculated as the mean of triplicate samples from each of three male and three female rabbits.
**Test material is a 0.06% solution of T6049, actual dose in parenthesis.

FC95 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
Liver blnk-1		0.835		0.1138			
Liver blnk-2		0.287		0.1347			
Liver spk-1	106%	1.140		0.1403			
Liver spk-2	99%	1.200		0.1248			
F53405-1		0.474		0.1141			
F53405-2		0.281	0.353	0.1270	78.27	27.65	0
F53405-3		0.305		0.1322			
F53356-1		0.212		0.1431			
F53356-2		0.249	0.220	0.1458	74.69	16.40	0
F53356-3		0.198		0.1367			
F53404-1		0.684		0.1279			
F53404-2		0.244	0.394	0.1186	70.28	27.68	0
F53404-3		0.254		0.1230			
F53353-1		0.232		0.1441			
F53353-2		0.205	0.206	0.1096	77.41	15.93	0
F53353-3		0.181		0.1355			
Liver blk 1		0.272		0.1165			
Liver blk 2		0.129		0.1550			
Liver spk 1	91%	1.111		0.1237			
Liver spk 2	102%	1.096		0.1408			
F53358-1		0.402		0.1346			
F53358-2		0.320	0.335	0.1164	81.38	27.27	0
F53358-3		0.283		0.1370			
F53359-1		0.336		0.1143			
F53359-2		0.209	0.247	0.1174	78.86	19.52	0
F53359-3		0.197		0.1142			
F53402-1		0.149		0.1518			
F53402-2		0.136	0.147	0.1645	78.73	11.61	5
F53402-3		0.157		0.1434			
F53364-1		0.275		0.1097			
F53364-2		0.285	0.298	0.1140	84.10	25.09	5
F53364-3		0.335		0.1085			
F53352-1		0.257		0.1685			
F53352-2		0.241	0.255	0.1418	67.61	17.26	5
F53352-3		0.267		0.1401			
F53349-1		0.180		0.1390			
F53349-2		0.242	0.234	0.1010	77.60	18.15	5
F53349-3		0.280		0.1437			
F53399-1		0.238		0.1014			
F53399-2		0.209	0.200	0.1021	65.82	13.20	5
F53399-3		0.155		0.1290			
F53346-1		0.167		0.1340			
F53346-2		0.172	0.222	0.1396	75.41	16.75	100
F53346-3		0.328		0.1283			
F53350-1		0.284		0.1296			
F53350-2		0.266	0.241	0.1477	74.05	17.87	100
F53350-3		0.174		0.1375			
F53397-1		0.238		0.1273			
F53397-2		0.291	0.253	0.1367	74.88	18.93	100
F53397-3		0.230		0.1128			

000083

FC95 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
F53400-1		0.157		0.1418			
F53400-2		0.191	0.171	0.1233	74.96	12.80	100
F53400-3		0.165		0.1356			
F53360-1		0.144		0.1420			
F53360-2		0.209	0.168	0.1057	76.35	12.86	100
F53360-3		0.151		0.1309			
Liver blk 1		0.432		0.1137			
Liver blk 2		0.366		0.1019			
Liver spk 1	**95%**	1.389		0.1036			
Liver spk 2	**95%**	1.266		0.1132			
F53347-1		0.344		0.1221			
F53347-2		0.268	0.269	0.1073	83.70	22.50	500
F53347-3		0.195		0.1415			
F53365-1		0.206		0.1301			
F53365-2		0.289	0.216	0.1212	82.60	17.88	500
F53365-3		0.154		0.1486			
F53345-1		0.261		0.1438			
F53345-2		0.251	0.282	0.1499	81.34	22.96	500
F53345-3		0.335		0.1352			
F53354-1		0.420		0.1120			
F53354-2		0.364	0.333	0.1091	70.97	23.66	500
F53354-3		0.216		0.1272			
F53403-1		0.323		0.1126			
F53403-2		0.344	0.336	0.1375	70.62	23.71	500
F53403-3		0.340		0.1378			
F53000-1		0.307		0.1407			
F53000-2		0.272	0.293	0.1384	79.62	23.36	500
F53000-3		0.301		0.1257			
F52999-1		0.243		0.1369			
F52999-2		0.236	0.230	0.1040	87.51	20.13	5
F52999-3		0.211		0.1218			
F53351-1		0.193		0.1419			
F53351-2		0.188	0.195	0.1449	80.69	15.71	100
F53351-3		0.203		0.1210			
liver spk-3	**94%**	1.079		0.1312			
liver spk-4	**89%**	0.997		0.1355			
liver spk-5	**71%**	3.467		0.1253			
liver spk-6	**119%**	5.930		0.1215			
liver spk-7	**100%**	4.330		0.1401			

000084

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

HWI# 6329-130

David Christenson
10-31-95

Study: Single-Dose Dermal Absorption
Protocol Number: TP3016.AB
Test Material: T-6049 in Rabbits (FC 95)
Matrix: Liver
R Squared Value: 0.9955
Response Factor Amount: 1.27E-05
Analyst: DLC
Date: 4/3/95
Method: AMDT-M-4
Instrument: Fisons VG 2000 Electrospray MS
LABBASE File: 040395A/B

Group Dose	Sample #	Ion Count Area	Extracted wt g	Dilution factor	Concentration µg/g **	Total mass of liver g	Total amount of FC-95 per liver mg
Group 1: 0 mg/kg Distilled Water	F53404	N/D	1.0422	1	N/D	69.156	N/D
	F53356	N/D	1.1273	1	N/D	73.403	N/D
	F53359	N/D	1.2009	1	N/D	72.193	N/D
	F53358	6571	1.0779	1	0.0618	69.308	0.004
	F53405	N/D	1.2166	1	N/D	77.164	N/D
	F53353	N/D	1.0734	1	N/D	75.915	N/D
Group 2: 5 mg /kg *	F53402	6645	1.1472	1	0.0587	76.184	0.004
	F53352	58373	1.1648	1	0.5080	81.324	0.041
	F53399	N/D	1.1776	1	N/D	63.494	N/D
	F53349	N/D	1.0143	1	N/D	76.006	N/D
	F53364	22296	1.0259	1	0.2203	80.666	0.018
Group 3: 100 mg/kg *	F53400	12849	1.3817	1	0.0943	73.828	0.007
	F53360	11111	1.054	1	0.1069	74.574	0.008
	F53350	18253	1.3119	1	0.1410	72.534	0.010
	F53346	N/D	1.3537	1	N/D	73.666	N/D
	F53397	N/D	1.2903	1	N/D	73.063	N/D
Group 4: 500 mg/kg	F53403	59686	1.0511	1	0.5756	69.012	0.040
	F53347	N/D	1.0447	1	N/D	83.100	N/D
	F53000	34395	1.1242	1	0.3101	77.255	0.024
	F53345	4623	1.2143	1	0.0386	77.250	0.003
	F53365	N/D	1.0281	1	N/D	66.252	N/D
	F53354	N/D	1.194	1	N/D	80.146	N/D

* Rabbits F52999 (G2) and F53351 (G3) were not analyzed

** The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.

Replot

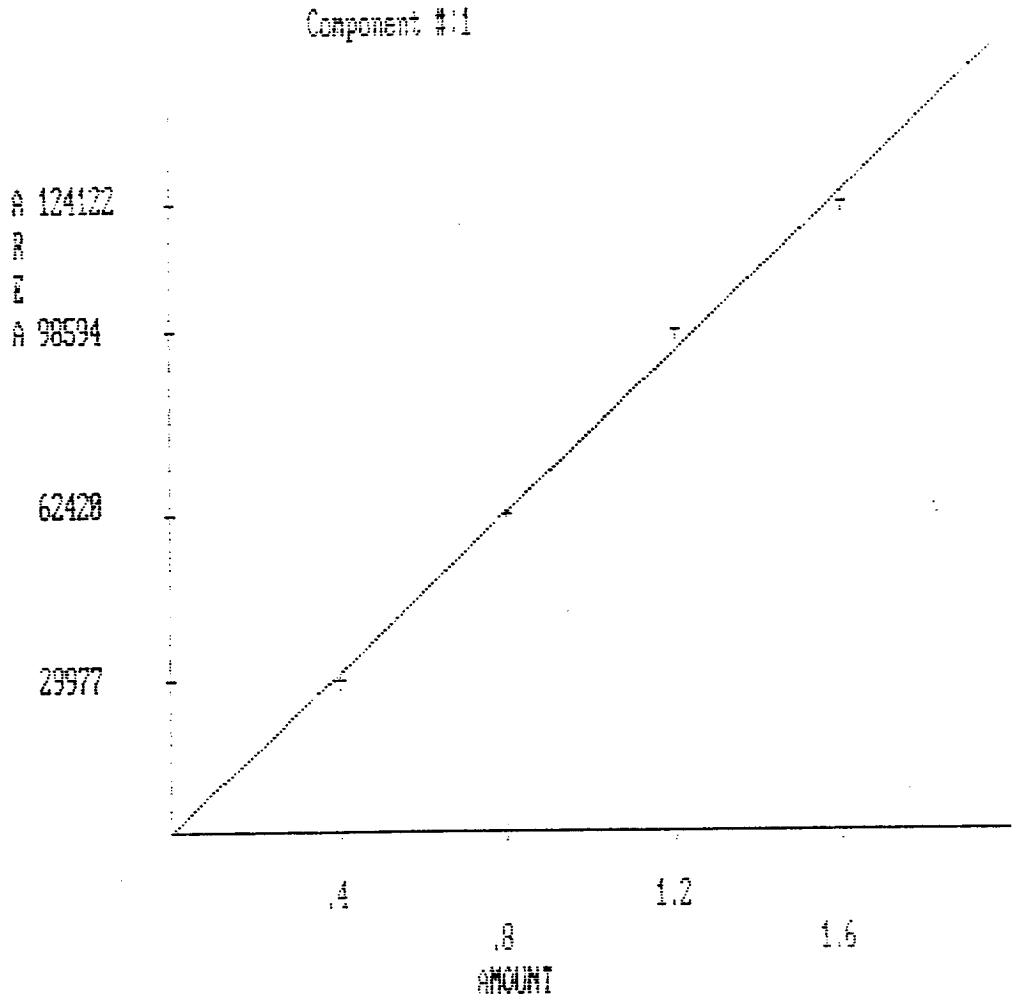
A-2

FILE: 040315A

4-NI * 6329-130

FC-95

Method DLCLIV
Sample DLCLIV
Operator DLC
Run date 05-09-1995 06:46:52 Version: 11
Printed on 05-09-1995 AT 06:47:07
Straight Line Fit forced through Origin.



Component 1 =
EXTERNAL STANDARD CALIBRATION

LEVEL	AMOUNT	AREA
1	0.4000	29977
2	0.8000	62420
3	1.2000	98594
4	1.6000	124122

Y = SLOPE * X + INTERCEPT

Area = 7.8924E+04 * Amount + 0.0000E+00
Amount = 1.2670E-05 * Area + 0.0000E+00
R squared = 0.9955

000087

HWI # 6329-130 Electrospray MS Analysis

FC-95

A-3

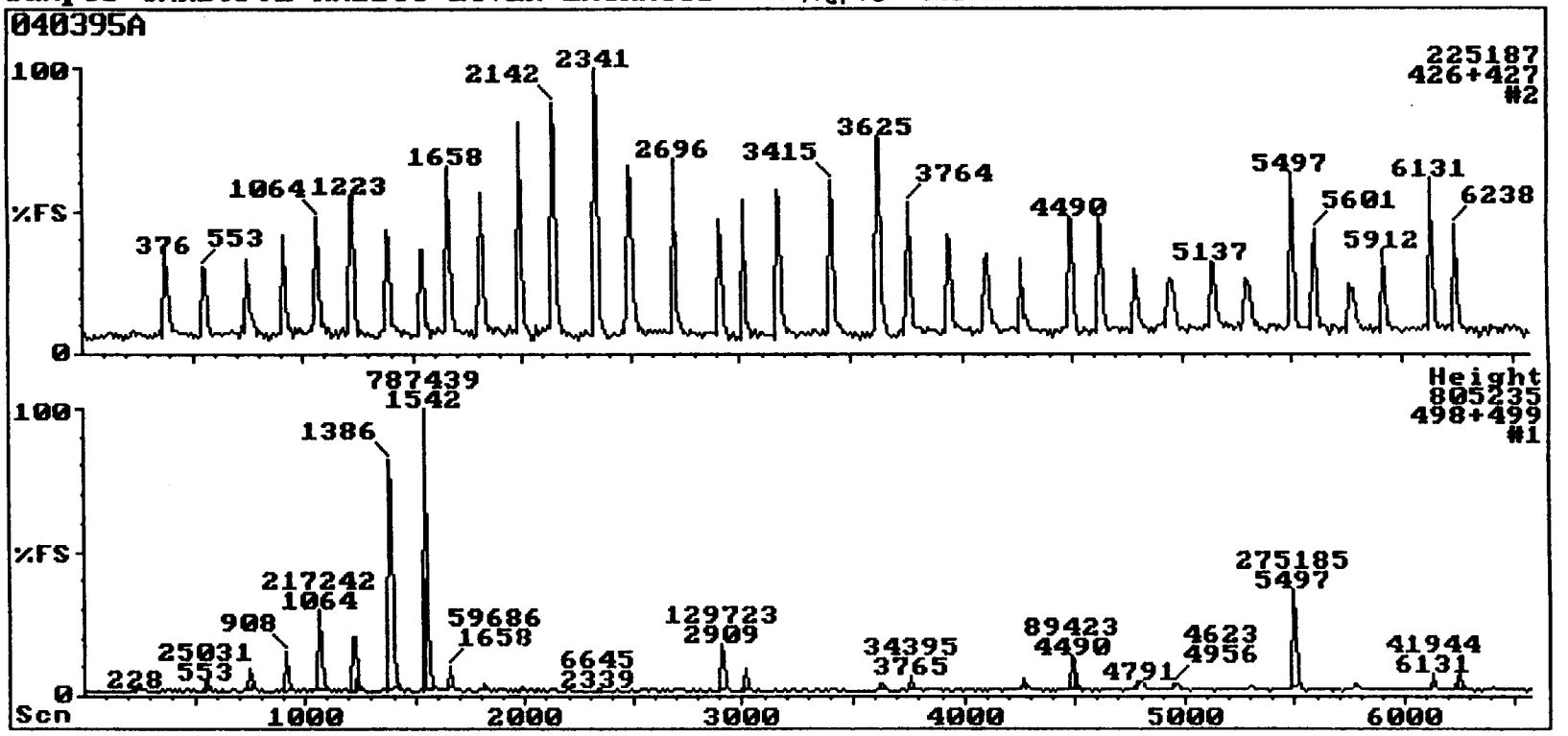
One sample misidentified (scan # 2696, F53357) actual animal number unknown.
10/18/95 DLC

Contains pages A-3 through A-10

000054

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

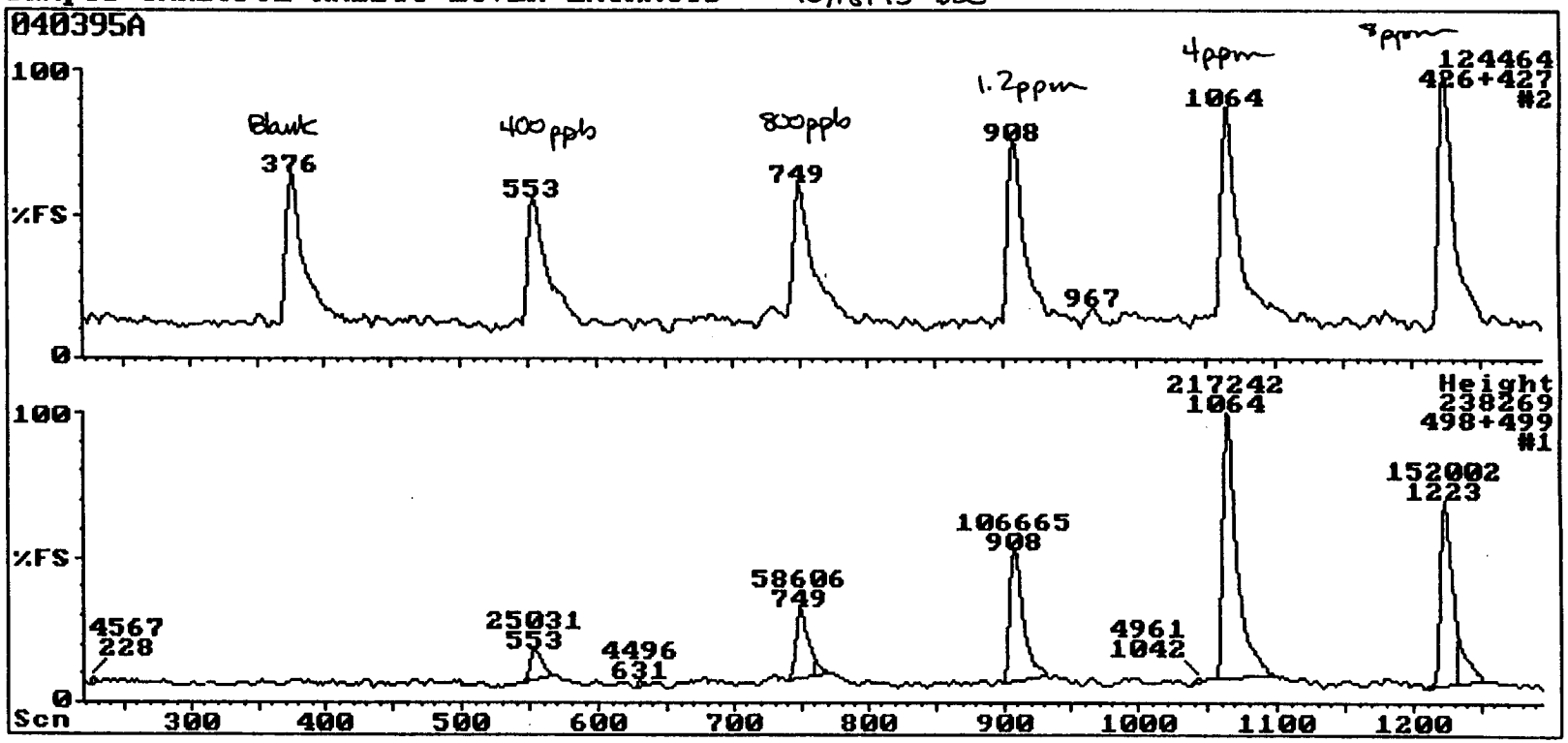
Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC



Beef liver standards

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC

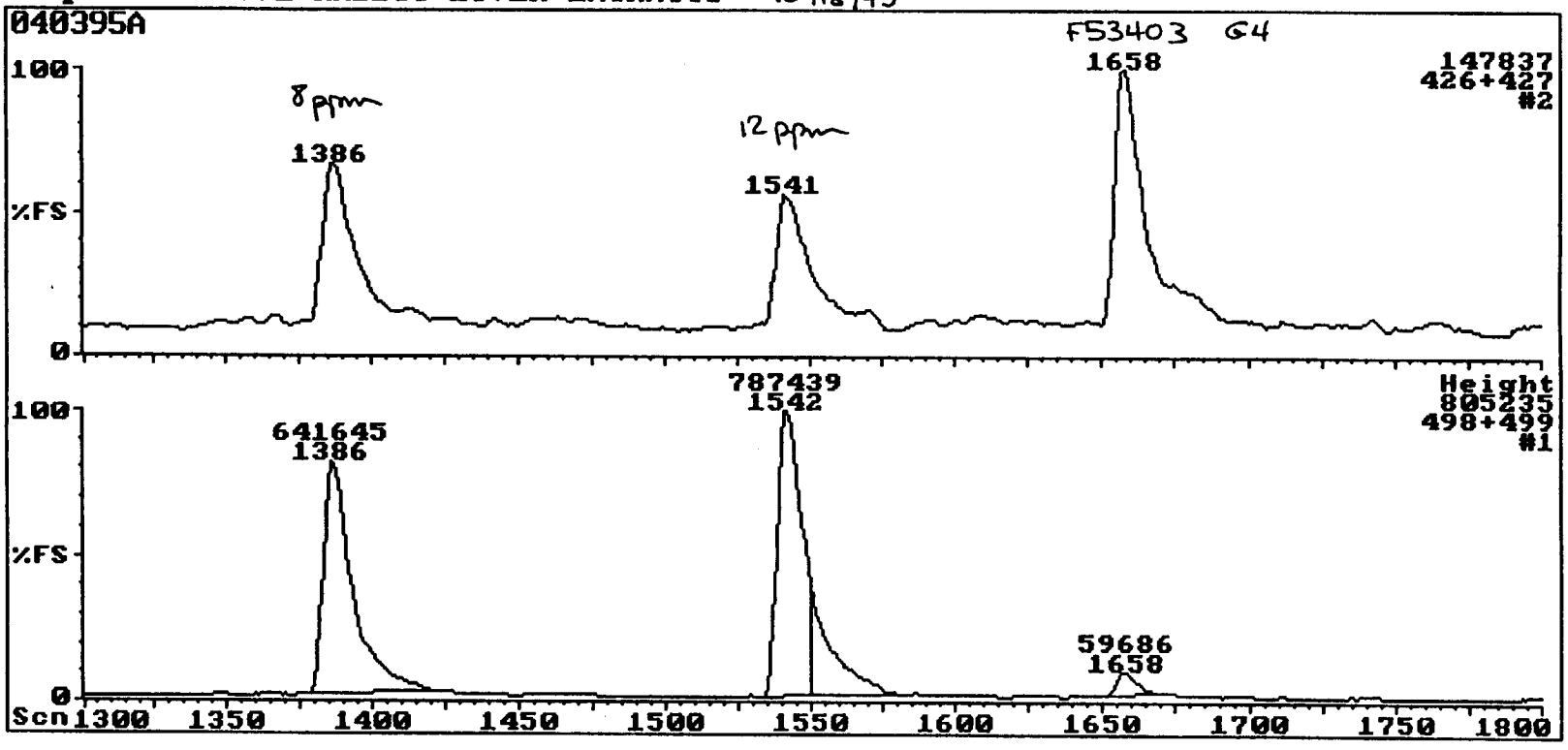


A-5

000050

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95

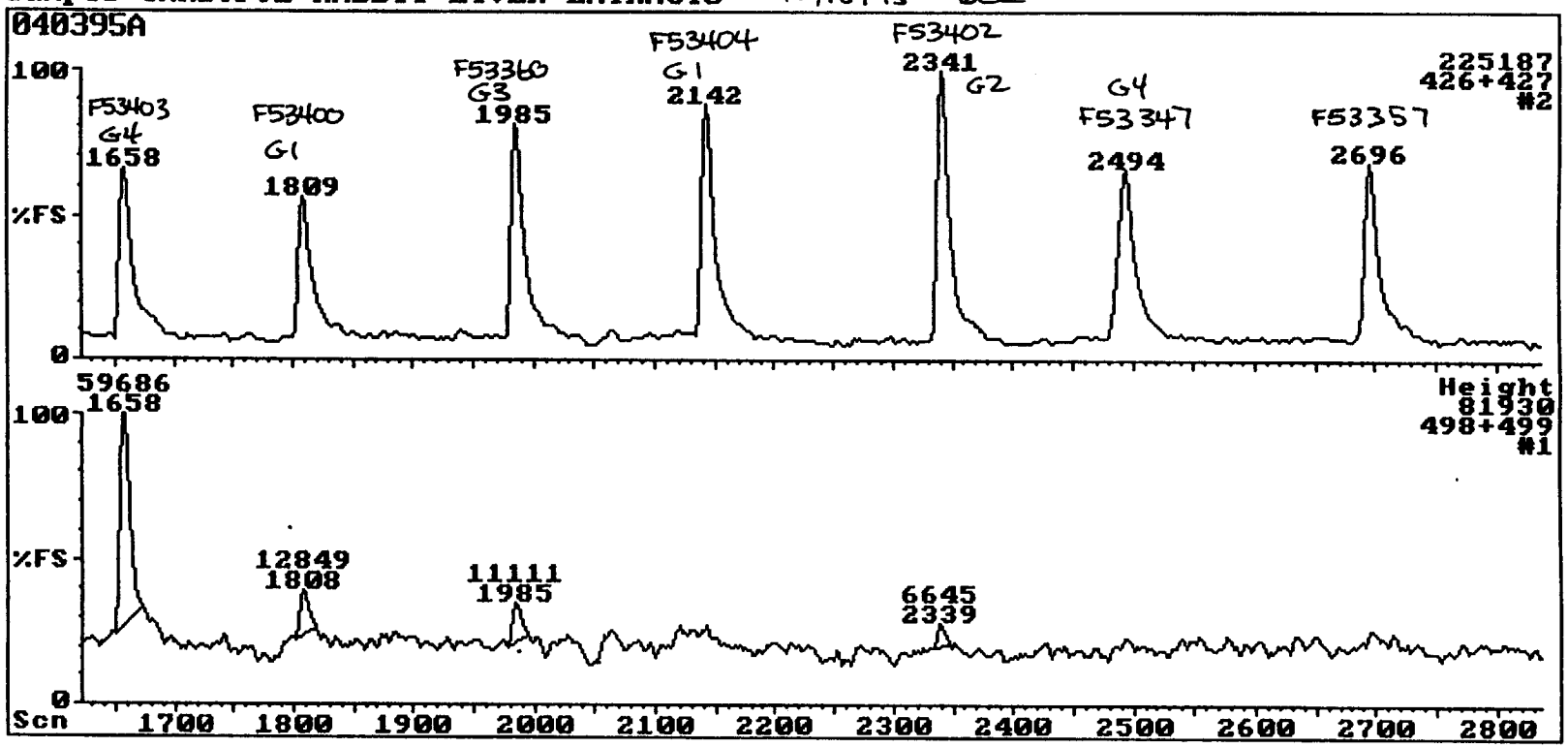


A-6

000091

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

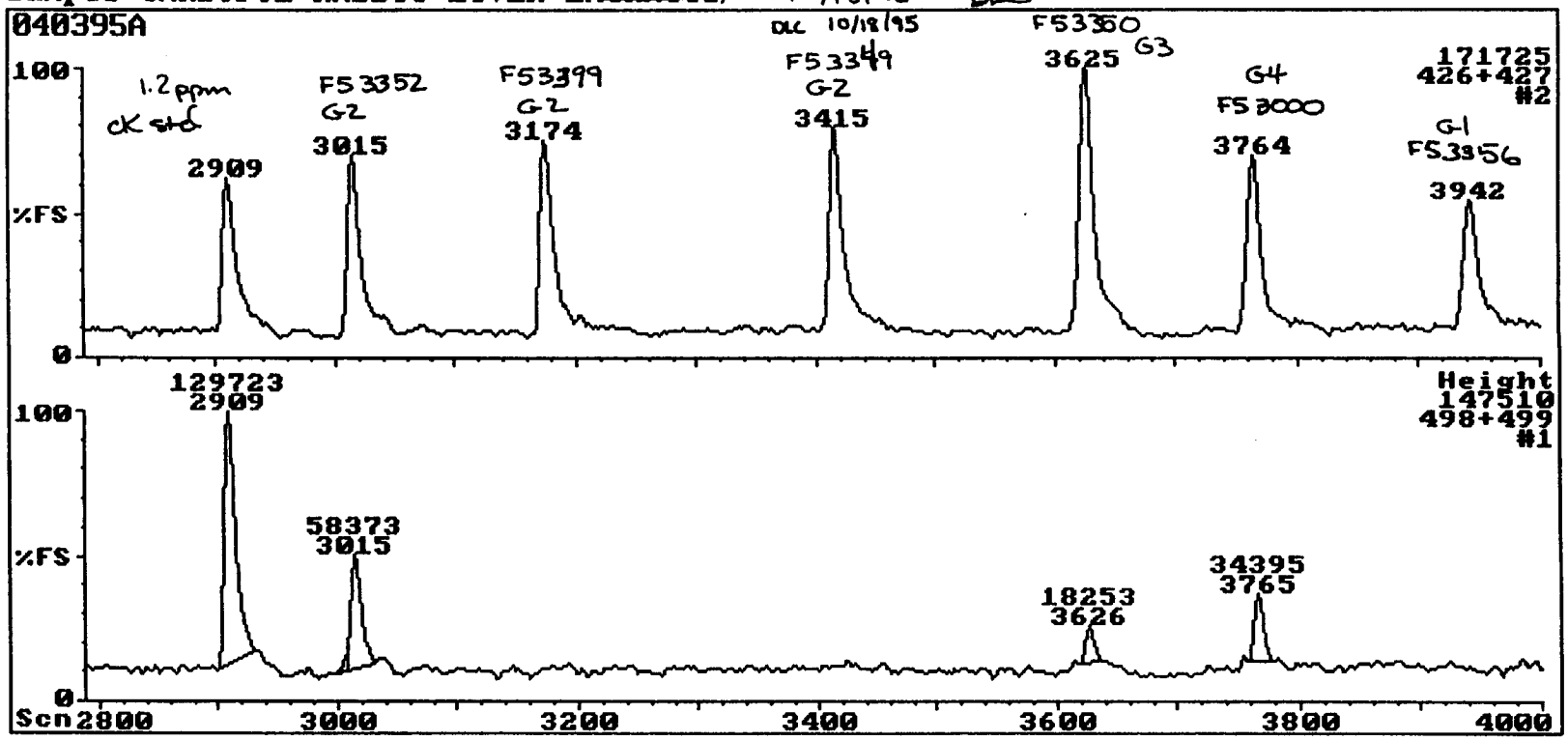
Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC



A-7

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

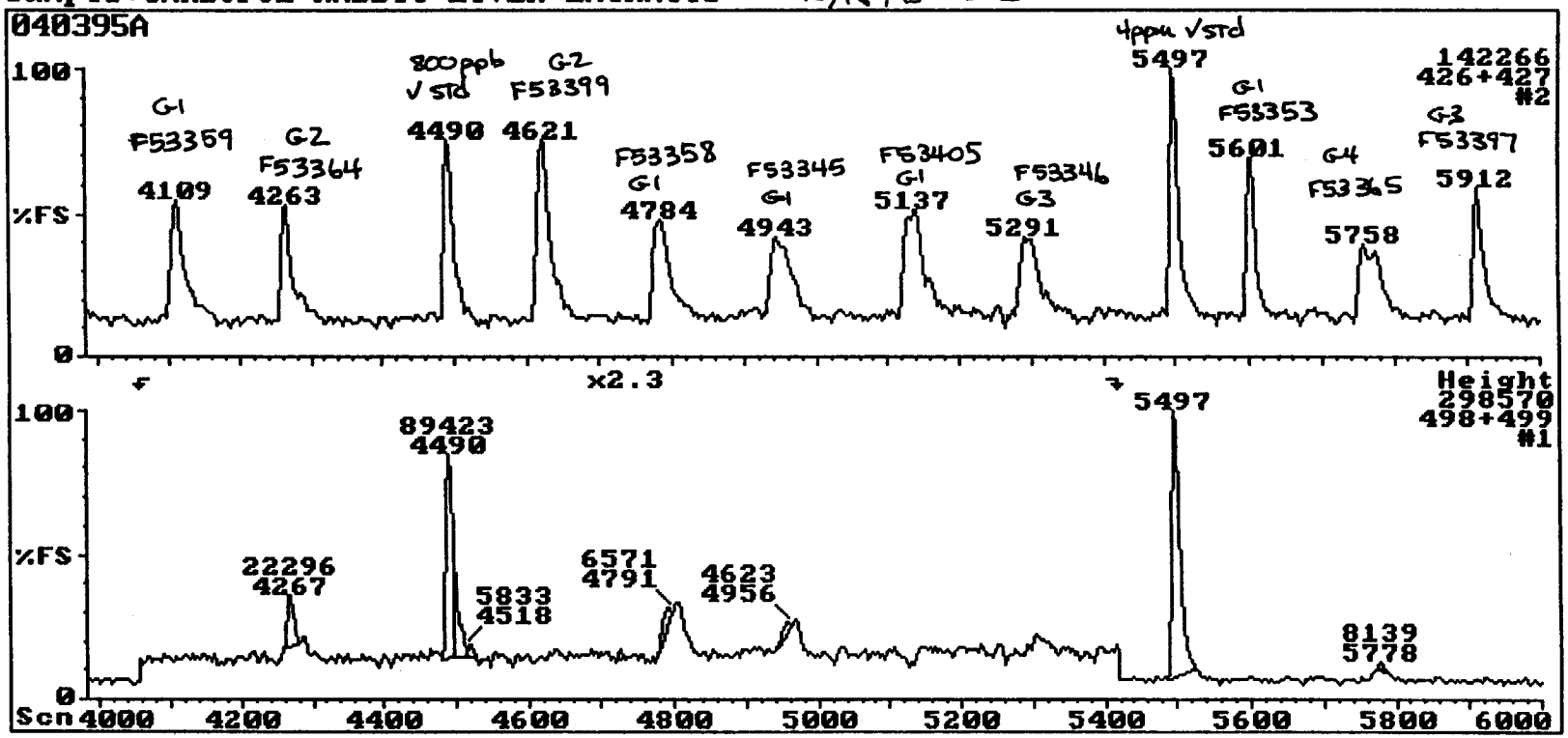
Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~, 10/18/95 DLC



000092

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

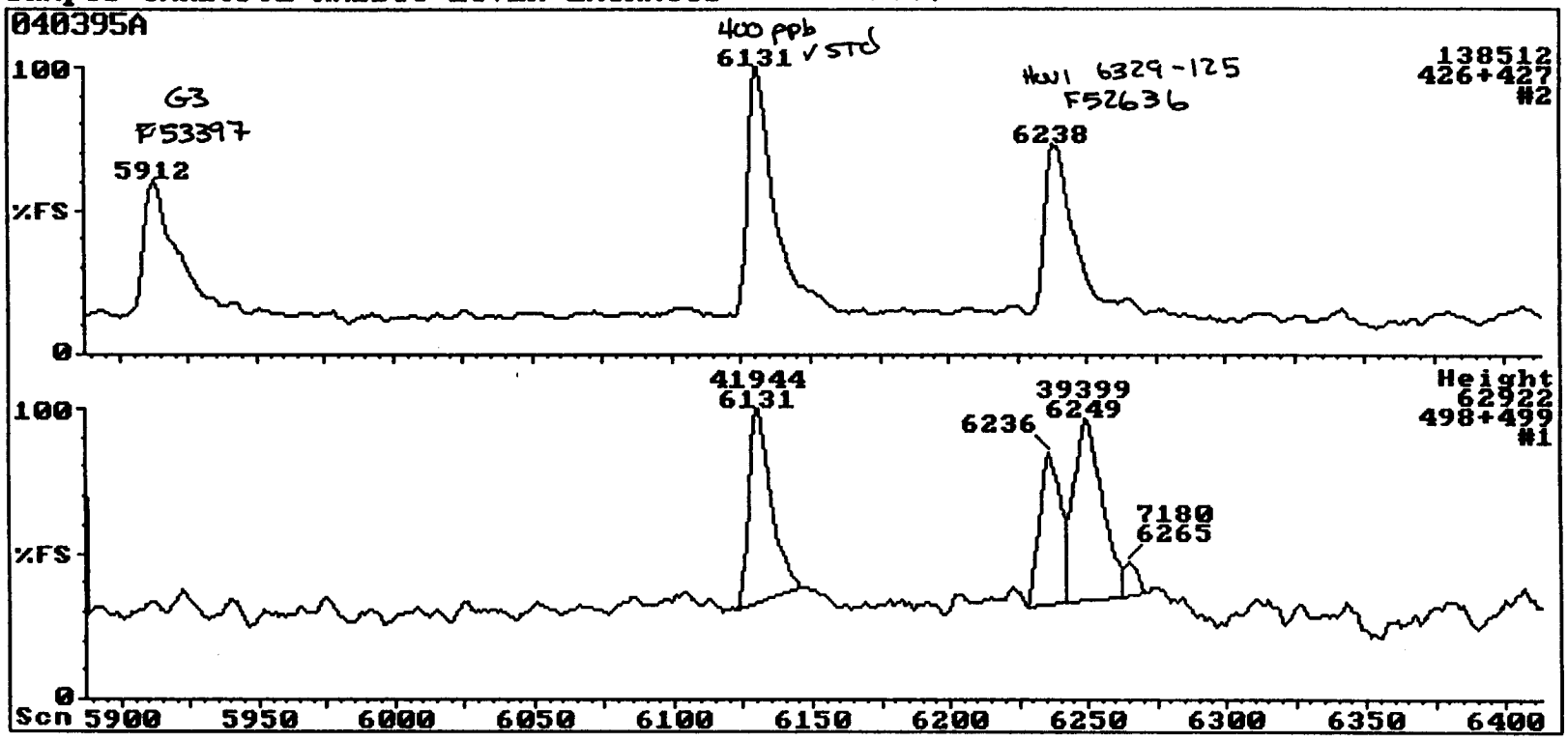
Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC



A-9

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC



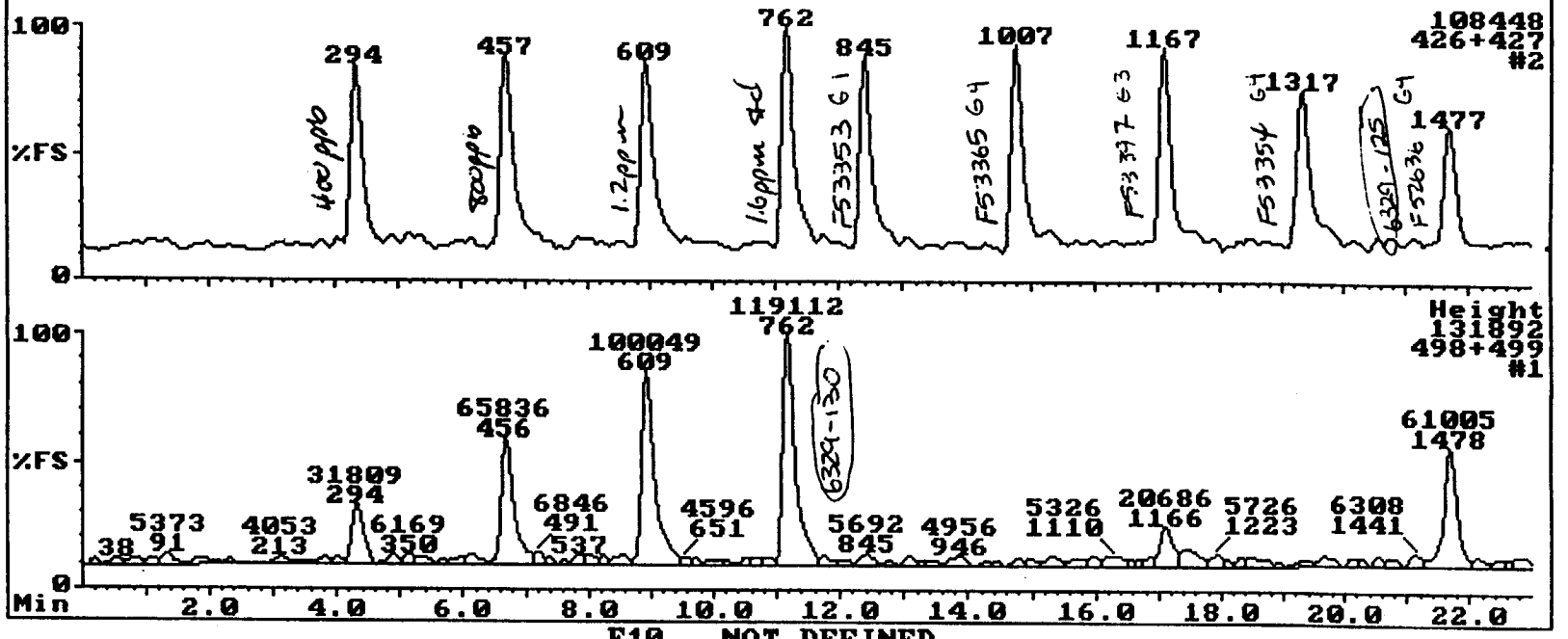
000094

A-10

File:040395B LAB-BASE - The MS Data System 03/04/1995 10:53

Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC

040395B



F10 NOT DEFINED

000095

3M Environmental Laboratory

Final Report- Analytical Study

Single-dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits

In-Vivo Study Reference Number: HWI#6329-129

Study Number: AMDT-112294.1
Test Substance: FC-95 (T-6049)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology & Services
935 Bush Avenue
St. Paul, MN 55106

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-14-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/20/95
Completion Date

1.0 SUMMARY

Rabbit serum and liver were analyzed for fluorine content at various times after rabbits were dosed intravenously with a single dose of FC-95 (T-6049). The fluorine in liver was detected after a single dose of 100 mg/kg (60 ug/kg FC-95) or after 500 mg/kg (300 ug/kg FC-95). The liver fluoride ion concentration in the Group 5 animal (300 ug/kg), at 48 hours post dose was 1.0 ppm and for the rabbit dosed with 60 ug/kg the concentration was about 0.4 ppm. The presence of perfluorooctanesulfonate was confirmed with electrospray mass spectrometry.

The method would probably be able to detect a dose somewhere between 10 and 60 ug/kg. Analysis of fluorine at 28 days in liver and serum will likely provide a marker for dermal absorption for dermally applied fluorochemicals that are biotransformed to perfluorooctanesulfonate anion. The data agree with a second pharmacokinetic study of FC-95 which was carried out to 28 days with more animals (HWI#6329-159).

2.0 INTRODUCTION

This study was designed to provide information as to whether the perfluoro-octanesulfonate anion does go to the liver and other tissues when the material is administered in an intravenous dose, and to ascertain the change in concentration with time after dose in serum and liver.

It is known from studies done previously with rats that the half-life of perfluoro-octanesulfonate anion is quite long (>1 month). It was expected that the half-life in rabbits would also be long. Perfluorooctanesulfonate anion is a likely biotrans-formation product of several fluorochemicals that are to be tested for dermal absorption in other studies. The pharmacokinetics of the perfluorooctanesulfonate anion is very relevant to these dermal absorption studies.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company

3.3. Purity and Strength of Reference Substance: Responsibility of Sponsor.

3.4 Stability of Reference Substance: To be determined by Sponsor.

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples the storage is -20 ± 10^0 C.

3.6 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

4.0 EXPERIMENTAL-Overview

Serum and tissues from animals dosed as described (HWI#6329-129), were available for analysis for fluorine compounds. Since perfluorooctanesulfonate anion is not biotransformed, the analysis was accomplished with combustion and subsequent analysis for fluorine. The fluorine data are related directly to perfluorooctane-sulfonate concentration. The fluorine analysis of serum collected at different time intervals after dosing provides data which can be interpreted pharmacokinetically.

5.0 EXPERIMENTAL - Methods

5.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

5.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

5.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

5.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

5.5 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

6.0 DATA ANALYSIS

The material T-6049 was a solution of FC-95 diluted to 0.06% in water. The doses of 5, 10, 100, and 500 mg/kg are thus 3, 6, 60, and 300 ug/kg. These are very low doses.

The liver fluoride ion concentration in the Group 5 animal (300 ug/kg), at 48 hours post dose was 1.0 ppm and for the rabbit dosed with 60 ug/kg the concentration was about 0.4 ppm. The control rabbit, the 5 mg/kg rabbit, and the 10 mg/kg rabbit concentrations were not above the practical limit of quantitation.

For livers of the two highest doses using analysis by electrospray mass spectrometry, the perfluorooctanesulfonate was detectable and the estimated amounts for the 100 and 500 mg/kg rabbits were 0.3 and 0.6 ppm, respectively.

These data show that perfluorooctanesulfonate will be a very sensitive marker for dermal absorption tests of FC-95 and for any fluorochemicals that are biotransformed to perfluorooctanesulfonate. The fact that the material can be observed in liver after a 60 ug/kg dose is suggestive that a very large portion of the dose will be in liver if the material is present systemically.

Other data was collected on serum samples using thermal extraction of fluoride by means of a modified Dohrmann organic halide analyzer and fluoride measurement by means of a Orion expandable ion analyzer (see appendices). This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated here and therefore is not discussed in detail.

6.1 Circumstances That May Affect the Quality of the Data: These values for concentration are from combustion analyses of the biological material. The recoveries are based on spiking the biological material with known amounts of FC-95. The fluorine is measured by selective ion electrode and the Orion meter is calibrated over a 5 point range. However, if there is a different response at different concentrations of FC-95 other than the point used as a calibration check, there could be a bias at those concentrations even though the amount of fluorine is being measured accurately. This can come from other variables in the method such as the efficiency of combustion. Thus, the values reported for concentration are not absolute values. The values are certainly sufficient to make the point that the perfluorooctanesulfonate anion is present at 48 hours in liver.

7.0 CONCLUSION

The method would probably be able to detect a dose somewhere between 10 and 60 ug/kg. Analysis of fluorine at 28 days in liver and serum will likely provide a marker for dermal absorption for dermally applied fluorochemicals that are biotransformed to perfluorooctanesulfonate anion. The data agree with a second pharmacokinetic study of FC-95 which was carried out to 28 days with more animals (HWI#6329-159).

8.0 MAINTENANCE OF RAW DATA AND RECORDS

8.1 Raw Data and Data: Raw data, approved protocol, approved final report, appropriate specimens, and electronic data will be maintained in the AMDT Archives.

9.0 APPENDICES

9.1 Protocol and Amendments

9.1.1 Protocol and Final Report: HWI#6329-129 "Single Dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-112294.1 including methods.

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-112294.1

9.10 Instrument Settings: See methods

9.11 Data: See attached:

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

9.11.3 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.1.1 Protocol and Final Report: HWI#6329-129 "Single Dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707 7545

a CORNING Company

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

February 1, 1995

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-129

000005

Phone 608-241-4471 Fax 608-241-7227
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD MADISON, WI 53704

HWI 6329-129

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
11/06/94	11/06/94	Protocol Review	11/08/94	12/10/94
11/16/94	11/16/94	Animal Observation	11/16/94	12/10/94
01/10/95	01/10/95	Data/Report Review	01/10/95	02/10/95
01/30/95	01/30/95	Report Rereview	01/30/95	02/10/95

Cecilia M. Danner
Representative, Quality Assurance Unit

2/1/95
Date

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits

Test Material	T-6049
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Experimental Start Date	November 15, 1994
Experimental Termination Date	November 17, 1994

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Francis (Bud) W. McDonald
Study Coordinator

Patricia Padgham
In-life Supervisor

Rose M. Bridge
Report Supervisor

Quality Assurance

Sherry R. W. Petsel
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Jack Serfort/
Deborah L. Pirkel
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

	Page
Quality Assurance Statement	2
Study Identification	3
Key Personnel	4
Summary	6
Objective	7
Regulatory Compliance	7
Test and Control Materials	7
Test System	8
Procedures	9
Results	11
Discussion	11
Signature	11
Reference	11
Table	
1 Individual Body Weights (g)	12
2 Individual Clinical Signs	13
Appendix A	14
Protocol TP8084.PK	15

SUMMARY

This study was done to assess the level of systemic exposure of T-6049 when administered by intravenous injection to rabbits.

Female Hra:(NZW)SPF rabbits were assigned at random to five groups (one/group). On Day 0, the animals received a single intravenous injection of the vehicle (sterile water for injection) or 5, 10, 100, or 500 mg of T-6049/kg of body weight (Groups 1 through 5, respectively). The dose volume was 0.5 mL/kg for all groups.

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection. Body weights were determined just before test material administration (Day 0). A blood sample (approximately 4 mL) was collected from an auricular artery or marginal ear vein of the animals at 2-, 4-, 6-, 8-, 12-, and 24-hours post-injection. In addition, at the time of experimental termination (48-hours post-injection), approximately 20 mL of blood was obtained from each animal. All samples were centrifuged, separated into serum and cellular fractions, and sent to the Sponsor. Approximately 48 hours post-injection, the animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not done, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and sent frozen to the Sponsor after termination of the in-life phase.

The animals treated with sterile water for injection, 5 mg T-6049/kg, and 10 mg T-6049/kg appeared normal throughout the study. At the 0.5, 2, and 4 hour post-dose observation, the animal treated with 100 mg T-6049/kg appeared hypoactive and the animal treated with 500 mg T-6049/kg exhibited a staggered gait. In addition, the animal treated with 500 mg/kg showed a decrease in food consumption at the 48-hour post-dose observation.

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6049, when administered as a single intravenous injection to rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test mixtures for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study were in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6049 and described as a clear, colorless liquid. The control material was Sterile Water for Injection, USP (Abbott Laboratories, Lot No. 86-748-DM-02; Exp. March 1, 1996), and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). A sample of the test material/vehicle mixtures for concentration, solubility, homogeneity, and stability analyses was not taken before administration as this was not requested by the Sponsor. The purity and stability of the USP grade control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test material was stored at room temperature. The control material was stored refrigerated. Any unused test material will be returned to the Sponsor after completion of all testing according to Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any remaining vehicle may be used for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were received from HRP, Inc., Kalamazoo, Michigan on October 19, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from the required temperature and humidity conditions existed, they were documented and considered to have had no adverse effect on the study outcome. Animal husbandry and housing at HWI complied with standards outlined in the "Guide for the Care and Use of Laboratory Animals".[1]

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were selected at random based on health and body weight requirements.

Study Design

Female animals weighing from 2,587 to 2,857 g at initiation of treatment were placed into the following study groups:

Group	Treatment	Dose Level (mg T-6049/kg)	Dose Volume (mL/kg)	Number of Animals
1 (Control)	*	0	0.5	1
2	T-6049	5	0.5	1
3	T-6049	10	0.5	1
4	T-6049	100	0.5	1
5	T-6049	500	0.5	1

* Sterile Water for Injection, USP.

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Dose Preparation and Administration

The test material was diluted with Sterile Water for Injection to achieve a specific concentration for each dose level in Groups 2 through 4. The test material was administered undiluted at the 500 mg/kg dose level, using the bulk density of 0.98 g/mL to determine the dose volume. An individual dose of each respective test solution or control was calculated for each animal based on its body weight on the day of treatment. The respective test solution was administered by intravenous injection into a marginal ear vein. The dose was given as a slow push (approximately 30 to 60 seconds in duration). The prepared test solutions were stored at room temperature until administered. After administration, any remaining test solutions were discarded.

Reason for Route of Administration

Intravenous injection is an acceptable route to assess systemic exposure.

Observations of Animals

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection.

Body weights were determined just before test material administration (Day 0).

Sample Collection

A blood sample (approximately 4 mL) was collected from either ear via the catheterization of the auricular artery or from the marginal ear vein of all animals at 2, 4, 6, 8, 12, and 24 hours post-injection. At the time of necropsy (approximately 48-hours post-injection), approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were stored at room temperature until centrifuged and separated into serum and cellular fractions. The blood samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the experimental phase (approximately 48-hours post-injection), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not conducted, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and immediately placed on dry ice, then frozen by placing in a freezer set to maintain a temperature of -20°C ±10°C. After tissue/bile collection, the animals were discarded.

Shipment of Tissues

After completion of the in-life phase the blood samples, livers, bile, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106). The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual body weights at initiation are in Table 1.

Clinical Observations

Individual clinical signs are in Table 2. The animals treated with sterile water for injection, 5 mg T-6049/kg, and 10 mg T-6049/kg appeared normal throughout the study. At the 0.5, 2, and 4 hour post-dose observation, the animal treated with 100 mg T-6049/kg appeared hypoactive and the animal treated with 500 mg T-6049/kg exhibited a staggered gait. In addition, the animal treated with 500 mg/kg showed a decrease in food consumption at the 48-hour post-dose observation.

Pathology

All animals survived to termination of the experimental phase and were not examined grossly when sacrificed.

DISCUSSION

The level of systemic exposure of T-6049 was evaluated in female albino rabbits when administered as a single intravenous injection at levels of 0, 5, 10, 100, and 500 mg/kg. Administration of this material resulted in hypoactivity at the 100 mg/kg dose level, and staggered gait and a decrease in food consumption at the 500 mg/kg dose level.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

2-1-95
Date

REFERENCE

1. NIH Publication No. 86-23 (revised 1985).

Table 1
Individual Body Weights (g)

Group	Dose Level (mg/kg)	Sex	Animal Number	Day 0
1	0	Female	F52652	2,634
2	5	Female	F52653	2,587
3	10	Female	F52654	2,620
4	100	Female	F52665	2,857
5	500	Female	F52666	2,794

Table 2
Individual Clinical Signs

Group	Dose Level (mg/kg)	Sex	Animal Number	Observation	Hour 0.5	2	4	24	48
1	0	Female	F52652	Appeared normal	✓	✓	✓	✓	✓
2	5	Female	F52653	Appeared normal	✓	✓	✓	✓	✓
3	10	Female	F52654	Appeared normal	✓	✓	✓	✓	✓
4	100	Female	F52665	Appeared normal	-	-	-	✓	✓
				Hypoactivity	✓	✓	✓	-	-
5	500	Female	F52666	Appeared normal	-	-	-	✓	-
				Staggered gait	✓	✓	✓	-	-
				Decreased food consumption	-	-	-	-	✓

✓ Indicates condition exists.
- Not applicable.

APPENDIX A

Protocol TP8084.PK

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707-7545

a CORNING Company

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP8084.PK

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-6049 in Rabbits

Date:

November 9, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-129

Phone 608 241 4471 Fax 608 241 7227
EXPRESS MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI 53704

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits

HWI No.	6329-129
Test Material	T-6049
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	Week of November 14, 1994
Experimental Termination Date	Week of November 14, 1994
Draft Report Date	Week of December 19, 1994

1. Study
Single-Dose Intravenous Pharmacokinetic Study in Rabbits

2. Purpose
To assess the level of systemic exposure when the test material is administered as a single intravenous injection to rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines with the exception that analysis of the test material mixtures for concentration, solubility, homogeneity, and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] 59 Nohsan No. 3850 (Japanese MAFF)
[ ] Notification No. 313 (Japanese MOHW)

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Material

A. Identification
T-6049

B. Physical Description
(To be documented in the raw data)

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Samples of test material/vehicle mixture(s) for concentration, solubility, homogeneity, and stability analyses will be taken before administration if requested by the Sponsor. These samples (if taken) will be sent to the Sponsor after experimental termination for possible analysis.

D. Storage
Room temperature

E. Reserve Samples
Reserve samples will not be required for this study.

F. Retention
Any unused test material will be discarded after issuance of the final report, unless directed otherwise by the Sponsor.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Sterile water for injection

B. Physical Description
Clear, colorless liquid

C. Purity and Stability
The purity and stability of this USP grade material is considered to be adequate for the purposes of this study.

D. Storage
Refrigerated

E. Reserve Samples
See Section, 5. E. Reserve Samples

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.5 to 3.5 kg

(5) Number and Sex
5 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Dose Level (mg/kg)[a]	Number of Females
1	0 (Control)	1
2	5	1
3	10	1
4	100	1
5	500	1

a The dose volume will be 0.5 ml/kg for Groups 1-4 and approximately 0.5 mL/kg of body weight (depending on the bulk density of the test material) for Group 5.

C. Dosing Procedures

(1) Dosing Route
Intravenous injection into a marginal ear vein over approximately 30 to 60 seconds.

(2) Reason for Dosing Route
Intravenous injection is an acceptable route to assess systemic exposure.

(3) Dosing Duration
Single dose

(4) Dose Preparation
The test material will be diluted with sterile water for injection to achieve a specific concentration for each dose level in Groups 1-4. The test material will be administered undiluted at the 500 mg/kg dose level, using the bulk density to determine the dose volume. Individual doses will be calculated based on the animal's body weight taken just before test material administration. The prepared test mixtures will be stored at room temperature until administration.

D. Observation of Animals

(1) Clinical Observations
The animals will be observed for clinical signs of toxicity at approximately 0.5, 2.0, 4.0, 24, and 48 hours after treatment.

(2) Body Weights
Just before test material administration.

(3) Sample Collections

(a) Frequency
2, 4, 6, 8, 12, 24, and 48 hours post-injection

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from either ear via the catheterization of the auricular artery or from the marginal ear vein at 2, 4, 6, 8, 12, and 24 hours post-injection. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal at the time of necropsy (48 hours post-injection). Approximately 20 mL of blood will be collected from moribund animals during the study, also, if possible. The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain a temperature of -20°C ±10°C. The separated serum and cellular fractions will be sent frozen to the Sponsor after experimental termination.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

E. Termination

(1) Unscheduled Sacrifices and Deaths
Any animal dying during the study or sacrificed in a moribund condition, will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital, bled via the vena cava, and exsanguinated.

(2) Scheduled Sacrifice
At approximately 48 hours post-injection, animals surviving to termination will be anesthetized with sodium pentobarbital, bled via the vena cava, and exsanguinated. An abbreviated gross necropsy examination will not be done, however, tissues will be collected.

(a) Sample Collection
The whole liver and bile from each animal dying during the study, sacrificed in a moribund condition, or surviving to termination will be collected. Both kidneys from each animal will also be collected. The tissues will be placed on dry ice immediately after collection and then placed in a freezer set to maintain a temperature of -20°C ±10°C.

The tissues (liver, bile, kidneys) will be sent frozen on dry ice to the Sponsor after experimental termination. The samples will be shipped to the person listed in Section 7.D.(3).(c). The Sponsor is responsible for the retention and disposition of the samples.

F. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Description of any toxic effects
Gross pathology findings/gross pathology report (if applicable)

9. Location of Raw Data, Records, and Final Report
Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Sample collection records
Pathology records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff — 11-18-94
John L. Butenhoff, PhD
Sponsor's Representative
3M
Date

Steven M. Glaza — 11-9-94
Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.
Date

Cecilia M. Dyer — 11/9/94
Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.
Date

(6329-129.protdsk1)

9.1.2 Analytical protocol AMDT-112294.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits

In-Vivo Study Reference Number: HWI#6329-129

Study Number: AMDT-112294.1
Test Substance: FC-95 (T-6049)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Initiation Date: July 25, 1995
Proposed Completion Date: August 25,1995

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-14-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

Author: James D. Johnson

Approved By:

James D. Johnson 11/16/95
James D. Johnson — Date
Study Director

John Z. Butenhoff 11/20/95
John Butenhoff, PhD — Date
Sponsor Representative

1.0 PURPOSE

This study is designed to provide information as to whether the perfluorooctanesulfonate anion does go to the rabbit liver and other tissues when the material is administered in an intravenous dose, and to ascertain the change in concentration with time after dose in serum and liver. These data will be used to interpret dermal absorption studies where perfluorooctanesulfonate is directly applied or is present as a biotransformation product. The persistence of perfluorooctanesulfonate as a marker in liver and serum or possibly other tissues is a critical part for the interpretation of these dermal studies.

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

2.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.
2.1.2 Analytical Reference Matrix: Bovine liver and bovine serum
2.1.3 Analytical Control Substance: None
2.1.4 Analytical Control Matrix: Bovine liver and bovine serum

2.2 Source of Materials: 3M ICP/PCP Division (2.1.1), grocery store (2.1.2, 2.1.4 liver), Sigma Chemical Company (2.1.2, 2.1.4 serum)

2.3 Number of Test and Control Samples: Liver and serum from 4 test animals and 1 control animal. Other biological tissues (kidney, bile, cellular fraction) will be available for analysis if deemed appropriate by the Study Director.

2.4 Identification of Test and Control Samples: The samples are identified using the HWI animal identification number which consists of a letter and five digit number, plus the tissue identity and day identity (serum).

2.5 Purity and Strength of Reference Substance: To be determined by Sponsor.

2.6 Stability of Reference Substance: To be determined by Sponsor.

2.7 Storage Conditions for Test Materials: Room temperature (2.1.1), -20 ± 10°C (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days. This study is in parallel with a 28 day dermal absorption study so all tissues will be retained.

2.9 Safety Precautions: Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting the samples.

3.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-129), are available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests can be performed. The screening for fluoride in liver via combustion (See Methods--next Section) is the appropriate analysis to present definitive data for fluorine in the liver. For these analyses, bovine liver appears to be a good approximation to rabbit liver for matrix spikes and recovery determinations. Since perfluorooctanesulfonate anion is not biotransformed, fluorine content will be an accurate estimate of the concentration of the compound.

4.0 EXPERIMENTAL - Methods

4.1 Liver and Serum screening methods: (attached)

4.1.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

4.1.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

4.1.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

4.1.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

4.1.5 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-95 per tissue or fluid. Half-life of disappearance from serum will be determined from the linear regression of the averages of the concentrations at different time points for the post distribution part of the serum concentration versus time curve.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

6.1 Raw Data and Records: Raw data, approved protocol, appropriate specimens, approved final report, and electronic data will be maintained in the AMDT Archives.

7.0 REFERENCES

7.1 AMDT-S-10-0, Sample Tracking System

8.0 ATTACHMENTS

8.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

8.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

8.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

8.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

8.5 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Liver

Method Identification Number: AMDT-M-1 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95 Date

Quality Assurance 10-4-95 Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological tissues, particularly liver.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 150 mg, depending on sample moisture content. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are not to be handled with bare hands. Fluoride may leach from the skin to the sample. Use forceps or probe to transfer tissues.
7.2 Samples of liver are cut from frozen liver and placed in a tared and labeled weigh boat. Use a clean scalpel and cutting board. The cutting board and scalpel should be cleaned with water, methanol, or methanol-water solution after each liver is cut.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit liver studies, use beef liver as the matrix. Cut a piece of frozen beef liver (100 - 150 mg) and weigh it in a labeled and tared weigh boat.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked liver curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef liver as the matrix. Prepare at least 3 samples of beef liver (100 - 150 mg) for blanks.
8.3.2.3 Put sample in Dohrmann boat. Combust each sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.

8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 Weigh out at least 15 matrix samples (5 standards with 3 replicates each) in tared and labeled weigh boats. For rabbit studies, weigh 100-150 mg beef liver samples. Record weights in study data. Store the matrix samples on dry ice or ice packs to keep them frozen until used.
8.3.3.2 Place weighed beef liver sample in Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef liver.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.
9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-SOLIDS program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control
10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved By:

Group Leader — Date: 10/3/95

Quality Assurance — Date: 10-4-95

Software: MS Word 5.1a

Affected Documents: AMDT-M-1 Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 SCOPE: This method is for the calibration and operation of an Orion EA940 Expandable Ion Analyzer.

1.2 APPLICABLE COMPOUNDS: Fluoride.

1.3 APPLICABLE MATRICES: Liquid samples in an appropriate buffer solution. Preferred pH of 6.0.

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

4.1 Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.
4.2 Orion Model 900001 electrode filling solution (AgCl) or equivalent.
4.3 Orion 940907 100 ppm fluoride standard or equivalent.
4.4 Milli-Q™ water or equivalent.
4.5 Magnetic stir bars.
4.6 Lab tissues.
4.7 Sample collection vials.
4.8 Plastic 100 mL volumetric flasks.
4.9 Polystyrene pipettes.
4.10 Miscellaneous laboratory glassware.

5.0 EQUIPMENT

5.1 Orion Model EA940 Expandable Ion Analyzer or equivalent.
5.2 Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.
5.3 Magnetic Stir Plate.
5.4 IBM compatible 386 or 486 computer (only needed if using Orion 3E software).
5.5 Orion RS232 interface cable (only needed if using Orion 3E software).
5.6 Microsoft Excel 5.0 (only needed if using Orion 3E software).

6.0 INTERFERENCES

6.1 It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.
6.2 Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.
6.3 The rate the samples are stirred at should be consistent with the rate the standards were stirred.

6.4 Air bubbles trapped under electrode can give erroneous readings. Make sure no air is trapped under electrode.

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards
8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.
8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.
8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.
8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.
8.1.5 Shake the flasks to mix the solutions.
8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.
8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.
8.1.8 Invert and shake the flasks for the final mixing.
8.1.9 Record standards in Standards Log Book.

8.2 Calibration
8.2.1 If necessary, remove tape from electrode filling hole.
8.2.2 Invert probe to wet top seal.
8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.
8.2.4 Fill the electrode with filling solution.
8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.
8.2.6 Record the slope in the appropriate log book.
8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

8.3 Storage Conditions for Standards
8.3.1 Calibration standards are stored at room temperature.

9.0 PROCEDURES

9.1 Calibration and Measurement, Standard method:
9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.
9.1.2 Place a stir bar in the sample and place the sample on the stir plate.
9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.
9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.
9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

9.2 Calibration And Measurement, Using Orion 3E Software:

9.2.1 Calibration:
9.2.1.1 Follow steps 8.2.1 to 8.2.4.
9.2.1.2 Press Function Key #8 (F8).
9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.
9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.
9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.
9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.
9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

9.2.2 Data Spreadsheet:
9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.
9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

9.2.3 Fluoride Measurement:
9.2.3.1 Follow steps 9.2.1 through 9.2.4
9.2.3.2 Enter the name of the sample in the appropriate place on the screen.
9.2.3.3 Click on the NEW SAMPLE button
9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

10.3 Other Validation Parameters According to Reference 13.2, the range of detection is 0.02 ppm fluoride up to a saturated solution of fluoride.

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Orion Model EA940 Expandable Ion Analyzer Instruction Manual, Orion Research Incorporated, 1991.
13.2 Orion Model 960900 Solid State Combination Fluoride Electrode Instruction Manual, Orion Research Incorporated, 1991.

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 10-31-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 10-31-95

Quality Assurance — Date: 10-31-95

Software: MS Word, 6.0
Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

000045

1.0 SCOPE

1.1 **Scope:** This method is for the extraction of fluorochemicals from rabbit livers. Ethyl acetate is used to extract fluorochemicals from the livers for analysis by electrospray mass spectroscopy.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

2.1 Fluorochemicals, rabbit livers, electrospray mass spectrometer, fluorinated compounds, extraction.

3.0 PRECAUTIONS

3.1 Use gloves when handling the rabbit livers, they may contain pathogens.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

- 4.1.1 Syringe, capable of measuring 100 µL
- 4.1.2 Eppendorf type or disposable pipets
- 4.1.3 Gloves
- 4.1.4 Plastic grinding tubes
- 4.1.5 Plastic centrifuge tubes, 15 mL
- 4.1.6 Labels
- 4.1.7 Nitrogen
- 4.1.8 Timer
- 4.1.9 Filters, Titan nylon syringe filters, 0.2 µm.
- 4.1.10 Analytical pipets: glass volumetric pipets.
- 4.1.11 Disposable plastic 3 cc syringes.
- 4.1.12 Crimp cap autovials.

4.2 **Reagents**

- 4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
- 4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.
- 4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
- 4.2.4 Ethyl Acetate
- 4.2.5 Methanol
- 4.2.6 Milli-Q water
- 4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
- 4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

5.1 Ultra-Turrax T25 Grinder for grinding liver samples.
5.2 Vortex mixer
5.3 Centrifuge
5.4 Shaker
5.5 Analytical Evaporator

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 The rabbit livers are received frozen, and must be kept frozen until the extraction is performed.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.
8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.
8.1.3 Bring it up to volume with methanol, this is the stock standard.
8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

$$\frac{\text{actual mg perfluoroctane-sulphonic acid}}{0.1\ \text{L}} \times \frac{3\ \text{mL}}{250\ \text{mL}} = \text{actual concentration, ppm}$$

8.2 Prepare FC-95 Anion Standards

8.2.1 Prepare FC-95 standards for the standard curve.
8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.
8.2.3 Bring up to volume with dilute acetonitrile.
8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.
8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 Prepare Beef Liver Homogenate to Use for Standards

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.
8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

8.3.3 Spike seven of the 1 mL aliquots with the following amounts of working standards in step 9.12 of the procedure. One 1 mL aliquot serves as the blank.

Working Standard (Approximate Conc.)	uL	Approximate final concentration of FC-95 in liver
-	-	Blank
500 ppb	100	0.292 ppm
500 ppb	200	0.584 ppm
500 ppb	300	0.877 ppm
500 ppb	400	1.168 ppm
1 ppm	500	2.924 ppm
5 ppm	200	5.848 ppm
5 ppm	300	8.772 ppm

8.4 Calculate the actual value of the standards:

$$\frac{\text{uL of standard} \times \text{concentration (in ppm)}}{\text{171 mg liver}^{*} / \text{1 ml homogenate}} = \text{final concentration (ppm) of FC -95 in liver}$$

*Average weight of bovine liver in solution as determined by weighing 1 mL homogenates of 40 mg liver in 200 mL of Milli-Q water. The amount of FC-95 is reported as equivalents of FC-95 potassium salt.

8.5 **Calibration**

8.5.1 Extract the spiked beef liver homogenate following 9.13 to 9.23 of this method. Use these standards to establish your curve on the mass spectrometer.

8.5.2 Alternatively, a standard curve may be generated using ratios of responses of the perfluorooctansulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

8.6 **Storage Conditions for Standards**

8.6.1 New standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectrometer is performed.

8.7 **Storage Conditions for Standards**

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

9.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

9.2 Use a dissecting scalpel and cut off approximately 1 g of liver.

9.3 Weigh the sample directly into a tared plastic grinding tube.

9.4 Record the liver weight in the study note book.

9.5 Put a label on the vial with the study number, weight, rabbit ID, date and analyst initials.

9.6 Add 2.5 mLs water.
9.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, until the sample is a homogeneous solution with no large chunks.
9.8 Rinse the probe off into the sample with 2.5 mLs water using a pipet.
9.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
9.10 Cap the sample and vortex for 15 seconds.
9.11 Pipet 1 mL into a 15 mL centrifuge tube. Label the centrifuge tube with the identical information as the grinding tube. (See AMDT-M-4 Worksheet for documenting the remaining steps.)
9.12 Spike the beef liver homogenates with the appropriate amount of FC-95 standard as described in 8.3.
9.13 Spike the samples and beef liver homogenates with 100 uL of internal standard.
9.14 Add 1 mL of the sodium carbonate/sodium bicarbonate buffer and 1 mL ammonium acetate.
9.15 Using an analytical pipet, add 5 mL ethyl acetate.
9.16 Cap the sample and vortex 20 to 30 seconds.
9.17 Put them in the shaker for 20 min.
9.18 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.19 Remove 4 mLs of the top organic layer to a fresh 15 mL centrifuge tube with a 5 mL graduated glass pipet. Transfer the label to the fresh tube.
9.20 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.21 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.22 Vortex 15 seconds.
9.23 Transfer the sample to a 3 mL syringe. Attach a 0.2 µm nylon mesh filter, and filter the sample into a fresh centrifuge tube or a autovial. Label the tube or vial with the study number and animal number.
9.24 Cap and hold for analysis by electrospray mass spectroscopy.
9.25 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 **Quality Control** - not applicable
10.2 **Precision and Accuracy**- not applicable
10.3 **Other Validation Parameters**- not applicable

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22 Routine Maintenance of Ultra-Turrax T-25

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W_____	FC-95 approx 1 ppm actual ppm #W_____	FC-95 approx. 5 ppm actual ppm #W_____	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
[1]		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

[1] study number where the original worksheet is located and place a copy.

Liver Extraction Process:	Date & Initials
Pipet 1 mL of Liver Solution	
Pipet 100 uL of 12 ppm Internal Standard Std. #	
Vortex 15 sec.	
Pipet 1 mL of 250 ppm Ammonium Acetate Std. #	
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	
Pipet 5 mL of Ethyl Acetate	
Vortex 20-30 sec.	
Shake 20 min.	
Centrifuge 20-25 min.	
Remove a 4 mL aliquot of organic layer	
Blow down to near dryness (<0.25 mL) with N_2	
Add 1 mL of 1:1 Acetonitrile/H_2O TN#	
Vortex 15 sec.	
Filter using a 3cc B-D syringe with a 0.2um SRI filter into a 1.5 mL autosample vial.	

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5 **Adoption Date:** 6-6-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 6/6/95

Quality Assurance — Date: 6/6/95

Software: MS Word, 6.0
Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

1.0 SCOPE

1.1 **Scope:** This method is for the analysis of extracts of rabbit liver or other tissues or fluids for fluorochemicals using the electrospray mass spectrometer. The analysis is performed by single ion monitoring of FC-95 anion, M/Z= 499, the internal standard M/Z = 427, and other appropriate masses.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers (samples), Beef Liver (standards), other tissues and fluids.

2.0 KEYWORDS

2.1 Fluorochemicals, fluorinated compounds, electrospray mass spectroscopy, mass spectrometer, rabbit livers.

3.0 PRECAUTIONS

3.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

3.2 Do not run the pump above it's capacity of 4000 psi. If pressure goes over 4000 psi stop and release pressure. The peak tubing may be plugged. Troubleshoot back to find the plug and replace the plugged tubing. See AMDT-EP-15

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

4.1.3 100 uL or 250 uL flat tip syringe for sample injection.

4.2 **Reagents**

4.2.1 Dilute acetonitrile mobile phase, dilute acetonitrile 1:1 with Milli-Q water.

4.2.2 Milli-Q water, all water used in this method should be Milli-Q water.

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 Keep the extracted samples in capped 15 mL centrifuge tubes or in capped autovials until ready for analysis.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Seven beef liver standards and one blank beef liver are prepared during the extraction procedure. (See AMDT-M-4, section 8.0)

8.2 Calibration

8.2.1 Run the seven beef liver standards twice, starting with the lowest standard to obtain the standard curve.

8.2.2 Typically one standard is run after each 5 to 7 samples. Choose a standard in the same range of concentration as the samples.

8.3 Storage Conditions for Standards

8.3.1 Fresh standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

9.1.1 Set software to "Operate on", Ion Mode ES^-.

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

9.1.4 Set the pump to "Run". Set the flow to 1000 uL/min. Observes droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

9.1.5 Check the fused silica at the end of the probe. Use an eye piece to check for chips. The tip should be flat with no jagged edges. If any chips are found cut off the tip of the silica with a column cutter and pull the silica through to the appropriate length.

9.1.6 Check your nitrogen supply. Turn on the nitrogen. There should be no nitrogen leaking around the tip of the probe. A fine mist should be coming out of the tip.

9.1.7 Carefully guide the probe into the opening. Insert it until it won't go any further. Connect the voltage cable to the probe.

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES^-, and the appropriate masses to 427 and 499.

9.1.9 If it is not in single ion mode go to "Option" and set SIR.

9.1.10 Start Acquisition. Assign a file name, MO-DAY-YR + letter. Record it in the log book.

9.1.11 Run the beef liver samples first, running each standard twice at the beginning of the run.. Run a QC check by running one standard after every 5 to 7 samples.

9.2 Manual Injection

9.2.1 Draw 150 uL of sample into a syringe. Inject the sample into the rheodyne injection port. Inject slowly. Record the sample ID in the log book.

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

9.2.4 Record the scan number for each sample in the logbook.

9.3 **Using the Autosampler**

9.3.1 Set up sample tray A, B, or C.

9.3.2 Record the samples and their positions in the instrument log book. Up to 17 vials may be in each run.

9.3.3 Set-up the sampler:

- 9.3.3.1 Push the sample button
- 9.3.3.2 Set sample loop size = 100 uL
- 9.3.3.3 Set inject/sample = 2
- 9.3.3.4 Set Cycle time = 0
- 9.3.3.5 Name the file: Livers
- 9.3.3.6 Identify the tray used
- 9.3.3.7 Add the samples to Queue by pressing "Enter"
- 9.3.3.8 Press "Run" to start

10.0 VALIDATION

10.1 **Quality Control**

10.1.1 Run a standard every 5 to 7 samples. If a significant change(± 50%) in peak height occurs stop the run. Only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 **Precision and Accuracy**

10.2.1 See Method Validation Report number AMDT-M-5.0.V1

10.3 **Other Validation Parameters**

10.4 Refer to Method Validation Report Number AMDT-M-5.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.2 Plot the standard curve, using the mean of the two values obtained for each standard.

11.2.1 Read peak heights or areas for the samples from the printout. Use linear regression to determine the sample concentrations.

11.2.2 Calculate the mg of FC-95 anion, or other fluorochemical in the total rabbit liver:

mg FC-95 anion in the total rabbit liver =

$$\frac{\text{mg FC-95 anion from std. curve}}{\text{gms of liver used for analysis}} \times \text{Total mass of liver, gms}$$

11.3 Make a results table and enter it in the study book.

11.4 Print a chromatogram for each sample, with the peaks labeled with the sample or standard ID. Write the study number on the printout, initial, date, and put it in the study folder. Staple all chromatograms together and number pages.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Serum

Method Identification Number: AMDT-M-14 **Adoption Date:** 10-3-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader — Date: 10/3/95

Quality Assurance — Date: 9-27-95

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological fluids, particularly serum.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 100 µl. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are to be handled with plastic pipettes. A new pipette is to be used for each sample.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit serum studies, use beef serum as the matrix.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked serum curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef serum as the matrix.
8.3.2.3 Put serum blank in Dohrmann boat. Combust sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.
8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 If beef serum is frozen, thaw at least enough to complete the standard curve analysis for the day (≈30 mL).
8.3.3.2 Pipette 100µL of beef serum into Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef serum.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.
9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and pipette 100µL of sample into the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.

9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-WATER program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator and/or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control

10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

Completed by: QAU Auditor Original to: Study Director Copies to: QAU Files

Study Title: **Single-dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits**

Study Number: AMDT-112294.1 Name of Auditor: Kari Rambo

This study has been inspected by the Quality Assurance Unit as indicated in the following table. The findings were reported to the study director and management.

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
10-11-95	11-03-95	Final Report	11-03-95	11-03-95

Kari Rambo 11-3-95
QAU Auditor Date

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:
Jim Johnson
Dave Christenson

Documentation and Reporting:
Jim Johnson
Rich Youngblom

Quality Assurance Unit:
Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

Summary of Combustion Data - Liver
AMDT-112294.1, HWI 6329-129
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group

ID	ug
Control group	23.37
5.0 mg/kg dose (T6049)	23.28
10.0 mg/kg dose (T6049)	16.01
100 mg/kg dose (T6049)	35.87
500 mg/kg dose (T6049)	92.03

FC95 PK ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
BLNK LIVER 1		1.052		0.1216			
BLNK LIVER 2		0.423		0.1551			
BLNK LIVER 3		0.758		0.1135			
BLNK LIVER 4		0.473		0.1502			
BLNK LIVER 5		0.308		0.1473			
LIVER SPK 63-1	77%	0.895		0.1300			
LIVER SPK 63-2	86%	1.024		0.1274			
LIVER SPK 63-3	83%	1.045		0.1205			
LIVER SPK 126-1	84%	2.192		0.1159			
LIVER SPK 126-2	83%	1.616		0.1554			
LIVER SPK 126-3	86%	2.353		0.1110			
LIVER SPK 253-1	82%	3.580		0.1399			
LIVER SPK 253-2	80%	3.966		0.1228			
LIVER SPK 253-3	86%	4.195		0.1242			
LIVER SPK 506-1	69%	6.493		0.1292			
LIVER SPK 506-2	68%	6.338		0.1297			
LIVER SPK 506-3	69%	7.100		0.1175			
LIVER SPK 1012-1	63%	12.884		0.1186			
LIVER SPK 1012-2	75%	14.244		0.1277			
LIVER SPK 1012-3	64%	13.635		0.1139			
BLANK LIVER-1		0.432		0.1144			
BLANK LIVER-2		0.303		0.1223			
BLANK LIVER-3		0.219		0.1402			
F52652-1		0.332		0.1329			
F52652-2		0.242	0.273	0.1202	85.62	23.37	0.0
F52652-3		0.245		0.1013			
F52653-1		0.319		0.1385			
F52653-2		0.217	0.254	0.1118	91.68	23.28	5.0
F52653-3		0.226		0.1155			
F52654-1		0.174		0.1464			
F52654-2		0.206	0.204	0.1173	78.65	16.01	10.0
F52654-3		0.231		0.1168			
F52665-1		0.480		0.1411			
F52665-2		0.384	0.434	0.1502	82.72	35.87	100.0
F52666-1		0.437		0.1325			
F52666-1		0.973		0.1415			
F52666-2		1.043	0.988	0.1146	93.11	92.03	500.0
F52666-3		0.949		0.1161			
LIVER SPK-A	66%	0.791		0.1269			
LIVER SPK-B	67%	0.895		0.1126			
LIVER SPK-C	73%			0.1290			
LIVER SPK-D	74%			0.1397			
LIVER SPK-E	76%			0.1457			
LIVER SPK-F	74%			0.1351			
LIVER BLNK-1				0.1232			

000071

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

A-1
Contains pages
A-1 through A-7
D. Christenson
11-10-95

HWI# 6329-129

Study: Single-Dose Intravenous Pharmacokinetic
Protocol Number: TP8084.PK
Test Material: T-6049 in Rabbits (FC-95)
Matrix: Liver
R Squared Value: 0.9717
Response Factor Amount: 1.07E-05
Analyst: DLC
Date: 3/28/95
Method:
Instrument: Fisons VG 2000 Electrospray MS
LABBASE File: 032895C

Group Dose	Sample #	Ion Count Area	Extracted wt g	Dilution factor	Concentration µg/g *	Total mass of liver g	Total amount of FC-95 per liver mg
Group 1: 0 mg/kg	F52652	N.D.	0.9979	1	N.D.	85.619	N.D.
Group 2: 5 mg /kg	F52653	N.D.	1.061	1	N.D.	91.683	N.D.
Group 3: 10 mg /kg	F52654	6056	1.1129	1	0.0466	78.656	0.004
Group 4: 100 mg/kg	F52665	39065	1.0209	1	0.3274	82.718	0.027
Group 5: 500 mg/kg	F52666	71977	1.1125	1	0.5536	93.111	0.052

* The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.

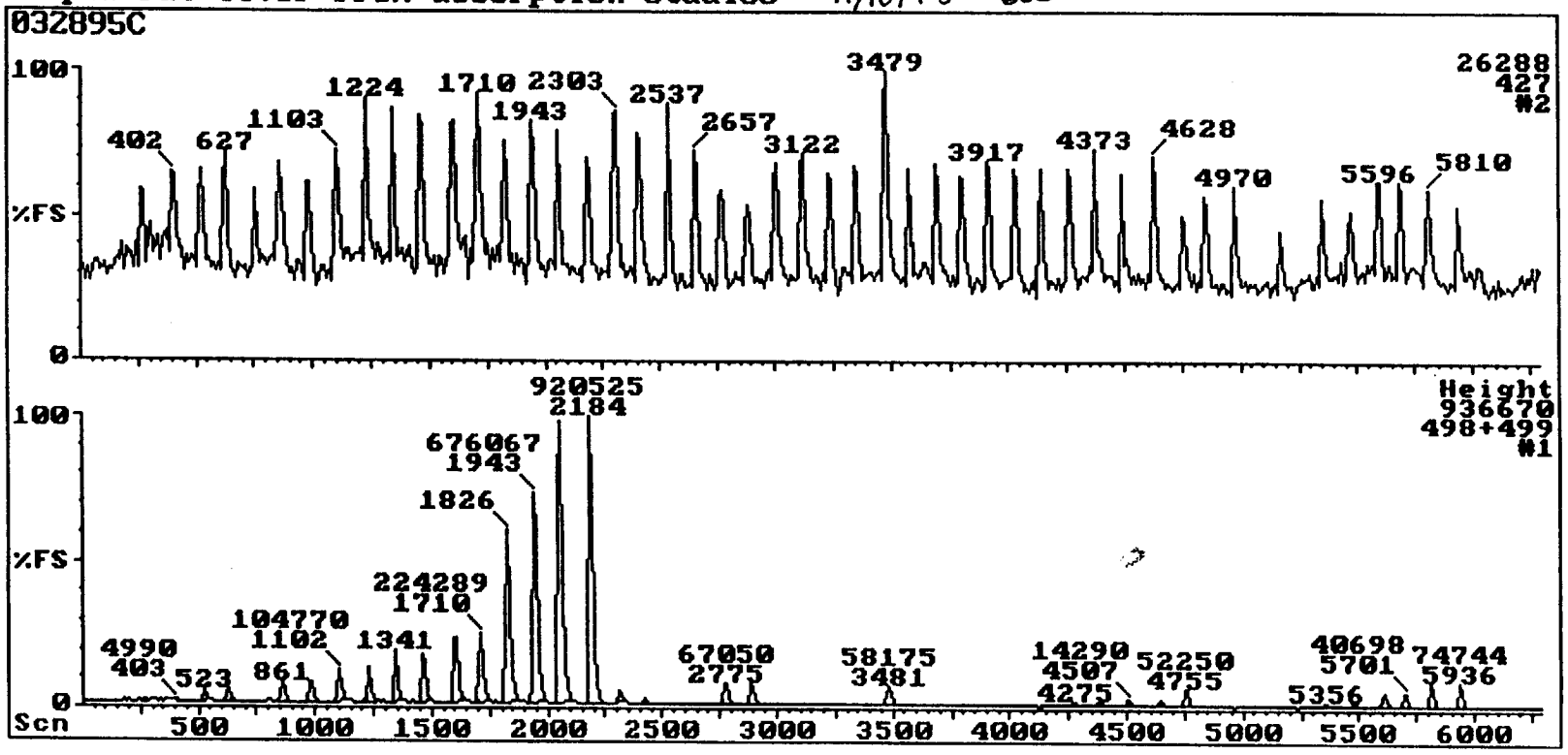
HWI # 6329-124 FX 1801

6329-129 FC 95

000027

File:032895C LAB-BASE - The MS Data System 28/03/1995 10:55

Sample: ~~rat liver from absorption studies~~ 11/10/95 QC

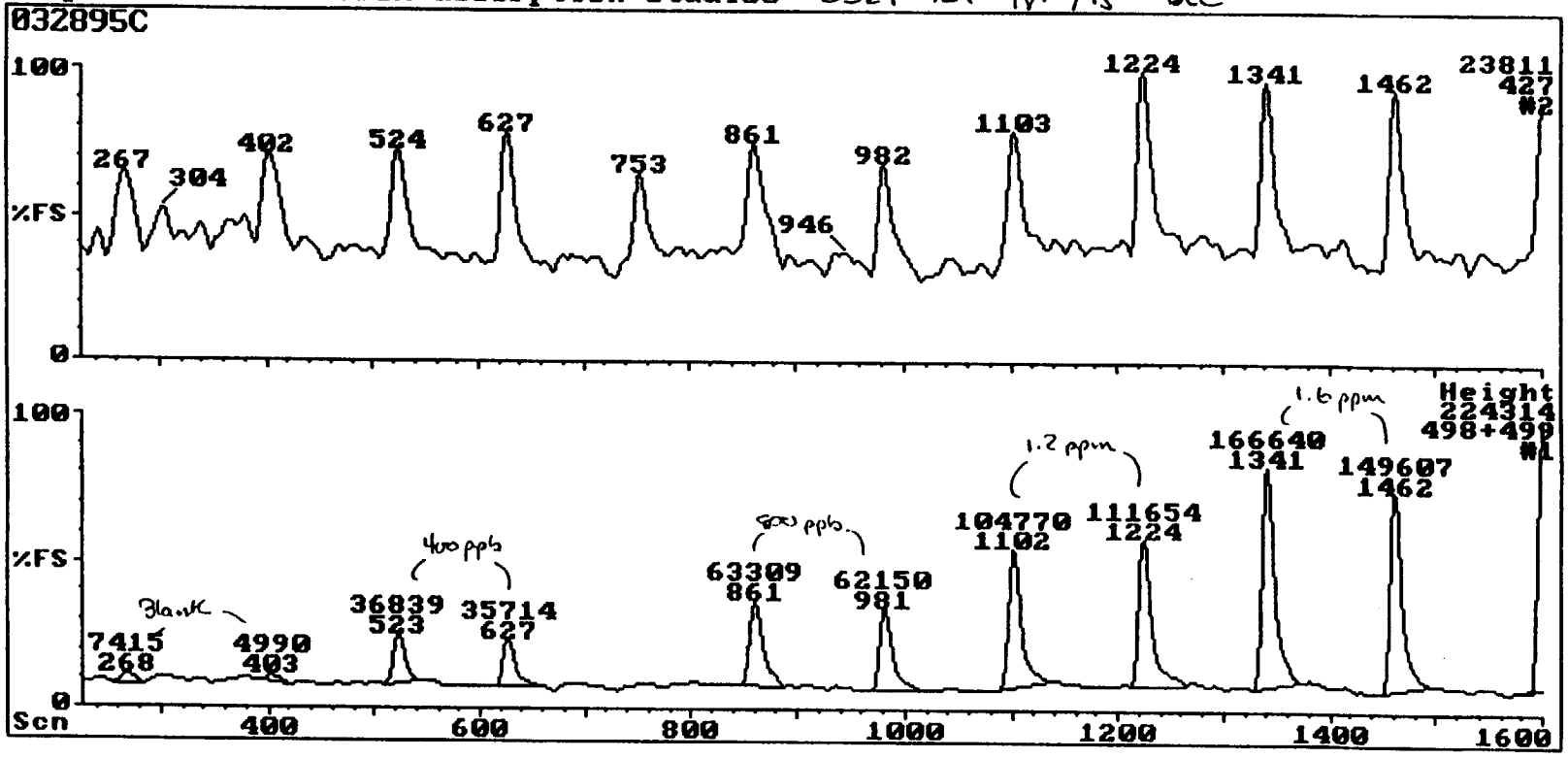


A-3

000025

File:032895C LAB-BASE - The MS Data System 28/03/1995 10:55

Sample: ~~rat liver from absorption studies~~ 6329-129 1/10/95 OLC

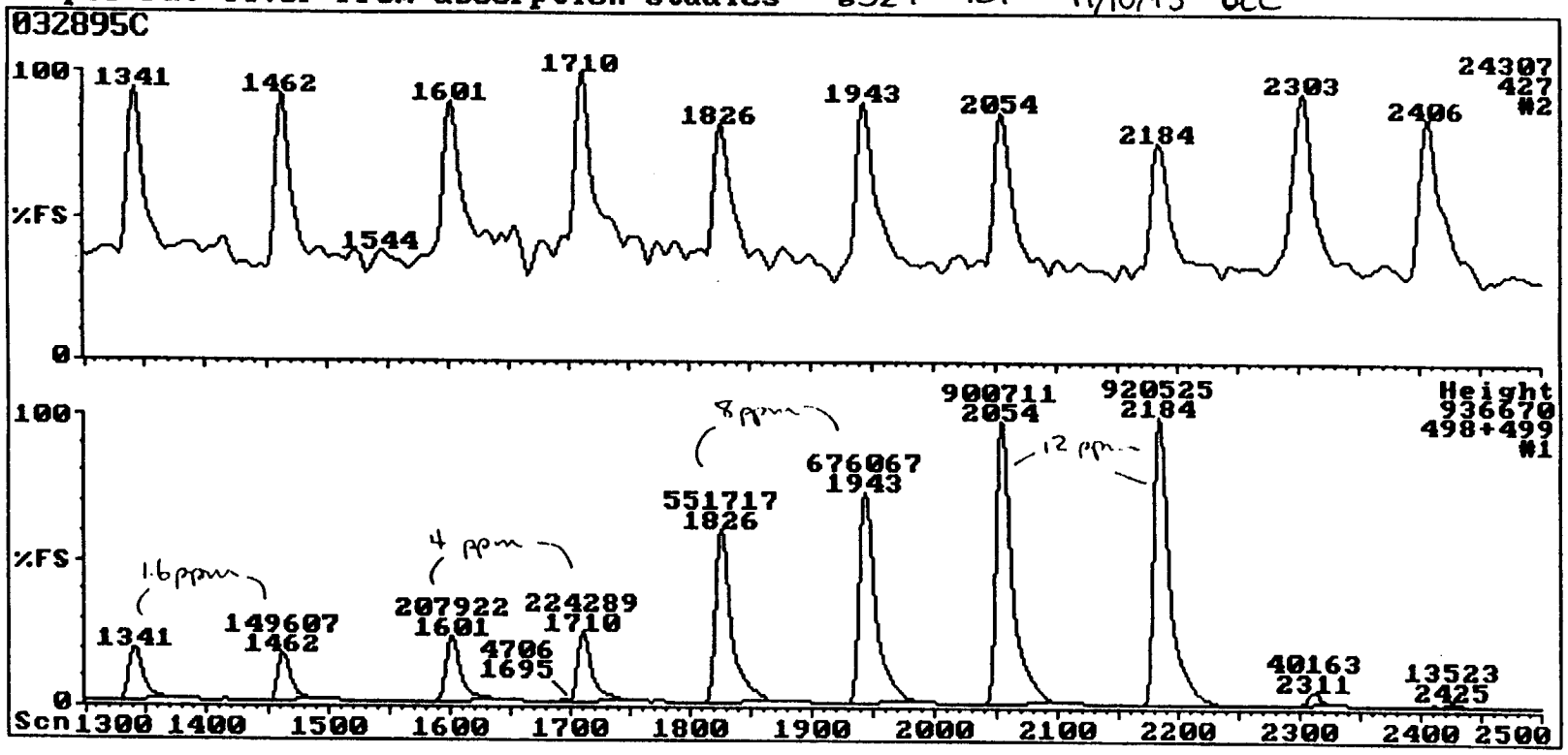


A-4

000076

File:032895C LAB-BASE - The MS Data System 28/03/1995 10:55

Sample: ~~rat liver from absorption studies~~ 6329 - 129 11/10/95 DLC

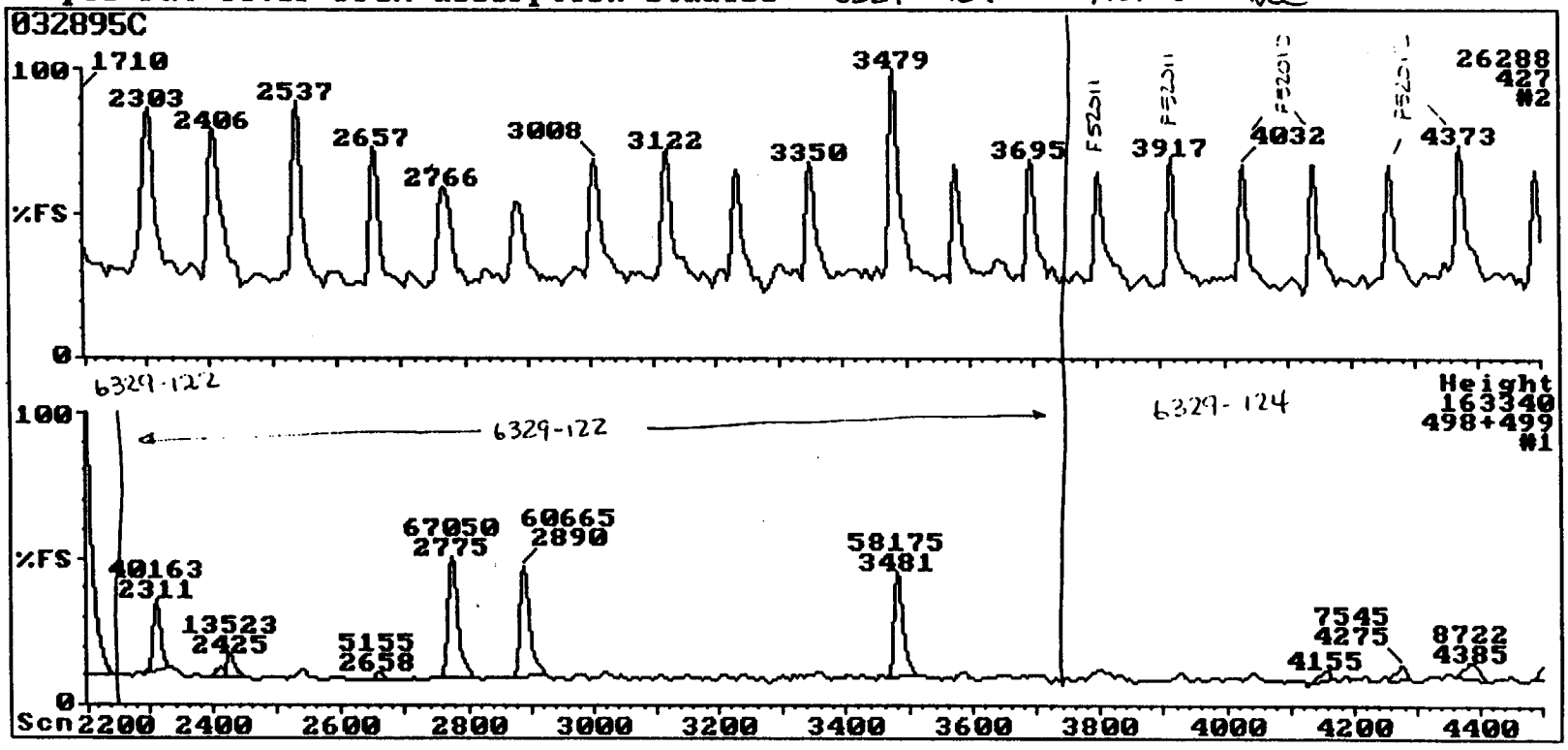


A-5

000022

File:032895C LAB-BASE - The MS Data System 28/03/1995 10:55

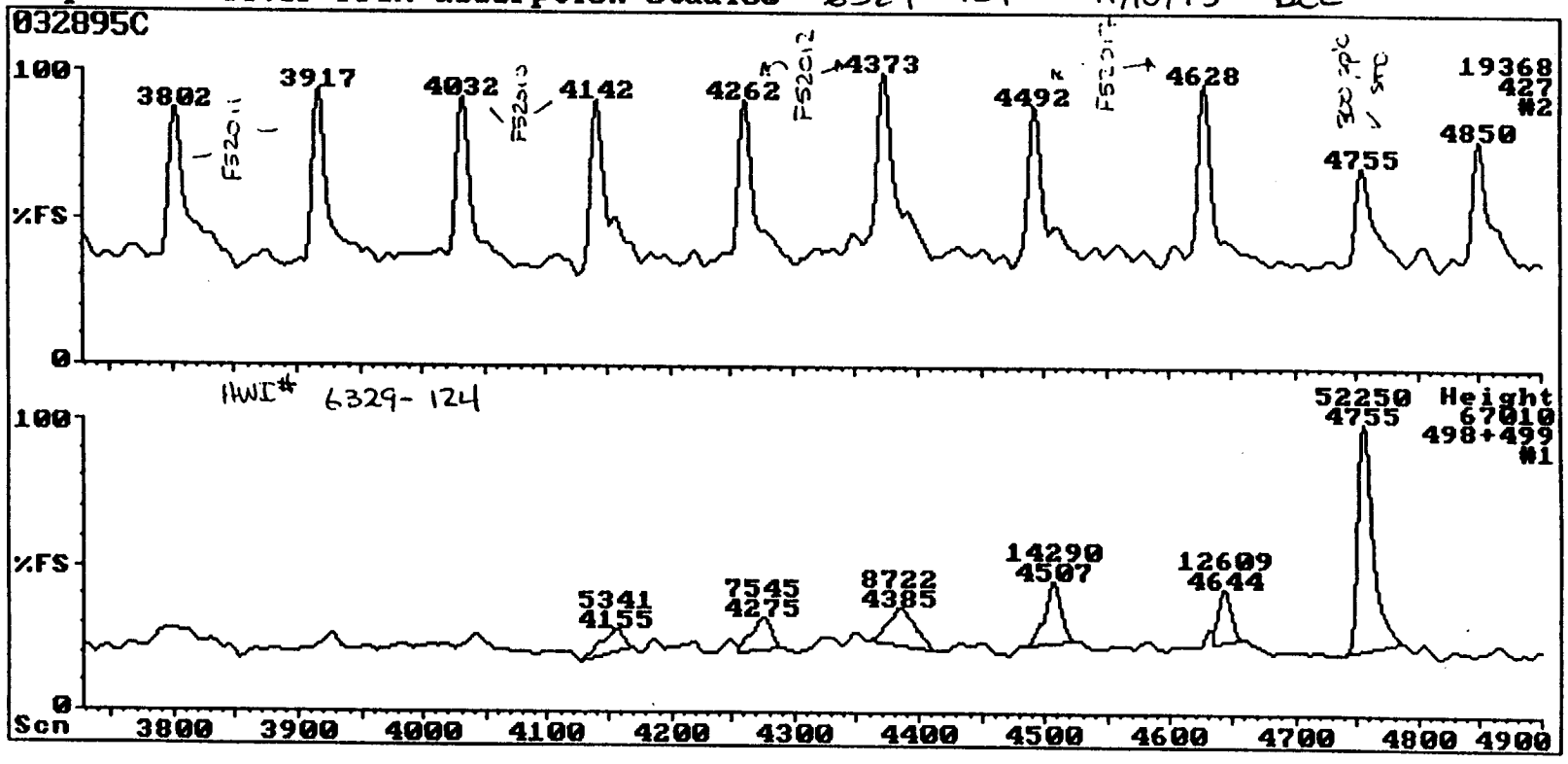
Sample: ~~rat liver from absorption studies~~ 6329 - 129 11/10/95 DLC



A-6

File:032895C LAB-BASE - The MS Data System 28/03/1995 10:55

Sample: ~~rat liver from absorption studies~~ 6329 - 129 11/10/95 DCC



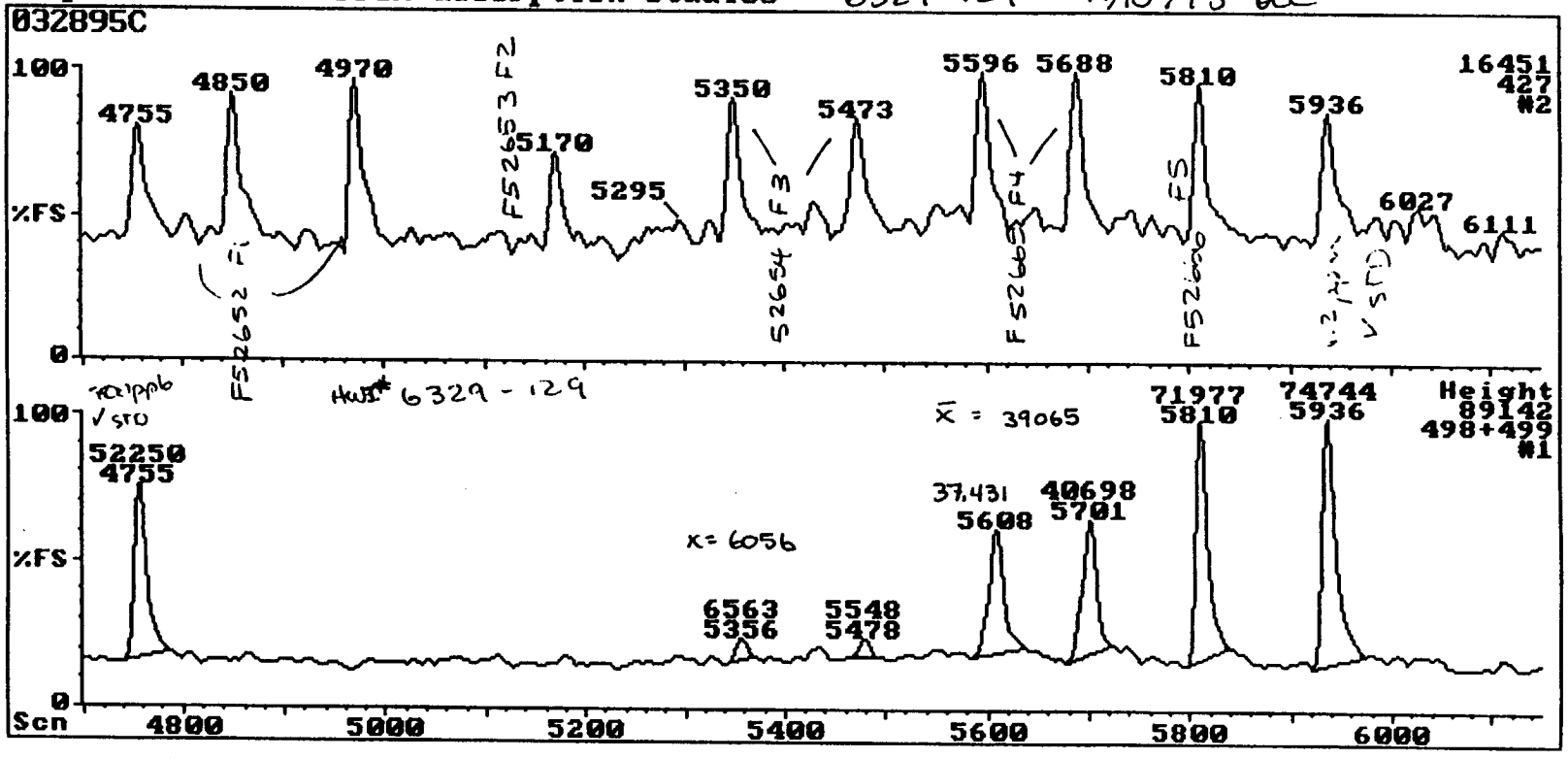
000027

A-7

000079

File:032895C LAB-BASE - The MS Data System 28/03/1995 10:55

Sample: ~~rat liver from absorption studies~~ 6329-129 11/10/95 OCC



9.11.3 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

Summary of Combustion Data - Liver
AMDT-112294.1, HWI 6329-129

Concentration of Fluoride in Serum
Mean per Dose Group per Sampling Interval*

		Average ppm F- in serum (W/W)
500 mg/kg dosed rabbit	48 HR	0.633
	24 HR	0.707
	12 HR	0.789
	8 HR	0.620
	6 HR	0.595
	4 HR	0.614
	2 HR	0.705
100 mg/kg dosed rabbit	48 HR	0.341
	24 HR	0.289
	12 HR	0.395
	8 HR	0.293
	6 HR	0.246
	4 HR	0.265
	2 HR	0.255

***2-24 hour calculated as the mean of duplicate samples, 48 hour analyzed in triplicate.**

FC95 PK ID	% rcvry	Actual ppm F- in serum (W/W)	Average ppm F- in serum (W/W)	serum burned (mL)	Dosage (mg/kg)
Serum blk 1		0.931		0.1	
Serum blk 2		0.590		0.1	
Serum blk 3		0.391		0.1	
Serum blk 4		0.321		0.1	
Serum SPK 1	69%	2.10		0.1	
Serum SPK 2	184%	5.58		0.1	
Serum SPK 3	79%	2.40		0.1	
Serum SPK 4	78%	2.36		0.1	
Serum SPK 5	103%	3.13		0.1	
Serum SPK 6	98%	2.99		0.1	
SERUM BLANK-1		0.511		0.1	
SERUM BLANK-2		0.375		0.1	
F52666-F5-48 HR - 1		0.689		0.1	
F52666-F5-48 HR - 2		0.582	0.633	0.1	500
F52666-F5-48 HR - 3		0.628		0.1	
F52666-F5-24 HR - 1		0.735		0.1	
F52666-F5-24 HR - 2		0.680	0.707	0.1	500
F52666-F5-12 HR - 1		0.835		0.1	
F52666-F5-12 HR - 2		0.743	0.789	0.1	500
F52666-F5-8 HR - 1		0.636		0.1	
F52666-F5-8HR - 2		0.605	0.620	0.1	500
F52666-F5-6HR - 1		0.617		0.1	
F52666-F5-6HR - 2		0.573	0.595	0.1	500
F52666-F5-4HR - 1		0.580		0.1	
F52666-F5-4HR - 2		0.649	0.614	0.1	500
F52666-F5-2HR - 1		0.755		0.1	
F52666-F5-2HR - 2		0.655	0.705	0.1	500
SERUM BLANK 1		0.575		0.1	
SERUM BLANK 2		0.314		0.1	
F52665-F4-48 HR-1		0.333		0.1	
F52665-F4-48 HR-2		0.350	0.341	0.1	100
F52665-F4-48 HR-3		0.339		0.1	
F52665-F4-24HR-1		0.303		0.1	
F52665-F4-24HR-2		0.276	0.289	0.1	100
F52665-F4-12HR-1		0.464		0.1	
F52665-F4-12HR-2		0.326	0.395	0.1	100
F52665-F4-8HR-1		0.288		0.1	
F52665-F4-8HR-2		0.299	0.293	0.1	100
F52665-F4-6HR-1		0.242		0.1	
F52665-F4-6HR-2		0.249	0.246	0.1	100
F52665-F4-4HR-1		0.272		0.1	
F52665-F4-4HR-2		0.257	0.265	0.1	100
F52665-F4-2HR-1		0.254		0.1	
F52665-F4-2HR-2		0.256	0.255	0.1	100

Medicine, Health Physics
Industrial Hygiene, Toxicology
3M Medical Department

3M Center, Building 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
612 733 1110

3M

Oral Developmental Toxicity (Teratology) in Rats and Rabbits

An oral teratology study in rats with potassium perfluorooctanesulfonate was conducted at Riker Laboratories (Riker Pharmaceutical Pathology / Toxicology Laboratory). Dose levels (oral) given to the pregnant rat dams were 0, 1, 5, and 10 mg/kg. Maternal toxicity (reduced weight gain) occurred at the high dose of 10 mg/kg on days 6 through 15 of gestation. Evidence of fetal toxicity was not found at any dose level. No skeletal and soft tissue teratogenic changes were found at any dose level with one exception. A change in the lens of the eye was found in all dose groups including the control but the incidence in high dose group was significantly higher. This change was reported as a developmental eye abnormality and the summary of the report states the compound was teratogenic. An outside consultant and teratology expert, Dr. E. Marshall Johnson from Jefferson Medical College, visited 3M and reviewed the rat pup eye specimens in question. He concluded that the eye/lens changes were, in fact, sectioning artifacts and not compound related teratology abnormalities (see enclosed letter). A repeat study was conducted at Hazleton Laboratories America (see below) which revealed no effects on the lens.

In the subsequent study mentioned above, potassium perfluorooctanesulfonate (suspended in corn oil) was administered on gestational days 6-15 by oral gavage to groups of 25 pregnant Sprague-Dawley CD rats at doses of 0 (control), 1, 5, and 10 mg/kg/day (Hazleton Laboratories America). Severe maternal toxicity occurred in the 5 mg/kg and 10 mg/kg dose groups, as evidenced by significant reductions in mean body weight gain, terminal body weight minus gravid uterine weight and food consumption compared to control dams, actual losses in body weight on commencement of treatment among numerous dams and death in two dams in the 10 mg/kg dose group prior to gestational day 20. Mean body weight gains (days 0-20) at 5 and 10 mg/kg were 104 ± 35 (S.D.) and 34 ± 73 (S.D.), respectively, as compared to 125 ± 24 (S.D.) in the control group. Mean food consumption values (days 0-20) at 5 and 10 mg/kg were 363 ± 60 (S.D.) and 264 ± 90 (S.D.), respectively, as compared to 421 ± 28 for the control group. Mean terminal body weight minus gravid uterine weight at 5 and 10 mg/kg was 293 ± 28 (S.D.) and 241 ± 60 (S.D.), respectively, as compared to 321 ± 23 (S.D.) in the control group. Clinical signs in surviving dams included hunching, lower body weight, alopecia, rough haircoat, anorexia. Gastrointestinal and kidney lesions were noted in the high-dose dams.
Treatment-related fetal effects that were attributed to maternal toxicity included: increased resorptions and fetal death, decreased fetal body weight, delayed skeletal

Medicine, Health Physics
Industrial Hygiene, Toxicology
3M Medical Department

3M Center, Building 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
612 733 1110

3M

ossification, cleft palate, subcutaneous edema and cryptorchism (undescended testicles). These effects occurred primarily in the high-dose group. The maternal and fetal NOAELs for this study were both 1 mg/kg/day.

An oral developmental toxicity (teratology) study in rabbits was conducted at Argus Laboratories. In this study, dose groups of 22 pregnant new zealand white rabbits were dosed on days 7 through 20 of gestation with either 0, 0.1, 1.0, 2.5, or 3.75 mg/kg/day PFOS.

Maternal toxic effects included: 1) decreased body weight at the highest three dose levels with a minimal effect at the 1.0 mg/kg dose; 2) decreased food consumption at the highest two doses; 3) frequent scant feces at the highest dose, and 4) increased abortions at the highest two doses.

Fetal toxic effects included reduced fetal weight and an increase in delayed ossification at the highest two doses. No teratogenic events were observed in the study.

Based on this study, PFOS was not teratogenic under conditions of the study and the maternal and fetal NOELs are 0.1 mg/kg/day and 1.0 mg/kg/day, respectively.

Thus, the weight of the evidence indicates that perfluorooctanesulfonate does not cause teratogenic effects in rats and rabbits when dosed at levels which are not maternally toxic. The lens change observed in rat pups in the Riker Pharmaceutical study was a sectioning artifact and was not found upon repeat studies at an independent laboratory.

HAZLETON LABORATORIES AMERICA, INC.
2300 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

RECEIVED
DEC 20 1983
TOXICOLOGY

RAT TERATOLOGY STUDY

T-3351

FINAL REPORT

Submitted to

3M Company

St. Paul, Minnesota

December 19, 1983

HAZLETON LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

OFFICE OF QUALITY ASSURANCE

Project Title: Rat Teratology Study

Project No.: 154-160

Quality Assurance inspections of the study and review of the final report of the above referenced project were conducted according to the standard operating procedures of the Office of Quality Assurance and according to the general requirements of the Good Laboratory Practice regulations that were issued on December 22, 1978, by the Food and Drug Administration for compliance on and after June 20, 1979. Findings from the inspections and final report review were reported to management and to the study director on the following dates:

Inspections/Review		Findings Reported	Inspector/Reviewer
Protocol	6/15/83	6/15/83	E. Burroughs
Study	7/6,8,11,12/83	7/15/83	E. Burroughs
Final report	12/6-9/83	12/16/83	P. Runge

Frederick G. Snyder
Director
Office of Quality Assurance

HAZLETON LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

SUBJECT: Rat Teratology Study
Project No. 154-160

We, the undersigned, hereby declare that the work was performed under our supervision, according to the procedures herein described.

Study Director:

LAWRENCE T. WETZEL, Ph.D.
Staff Scientist
Department of Toxicology

Laboratory Supervision:

GEORGE A. BURDOCK, Ph.D.
Rodent Toxicology I
Department of Toxicology

Teratology:

RUTH S. DURLOO, B.S.
Research Associate
Department of Toxicology

Report Preparation:

BARBARA R. COLPEAN, B.A., B.S.
Technical Writer
Scientific Resources Department

tls

HAZLETON LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

TABLE OF CONTENTS

	Page
SUMMARY	1
INTRODUCTION	3
CONTROL AND TEST MATERIALS	3
TEST ANIMALS	4
METHODS	5
Mating Period	5
Groups and Dosage Levels	5
Compound Preparation and Administration	6
Maternal Observations and Records	6
Cesarean Sacrifice	7
Visceral Examination of Fetuses	8
Skeletal Examination of Fetuses	8
Tissue Preservation	8
Statistical Analyses	9
Specimen, Raw Data, and Final Report Storage	11
RESULTS - MATERNAL DATA	12
Mortality and Clinical Observations	12
Body Weights and Food Consumption	12
Uterine Weights	13
Gross Pathology	13
RESULTS - CESAREAN DATA	14
Pregnancy Rates, Corpora Lutea, and Uterine Implantations	14
Fetal Viability, Weight, and Sex	14
Fetal Development	15
TABLES	
Table 1 - Summary of Maternal Clinical Observations	17
Table 2 - Mean Maternal Body Weights and Body Weight Changes	18
Table 3 - Mean Maternal Food Consumption	19
Table 4 - Mean Terminal Body Weights, Gravid Uterine Weights, and Terminal Body Weights Minus Gravid Uterine Weights	20
Table 5 - Summary of Maternal Gross Pathology Findings	21
Key to Tables 6 - 14	23
Table 6 - Summary of Mean Ovarian and Uterine Data	25

TABLE OF CONTENTS - CONTINUED

	Page
Table 7 - Summary of Mean Litter Data	26
Table 8 - Summary of External Findings	27
Table 9 - Summary of Visceral Findings	28
Table 10 - Summary of Skeletal Findings	29
Table 11 - Incidence of External Findings Per Litter	30
Table 12 - Incidence of Visceral Findings Per Litter	31
Table 13 - Incidence of Skeletal Findings Per Litter	32
Table 14 - Mean Incidence of External, Visceral, and Skeletal Findings Per Litter	33
APPENDICES	
Appendix 1 - Individual Clinical Signs	34
Key to Appendices 2, 3, and 4	38
Appendix 2 - Individual and Mean Maternal Body Weights and Body Weight Changes	39
Appendix 3 - Individual and Mean Food Consumption	43
Appendix 4 - Individual and Mean Terminal Body Weights, Gravid Uterine Weights, and Terminal Body Weights Minus Gravid uterine Weights	47
Appendix 5 - Individual and Mean Ovarian and Uterine Data	51
Appendix 6 - Individual and Mean Litter Data	55
Appendix 7 - Individual Visceral Findings in Fetuses	59
Appendix 8 - Individual Skeletal Findings in Fetuses	67
Appendix 9 - References	75

HAZLETON LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

SPONSOR: 3M Company

DATE: December 19, 1983

MATERIAL: T-3351

SUBJECT: FINAL REPORT
Rat Teratology Study
Project No. 154-160

SUMMARY

T-3351, suspended in corn oil at concentrations intended to deliver 1, 5, or 10 mg/kg/day (Groups 2, 3, and 4, respectively), was administered by oral intubation to mated female Sprague-Dawley CD® rats on presumed gestation Days 6-15. A fourth group (Group 1) of mated females served as the control group and received the vehicle only.

Two Group 4 females died. Treatment-related clinical observations noted in Groups 3 and 4 included thinness, hunched appearances, rough haircoats, alopecia, and anorexia. Food consumed by Groups 3 and 4 animals was significantly less than control both during treatment and throughout gestation. Compared to control body weight gains, significantly less body weight was gained by Groups 3 and 4 females throughout gestation. Actual loss of body weight occurred in Groups 3 and 4 animals once treatment had been initiated. Terminal body weight minus gravid uterine weight was also significantly decreased in

Groups 3 and 4. Treatment-related gross pathology in Group 4 animals included incidences of gastrointestinal tract effects.

Statistical analysis revealed a positive treatment-related trend in fetal loss, attributable primarily to an increased incidence of late resorbing fetuses and dead fetuses in litters from Group 4 dams. Mean male and female fetal body weights were significantly decreased in fetuses Groups 3 and 4 litters. Significant increases in external and visceral anomalies occurred in fetuses from Group 4 dams and included subcutaneous edema, cleft palate, and cryptorchism. Anomalies were observed primarily in fetuses from Group 4 dams exhibiting signs of severe maternal toxicity, i.e., decreased food consumption and weight loss. Fetal toxicity manifested as increased numbers of late resorbing fetuses and dead fetuses, decreased fetal body weights, and delays in skeletal system ossification occurred in litters derived from that group as well.

The specificity and frequency of these results indicate that administration of T-3351 to pregnant rats during Days 6-15 of gestation at a dose of 10 mg/kg results in maternal toxicity, fetotoxicity and an increased incidence of external and visceral anomalies and skeletal variants.

T-3351 did not appear to be teratogenic in rats at dose levels less than or equal to 5 mg/kg.

INTRODUCTION

This study was designed to evaluate the embryo/fetal toxicity and teratogenic potential of T-3351 when administered by oral gavage to pregnant rats during the period of fetal organogenesis. The rats were placed in breeding on June 27, 1983, and cesarean sections were completed on July 26, 1983. The study was conducted according to a protocol designed to satisfy existing guidelines of the Food and Drug Administration as well as Good Laboratory Practice Regulations. This report presents and discusses the methods and results from the study.

CONTROL AND TEST MATERIALS

The vehicle and control material, Duke's® Corn Oil (Lot No. 80275), a slightly viscous, yellow liquid, was received from the C. F. Sauer Co., Richmond, Virginia, and was stored at room temperature.

The test material, T-3351, a white powder, was received from the sponsor on January 5, 1983, and was stored at room temperature. The test material was assumed to be 100% active compound. Information on the methods of synthesis, stability, as well as data on composition, or other characteristics which define the test material, are on file with the sponsor.

TEST ANIMALS

Two hundred sixty (one hundred thirty/sex) sexually mature, cesarean-derived Sprague-Dawley Crl:CD● (SD)BR rats were received from Charles River Breeding Laboratories, Inc., Kingston, New York, on June 8, 1983. The rat was selected for use in this study because historically this species has been used in teratology studies and is required by appropriate regulatory agencies. The animals were housed individually in elevated stainless-steel cages and acclimated to laboratory conditions for approximately three weeks prior to initiation of the study. During this time, two male rats were euthanized due to an unthrifty appearance. Following an examination by a staff veterinarian for health status, one hundred twenty eight rats/sex were placed in breeding.

The rats were housed one male and one female per cage during breeding. Following confirmation of mating, the females were uniquely identified by ear tags and individually housed in elevated stainless-steel cages. Food (Purina Rodent Laboratory Chow● 5001) and tap water (via an automatic watering system) were available ad libitum. The mean room temperature ± the standard deviation was 72.9°F ± 1.36 and the mean relative humidity ± the standard deviation was 69.1% ± 3.16 during the study. A twelve-hour light/dark cycle was maintained.

METHODS

Mating Period

During the mating period, one female was paired with one male until mating was confirmed or until two weeks had elapsed. A sufficient number of mated females, during the course of this study, were obtained after nine days of cohabitation and mating was discontinued. Daily vaginal examinations of each female were performed to detect the presence and viability of sperm or the presence of a copulatory plug. The day of observation of sperm or copulatory plug was designated as "Day 0" of gestation.

Groups and Dosage Levels

Upon confirmation of mating, each female was assigned to one of the following groups by use of a table of random numbers (Cochran and Cox, 1957).

Group	Number of Females	Dose[a] mg/kg/day
1	25	0
2	25	1
3	25	5
4	25	10

[a] Based on individual animal body weights at each weighing interval during the dosing period. Animals were dosed on Days 6 through 15 of presumed gestation.

All males and all remaining females were sacrificed via carbon dioxide asphyxiation and discarded without necropsy once a sufficient number of matings were confirmed.

Compound Preparation and Administration

Suspensions of the test material in the corn oil vehicle were prepared on a weight per volume basis. The required amount of compound for each level was weighed on an electronic Arbor 126 balance and transferred to a 7 ml tissue grinder. Approximately 3 ml of corn oil was added and the test material was ground into a fine suspension. This suspension was transferred to a precalibrated beaker and any remaining compound in the tissue grinder was rinsed into the beaker. Additional corn oil was added to the suspension to produce the desired concentration and the suspension was mixed on a magnetic stirrer. Prepared suspensions were mixed with a magnetic stirrer during dosing.

Females were given the appropriate dosing suspension or vehicle by oral intubation on a daily basis beginning on Day 6 and continuing through Day 15 of presumed gestation. The dose administered to each female was based on the most recently recorded individual body weight. The test material was administered orally because of the relative ease and accuracy of dosing.

Fresh dosing suspensions were prepared weekly and stored under refrigeration (approximately 40°F). Samples of each test mixture as well as the vehicle were sent to the sponsor for analysis.

Maternal Observations and Records

All animals were examined twice daily for mortality and moribundity (from day of receipt through animal sacrifice) and once daily for clinical signs of toxicity and pharmacologic effects (throughout gestation). Individual body weights were recorded at initiation and on Days

6, 8, 12, 16, and 20 of presumed gestation. Food consumption during gestation was determined on Days 6, 8, 12, 16, and 20.

Cesarean Sacrifice

A gross necropsy was performed on all females found dead or terminally sacrificed. The uterus and ovaries from found dead females were removed and examined for the number of implantation sites and corpora lutea. Prior to terminal sacrifice and cesarean section on Day 20 of gestation, all surviving females were assigned random numbers so that all personnel performing cesarean sections and/or subsequent external, visceral, or skeletal examinations of the fetuses were unaware of the dose level from which the animals were derived.

On Day 20 of gestation, all surviving females were weighed and sacrificed by carbon dioxide asphyxiation. The uterus from each female was excised, weighed, and examined for the number and placement of uterine implantation sites, number of live and dead fetuses, number of early and late resorbing fetuses, and any abnormalities. The uterus was not reweighed after the contents were removed. The ovaries were examined for the number of corpora lutea. Each female was examined grossly for any abnormalities.

Each fetus was sexed, weighed, examined externally, and tagged for identification. Beginning at the ovarian end of the left uterine horn and continuing past the cervix into the right uterine horn, the first available fetus was fixed in Bouin's solution for visceral evaluation. The next two fetuses were processed for skeletal evaluation. This

process was repeated until all available fetuses were selected for evaluation. Dead fetuses were discarded without examination.

Visceral Examination of Fetuses

Approximately one-third of all live fetuses from each litter was fixed in Bouin's solution and sectioned by the Wilson Technique for assessing soft tissue development (Wilson and Warkany, 1965). During visceral examination of the fetuses, particular attention was paid to examination of the eyes, palate, and brain. Stainless-steel razor blades were used for sectioning procedures.

Skeletal Examination of Fetuses

The remaining fetuses were eviscerated and placed in 95% ethyl alcohol. After proper fixation and dehydration, the skeletons were macerated and stained in 1% KOH with an alizarin red solution, and cleared in 30% glycerol (modified Staples and Schnell, 1964). This evaluation included examination of the skull, long bones, vertebral column, rib cage, extremities, and pectoral and pelvic girdles. Bone alignment and degree of ossification were assessed.

Tissue Preservation

The following are the procedures for preservation of the various tissues: (1) The uterus and ovaries of each animal were preserved in 10% neutral buffered formalin; (2) The fetuses examined by Wilson's technique were placed in tissue capsules, preserved in 95% ethyl alcohol and sealed in plastic bags after sectioning; (3) Fetuses stained for skeletal

examination were preserved in plastic bags containing a glycerol:ethyl alcohol (1:1) solution with several crystals of thymol to retard bacterial and mold growth.

Statistical Analyses

Maternal body weight changes (Days 6-8, 6-16, 16-20, and 0-20), total food consumption (Days 7-8, 7-16, 17-20, and 0-20), gravid uterine weight, and terminal body weight minus gravid uterine weight data of the control group were compared statistically against the data of the compound-treated groups of the same sex.

Analyses of the above data were performed in the following order: Levene's test for homogeneity of variance (Levene, 1960; Draper and Hunter, 1969) was performed and if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA) (Winer, 1971). If the variance proved to be heterogeneous, a series of transformations was performed until variance homogeneity was achieved. These transformations were $\log_{10}$, square (X^2), square root ($X^{1/2}$), reciprocal (1/X), angular (arcsine $X^{1/2}$), and rank, in that order. If rank transformation was ineffective in removing variance heterogeneity, ANOVA of ranked data was completed. If ANOVA of untransformed or transformed data was significant, Dunnett's t-test (Dunnett, 1955 and 1964) was used for control vs. compound-treated group mean comparisons. If ANOVA was not significant, the analysis was complete.

Data transformations are designated throughout this report as presented on the following page.

A = Data analyzed following $\log_{10}$ transformation.

B = Data analyzed following square (X^2) transformation.

C = Data analyzed following square root ($X^{1/2}$) transformation.

D = Data analyzed following reciprocal (1/X) transformation.

E = Data analyzed following angular (arcsine $X^{1/2}$) transformation.

F = Data analyzed following rank transformation.

Mean fetal body weights per litter were statistically analyzed as follows: Bartlett's test for homogeneity of variances (Bartlett, 1937) was performed by one-way classification of covariance (ANCOVA) (Winer, 1971). If ANCOVA was significant, control vs. treatment group comparisons were analyzed using the Games and Howell modification of the Tukey-Kramer honestly significant difference test (Games and Howell, 1976).

Tests for homogeneity of variances, ANOVA, and ANCOVA were evaluated at the 5.0% one-tailed probability level. Control vs. compound-treated group mean comparisons of the above data were evaluated at the 5.0% two-tailed probability level.

In addition to the above data, percent fetal viability, percent fetal loss (dead and resorbing fetuses), percent early, late, and total resorptions, and the number of dead fetuses were analyzed by nonparametric one-way ANOVA (Kruskal and Wallis, 1952, 1953; Miller, 1966) and the Terpstra-Jonckheere test for trend (Terpstra, 1952; Jonckheere, 1954). The litter was used as the experimental unit. The teratology data listed on the next page were analyzed using the

Cochran-Armitage test for linear trend in proportions (Cochran, 1954; Armitage, 1955; Fleiss, 1981) and Fisher's "exact" test (Fisher, 1950).

Visceral Anomalies	Skeletal Variants
Subcutaneous edema	Skull
Cleft palate	Pectoral girdle
Cryptorchism	Rib cage
Number of litters affected	Vertebral column
Number of fetuses affected	Pelvic girdle
	Limbs
	Bone alignment
	Number of litters affected
	Number of fetuses affected

If a significant trend was noted, the results of Fisher's "exact" test were evaluated at the one-tailed, 5% level. If a significant trend was not observed, or if there was a significant trend with severe departure from it, the results of Fisher's "exact" test were evaluated at the two-tailed, 5% level. When using these statistical tests on indices, the actual proportions prior to conversion to percentages were the data that were analyzed.

Statistical references are appended to this report, and statistically significant differences, as indicated by the aforementioned tests, are designated throughout this report by the term "significant" and/or as follows:

S+ = Significantly higher than the control value.

S- = Significantly lower than the control value.

Specimen, Raw Data, and Final Report Storage

All specimens, raw data, and the final report are stored in the archives of Hazleton Laboratories America, Inc.

RESULTS - MATERNAL DATA

Mortality and Clinical Observations

Individual clinical observations are presented in Appendix 1. A summary of clinical observations is presented in Table 1.

Two Group 4 females were found dead on Day 17 prior to cesarean section on Day 20 of gestation. No other animals were found dead or sacrificed *in extremis* during the study.

Treatment-related clinical observations were noted in several of the Groups 3 and 4 animals during the treatment and posttreatment phases. These observations included hunched and thin appearances, anorexia, bloody discharge from vagina, urine stains, alopecia, rough haircoat and bloody crust.

Body Weights and Food Consumption

Individual and mean body weights and body weight changes are presented in Appendix 2 and Table 2, respectively. Individual and mean food consumption values are presented in Appendix 3 and Table 3, respectively.

Statistical analysis revealed a significantly lower than control mean body weight gain in the Groups 3 and 4 females for Days 6-8, 6-16, and 0-20 of presumed gestation. These findings were considered to be treatment related since, compared to the treatment phase, there was a proportionally greater than control increase in mean body weight gain in the treated females during the posttreatment phase (Days 16-20).

Mean total food consumption was significantly lower in the Group 4 females during Days 17-20. Significant decreases in mean food consumption were also noted in the Groups 3 and 4 females during Days 7-16 and 0-20 of presumed gestation when compared to control, concomitant with the decrease in body weight gain during the same intervals.

Uterine Weights

Individual and mean terminal body weights, gravid uterine weights, and terminal body weights minus gravid uterine weights are presented in Appendix 4 and Table 4, respectively.

The mean gravid uterine weight of the Group 4 females was significantly lower than control. The mean terminal body weights minus the gravid uterine weights were lower in the treated groups when compared to the control group, with significant decreases noted in the Groups 3 and 4 females.

Gross Pathology

A summary of maternal gross pathology findings is presented in Table 5.

An increased incidence of stomach lesions was noted in the Group 4 animals. Remaining gross pathology findings were considered incidental.

RESULTS - CESAREAN DATA

Individual and mean ovarian and uterine data are presented in Appendix 5. Individual and mean litter data are presented in Appendix 6. Individual visceral and skeletal findings in fetuses are presented in Appendices 7 and 8, respectively. Summary ovarian and uterine data are presented in Table 6. Summary litter data are presented in Table 7. Summaries of the external, visceral, and skeletal findings are presented in Tables 8 through 14.

Pregnancy Rates, Corpora Lutea, and Uterine Implantations

Pregnancy rates, number of corpora lutea, and implantation efficiencies were comparable for all groups.

Fetal Viability, Weight, and Sex

The mean incidence of early resorptions was comparable between the control and treated groups. Nonparametric analyses of the mean incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss did not indicate statistical significance. However, there was a significant linear trend towards an increased incidence in these data with respect to control. The primary trend component was contributed by the high-dose group. The mean fetal weights of the Groups 3 and 4 males and females were significantly lower than their respective controls. The percent of male fetuses was 52.29%, 54.24%, and 60.38% for Groups 2, 3, and 4, respectively, and 44.01% for the control group.

Fetal Development

Statistically significant findings in the teratology data are presented in the following chart:

Statistically Significant Teratology Data

Teratology Observation	Group: 2	3	4	Trend	Departure from Trend
Visceral anomalies					
Subcutaneous edema			S+	S	S
Cleft palate			S+	S	S
Cryptorchism			S+	S	NS
Number of litters affected			S+	S	NS
Number of fetuses affected			S+	S	S
Skeletal variants					
Skull	S+		S+	S	S
Pectoral girdle			S+	S	S
Rib cage		S+	S+	S	S
Vertebral column			S+	S	S
Pelvic girdle	S-		S+	S	S
Limbs			S+	S	S
Bone alignment			S+	S	NS
Number of litters affected				S	NS
Number of fetuses affected			S+	S	S

S = Significant
NS = Not significant

Significant treatment-related increases in the incidences of visceral anomalies and skeletal variants were noted in the Group 4 fetuses.

The number of Group 4 litters containing anomalous fetuses was also significantly increased compared to control. External and visceral anomalies observed included cleft palate, subcutaneous edema, and cryptorchism. Those anomalies occurred primarily in fetuses derived from litters whose mean male or female pup body weights were significantly below those of the corresponding control litters. Dams producing affected litters had significantly decreased food consumption throughout treatment and decreased body weight gain or actual body weight loss during gestation. Numerous clinical signs of toxicity were also present in these animals.

Table 1
Summary of Maternal Clinical Observations[a]
Rat Teratology Study of T-3351

	Pretreatment Days 0-5				Treatment Days 6-15				Posttreatment Days 16-20			
Group:	1	2	3	4	1	2	3	4	1	2	3	4
Observation / Dose Level (mg/kg):	0	1	5	10	0	1	5	10	0	1	5	10
Number of females examined	25	25	25	25	25	25	25	25	25	25	25	25
Number of females appearing normal	25	25	25	25	21	21	16	11	21	20	17	5
Number of females found dead or sacrificed prior to Day 20	0	0	0	0	0	0	0	0	0	0	0	2
Hunched							2	7			2	16
Thin						1	1	7			2	15
Anorexia								1			1	11
Ataxia												1
Sensitive to touch								1				1
Soft feces								1				
Bloody discharge from vagina												3
Urine stains								1	1		1	5
Alopecia					3	3	5	9	3	5	4	12
Rough haircoat							6	6	1		2	13
Bloody crust									1		1	6
Small eye							1				1	
Swollen neck					1							

[a]Numerals indicate the number of animals with that particular finding.

Table 2
Mean Maternal Body Weights and Body Weight Changes
Rat Teratology Study of T-3351

Body Weight	Day(s)		Group 1 0 mg/kg	Group 2 1 mg/kg	Group 3 5 mg/kg	Group 4 10 mg/kg
Mean Weight (g)	0		270.08	271.03	263.25	269.30
		N:	24	23	23	22
	6		298.47	296.05	292.80	297.72
		N:	24	23	23	22
	8		301.39	298.04	288.22	293.63
		N:	24	23	23	22
	12		318.31	308.18	290.09	276.25
		N:	24	23	23	22
	16		338.53	329.20	305.34	265.12
		N:	24	23	23	22
	20		395.41	389.69	367.11	301.91
		N:	24	23	23	22
Mean Change (g)	0-6		28.38	25.03	29.54	28.43
	6-8		2.93	1.99	-4.58^{S-}	-4.05^{S-}
	6-12		19.84	12.13	-2.70	-21.48
	6-16^{F}		40.06	33.15	12.54^{S-}	-32.60^{S-}
	6-20		96.95	93.64	74.32	5.40
	16-20^{F}		56.88	60.45	61.77	36.63
	0-20^{F}		125.33	118.67	103.86^{S-}	33.45^{S-}

N: Indicates the sample size on which the mean calculations were based. Only pregnant females were used in mean calculations.

Table 3
Mean Maternal Food Consumption(g)
Rat Teratology Study of T-3351

Interval Days		Group 1 0 mg/kg	Group 2 1 mg/kg	Group 3 5 mg/kg	Group 4 10 mg/kg
0-6		132.67	128.01	134.92	133.33
	N:	24	23	23	22
7-8		37.48	35.03	31.53	37.64
	N:	24	23	22	22
9-12		74.58	68.38	59.58	36.86
	N:	24	23	20	18
13-16		78.52	68.45	57.89	22.75
	N:	24	23	20	19
17-20[F]		97.25	97.17	87.87	39.33[S-]
	N:	24	23	19	14
7-16[B]		190.58	171.85	147.91[S-]	97.63[S-]
	N:	24	23	19	17
0-20[B]		420.49	397.03	363.14[S-]	262.89[S-]
	N:	24	23	18	13

N: Indicates the sample size on which the mean calculations were based. Only pregnant females were used in mean calculations. Invalid food consumption values were not included in mean calculations.

Table 4
Mean Terminal Body Weights, Gravid Uterine Weights, and Terminal Body Weights
Minus Gravid Uterine Weights
Rat Teratology Study of T-3351

Group and Dose Level		Terminal Body Weight	Gravid Uterine Weight[B]	Terminal Body Weight Minus Gravid Uterine Weight[F]
1		395.41	74.35	321.07
0 mg/kg	N:	24	24	24
2		389.69	75.59	314.10
1 mg/kg	N:	23	23	23
3		367.11	73.46	293.38[S-]
5 mg/kg	N:	23	22[a]	22[a]
4		301.91	60.51[S-]	241.40[S-]
10 mg/kg	N:	20	20	20

[a]The gravid uterine weight of one Group 3 female was inadvertently not recorded.
N: Indicates the sample size on which the mean calculations were based. Only pregnant females were used in mean calculations.

Table 5
Summary of Maternal Gross Pathology Findings
Rat Teratology Study of T-3351

Organ and Description	Group: 1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Number of animals examined	25	25	25	23 (2)
Number with no gross pathology	22	24	19	16 (0)
THYMUS				
Firm	1			
Enlarged	1			
LUNG				
Focal areas - dark red				1
HEART				
Right atrium enlarged				1
SPLEEN				
Enlarged	1			
Cystic				1
KIDNEY(S)				
Enlarged	1			
Irregularly shaped	1			
Pale	1		2	
Pelvis dilated		1	5	2 (1)
Constricted across midline			1	
Mottled tan and dark red			1	
Pelvis had a firm, white calculus		1		
ABDOMEN				
Tissue mass	1			

( ) = Findings in animals found dead during the study.

Table 5 - Continued
Summary of Maternal Gross Pathology Findings
Rat Teratology Study of T-3351

Organ and Description	Group: 1	2	3	4
	Dose Level (mg/kg): 0	1	5	10
STOMACH				
Glandular mucosa - pitted areas				1
- reddened				(1)
- depressed area - red				(1)
Nonglandular mucosa - raised area - dark (thin section in wall)				1
- pinpoint, depressed areas				1
- walls thin				(1)
- thickened area - red				(1)
- dark foci				1
Distended with dark red material				(1)
Contained yellow mucous				1
Walls thickened				1
Walls thin				(1)
PEYER'S PATCHES				
Enlarged/prominent	1			1
MANDIBULAR LYMPH NODES				
Reddened				1
ALL LYMPH NODES				
Enlarged	1			
URINARY BLADDER				
Walls thickened				1
Contained thick white fluid				1
OVARY(IES)				
Clear fluid-filled cyst	1			

( ) = Findings in animals found dead during the study.

Key to Tables 6-14

Pregnancy rate (percent) - (number of pregnant rats/number of rats mated) x 100.

Survival Rate (percent) - (number of rats surviving to Day 20/number of rats placed on study at initiation) x 100.

Mean Implantation Efficiency (percent) - Group mean of ([implantations per litter/corpora lutea per litter] x 100).

Mean Incidence of Resorptions (percent)[a] - Group mean of ([resorptions per litter/implantations per litter] x 100).

Mean Incidence of Fetal Mortality (percent) - Group mean of ([dead and resorbing fetuses per litter/implantations per litter] x 100).

Mean Incidence of Fetal Viability (percent) - Group mean of ([live fetuses per litter/implantations per litter] x 100).

Mean Incidence of External Variants (percent) - Group mean of ([number of fetuses per litter with external variants/number of fetuses per litter examined externally] x 100).

Mean Incidence of External Anomalies (percent) - Group mean of ([number of fetuses per litter with external anomalies/number of fetuses per litter examined externally] x 100).

Mean Incidence of Visceral Variants (percent) - Group mean of ([number of fetuses per litter with visceral variants/number of fetuses per litter examined viscerally] x 100).

[a]Values calculated separately for early, late, and total resorptions.

Key to Tables 6-14 - Continued

Mean Incidence of Visceral Anomalies (percent) - Group mean of ([number of fetuses per litter with visceral anomalies/number of fetuses per litter examined viscerally] x 100).

Mean Incidence of Skeletal Variants (percent) - Group mean of ([number of fetuses per litter with skeletal variants/number of fetuses per litter examined skeletally] x 100).

Mean Incidence of Skeletal Anomalies (percent) - Group mean of ([number of fetuses per litter with skeletal anomalies/number of fetuses per litter examined skeletally] x 100).

Table 6
Summary of Mean Ovarian and Uterine Data
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Number of females with mating confirmed	25	25	25	25
Number of pregnant females	24	23	23	22
Pregnancy rate (%)	96	92	92	88
Number of females surviving to Day 20	25	25	25	23
Survival rate (%)	100	100	100	92
Mean number of:				
Corpora lutea	16.6	15.9	15.8	16.6
Implantations	14.5	14.4	14.6	14.4
Early resorptions	1.0	0.8	0.7	1.6
Late resorptions	0.0	0.0	0.0[a]	0.9
Total resorptions	1.0	0.8	0.7	2.4
Indices calculated on a per litter basis:				
Mean implantation efficiency (%)	87.90	91.14	92.70	87.72
Mean incidence of early resorptions (%)	7.00	5.53	4.70	11.32
Mean incidence of late resorptions (%)	0.00	0.00	0.29	5.10
Mean incidence of total resorptions (%)	7.00	5.53	5.00	16.42

[a]One late resorption was noted for female No. 27086, therefore, the actual mean was 0.04.

Table 7
Summary of Mean Litter Data[a]
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Mean number of:				
Fetuses - dead	0.0	0.0	0.0[b]	0.5
- live	13.5	13.6	13.8	11.6
Indices calculated on a per litter basis:				
Mean incidence of fetal mortality (%)[c]	7.00	5.53	5.28	19.43
Mean incidence of fetal viability (%)	93.00	94.47	94.72	80.59
Mean percent males (%)	44.01	52.29	54.24	60.38
Live fetuses				
Mean body weight (g)[d]				
Males - unadjusted mean	3.28	3.23	3.10	2.64
- covariance adjusted mean	3.28	3.23	3.10S-	2.63S-
Females - unadjusted mean	3.17	3.10	2.97	2.57
- covariance adjusted mean	3.16	3.10	2.97S-	2.59S-

[a]Sex, percent males, and mean body weight data include values for live fetuses only.
[b]One dead fetus was noted for female No. 27101, therefore, the actual mean was 0.04.
[c]Percent mortality includes resorptions as well as dead fetuses.
[d]Statistical analysis performed on covariance adjusted means.

Table 8
Summary of External Findings
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Number of fetuses examined	325	313	317	231
Number of fetuses within normal limits	325	313	317	196
Number of fetuses with variants	0	0	0	0
Number of fetuses with anomalies	0	0	0	35
Number and type of anomalies	0	0	0	45
Skin - edematous				32
Palate - cleft				13

Table 9
Summary of Visceral Findings
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Number of fetuses examined	115	113	115	81
Number of fetuses within normal limits	111	105	108	56
Number of fetuses with variants	4	8	5	1
Number of fetuses with anomalies	0	1	2	24
Number and type of variants				
Brain - cerebral hemisphere contained dark area	4	10	5	1
Eye(s) - contained dark material	1			
Liver - dark areas				1
Kidney(s) - pelvis dilated			1	
Ureter(s) - distended		4	2	
- undulated	3	5	2	
		1		
Number and type of anomalies				
Skin - edematous	0	1	3	44
Palate - cleft		1	1	21[a]
Cryptorchism				15[b]
			2	8

[a]Twelve fetuses with edematous skin seen externally were confirmed at visceral examination.
[b]Four fetuses with cleft palate seen externally were confirmed at visceral examination.

Table 10
Summary of Skeletal Findings
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Number of fetuses examined	210	200	202	150
Number of fetuses within normal limits	134	113	135	63
Number of fetuses with variants	76	87	67	87
Number of fetuses with anomalies	0	0	0	0
Number and type of variants	150	193	147	349
Lagging ossification				
Skull - incomplete closure (<75%)	6	15	17	46
- interparietal ossification incomplete/nonossified	30	40	15	29
- parietals ossification irregular	1	14	1	
- supraoccipital ossification incomplete/nonossified	23	31	23	36
- supraoccipital nonfused	1			17
- hyoid ossification incomplete/nonossified	22	46	13	8
Pectoral girdle - scapula ossification incomplete				6
Rib cage - sternebrae bipartite		1	14	62
- sternebrae 1 and 2 fused	1			
- sternebrae <3 ossified	5	1	4	9
- ribs <13 ossified[a]		1		
- ribs >13 ossified (cervical region)			2	
- ribs 13th pair small[a]	4			
- ribs ossification irregular	1	2	1	
Vertebral column - sacral arches nonossified	2		2	13
- thoracic centra ossification incomplete/nonossified	18	20	18	15
- thoracic centra nonfused	10	12	5	6
- caudals <3 ossified	13	5	14	34
Pelvic girdle - pubis ossification incomplete/nonossified	8		4	15
- ischium nonossified	1		2	2
Forelimbs - <3 ossified	1		2	5
Hindlimbs - <4 ossified	2	1	6	36
Bone Alignment				
Ribs - angulated	1	4	4	9
Sternebrae - checkered				1

[a]Possible preparation damage at time of evisceration.

Table 11
Incidence of External Findings Per Litter
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Number of litters examined	24	23	23	18
Number of litters within normal limits	24	23	23	12
Number and percent of litters with variants	0/0.00	0/0.00	0/0.00	0/0.00
Number and percent of litters with anomalies	0/0.00	0/0.00	0/0.00	6/33.33
Number and percent of litters with anomalies				
Skin - edematous				4/22.22
Palate - cleft				6/33.33

Table 12
Incidence of Visceral Findings Per Litter
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Number of litters examined	24	23	23	18
Number of litters within normal limits	20	16	18	9
Number and percent of litters with variants	4/16.67	7/30.43	4/17.39	1/5.56
Number and percent of litters with anomalies	0/0.00	1/4.35	1/4.35	8/44.44
Number and percent with variants				
Brain - cerebral hemisphere contained dark area	1/4.17			
Eye(s) - contained dark material				1/5.56
Liver - dark areas			1/4.35	
Kidney(s) - pelvis dilated		4/17.39	2/8.70	
Ureter(s) - distended	3/12.50	5/21.74	2/8.70	
- undulated		1/4.35		
Number and percent with anomalies				
Skin - edematous		1/4.35	1/4.35	5/27.78
Palate - cleft				7/38.89
Cryptorchism			1/4.35	4/22.22

Table 13
Incidence of Skeletal Findings Per Litter
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
Number of litters examined	24	23	23	18
Number of litters within normal limits	1	0	4	3
Number and percent of litters with variants	23/95.83	23/100.00	19/82.61	15/83.33
Number and percent of litters with anomalies	0/0.00	0/0.00	0/0.00	0/0.00
Number and percent with variants				
Lagging ossification				
Skull - incomplete closure (<75%)	4/16.67	5/21.74	6/26.09	9/50.00
- interparietal ossification incomplete	15/62.50	11/47.83	8/34.78	9/50.00
- parietals ossification irregular	1/4.17	4/17.39	1/4.35	
- supraoccipital ossification incomplete	12/50.00	12/52.17	10/43.48	11/61.11
- supraoccipital nonfused	1/4.17			4/22.22
- hyoid ossification incomplete/nonossified	10/41.67	16/69.57	9/39.13	6/33.33
Pectoral girdle - scapula ossification incomplete				2/11.11
Rib cage - sternebrae bipartite		1/4.35	4/17.39	13/72.22
- sternebrae 1 and 2 fused	1/4.17			
- sternebrae <3 ossified	3/12.50	1/4.35	2/8.70	3/16.67
- ribs <13 ossified[a]		1/4.35		
- ribs >13 ossified (cervical region)			1/4.35	
- ribs 13th pair small[a]	1/4.17			
- ribs ossification irregular	1/4.17	1/4.35	1/4.35	
Vertebral column - sacral arches nonossified	2/8.33		1/4.35	6/33.33
- thoracic centra ossification incomplete/ nonossified	9/37.50	16/69.57	11/47.83	9/50.00
- thoracic centra nonfused	6/25.00	7/30.43	2/8.70	4/22.22
- caudals <3 ossified	6/25.00	4/17.39	5/21.74	7/38.89
Pelvic girdle - pubis ossification incomplete/nonossified	4/16.67		1/4.35	4/22.22
- ischium nonossified	1/4.17		1/4.35	1/5.56
Forelimbs - <3 ossified	1/4.17		1/4.35	3/16.67
Hindlimbs - <4 ossified	2/8.33	1/4.35	2/8.70	7/38.89
Bone alignment				
Ribs - angulated	1/4.17	2/8.70	2/8.70	4/22.22
Sternebrae - checkered				1/5.56

[a] [illegible] preparation damage at time of evisceration

Table 14
Mean Incidence of External, Visceral, and Skeletal Findings Per Litter
Rat Teratology Study of T-3351

Group:	1	2	3	4
Dose Level (mg/kg):	0	1	5	10
External				
Mean values calculated on a per litter basis:				
Number with variants	0.0	0.0	0.0	0.0
Number with anomalies	0.0	0.0	0.0	1.9
Incidence of variants (%)	0.0	0.0	0.0	0.0
Incidence of anomalies (%)	0.0	0.0	0.0	18.9
Visceral				
Mean values calculated on a per litter basis:				
Number with variants	0.2	0.3	0.2	0.1
Number with anomalies	0.0	0.0	0.1	1.3
Incidence of variants (%)	4.0	7.0	4.3	1.1
Incidence of anomalies (%)	0.0	0.7	1.5	31.7
Skeletal				
Mean values calculated on a per litter basis:				
Number with variants	3.2	3.8	2.9	4.8
Number with anomalies	0.0	0.0	0.0	0.0
Incidence of variants (%)	36.3	44.6	32.1	60.2
Incidence of anomalies (%)	0.0	0.0	0.0	0.0

Appendix 1
Individual Clinical Signs[a]
Rat Teratology Study of T-3351

Maternal Number	Group	Observation	Body Area	Day(s)
27032	1	Alopecia	Paw-fore-right	15-17
27034	1	Urine stains		18-20
		Bloody crust	Nose	20
		Swollen	Neck	9-20
27042	1	Alopecia	Paws-fore-both	8-20
27046	1	Alopecia	Paws-fore-both	10-11
27049	1	Alopecia	Abdomen	18-20
		Alopecia	Paw-fore-right	19-20
27057	2	Alopecia	Paw-fore-left	15
		Alopecia	Paw-fore-both	16-20
27059	2	Alopecia	Hip-right	10-14
		Alopecia	Leg-hind-right	15-20
		Alopecia	Side-right	10-20
27062	2	Thin		6
27063	2	Alopecia	Paw-fore-right	17-20
27065	2	Alopecia	Paw-fore-right	17-20
27066	2	Alopecia	Abdomen	15-20
27082	3	Alopecia	Hips-both	15-20
27083	3	Alopecia	Hip-right	14-20
27084	3	Hunched		15-19
		Thin		10, 15-19
		Alopecia	Leg-fore-right	10-14
		Alopecia	Leg-hind-right	10-12,14
		Rough haircoat		10-12
27085	3	Rough haircoat		7-12
27090	3	Rough haircoat		9-11
27091	3	Alopecia	Paws-fore-both	14-20
		Rough haircoat		8-11,13
27095	3	Hunched		8-9,11
		Rough haircoat		10-12,19-20
27099	3	Alopecia	Hips-both	13-20
		Alopecia	Legs-hind-both	16-20
27100	3	Hunched		16-17,20
		Thin		16,20
		Anorexia		16,20
		Urine stains		18-20
		Rough haircoat		16,20
		Bloody crust	Eyelids-both	20
		Bloody crust	Mouth	20
		Bloody crust	Nose	20
		Bloody crust	Paws-fore-both	20
27101	3	Rough haircoat		8-9
		Small	Eye-left	15-20

[a]Animals not included in this table appeared normal throughout the study.

Appendix 1 - Continued
Individual Clinical Signs[a]
Rat Teratology Study of T-3351

Maternal Number	Group	Observation	Body Area	Day(s)
27107	4	Hunched		18-20
		Thin		13-17
		Rough haircoat		13-20
27109	4	Hunched		9-12,14-20
		Thin		10-20
		Anorexia		16,18-20
		Sensitive to touch		10-14,16-18,20
		Alopecia	Hips-both	11-20
		Alopecia	Hip-left	10
		Rough haircoat		13-20
		Bloody crust	Mouth	20
		Bloody crust	Nose	20
27111	4	Alopecia	Abdomen	10-20
		Alopecia	Hips-both	10-20
27112	4	Hunched		18-20
		Thin		15-20
		Anorexia		17-20
		Urine stains		19-20
		Rough haircoat		16-20
		Bloody crust	Mouth	20
		Bloody crust	Nose	20
27113	4	Hunched		20
		Thin		12,20
		Anorexia		12,17-18,20
		Urine stains		14-18,20
		Alopecia	Abdomen	11-19
		Alopecia	Hips-both	9-18
		Alopecia	Hip-left	8,19-20
		Alopecia	Neck	8-18
		Rough haircoat		15-20
		Bloody crust	Mouth	16-20
		Bloody crust	Nose	16-20
		Bloody crust	Paws-fore-both	16-20
		Bloody crust	Vagina	18-19
27114	4	Hunched		13-20
		Thin		12,20
		Anorexia		17-20
		Rough haircoat		18-19
27115	4	Alopecia	Sides-both	8-13
		Alopecia	Side-right	14-20
27116	4	Alopecia	Paw-fore-left	19-20
27117	4	Hunched		14-20
		Thin		17,19-20
		Anorexia		16-20

[a]Animals not included in this table appeared normal throughout the study.

Appendix 1 - Continued
Individual Clinical Signs[a]
Rat Teratology Study of T-3351

Maternal Number	Group	Observation	Body Area	Day(s)
27117	4	Urine stains		16-20
		Rough haircoat		9-20
		Bloody crust	Genitals	20
		Bloody crust	Mouth	16-20
		Bloody crust	Nose	18-20
		Bloody crust	Paws-fore-both	16-20
27118	4	Hunched		16-20
		Thin		16-17,19-20
		Anorexia		19
		Rough haircoat		16-18
27119	4	Hunched		12-20
		Thin		16-20
		Anorexia		16-18,20
		Ataxia		18-20
		Soft feces		8-10
		Bloody discharge	Vagina	19-20
		Urine stains		18-20
		Alopecia	Hip-left	11
		Alopecia	Hip-right	12-20
		Alopecia	Inguinal-right	13-18
		Alopecia	Side-right	15-20
		Rough haircoat		20
27120	4	Hunched		16
		Thin		16
		Anorexia		16
		Bloody discharge	Vagina	16
		Rough haircoat		16
		Found dead		17
27121	4	Hunched		7-9,11-17
		Thin		7-16
		Rough haircoat		7-11
27122	4	Hunched		16-17
27123	4	Alopecia	Abdomen	10-20
		Alopecia	Hip-right	12-20
		Alopecia	Leg-hind-right	20
		Alopecia	Side-right	18-20
27125	4	Hunched		16-20
		Thin		16,18-20
		Anorexia		16-17,20
		Alopecia	Abdomen	15-20
		Rough haircoat		8-9
		Bloody crust	Paws-fore-both	19-20

[a]Animals not included in this table appeared normal throughout the study.

Appendix 1 - Continued
Individual Clinical Signs[a]
Rat Teratology Study of T-3351

Maternal Number	Group	Observation	Body Area	Day(s)
27126	4	Hunched		18,20
		Thin		19
		Anorexia		16-17,20
		Alopecia	Hip-left	17-20
27127	4	Hunched		16
		Thin		16
		Bloody discharge	Vagina	16
		Rough haircoat		16
		Found dead		17
27128	4	Hunched		14-20
		Thin		14-20
		Anorexia		17-18,20
		Alopecia	Paw-fore-right	15-17
		Rough haircoat		18-20
27129	4	Hunched		14-20
		Thin		16-20
		Anorexia		16
		Urine stains		19
		Rough haircoat		16-20
		Bloody crust	Paws-fore-both	16

[a]Animals not included in this table appeared normal throughout the study.

Key to Appendices 2, 3, and 4

P = Pregnant

NP = Not Pregnant

C = Cesarean Section Performed (preceded by day of gestation)

D = Death (preceded by day of gestation)

MS = Moribund Sacrifice (preceded by day of gestation)

ND = Natural Delivery (preceded by day of gestation)

() = Data excluded from mean calculation.

Explanation of Footnotes

[a]Only data from pregnant animals surviving to Day 20 cesarean section were used in mean calculations.

[b]Invalid food consumption value not included in mean calculations.

[c]Gavid uterine weight inadvertently not recorded.

Appendix 2
Individual and Mean Maternal Body Weights and Body Weight Changes[a]
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number	Disposition	Body Weight (g) Day of Gestation 0	6	8	12	16	20	Body Weight Change (g) Days 0-6	6-8	6-12	6-16[F]	6-20	16-20[F]	0-20[F]
27030	P, 20-C	277.3	303.8	306.8	340.3	358.6	422.5	26.5	3.0	36.5	54.8	118.7	63.9	145.2
27031	P, 20-C	261.9	288.8	284.4	314.1	329.2	388.2	26.9	-4.4	25.3	40.4	99.4	59.0	126.3
27032	P, 20-C	245.7	282.0	292.1	317.5	338.6	404.3	36.3	10.1	35.5	56.6	122.3	65.7	158.6
27033	P, 20-C	256.5	274.3	275.5	299.9	314.4	367.8	17.8	1.2	25.6	40.1	93.5	53.4	111.3
27034	P, 20-C	250.1	263.4	259.0	281.1	300.9	341.4	13.3	-4.4	17.7	37.5	78.0	40.5	91.3
27035	P, 20-C	312.7	351.5	351.0	362.4	353.6	446.6	38.8	-0.5	10.9	2.1	95.1	93.0	133.9
27036	P, 20-C	248.7	279.5	283.3	312.9	340.2	409.0	30.8	3.8	33.4	60.7	129.5	68.8	160.3
27037	NP, 20-C	(266.4)	(260.8)	(278.7)	(272.8)	(279.2)	(287.1)	(-5.6)	(17.9)	(12.0)	(18.4)	(26.3)	(7.9)	(20.7)
27038	P, 20-C	253.8	283.4	284.7	296.4	309.1	373.1	29.6	1.3	13.0	25.7	89.7	64.0	119.3
27039	P, 20-C	291.2	321.4	330.6	343.5	365.1	427.1	30.2	9.2	22.1	43.7	105.7	62.0	135.9
27040	P, 20-C	241.6	273.8	272.0	289.2	310.9	348.8	32.2	-1.8	15.4	37.1	75.0	37.9	107.2
27041	P, 20-C	241.1	277.3	281.2	293.5	319.9	374.1	36.2	3.9	16.2	42.6	96.8	54.2	133.0
27042	P, 20-C	259.8	286.6	294.7	307.2	333.2	381.9	26.8	8.1	20.6	46.6	95.3	48.7	122.1
27043	P, 20-C	287.1	310.9	318.1	334.8	345.6	368.2	23.8	7.2	23.9	34.7	57.3	22.6	81.1
27044	P, 20-C	278.7	297.4	297.9	302.5	336.5	390.8	18.7	0.5	5.1	39.1	93.4	54.3	112.1
27045	P, 20-C	278.0	316.0	312.9	328.4	342.8	381.8	38.0	-3.1	12.4	26.8	65.8	39.0	103.8
27046	P, 20-C	269.8	293.6	294.1	313.2	336.0	384.5	23.8	0.5	19.6	42.4	90.9	48.5	114.7
27047	P, 20-C	294.0	323.6	320.1	314.5	317.3	371.1	29.6	-3.5	-9.1	-6.3	47.5	53.8	77.1
27048	P, 20-C	298.5	327.7	330.5	352.0	378.8	438.8	29.2	2.8	24.3	51.1	111.1	60.0	140.3
27049	P, 20-C	270.1	310.1	327.1	343.1	376.1	447.0	40.0	17.0	33.0	66.0	136.9	70.9	176.9
27050	P, 20-C	260.5	285.3	293.6	316.8	334.3	392.5	24.8	8.3	31.5	49.0	107.2	58.2	132.0
27051	P, 20-C	276.0	306.6	307.2	321.4	345.4	399.4	30.6	0.6	14.8	38.8	92.8	54.0	123.4
27052	P, 20-C	275.2	299.0	306.6	327.1	356.8	422.2	23.8	7.6	28.1	57.8	123.2	65.4	147.0
27053	P, 20-C	280.8	306.0	303.6	294.2	329.6	396.2	25.2	-2.4	-11.8	23.6	90.2	66.6	115.4
27054	P, 20-C	272.9	301.2	306.4	333.4	351.8	412.6	28.3	5.2	32.2	50.6	111.4	60.8	139.7
N		24	24	24	24	24	24	24	24	24	24	24	24	24
Mean		270.8	298.47	301.39	318.31	338.53	395.41	28.38	2.93	19.84	40.06	96.95	56.88	125.33
S.D.		18.790	20.580	21.463	21.071	20.479	28.666	6.658	5.364	12.585	16.908	21.937	13.752	24.174

Appendix 2 - Continued
Individual and Mean Maternal Body Weights and Body Weight Changes[a]
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number	Disposition	Body Weight (g) Day of Gestation 0	6	8	12	16	20	Body Weight Change (g) Days 0-6	6-8	6-12	6-16[F]	6-20	16-20[F]	0-20[F]
27055	P, 20-C	268.7	290.8	294.2	310.5	329.2	381.3	22.1	3.4	19.7	38.4	90.5	51.2	112.6
27056	P, 20-C	294.4	318.8	323.2	343.3	360.2	429.0	24.4	4.4	24.5	41.4	110.2	68.8	134.6
27057	P, 20-C	262.2	286.5	282.8	295.7	301.7	372.2	24.3	-3.7	9.2	15.2	85.7	70.5	110.0
27058	P, 20-C	282.2	312.0	300.5	327.1	344.7	401.1	29.8	-11.5	15.1	32.7	89.1	56.4	118.9
27059	P, 20-C	267.8	304.9	302.4	312.6	324.8	388.4	37.1	-2.5	7.7	19.9	83.5	63.6	120.6
27060	NP, 20-C	(253.4)	(272.7)	(272.7)	(289.5)	(269.3)	(272.1)	(19.3)	(0.0)	(16.8)	(-3.4)	(-0.6)	(2.8)	(18.7)
27061	P, 20-C	270.1	301.0	296.4	303.2	319.8	379.4	30.9	-4.6	2.2	18.8	78.4	59.6	109.3
27062	P, 20-C	267.0	228.1	271.8	290.7	307.7	356.8	-38.9	43.7	62.6	79.6	128.7	49.1	89.8
27063	P, 20-C	267.9	298.0	294.7	295.1	317.3	378.7	30.1	-3.3	-2.9	19.3	80.7	61.4	110.8
27064	P, 20-C	261.8	296.0	301.8	314.7	325.3	388.7	34.2	5.8	18.7	29.3	92.7	63.4	126.9
27065	P, 20-C	259.8	299.4	294.0	302.8	329.0	397.8	39.6	-5.4	3.4	29.6	98.4	68.8	138.0
27066	P, 20-C	279.1	296.2	304.7	304.7	323.8	362.4	17.1	8.5	8.5	27.6	66.2	38.6	83.3
27067	P, 20-C	275.5	307.7	304.5	305.8	336.6	385.5	32.2	-3.2	-1.9	28.9	77.8	48.9	110.0
27068	P, 20-C	271.8	282.0	279.8	298.2	320.1	386.8	10.2	-2.2	16.2	38.1	104.8	66.7	115.0
27069	P, 20-C	283.0	314.4	317.6	329.3	355.7	413.7	31.4	3.2	14.9	41.3	99.3	58.0	130.7
27070	P, 20-C	263.6	289.4	299.7	292.4	329.6	397.6	25.8	10.3	3.0	40.2	108.2	68.0	134.0
27071	P, 20-C	265.6	291.9	293.7	295.5	300.2	377.8	26.3	1.8	3.6	8.3	85.9	77.6	112.2
27072	P, 20-C	286.2	310.4	316.6	311.1	338.9	413.3	24.2	6.2	0.7	28.5	102.9	74.4	127.1
27073	P, 20-C	275.6	301.6	290.7	306.7	334.3	407.8	26.0	-10.9	5.1	32.7	106.2	73.5	132.2
27074	P, 20-C	278.4	324.3	324.8	350.4	380.5	436.3	45.9	0.5	26.1	56.2	112.0	55.8	157.9
27075	P, 20-C	265.7	288.3	292.0	302.4	324.4	372.3	22.6	3.7	14.1	36.1	84.0	47.9	106.6
27076	P, 20-C	259.5	290.7	285.9	294.6	316.1	370.8	31.2	-4.8	3.9	25.4	80.1	54.7	111.3
27077	P, 20-C	262.8	293.6	292.5	304.5	328.6	388.4	30.8	-1.1	10.9	35.0	94.8	59.8	125.6
27078	P, 20-C	264.9	283.2	290.6	296.8	323.2	276.8	18.3	7.4	13.6	40.0	93.6	53.6	111.9
27079	NP, 20-C	(258.5)	(281.7)	(275.7)	(275.5)	(274.5)	(273.1)	(23.2)	(-6.0)	(-6.2)	(-7.2)	(-8.6)	(-1.4)	(14.6)
N		23	23	23	23	23	23	23	23	23	23	23	23	23
Mean		271.03	296.05	298.04	308.18	329.20	389.69	25.03	1.99	12.13	33.15	93.64	60.45	118.67
S.D.		9.265	18.650	13.221	15.771	18.312	20.073	15.895	10.774	13.617	14.547	14.164	9.823	16.015

Appendix 2 - Continued
Individual and Mean Maternal Body Weights and Body Weight Changes[a]
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number	Disposition	Body Weight (g) Day of Gestation 0	6	8	12	16	20	Body Weight Change (g) Days 0-6	6-8	6-12	6-16[F]	6-20	16-20[F]	0-20[F]
27080	P, 20-C	277.9	309.2	305.7	310.1	343.9	425.3	31.3	-3.5	0.9	34.7	116.1	81.4	147.4
27081	P, 20-C	267.1	291.4	286.9	289.2	299.9	355.0	24.3	-4.5	-2.2	8.5	63.6	55.1	87.9
27082	P, 20-C	256.1	285.0	273.2	279.5	285.2	352.7	28.9	-11.8	-5.5	0.2	67.7	67.5	96.6
27083	P, 20-C	272.9	301.1	300.1	306.6	306.0	361.1	28.2	-1.0	5.5	4.9	60.0	55.1	88.2
27084	NP, 20-C	(258.7)	(300.9)	(276.1)	(286.3)	(246.5)	(279.4)	(42.2)	(-24.8)	(-14.6)	(-54.4)	(-21.5)	(32.9)	(20.7)
27085	P, 20-C	277.1	295.2	284.7	307.1	329.1	382.6	18.1	-10.5	11.9	33.9	87.4	53.5	105.5
27086	P, 20-C	261.3	286.3	282.7	296.2	312.2	372.1	25.0	-3.6	9.9	25.9	85.8	59.9	110.8
27087	P, 20-C	274.0	305.8	294.1	304.9	318.0	415.7	31.8	-11.7	-0.9	12.2	109.9	97.7	141.7
27088	P, 20-C	229.6	251.5	251.1	263.7	269.9	342.9	21.9	-0.4	12.2	18.4	91.4	73.0	113.3
27089	P, 20-C	261.6	293.4	285.4	298.2	317.1	374.7	31.8	-8.0	4.8	23.7	81.3	57.6	113.1
27090	P, 20-C	224.7	243.2	245.8	242.7	255.1	308.6	18.5	2.6	-0.5	11.9	65.4	53.5	83.9
27091	P, 20-C	252.6	287.0	280.5	275.3	302.8	360.9	34.4	-6.5	-11.7	15.8	73.9	58.1	108.3
27092	P, 20-C	276.0	309.1	302.2	290.6	316.9	391.3	33.1	-6.9	-18.5	7.8	82.2	74.4	115.3
27093	P, 20-C	265.5	297.3	286.9	291.6	312.3	373.2	31.8	-10.4	-5.7	15.0	75.9	60.9	107.7
27094	P, 20-C	251.7	281.2	289.4	299.6	312.2	372.0	29.5	8.2	18.4	31.0	90.8	59.8	120.3
27095	P, 20-C	276.7	305.1	297.7	300.1	336.2	410.4	28.4	-7.4	-5.0	31.1	105.3	74.2	133.7
27096	P, 20-C	270.6	301.2	298.7	278.9	278.3	358.6	30.6	-2.5	-22.3	-22.9	57.4	80.3	88.0
27097	P, 20-C	290.8	319.7	320.5	314.7	335.8	398.7	28.9	0.8	-5.0	16.1	79.0	62.9	107.9
27098	P, 20-C	273.0	299.8	292.0	293.6	313.4	371.5	26.8	-7.8	-6.2	13.6	71.7	58.1	98.5
27099	P, 20-C	259.1	290.0	273.1	287.4	302.7	366.1	30.9	-16.9	-2.6	12.7	76.1	63.4	107.0
27100	P, 20-C	282.8	320.2	310.5	295.4	269.8	254.1	37.4	-9.7	-24.8	-50.4	-66.1	-15.7	-28.7
27101	P, 20-C	276.5	294.0	293.3	280.3	305.1	357.0	17.5	-0.7	-13.7	11.1	63.0	51.9	80.5
27102	P, 20-C	260.4	280.0	281.7	289.9	308.9	369.0	19.6	1.7	9.9	28.9	89.0	60.1	108.6
27103	P, 20-C	216.8	287.6	292.8	276.5	292.0	370.1	70.8	5.2	-11.1	4.4	82.5	78.1	153.3
27104	NP, 20-C	(272.7)	(282.9)	(274.9)	(251.1)	(253.7)	(275.4)	(10.2)	(-8.0)	(-31.8)	(-29.2)	(-7.5)	(21.7)	(2.7)
N		23	23	23	23	23	23	23	23	23	23	23	23	23
Mean		263.25	292.80	288.22	290.09	305.34	367.11	29.54	-4.58[S-]	-2.70	12.54[S-]	74.32	61.77	103.86[S-]
S.D.		18.525	18.070	16.876	16.252	22.346	35.036	10.523	6.103	11.235	18.834	34.272	20.386	34.851

Appendix 2 - Continued
Individual and Mean Maternal Body Weights and Body Weight Changes[a]
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number	Disposition	Body Weight (g) Day of Gestation 0	6	8	12	16	20	Body Weight Change (g) Days 0-6	6-8	6-12	6-16[F]	6-20	16-20[F]	0-20[F]
27105	P, 20-C	279.5	303.4	293.2	286.8	281.4	364.7	23.9	-10.2	-16.6	-22.0	61.3	83.3	85.2
27106	P, 20-C	274.9	310.7	302.2	307.5	322.7	378.4	35.8	-8.5	-3.2	12.0	67.7	55.7	103.5
27107	P, 20-C	239.1	255.2	255.3	228.0	212.7	271.7	16.1	0.1	-27.2	-42.5	16.5	59.0	32.6
27108	P, 20-C	285.4	315.6	312.0	299.7	291.7	377.2	30.2	-3.6	-15.9	-23.9	61.6	85.5	91.8
27109	P, 20-C	273.2	301.8	287.7	250.3	230.3	209.3	28.6	-14.1	-51.5	-71.5	-92.5	-21.0	-63.9
27110	P, 20-C	254.7	293.1	279.3	273.3	261.4	325.9	38.4	-13.8	-19.8	-31.7	32.8	64.5	71.2
27111	P, 20-C	269.9	300.4	301.1	305.9	309.8	386.7	30.5	0.7	5.5	9.4	86.3	76.9	116.8
27112	P, 20-C	251.7	281.3	281.0	274.7	238.2	222.8	29.6	-0.3	-6.6	-43.1	-58.5	-15.4	-28.9
27113	P, 20-C	294.6	334.6	318.1	274.5	254.1	235.3	40.0	-16.5	-60.1	-80.5	-99.3	-18.8	-59.3
27114	P, 20-C	287.0	318.2	313.0	282.1	254.4	240.5	31.2	-5.2	-36.1	-63.8	-77.7	-13.9	-46.5
27115	P, 20-C	242.9	274.3	271.1	263.1	265.6	351.6	31.4	-3.2	-11.2	-8.7	77.3	86.0	108.7
27116	P, 20-C	241.8	275.6	278.7	274.7	270.3	340.0	33.8	3.1	-0.9	-5.3	64.4	69.7	98.2
27117	P, 20-C	303.0	339.4	339.1	289.8	271.4	247.2	36.4	-0.3	-49.6	-68.0	-92.2	-24.2	-55.8
27118	NP, 20-C	(272.6)	(273.3)	(275.3)	(256.5)	(224.9)	(206.6)	(0.7)	(2.0)	(-16.8)	(-48.4)	(-66.7)	(-18.3)	(-66.0)
27119	P, 20-C	255.6	286.4	279.3	244.3	221.8	180.7	30.8	-7.1	-42.1	-64.6	-105.7	-41.1	-74.9
27120	P, 17-D	295.4	326.3	329.7	292.4	271.0	-	30.9	3.4	-33.9	-55.3	-	-	-
27121	P, 20-C	264.4	281.4	269.3	268.4	265.2	349.6	17.0	-12.1	-13.0	-16.2	68.2	84.4	85.2
27122	P, 20-C	253.4	275.6	277.7	270.4	265.5	331.3	22.2	2.1	-5.2	-10.1	55.7	65.8	77.9
27123	P, 20-C	310.8	332.5	338.2	319.2	335.4	414.9	21.7	5.7	-13.3	2.9	82.4	79.5	104.1
27124	P, 20-C	264.3	283.2	292.5	278.9	296.7	366.8	18.9	9.3	-4.3	13.5	83.6	80.1	102.5
27125	P, 20-C	251.8	277.0	275.9	248.9	223.2	211.4	25.2	-1.1	-28.1	-53.8	-65.6	-11.8	-40.4
27126	P, 20-C	271.2	290.5	286.2	270.0	243.9	232.2	19.3	-4.3	-20.5	-46.6	-58.3	-11.7	-39.0
27127	P, 17-D	259.9	293.4	280.2	274.5	245.9	-	33.5	-13.2	-18.9	-47.5	-	-	-
27128	NP, 20-C	(263.6)	(286.2)	(283.9)	(270.1)	(225.1)	(189.7)	(22.6)	(-2.3)	(-16.1)	(-61.1)	(-96.5)	(-35.4)	(-73.9)
27129	NP, 20-C	(297.0)	(299.5)	(293.5)	(259.4)	(234.6)	(256.8)	(2.5)	(-6.0)	(-40.1)	(-64.9)	(-42.7)	(22.2)	(-40.2)
N		22	22	22	22	22	20	22	22	22	22	20	20	20
Mean		269.30	297.72	293.63	276.25	265.12	301.91	28.43	-4.05[S-]	-21.48	-32.60[S-]	5.40	36.63	33.45[S-]
S.D.		20.329	22.698	22.916	21.762	32.153	73.253	6.951	7.115	17.769	29.333	75.133	48.285	73.412

Appendix 3
Individual and Mean Food Consumption[a]
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number	Food Consumption (g) Interval (Days of Gestation)						
	0-6	7-8	9-12	13-16	17-20[F]	7-16[B]	0-20[B]
27030	140.9	42.8	92.5	87.1	101.7	222.4	465.0
27031	130.0	33.1	73.2	71.3	91.1	177.6	398.7
27032	140.6	45.5	86.1	82.2	106.4	213.8	460.8
27033	123.7	32.0	75.8	80.6	96.6	188.4	408.7
27034	80.6	18.6	48.8	51.9	67.8	119.3	267.7
27035	166.0	44.7	69.8	44.8	109.1	159.3	434.4
27036	139.2	36.2	80.9	86.8	106.4	203.9	449.5
27037	(113.8)	(30.1)	(52.2)	(55.2)	(69.7)	(137.5)	(321.0)
27038	134.9	33.4	75.9	77.9	107.0	187.2	429.1
27039	145.4	43.3	76.8	91.6	107.8	211.7	464.9
27040	122.3	31.8	69.7	71.1	84.5	172.6	379.4
27041	129.9	37.2	72.2	73.0	86.5	182.4	398.8
27042	136.1	42.9	79.5	80.1	95.8	202.5	434.4
27043	142.4	44.6	89.5	81.5	93.0	215.6	451.0
27044	141.2	34.2	60.6	78.7	99.0	173.5	413.7
27045	146.9	39.1	79.1	69.4	78.4	187.6	412.9
27046	115.9	37.6	75.8	78.1	95.4	191.5	402.8
27047	123.4	29.7	41.8	82.6	71.8	154.1	349.3
27048	144.5	42.4	91.7	93.5	113.4	227.6	485.5
27049	149.3	51.3	90.8	97.7	116.4	239.8	505.5
27050	119.5	36.5	77.6	72.1	92.5	186.2	398.2
27051	132.7	36.6	74.9	74.4	112.2	185.9	430.8
27052	127.1	39.8	81.3	85.5	106.4	206.6	440.1
27053	125.0	36.2	47.1	97.3	96.7	180.6	402.3
27054	126.5	30.1	78.4	75.2	98.1	183.7	408.3
N	24	24	24	24	24	24	24
Mean	132.67	37.48	74.58	78.52	97.25	190.58	420.49
S.D.	15.924	6.812	13.416	12.317	12.693	25.916	47.701

Appendix 3 - Continued
Individual and Mean Food Consumption[a]
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number	Food Consumption (g) Interval (Days of Gestation)						
	0-6	7-8	9-12	13-16	17-20[F]	7-16[B]	0-20[B]
27055	122.0	35.2	63.7	71.0	92.5	169.9	384.4
27056	136.0	42.1	74.1	69.7	108.9	185.9	430.8
27057	130.8	35.2	68.0	49.6	88.6	152.8	372.2
27058	136.5	29.1	70.2	69.0	98.0	168.3	402.8
27059	141.7	38.2	64.5	71.2	112.1	173.9	427.7
27060	(108.2)	(30.2)	(67.9)	(39.8)	(77.9)[b]	(137.9)	(324.0)[b]
27061	131.5	31.6	62.8	66.0	91.3	160.4	383.2
27062	55.7	38.6	81.8	68.6	70.3	189.0	315.0
27063	135.6	34.2	61.4	70.9	98.7	166.5	400.8
27064	143.0	39.1	73.5	55.3	99.4	167.9	410.3
27065	145.9	34.7	63.1	81.3	116.9	179.1	441.9
27066	99.3	33.6	60.3	63.0	92.1	156.9	348.3
27067	144.9	35.2	63.6	72.8	88.5	171.6	405.0
27068	111.5	22.7	64.2	59.2	97.7	146.1	355.3
27069	145.8	40.4	72.6	77.0	94.0	190.0	429.8
27070	133.0	42.8	61.4	67.6	98.5	171.8	403.3
27071	119.6	34.9	60.6	41.5	101.4	137.0	358.0
27072	145.4	41.9	62.1	68.2	109.2	172.2	426.8
27073	105.6	24.4	62.8	100.6	107.1	187.8	400.5
27074	147.3	41.9	93.2	88.8	113.6	223.9	484.8
27075	127.0	40.7	72.7	68.2	84.3	181.6	392.9
27076	131.2	27.8	87.8	59.0	79.5	174.6	385.3
27077	126.4	28.1	63.2	70.0	95.9	161.3	383.6
27078	128.5	33.2	65.1	65.8	96.4	164.1	389.0
27079	(156.8)	(23.8)	(53.4)	(56.3)	(64.9)	(133.5)	(355.2)
N	23	23	23	23	23	23	23
Mean	128.01	35.03	68.38	68.45	97.17	171.85	397.03
S.D.	20.444	5.735	8.893	12.065	11.147	17.544	35.568

Appendix 3 - Continued
Individual and Mean Food Consumption[a]
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number	Food Consumption (g) Interval (Days of Gestation)						
	0-6	7-8	9-12	13-16	17-20[F]	7-16[B]	0-20[B]
27080	139.2	36.9	60.3	70.0	104.7	167.2	411.1
27081	139.9	34.5	48.3	60.9	82.8	143.7	366.4
27082	138.5	26.3	46.7	38.5	83.1	111.5	333.1
27083	136.1	42.7	64.6	39.5	73.4	146.8	356.3
27084	(141.5)	(21.4)	(53.2)	(11.2)	(74.2)	(85.8)	(301.5)
27085	127.7	23.3	55.7	66.7	80.5	145.7	353.9
27086	113.8	28.3	53.5	59.6	88.0	141.4	343.2
27087	146.0	32.2	(116.4)[b]	72.5	123.9	(221.1)[b]	(491.0)[b]
27088	118.1	(156.2)[b]	(86.5)[b]	(88.1)[b]	(109.3)[b]	(330.8)[b]	(558.2)[b]
27089	134.4	35.0	61.0	61.3	87.8	157.3	379.5
27090	97.9	28.3	46.9	52.3	74.7	127.5	300.1
27091	132.3	26.8	44.6	60.5	85.4	131.9	349.6
27092	147.3	36.2	153.0	67.1	95.0	256.3	498.6
27093	130.5	24.4	51.1	79.8	100.9	155.3	386.7
27094	168.5	40.4	72.5	69.0	96.1	181.9	446.5
27095	120.0	24.9	56.5	55.2	114.6	136.6	371.2
27096	136.2	34.6	(119.9)[b]	(160.8)[b]	(100.6)[b]	(315.3)[b]	(552.1)[b]
27097	145.1	40.9	57.8	53.6	100.9	152.3	398.3
27098	147.3	32.9	75.0	89.4	(135.8)[b]	197.3	(480.4)[b]
27099	177.4	26.6	67.0	(110.3)[b]	(108.9)[b]	(203.9)[b]	(490.2)[b]
27100	141.8	28.9	34.2	1.5	0.0	64.6	206.4
27101	118.9	30.1	44.0	65.1	91.9	139.2	350.0
27102	115.4	28.4	56.5	51.9	90.5	136.8	342.7
27103	130.8	31.1	42.4	43.4	95.3	116.9	343.0
27104	(138.8)	(24.5)	(27.8)	(36.9)	(64.3)	(89.2)	(292.3)
N	23	22	20	20	19	19	18
Mean	134.92	31.53	59.58	57.89	87.87	147.91[S-]	363.14[S-]
S.D.	17.356	5.567	24.302	18.350	24.771	38.131	59.846

Appendix 3 - Continued
Individual and Mean Food Consumption[a]
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number	Food Consumption (g) Interval (Days of Gestation)						
	0-6	7-8	9-12	13-16	17-20[F]	7-16[B]	0-20[B]
27105	126.5	30.6	41.5	17.9	80.0	90.0	296.5
27106	148.7	40.0	58.6	67.4	99.5	166.0	414.2
27107	110.0	144.4	23.6	54.2	(200.2)[b]	222.2	(532.4)[b]
27108	138.3	37.3	40.2	23.0	91.3	100.5	330.1
27109	140.5	31.7	(145.5)[b]	0.0	2.3	(177.2)[b]	(320.0)[b]
27110	143.2	31.3	55.7	35.6	85.0	122.6	350.8
27111	136.7	36.7	48.3	75.1	100.9	160.1	397.7
27112	128.9	33.8	40.1	1.6	0.0	75.5	204.4
27113	150.0	21.2	1.4	0.0	0.0	22.6	172.6
27114	142.9	30.8	21.3	0.0	1.1	52.3	196.1
27115	123.7	29.7	39.2	26.3	90.5	95.2	309.4
27116	117.4	29.0	86.5	57.1	(110.0)[b]	172.6	(400.0)[b]
27117	164.1	44.5	10.6	0.0	0.0	55.1	219.2
27118	(115.8)	(32.5)	(57.2)	(41.1)[b]	(24.9)	(130.8)[b]	(271.5)[b]
27119	124.6	28.9	15.2	0.2	0.0	44.3	168.9
27120	150.7	42.3	31.5	0.0	-	73.8	-
27121	98.5	24.3	(48.0)[b]	(126.3)[b]	(96.8)[b]	(198.6)[b]	(393.9)[b]
27122	120.6	33.5	44.3	(164.9)[b]	(152.0)[b]	(242.7)[b]	(515.3)[b]
27123	158.2	41.4	(66.2)[b]	54.5	(119.8)[b]	(162.1)[b]	(440.1)[b]
27124	137.6	34.5	(129.3)[b]	(196.3)[b]	(117.8)[b]	(360.1)[b]	(615.5)[b]
27125	115.0	28.1	17.1	0.0	0.0	45.2	160.2
27126	123.5	26.6	39.9	7.5	0.0	74.0	197.5
27127	133.7	27.5	48.4	11.8	-	87.7	-
27128	(122.7)	(31.5)	(41.1)	(20.4)[b]	(0.4)	(93.0)[b]	(216.1)[b]
27129	(113.0)	(27.9)	(15.7)	(0.0)	(72.2)	(43.6)	(228.8)
N	22	22	18	19	14	17	13
Mean	133.33	37.64	36.86	22.75	39.33[S-]	97.63[S-]	262.89[S-]
S.D.	16.209	24.571	20.179	26.380	46.891	54.321	90.464

Appendix 4
Individual and Mean Terminal Body Weights, Gravid Uterine Weights, and Terminal Body Weights Minus Gravid Uterine Weights (g)[a]
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number	Terminal Body Weight	Gravid Uterine Weight[B]	Terminal Body Weight Minus Gravid Uterine Weight[F]
27030	422.5	90.4	332.1
27031	388.2	90.9	297.3
27032	404.3	88.2	316.1
27033	367.8	67.8	300.0
27034	341.4	66.6	274.8
27035	446.6	97.3	349.3
27036	409.0	86.9	322.1
27037	Not Pregnant		
27038	373.1	69.2	303.9
27039	427.1	76.2	350.9
27040	348.8	54.7	294.1
27041	374.1	82.4	291.7
27042	381.9	80.1	301.8
27043	368.2	30.8	337.4
27044	390.8	80.6	310.2
27045	381.8	52.4	329.4
27046	384.5	72.3	312.2
27047	371.1	64.5	306.6
27048	438.8	74.0	364.8
27049	447.0	84.1	362.9
27050	392.5	74.8	317.7
27051	399.4	60.3	339.1
27052	422.2	83.1	339.1
27053	396.2	74.6	321.6
27054	412.6	82.1	330.5
N	24	24	24
Mean	395.41	74.35	321.07
S.D.	28.666	14.704	23.215

Appendix 4 - Continued
Individual and Mean Terminal Body Weights, Gravid Uterine Weights, and Terminal Body Weights Minus Gravid Uterine Weights (g)[a]
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number	Terminal Body Weight	Gravid Uterine Weight[B]	Terminal Body Weight Minus Gravid Uterine Weight[F]
27055	381.3	72.5	308.8
27056	429.0	88.5	340.5
27057	372.2	73.3	298.9
27058	401.1	86.5	314.6
27059	388.4	69.1	319.3
27060	Not Pregnant		
27061	379.4	69.2	310.2
27062	356.8	64.0	292.8
27063	378.7	67.4	311.3
27064	388.7	74.6	314.1
27065	397.8	80.0	317.8
27066	362.4	37.9	324.5
27067	385.5	76.1	309.4
27068	386.8	80.4	306.4
27069	413.7	83.7	330.0
27070	397.6	90.0	307.6
27071	377.8	77.8	300.0
27072	413.3	85.3	328.0
27073	407.8	84.7	323.1
27074	436.3	84.8	351.5
27075	372.3	77.3	295.0
27076	370.8	60.7	310.1
27077	388.4	83.2	305.2
27078	376.8	71.6	305.2
27079	Not Pregnant		
N	23	23	23
Mean	389.69	75.59	314.10
S.D.	20.073	11.458	14.093

Appendix 4 - Continued
Individual and Mean Terminal Body Weights, Gravid Uterine Weights, and Terminal Body Weights Minus Gravid Uterine Weights (g)[a]
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number	Terminal Body Weight	Gravid Uterine Weight[B]	Terminal Body Weight Minus Gravid Uterine Weight[F]
27080	425.3	103.9	321.4
27081	355.0	72.2	282.8
27082	352.7	72.4	280.3
27083	361.1	68.5	292.6
27084	Not Pregnant		
27085	382.6	89.8	292.8
27086	372.1	79.4	292.7
27087	415.7	76.6	339.1
27088	342.9	73.5	269.4
27089	374.7	71.2	303.5
27090	308.6	61.6	247.0
27091	360.9	75.3	285.6
27092	391.3	88.0	303.3
27093[C]	373.2	-	-
27094	372.0	71.2	300.8
27095	410.4	87.1	323.3
27096	358.6	65.4	293.2
27097	398.7	65.2	333.5
27098	371.5	68.0	303.5
27099	366.1	61.6	304.5
27100	254.1	48.2	205.9
27101	357.0	73.9	283.1
27102	369.0	72.9	296.1
27103	370.1	70.2	299.9
27104	Not Pregnant		
N	23	22	22
Mean	367.11	73.46	293.38[S-]
S.D.	35.036	11.486	28.235

Appendix 4 - Continued
Individual and Mean Terminal Body Weights, Gravid Uterine Weights, and Terminal Body Weights Minus Gravid Uterine Weights (g)[a]
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number	Terminal Body Weight	Gravid Uterine Weight[B]	Terminal Body Weight Minus Gravid Uterine Weight[F]
27105	364.7	82.3	282.4
27106	378.4	58.9	319.5
27107	271.7	54.6	217.1
27108	377.2	79.8	297.4
27109	209.3	72.2	137.1
27110	325.9	64.4	261.5
27111	386.7	86.5	300.2
27112	222.8	40.8	182.0
27113	235.3	43.3	192.0
27114	240.5	42.3	198.2
27115	351.6	82.4	269.2
27116	340.0	55.4	284.6
27117	247.2	38.5	208.7
27118	Not Pregnant		
27119	180.7	32.4	148.3
27120	Found Dead		
27121	349.6	71.7	277.9
27122	331.3	66.0	265.3
27123	414.9	79.4	335.5
27124	366.8	76.0	290.8
27125	211.4	39.7	171.7
27126	232.2	43.6	188.6
27127	Found Dead		
27128	Not Pregnant		
27129	Not Pregnant		
N	20	20	20
Mean	301.91	60.51[S-]	241.40[S-]
S.D.	73.253	17.739	59.517

Appendix 5
Individual and Mean Ovarian and Uterine Data
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number	Ovarian Corpora Lutea	Uterine Implantations	Implantation Efficiency (%)	Early Resorptions	Early Resorptions (%)	Late Resorptions	Late Resorptions (%)	Total Resorptions	Total Resorptions (%)
27030	15	15	100.0	0	0.0	0	0.0	0	0.0
27031	17	17	100.0	1	5.9	0	0.0	1	5.9
27032	19	15	78.9	1	6.7	0	0.0	1	6.7
27033	19	15	78.9	3	20.0	0	0.0	3	20.0
27034	16	15	93.8	1	6.7	0	0.0	1	6.7
27035	20	18	90.0	1	5.6	0	0.0	1	5.6
27036	14	14	100.0	0	0.0	0	0.0	0	0.0
27037	Not Pregnant								
27038	16	14	87.5	2	14.3	0	0.0	2	14.3
27039	19	17	89.5	4	23.5	0	0.0	4	23.5
27040	16	13	81.3	1	7.7	0	0.0	1	7.7
27041	15	15	100.0	0	0.0	0	0.0	0	0.0
27042	17	15	88.2	1	6.7	0	0.0	1	6.7
27043	14	7	50.0	2	28.6	0	0.0	2	28.6
27044	18	16	88.9	1	6.3	0	0.0	1	6.3
27045	19	10	52.6	0	0.0	0	0.0	0	0.0
27046	13	13	100.0	0	0.0	0	0.0	0	0.0
27047	12	12	100.0	1	8.3	0	0.0	1	8.3
27048	15	15	100.0	1	6.7	0	0.0	1	6.7
27049	19	18	94.7	1	5.6	0	0.0	1	5.6
27050	17	14	82.4	0	0.0	0	0.0	0	0.0
27051	16	13	81.3	2	15.4	0	0.0	2	15.4
27052	16	15	93.8	0	0.0	0	0.0	0	0.0
27053	18	15	83.3	0	0.0	0	0.0	0	0.0
27054	18	17	94.4	0	0.0	0	0.0	0	0.0
Total	398	348		23		0		23	
N	24	24	24	24	24	24	24	24	24
Mean	16.6	14.5	87.90	1.0	7.00	0.0	0.00	1.0	7.00
S.D.	2.12	2.45	13.472	1.04	8.024	-	-	1.04	8.024

Appendix 5 - Continued
Individual and Mean Ovarian and Uterine Data
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number	Ovarian Corpora Lutea	Uterine Implantations	Implantation Efficiency (%)	Early Resorptions	Early Resorptions (%)	Late Resorptions	Late Resorptions (%)	Total Resorptions	Total Resorptions (%)
27055	15	13	86.7	1	7.7	0	0.0	1	7.7
27056	17	16	94.1	1	6.3	0	0.0	1	6.3
27057	13	13	100.0	0	0.0	0	0.0	0	0.0
27058	15	15	100.0	1	6.7	0	0.0	1	6.7
27059	14	14	100.0	2	14.3	0	0.0	2	14.3
27060	Not Pregnant								
27061	12	11	91.7	0	0.0	0	0.0	0	0.0
27062	15	13	86.7	0	0.0	0	0.0	0	0.0
27063	14	13	92.9	1	7.7	0	0.0	1	7.7
27064	15	15	100.0	1	6.7	0	0.0	1	6.7
27065	17	16	94.1	0	0.0	0	0.0	0	0.0
27066	11	7	63.6	1	14.3	0	0.0	1	14.3
27067	16	16	100.0	2	12.5	0	0.0	2	12.5
27068	19	16	84.2	1	6.3	0	0.0	1	6.3
27069	25	17	68.0	2	11.8	0	0.0	2	11.8
27070	17	16	94.1	0	0.0	0	0.0	0	0.0
27071	15	14	93.3	0	0.0	0	0.0	0	0.0
27072	18	18	100.0	1	5.6	0	0.0	1	5.6
27073	17	16	94.1	0	0.0	0	0.0	0	0.0
27074	18	16	88.9	1	6.3	0	0.0	1	6.3
27075	18	15	83.3	1	6.7	0	0.0	1	6.7
27076	15	13	86.7	1	7.7	0	0.0	1	7.7
27077	13	13	100.0	0	0.0	0	0.0	0	0.0
27078	16	15	93.8	1	6.7	0	0.0	1	6.7
27079	Not Pregnant								
Total	365	331		18		0		18	
N	23	23	23	23	23	23	23	23	23
Mean	15.9	14.4	91.14	0.8	5.53	0.0	0.00	0.8	5.53
S.D.	2.85	2.31	9.694	0.67	4.804	-	-	0.67	4.804

Appendix 5 - Continued
Individual and Mean Ovarian and Uterine Data
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number	Ovarian Corpora Lutea	Uterine Implantations	Implantation Efficiency (%)	Early Resorptions	Early Resorptions (%)	Late Resorptions	Late Resorptions (%)	Total Resorptions	Total Resorptions (%)
27080	16	16	100.0	0	0.0	0	0.0	0	0.0
27081	14	13	92.9	0	0.0	0	0.0	0	0.0
27082	15	14	93.3	1	7.1	0	0.0	1	7.1
27083	14	14	100.0	2	14.3	0	0.0	2	14.3
27084	Not Pregnant								
27085	20	17	85.0	0	0.0	0	0.0	0	0.0
27086	15	15	100.0	0	0.0	1	6.7	1	6.7
27087	15	15	100.0	0	0.0	0	0.0	0	0.0
27088	15	14	93.3	1	7.1	0	0.0	1	7.1
27089	13	13	100.0	1	7.7	0	0.0	1	7.7
27090	13	12	92.3	0	0.0	0	0.0	0	0.0
27091	15	15	100.0	0	0.0	0	0.0	0	0.0
27092	19	17	89.5	1	5.9	0	0.0	1	5.9
27093	15	14	93.3	1	7.1	0	0.0	1	7.1
27094	14	13	92.9	0	0.0	0	0.0	0	0.0
27095	17	17	100.0	1	5.9	0	0.0	1	5.9
27096	17	16	94.1	3	18.8	0	0.0	3	18.8
27097	16	15	93.8	2	13.3	0	0.0	2	13.3
27098	18	14	77.8	2	14.3	0	0.0	2	14.3
27099	16	12	75.0	0	0.0	0	0.0	0	0.0
27100	19	16	84.2	0	0.0	0	0.0	0	0.0
27101	16	15	93.8	1	6.7	0	0.0	1	6.7
27102	16	14	87.5	0	0.0	0	0.0	0	0.0
27103	15	14	93.3	0	0.0	0	0.0	0	0.0
27104	Not Pregnant								
Total	363	335		16		1		17	
N	23	23	23	23	23	23	23	23	23
Mean	15.8	14.6	92.70	0.7	4.70	0.0	0.29	0.7	5.00
S.D.	1.86	1.47	7.017	0.88	5.859	0.21	1.397	0.86	5.781

Appendix 5 - Continued
Individual and Mean Ovarian and Uterine Data
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number	Ovarian Corpora Lutea	Uterine Implantations	Implantation Efficiency (%)	Early Resorptions	Early Resorptions (%)	Late Resorptions	Late Resorptions (%)	Total Resorptions	Total Resorptions (%)
27105	17	15	88.2	0	0.0	0	0.0	0	0.0
27106	17	11	64.7	1	9.1	0	0.0	1	9.1
27107	14	13	92.9	3	23.1	0	0.0	3	23.1
27108	17	16	94.1	1	6.3	0	0.0	1	6.3
27109	18	17	94.4	1	5.9	15	88.2	16	94.1
27110	21	14	66.7	0	0.0	0	0.0	0	0.0
27111	17	16	94.1	1	6.3	0	0.0	1	6.3
27112	14	13	92.9	1	7.7	0	0.0	1	7.7
27113	15	15	100.0	2	13.3	1	6.7	3	20.0
27114	18	15	83.3	2	13.3	0	0.0	2	13.3
27115	16	15	93.8	0	0.0	0	0.0	0	0.0
27116	14	12	85.7	1	8.3	0	0.0	1	8.3
27117	22	14	63.6	0	0.0	1	7.1	1	7.1
27118	Not Pregnant								
27119	15	13	86.7	13	100.0	0	0.0	13	100.0
27120[a]	17	16	94.1						
27121	15	15	100.0	0	0.0	0	0.0	0	0.0
27122	15	15	100.0	3	20.0	0	0.0	3	20.0
27123	18	15	83.3	0	0.0	0	0.0	0	0.0
27124	16	16	100.0	1	6.3	0	0.0	1	6.3
27125	16	13	81.3	0	0.0	0	0.0	0	0.0
27126	18	15	83.3	1	6.7	0	0.0	1	6.7
27127[a]	15	13	86.7						
27128	Not Pregnant								
27129	Not Pregnant								
Total	365	317		31		17		48	
N	22	22	22	20	20	20	20	20	20
Mean	16.6	14.4	87.72	1.6	11.32	0.9	5.10	2.4	16.42
S.D.	2.09	1.50	10.955	2.86	21.929	3.34	19.674	4.28	28.471

[a]Found dead on Day 17, therefore, fetal data not available.

Appendix 6
Individual and Mean Litter Data[a]
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number	Total Resorptions	Number of Fetuses: Dead	Fetal Loss (%)[b]	Live	Live (%)	Sex: Males	Females	Percent Males	Mean Weight (g)[c]: Males	Females
27030	0	0	0.0	15	100.0	7	8	46.7	3.7	3.6
27031	1	0	5.9	16	94.1	8	8	50.0	3.5	3.2
27032	1	0	6.7	14	93.3	7	7	50.0	3.9	3.4
27033	3	0	20.0	12	80.0	7	5	58.3	3.3	3.2
27034	1	0	6.7	14	93.3	5	9	35.7	2.5	2.8
27035	1	0	5.6	17	94.4	12	5	70.6	3.3	3.2
27036	0	0	0.0	14	100.0	7	7	50.0	4.1	3.9
27037	Not Pregnant									
27038	2	0	14.3	12	85.7	5	7	41.7	3.5	3.3
27039	4	0	23.5	13	76.5	6	7	46.2	3.6	3.4
27040	1	0	7.7	12	92.3	7	5	58.3	2.7	2.4
27041	0	0	0.0	15	100.0	4	11	26.7	3.4	3.3
27042	1	0	6.7	14	93.3	4	10	28.6	3.5	3.4
27043	2	0	28.6	5	71.4	1	4	20.0	2.2	3.2
27044	1	0	6.3	15	93.8	12	3	80.0	3.2	2.9
27045	0	0	0.0	10	100.0	3	7	30.0	3.2	3.2
27046	0	0	0.0	13	100.0	5	8	38.5	3.3	3.2
27047	1	0	8.3	11	91.7	5	6	45.5	3.0	2.9
27048	1	0	6.7	14	93.3	6	8	42.9	3.3	3.2
27049	1	0	5.6	17	94.4	9	8	52.9	3.2	2.9
27050	0	0	0.0	14	100.0	6	8	42.9	3.1	3.1
27051	2	0	15.4	11	84.6	5	6	45.5	3.2	3.2
27052	0	0	0.0	15	100.0	7	8	46.7	3.5	3.4
27053	0	0	0.0	15	100.0	2	13	13.3	3.3	3.0
27054	0	0	0.0	17	100.0	6	11	35.3	3.1	2.8
Total	23	0		325		146	179			
N	24	24	24	24	24	24	24	24	24	24
Unadjusted Mean	1.0	0.0	7.00	13.5	93.00	6.1	7.5	44.01	3.28	3.17
Unadjusted S.D.	1.04	-	8.024	2.64	8.024	2.59	2.30	14.828	0.408	0.301
Covariance Adjusted Mean									3.28	3.16
Covariance Adjusted S.D.									0.092	0.084

[a] Sex, percent males, and mean weight data include values for live fetuses only.
[b] Percent fetal loss includes resorptions as well as dead fetuses.
[c] Statistical analysis performed on covariance adjusted means.

Appendix 6 - Continued
Individual and Mean Litter Data[a]
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number	Total Resorptions	Number of Fetuses: Dead	Fetal Loss (%)[b]	Live	Live (%)	Sex: Males	Females	Percent Males	Mean Weight (g)[c]: Males	Females
27055	1	0	7.7	12	92.3	8	4	66.7	3.4	3.4
27056	1	0	6.3	15	93.8	8	7	53.3	3.3	3.3
27057	0	0	0.0	13	100.0	9	4	69.2	3.4	3.4
27058	1	0	6.7	14	93.3	8	6	57.1	3.4	3.2
27059	2	0	14.3	12	85.7	5	7	41.7	3.3	3.0
27060	Not Pregnant									
27061	0	0	0.0	11	100.0	7	4	63.6	3.8	3.6
27062	0	0	0.0	13	100.0	8	5	61.5	2.7	2.8
27063	1	0	7.7	12	92.3	3	9	25.0	3.5	3.2
27064	1	0	6.7	14	93.3	6	8	42.9	3.3	3.0
27065	0	0	0.0	16	100.0	8	8	50.0	2.8	2.8
27066	1	0	14.3	6	85.7	2	4	33.3	3.2	3.4
27067	2	0	12.5	14	87.5	5	9	35.7	2.9	2.8
27068	1	0	6.3	15	93.8	7	8	46.7	3.0	3.2
27069	2	0	11.8	15	88.2	10	5	66.7	3.3	3.3
27070	0	0	0.0	16	100.0	15	1	93.8	3.4	3.0
27071	0	0	0.0	14	100.0	7	7	50.0	3.1	2.9
27072	1	0	5.6	17	94.4	9	8	52.9	2.8	2.7
27073	0	0	0.0	16	100.0	6	10	37.5	2.9	2.9
27074	1	0	6.3	15	93.8	5	10	33.3	3.6	3.3
27075	1	0	6.7	14	93.3	8	6	57.1	3.2	3.1
27076	1	0	7.7	12	92.3	10	2	83.3	3.1	3.0
27077	0	0	0.0	13	100.0	5	8	38.5	3.5	3.2
27078	1	0	6.7	14	93.3	6	8	42.9	3.3	2.9
27079	Not Pregnant									
Total	18	0		313		165	148			
N	23	23	23	23	23	23	23	23	23	23
Unadjusted Mean	0.8	0.0	5.53	13.6	94.48	7.2	6.4	52.29	3.23	3.10
Unadjusted S.D.	0.67	-	4.804	2.27	4.802	2.66	2.45	16.688	0.278	0.240
Covariance Adjusted Mean									3.23	3.10
Covariance Adjusted S.D.									0.092	0.083

[a] Sex, percent males, and mean weight data include values for live fetuses only.
[b] Percent fetal loss includes resorptions as well as dead fetuses.
[c] Statistical analysis performed on covariance adjusted means.

Appendix 6 - Continued
Individual and Mean Litter Data[a]
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number	Total Resorptions	Number of Fetuses: Dead	Fetal Loss (%)[b]	Live	Live (%)	Sex: Males	Females	Percent Males	Mean Weight (g)[c]: Males	Females
27080	0	0	0.0	16	100.0	8	8	50.0	3.7	3.4
27081	0	0	0.0	13	100.0	8	5	61.5	3.2	2.9
27082	1	0	7.1	13	92.9	8	5	61.5	3.2	3.1
27083	2	0	14.3	12	85.7	3	9	25.0	3.1	2.8
27084	Not Pregnant									
27085	0	0	0.0	17	100.0	5	12	29.4	3.0	2.9
27086	1	0	6.7	14	93.3	7	7	50.0	3.1	3.0
27087	0	0	0.0	15	100.0	11	4	73.3	2.8	2.8
27088	1	0	7.1	13	92.9	6	7	46.2	3.4	3.3
27089	1	0	7.7	12	92.3	9	3	75.0	3.4	3.3
27090	0	0	0.0	12	100.0	8	4	66.7	3.0	2.9
27091	0	0	0.0	15	100.0	9	6	60.0	3.1	2.9
27092	1	0	5.9	16	94.1	12	4	75.0	3.2	3.2
27093	1	0	7.1	13	92.9	9	4	69.2	3.3	2.9
27094	0	0	0.0	13	100.0	7	6	53.8	3.2	3.1
27095	1	0	5.9	16	94.1	8	8	50.0	3.2	3.1
27096	3	0	18.8	13	81.3	6	7	46.2	3.2	3.1
27097	2	0	13.3	13	86.7	7	6	53.8	3.0	3.0
27098	2	0	14.3	12	85.7	6	6	50.0	3.0	3.0
27099	0	0	0.0	12	100.0	6	6	50.0	3.1	3.1
27100	0	0	0.0	16	100.0	10	6	62.5	1.5	1.7
27101	1	1	13.3	13	86.7	5	8	38.5	3.2	3.0
27102	0	0	0.0	14	100.0	6	8	42.9	3.1	2.8
27103	0	0	0.0	14	100.0	8	6	57.1	3.3	3.1
27104	Not Pregnant									
Total	17	1		317		172	145			
N	23	23	23	23	23	23	23	23	23	23
Unadjusted Mean	0.7	0.0	5.28	13.8	94.72	7.5	6.3	54.24	3.10	2.97
Unadjusted S.D.	0.86	0.21	6.028	1.57	6.018	2.04	2.01	13.293	0.392	0.322
Covariance Adjusted Mean									3.10[S-]	2.97[S-]
Covariance Adjusted S.D.									0.092	0.083

[a] Sex, percent males, and mean weight data include values for live fetuses only.
[b] Percent fetal loss includes resorptions as well as dead fetuses.
[c] Statistical analysis performed on covariance adjusted means.

Appendix 6 - Continued
Individual and Mean Litter Data[a]
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number	Total Resorptions	Number of Fetuses: Dead	Fetal Loss (%)[b]	Live	Live (%)	Sex: Males	Females	Percent Males	Mean Weight (g)[c]: Males	Females
27105	0	0	0.0	15	100.0	10	5	66.7	3.1	3.0
27106	1	0	9.1	10	90.9	6	4	60.0	3.2	3.2
27107	3	0	23.1	10	76.9	4	6	40.0	3.1	3.0
27108	1	0	6.3	15	93.8	9	6	60.0	3.2	3.2
27109	16	1	100.0	0	0.0					
27110	0	0	0.0	14	100.0	8	6	57.1	2.7	2.5
27111	1	0	6.3	15	93.8	7	8	46.7	3.2	3.2
27112	1	0	7.7	12	92.3	10	2	83.3	1.7	1.7
27113	3	6	60.0	6	40.0	4	2	66.7	1.9	2.0
27114	2	0	13.3	13	86.7	6	7	46.2	1.8	1.7
27115	0	0	0.0	15	100.0	9	6	60.0	3.3	3.0
27116	1	0	8.3	11	91.7	8	3	72.7	2.8	2.6
27117	1	2	21.4	11	78.6	8	3	72.7	1.6	1.5
27118	Not Pregnant									
27119	13	0	100.0	0	0.0					
27120	Found Dead									
27121	0	0	0.0	15	100.0	9	6	60.0	3.1	3.0
27122	3	0	20.0	12	80.0	6	6	50.0	3.2	3.4
27123	0	0	0.0	15	100.0	10	5	66.7	3.2	3.0
27124	1	0	6.3	15	93.8	9	6	60.0	2.8	2.9
27125	0	0	0.0	13	100.0	7	6	53.8	1.8	1.7
27126	1	0	6.7	14	93.3	9	5	64.3	1.8	1.7
27127	Found Dead									
27128	Not Pregnant									
27129	Not Pregnant									
Total	48	9		231		139	92			
N	20	20	20	20	20	18	18	18	18	18
Unadjusted Mean	2.4	0.5	19.43	11.6	80.59	7.7	5.1	60.38	2.64	2.57
Unadjusted S.D.	4.28	1.39	30.777	4.61	30.801	1.90	1.68	10.655	0.656	0.660
Covariance Adjusted Mean									2.63S-	2.59S-
Covariance Adjusted S.D.									0.105	0.097

[a] Sex, percent males, and mean weight data include values for live fetuses only.
[b] Percent fetal loss includes resorptions as well as dead fetuses.
[c] Statistical analysis performed on covariance adjusted means.

Appendix 7
Individual Visceral Findings in Fetuses
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number:	270	271	272	273	274	275	276	277	278	279	280	281	282
Number of fetuses examined	5	6	5	4	5	6	5	NP	4	5	4	5	5
Number of fetuses within normal limits	5	6	5	3	5	6	5		4	5	4	5	5
Number of fetuses with one or more visceral variants	0	0	0	1	0	0	0		0	0	0	0	0
Percent of fetuses with one or more skeletal variants (%)	0.00	0.00	0.00	25.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:													
Brain - cerebral hemisphere contained dark area				I									
Eye(s) - contained dark material													
Liver - dark areas													
Kidney(s) - pelvis dilated													
Ureter(s) - distended													
- undulated													
Number of fetuses with one or more visceral anomalies	0	0	0	0	0	0	0		0	0	0	0	0
Percent of fetuses with one or more visceral anomalies (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:													
Skin - edematous													
Palate - cleft													
Cryptorchism													

NP = Not Pregnant.

Appendix 7 - Continued
Individual Visceral Findings in Fetuses
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number:	2703	2704	2705	2706	2707	2708	2709	2710	2711	2712	2713	2714
Number of fetuses examined	2	5	4	5	4	5	6	5	4	5	5	6
Number of fetuses within normal limits	2	4	3	5	3	5	6	5	4	5	5	6
Number of fetuses with one or more visceral variants	0	1	1	0	1	0	0	0	0	0	0	0
Percent of fetuses with one or more skeletal variants (%)	0.00	20.00	25.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:												
Brain - cerebral hemisphere contained dark area												
Eye(s) - contained dark material												
Liver - dark areas												
Kidney(s) - pelvis dilated												
Ureter(s) - distended												
- undulated		A	J		K							
Number of fetuses with one or more visceral anomalies	0	0	0	0	0	0	0	0	0	0	0	0
Percent of fetuses with one or more visceral anomalies (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:												
Skin - edematous												
Palate - cleft												
Cryptorchism												

Appendix 7 - Continued
Individual Visceral Findings in Fetuses
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number:	270055	270056	270057	270058	270059	270060	270061	270062	270063	270064	270065	270066	270067
Number of fetuses examined	4	5	4	5	4	NP	4	8	4	5	6	2	5
Number of fetuses within normal limits	4	5	4	5	4		4	8	3	5	6	2	5
Number of fetuses with one or more visceral variants	0	0	0	0	0		0	0	1	0	0	0	0
Percent of fetuses with one or more skeletal variants (%)	0.00	0.00	0.00	0.00	0.00		0.00	0.00	25.00	0.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:													
Brain - cerebral hemisphere contained dark area													
Eye(s) - contained dark material													
Liver - dark areas													
Kidney(s) - pelvis dilated													
Ureter(s) - distended													
- undulated									A				
Number of fetuses with one or more visceral anomalies	0	0	0	0	0		0	0	0	0	0	0	0
Percent of fetuses with one or more visceral anomalies (%)	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:													
Skin - edematous													
Palate - cleft													
Cryptorchism													

NP = Not Pregnant.

Appendix 7 - Continued
Individual Visceral Findings in Fetuses
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number:	270668	270669	270670	270671	270672	270673	270674	270675	270676	270677	270678	270679
Number of fetuses examined	5	5	6	5	6	6	5	5	4	5	5	NP
Number of fetuses within normal limits	5	5	5	5	4	6	4	4	3	4	5	
Number of fetuses with one or more visceral variants	0	0	1	0	2	0	1	1	1	1	0	
Percent of fetuses with one or more skeletal variants (%)	0.00	0.00	16.67	0.00	33.33	0.00	20.00	20.00	25.00	20.00	0.00	
Identification letter(s) of fetuses with:												
Brain - cerebral hemisphere contained dark area												
Eye(s) - contained dark material												
Liver - dark areas												
Kidney(s) - pelvis dilated					Q		A		A	G		
Ureter(s) - distended			A		N				A	G		
- undulated								A				
Number of fetuses with one or more visceral anomalies	0	0	0	0	1	0	0	0	0	0	0	
Percent of fetuses with one or more visceral anomalies (%)	0.00	0.00	0.00	0.00	16.67	0.00	0.00	0.00	0.00	0.00	0.00	
Identification letter(s) of fetuses with:												
Skin - edematous					M							
Palate - cleft												
Cryptorchism												

NP = Not Pregnant.

Appendix 7 - Continued
Individual Visceral Findings in Fetuses
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number:	270880	270881	270882	270883	270884	270885	270886	270887	270888	270889	270890	270891	270892
Number of fetuses examined	6	5	5	4	NP	6	5	5	5	4	4	5	6
Number of fetuses within normal limits	4	5	4	4		6	5	5	5	3	4	5	6
Number of fetuses with one or more visceral variants	2	0	1	0		0	0	0	0	1	0	0	0
Percent of fetuses with one or more skeletal variants (%)	33.33	0.00	20.00	0.00		0.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:													
Brain - cerebral hemisphere contained dark area													
Eye(s) - contained dark material													
Liver - dark areas													
Kidney(s) - pelvis dilated	J									A			
Ureter(s) - distended	A												
- undulated			N										
Number of fetuses with one or more visceral anomalies	0	0	0	0		0	0	0	0	0	0	0	0
Percent of fetuses with one or more visceral anomalies (%)	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:													
Skin - edematous													
Palate - cleft													
Cryptorchism													

NP = Not Pregnant.

Appendix 7 - Continued
Individual Visceral Findings in Fetuses
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number:	270393	270394	270395	270396	270397	270398	270399	271002	271001	271002	271003	271004
Number of fetuses examined	5	5	6	5	5	4	4	6	5	5	5	NP
Number of fetuses within normal limits	5	5	6	5	5	4	4	4	5	5	4	
Number of fetuses with one or more visceral variants	0	0	0	0	0	0	0	0	0	0	1	
Percent of fetuses with one or more skeletal variants (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.00	
Identification letter(s) of fetuses with:												
Brain - cerebral hemisphere contained dark area												
Eye(s) - contained dark material												
Liver - dark areas												
Kidney(s) - pelvis dilated												
Ureter(s) - distended											M	
- undulated												
Number of fetuses with one or more visceral anomalies	0	0	0	0	0	0	0	2	0	0	0	
Percent of fetuses with one or more visceral anomalies (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.00	0.00	0.00	
Identification letter(s) of fetuses with:												
Skin - edematous								A				
Palate - cleft												
Cryptorchism								AG				

NP = Not Pregnant.

Appendix 7 - Continued
Individual Visceral Findings in Fetuses
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number:	27105	27106	27107	27108	27109	27110	27111	27112	27113	27114	27115	27116	27117
Number of fetuses examined	5	4	4	5	0[a]	5	5	4[b]	2[c]	5[d]	5	4[e]	4[f]
Number of fetuses within normal limits	4	4	4	5		5	5	3	0	0	5	4	0
Number of fetuses with one or more visceral variants	1	0	0	0		0	0	0	0	0	0	0	0
Percent of fetuses with one or more skeletal variants (%)	20.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Identification letter(s) of fetuses with:													
Brain - cerebral hemisphere contained dark area													
Eye(s) - contained dark material	H												
Liver - dark areas													
Kidney(s) - pelvis dilated													
Ureter(s) - distended													
- undulated													
Number of fetuses with one or more visceral anomalies	0	0	0	0		0	0	1	2	5	0	0	4
Percent of fetuses with one or more visceral anomalies (%)	0.00	0.00	0.00	0.00		0.00	0.00	25.00	100.00	100.00	0.00	0.00	100.00
Identification letter(s) of fetuses with:													
Skin - edematous									BE	ADGJN			AEJM
Palate - cleft								A	BE	JN			AEJ
Cryptorchism													AEM

[a] All fetuses resorbed and/or dead.

[b] Fetus E was noted to have cleft palate during external examination.

[c] All fetuses were noted to have edematous skin during external examination. Fetuses B and E were confirmed at visceral examination. Fetuses C and E were noted to have cleft palate during external examination. Fetus E was confirmed at visceral examination.

[d] Fetuses H and N were noted to have edematous skin during external examination. Fetus N was confirmed at visceral examination. Fetuses H and I were noted to have cleft palate during external examination.

[e] Fetus E was noted to have cleft palate during external examination.

[f] All fetuses were noted to have edematous skin during external examination. Fetuses A, E, J, and M were confirmed at visceral examination. Fetuses A, J, and L were noted to have cleft palate during external examination. Fetuses A and J were confirmed at visceral examination.

Appendix 7 - Continued
Individual Visceral Findings in Fetuses
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number:	27118	27119	27120	27121	27122	27123	27124	27125	27126	27127	27128	27129
Number of fetuses examined	NP	0[a]	FD	5	4	5	5	5[b]	5	FD	NP	NP
Number of fetuses within normal limits				4	3	5	5	0	0			
Number of fetuses with one or more visceral variants				0	0	0	0	0	0			
Percent of fetuses with one or more skeletal variants (%)				0.00	0.00	0.00	0.00	0.00	0.00			
Identification letter(s) of fetuses with:												
Brain - cerebral hemisphere contained dark area												
Eye(s) - contained dark material												
Liver - dark areas												
Kidney(s) - pelvis dilated												
Ureter(s) - distended												
- undulated												
Number of fetuses with one or more visceral anomalies				1	1	0	0	5	5			
Percent of fetuses with one or more visceral anomalies (%)				20.00	25.00	0.00	0.00	100.00	100.00			
Identification letter(s) of fetuses with:												
Skin - edematous								ADGJM	BEHKN			
Palate - cleft					E			DJM	EHN			
Cryptorchism				M				G	BHK			

NP = Not Pregnant.
FD = Found Dead.

[a] All fetuses resorbed and/or dead.

[b] All fetuses were noted to have edematous skin during external examination. Fetuses A, D, G, J, and M were confirmed at visceral examination. Fetuses B, F, J, and L were noted to have cleft palate during external examination. Fetus J was confirmed at visceral examination.

Appendix 8
Individual Skeletal Findings in Fetuses[a]
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number:	270	271	272	273	274	275	276	277	278	279	280	281	282
Number of fetuses examined	10	10	9	8	9	11	9	NP	8	8	8	10	9
Number of fetuses within normal limits	5	6	6	7	4	8	7		5	8	0	7	6
Number of fetuses with one or more skeletal variants	5	4	3	1	5	3	2		3	0	8	3	3
Percent of fetuses with one or more skeletal variants (%)	50.00	40.00	33.33	12.50	55.56	27.27	22.22		37.50	0.00	100.00	30.00	33.33
Identification letter(s) of fetuses with:													
Lagging Ossification													
Skull - incomplete closure (<75%)											BGM		
- interparietal ossification incomplete/nonossified		DI	J		FH		L		JN		M		
- parietals ossification irregular													
- supraoccipital ossification incomplete/nonossified			J	N	FK				J		BM		
- supraoccipital nonfused													
- hyoid ossification incomplete/nonossified	FN	GI	DJ		FIK	H	B		J		GLM		
Pectoral girdle - scapula ossification incomplete													
Rib cage - sternebrae bipartite													
- sternebrae 1 and 2 fused													F
- sternebrae <3 ossified					EF								
- ribs <13 ossified[b]													
- ribs >13 ossified (cervical region)													
- ribs 13th pair small[b]	CFHI												
- ribs ossification irregular													
Vertebral column - sacral arches nonossified					E						M		
- thoracic centra ossification incomplete/ nonossified					K				B		JLM		FG
- thoracic centra nonfused			I			BF					BG	CKO	D
- caudals <3 ossified					E						BCFGIL		
											M		
Pelvic girdle - pubis ossification incomplete/ nonossified					FIK								
- ischium nonossified					F								
Forelimbs - <3 ossified											M		
Hindlimbs - <4 ossified					E								
Bone Alignment													
Ribs - angulated													
Sternebrae - checkered													

NP = Not Pregnant.
[a] There were no skeletal anomalies noted in any fetuses.
[b] Possible preparation damage at time of evisceration.

Appendix 8 - Continued
Individual Skeletal Findings in Fetuses[a]
Rat Teratology Study of T-3351
Group 1 - 0 mg/kg

Maternal Number:	270403	270404	270405	270406	270407	270408	270409	270501	270502	270503	270504
Number of fetuses examined	3	10	6	8	7	9	11	9	7	10	11
Number of fetuses within normal limits	1	8	5	7	3	5	7	8	6	9	1
Number of fetuses with one or more skeletal variants	2	2	1	1	4	4	4	1	1	1	10
Percent of fetuses with one or more skeletal variants (%)	66.67	20.00	16.67	12.50	57.14	44.44	36.36	11.11	14.29	10.00	90.91
Identification letter(s) of fetuses with:											
Lagging Ossification											
Skull - incomplete closure (<75%)	D						R				E
- interparietal ossification incomplete/nonossified	DE	H	C	L	F	IL		B		EHI	BCEHIK LNQ
- parietals ossification irregular											E
- supraoccipital ossification incomplete/nonossified	D	J			F	I	ILR			EI	BFHIKL Q
- supraoccipital nonfused											E
- hyoid ossification incomplete/nonossified										EI	BEFHN
Pectoral girdle - scapula ossification incomplete											
Rib cage - sternebrae bipartite											
- sternebrae 1 and 2 fused											
- sternebrae <3 ossified							R				HI
- ribs <13 ossified[b]											
- ribs >13 ossified (cervical region)											
- ribs 13th pair small[b]											
- ribs ossification irregular											E
Vertebral column - sacral arches nonossified											
- thoracic centra ossification incomplete/ nonossified					BCFL	FH	N		J	FIK	
- thoracic centra nonfused									F		
- caudals <3 ossified	D						R			H	HI
Pelvic girdle - pubis ossification incomplete/ nonossified		J					R				HIL
- ischium nonossified											
Forelimbs - <3 ossified											
Hindlimbs - <4 ossified							R				
Bone Alignment											
Ribs - angulated											E
Sternebrae - checkered											

[a] There were no skeletal anomalies noted in any fetuses.
[b] Possible preparation damage at time of evisceration.

Appendix B - Continued
Individual Skeletal Findings in Fetuses[a]
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number:	270505	270506	270507	270508	270509	270606	270601	270602	270603	270604	270605	270606	270607
Number of fetuses examined	8	10	9	9	8	NP	7	5	8	9	10	4	9
Number of fetuses within normal limits	6	9	7	5	1		2	4	0	6	5	1	5
Number of fetuses with one or more skeletal variants	2	1	2	4	7		5	1	8	3	5	3	4
Percent of fetuses with one or more skeletal variants (%)	25.00	10.00	22.22	44.44	87.50		71.43	20.00	100.00	33.33	50.00	75.00	44.44
Identification letter(s) of fetuses with:													
Lagging Ossification													
Skull - incomplete closure (<75%)					B				E		BFNO		IP
- interparietal ossification incomplete/nonossified		P			BEGHJK				CEHLM	EFK			P
- parietals ossification irregular					BEGJK				CE				I
- supraoccipital ossification incomplete/nonossified		P			BGHJ				BCEFH		B		P
- supraoccipital nonfused													
- hyoid ossification incomplete/nonossified	M	P	I		BEGHJK				BCEFHI		BF		I
Pectoral girdle - scapula ossification incomplete					M				M				
Rib cage - sternebrae bipartite	M												
- sternebrae 1 and 2 fused													
- sternebrae <3 ossified													
- ribs <13 ossified[b]													
- ribs >13 ossified (cervical region)													
- ribs 13th pair small[b]													
- ribs ossification irregular													
Vertebral column - sacral arches nonossified									CE				
- thoracic centra ossification incomplete/ nonossified	I		F	BFFO	K				L		H	DF	I
- thoracic centra nonfused							BCEHK		C		O	G	M
- caudals <3 ossified								D					
Pelvic girdle - pubis ossification incomplete/ nonossified													
- ischium nonossified													
Forelimbs - <3 ossified													
Hindlimbs - <4 ossified													
Bone Alignment													
Ribs - angulated													
Sternebrae - checkered					J				CEH				

NP = Not Pregnant.
[a] There were no skeletal anomalies noted in any fetuses.
[b] Possible preparation damage at time of evisceration.

Appendix 8 - Continued
Individual Skeletal Findings in Fetuses[a]
Rat Teratology Study of T-3351
Group 2 - 1 mg/kg

Maternal Number:	2707608	2707609	2707807	2707901	2708002	2708103	2708204	2708305	2708406	2708507	2708608	2708709
Number of fetuses examined	10	10	10	9	11	10	10	9	8	8	9	NP
Number of fetuses within normal limits	8	9	7	7	0	8	8	0	3	6	6	
Number of fetuses with one or more skeletal variants	2	1	3	2	11	2	2	9	5	2	3	
Percent of fetuses with one or more skeletal variants (%)	20.00	10.00	30.00	22.22	100.00	20.00	20.00	100.00	62.50	25.00	33.33	
Identification letter(s) of fetuses with:												
Lagging Ossification												
Skull - incomplete closure (<75%)					CEFHIKL							
- interparietal ossification incomplete/nonossified				H	BECFHKLNR			BCEFHJLMO	IL	L	CO	
- parietals ossification irregular					CEFIKL							
- supraoccipital ossification incomplete/nonossified				H	BCEFKLNR	N	F	FJMO	IL	L	C	
- supraoccipital nonfused												
- hyoid ossification incomplete/nonossified	I		BHI	H	BCEFLOR		P	CFHJLM	EIL	EL	IO	
Pectoral girdle - scapula ossification incomplete												
Rib cage - sternebrae bipartite												
- sternebrae 1 and 2 fused												
- sternebrae <3 ossified					R							
- ribs <13 ossified[b]									F			
- ribs >13 ossified (cervical region)												
- ribs 13th pair small[b]												
- ribs ossification irregular												
Vertebral column - sacral arches nonossified												
- thoracic centra ossification incomplete/nonossified	O	C		L	H		F	H	B		I	
- thoracic centra nonfused	I							EJ				
- caudals <3 ossified					HR	F		O				
Pelvic girdle - pubis ossification incomplete/nonossified												
- ischium nonossified												
Forelimbs - <3 ossified												
Hindlimbs - <4 ossified					R							
Bone Alignment												
Ribs - angulated												
Sternebrae - checkered												

NP = Not Pregnant.
[a] There were no skeletal anomalies noted in any fetuses.
[b] Possible preparation damage at time of evisceration.

Appendix B - Continued
Individual Skeletal Findings in Fetuses[a]
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number:	270801	270802	270803	270804	270805	270806	270807	270808	270809	270901	270902
Number of fetuses examined	10	8	8	8	NP	11	9	10	8	8	8	10	10
Number of fetuses within normal limits	8	5	7	7		8	7	1	8	7	6	6	6
Number of fetuses with one or more skeletal variants	2	3	1	1		3	2	9	0	1	2	4	4
Percent of fetuses with one or more skeletal variants (%)	20.00	37.50	12.50	12.50		27.27	22.22	90.00	0.00	12.50	25.00	40.00	40.00
Identification letter(s) of fetuses with:													
Lagging Ossification													
Skull - incomplete closure (<75%)													
- interparietal ossification incomplete/nonossified		B	H					BE			EL	O	O
- parietals ossification irregular								B					FJOP
- supraoccipital ossification incomplete/nonossified						H	O	B				BC	JO
- supraoccipital nonfused													
- hyoid ossification incomplete/nonossified	B					H	F	I				BCK	
Pectoral girdle - scapula ossification incomplete													
Rib cage - sternebrae bipartite						E		BEFHLO					
- sternebrae 1 and 2 fused													
- sternebrae <3 ossified								B					
- ribs <13 ossified[b]													
- ribs >13 ossified (cervical region)													
- ribs 13th pair small[b]													
- ribs ossification irregular													
Vertebral column - sacral arches nonossified													
- thoracic centra ossification incomplete/ nonossified	I	CH		D		HK		INO	C				
- thoracic centra nonfused								BCF					
- caudals <3 ossified								B					O
Pelvic girdle - pubis ossification incomplete/ nonossified													
- ischium nonossified													
Forelimbs - <3 ossified													
Hindlimbs - <4 ossified								B					
Bone Alignment													
Ribs - angulated													
Sternebrae - checkered													

NP = Not Pregnant.
[a] There were no skeletal anomalies noted in any fetuses.
[b] Possible preparation damage at time of evisceration.

Appendix 8 - Continued
Individual Skeletal Findings in Fetuses[a]
Rat Teratology Study of T-3351
Group 3 - 5 mg/kg

Maternal Number:	270903	270904	270905	270906	270907	270908	270909	271000	271001	271002	271003	271004
Number of fetuses examined	8	8	10	8	8	8	8	10	8	9	9	NP
Number of fetuses within normal limits	3	8	7	3	5	8	4	0	8	7	6	
Number of fetuses with one or more skeletal variants	5	0	3	5	3	0	4	10	0	2	3	
Percent of fetuses with one or more skeletal variants (%)	62.50	0.00	30.00	62.50	37.50	0.00	50.00	100.00	0.00	22.22	33.33	
Identification letter(s) of fetuses with:												
Lagging Ossification												
Skull - incomplete closure (<75%)								BCEFHI KLNO		N		
- interparietal ossification incomplete/nonossified	HK		FI					O			EFN	
- parietals ossification irregular			I									
- supraoccipital ossification incomplete/nonossified	CEI						L	BCEFHI KLNO		N	F	
- supraoccipital nonfused												
- hyoid ossification incomplete/nonossified				D			HL	LN			F	
Pectoral girdle - scapula ossification incomplete												
Rib cage - sternebrae bipartite					JN			BEFIK				
- sternebrae 1 and 2 fused												
- sternebrae <3 ossified								BLN				
- ribs <13 ossified[b]												
- ribs >13 ossified (cervical region)				DG								
- ribs 13th pair small[b]												
- ribs ossification irregular	K											
Vertebral column - sacral arches nonossified								LN				
- thoracic centra ossification incomplete/ nonossified				N			EK	ILN		L	N	
- thoracic centra nonfused				FI								
- caudals <3 ossified					D			BCEFHI KLNO		N		
Pelvic girdle - pubis ossification incomplete/ nonossified								BILN				
- ischium nonossified								LN				
Forelimbs - <3 ossified								LN				
Hindlimbs - <4 ossified								BEIKL				
Bone Alignment												
Ribs - angulated	IK		EI									
Sternebrae - checkered												

NP = Not Pregnant.
[a] There were no skeletal anomalies noted in any fetuses.
[b] Possible preparation damage at time of evisceration.

Appendix 8 - Continued
Individual Skeletal Findings in Fetuses[a]
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number:	270105	270106	270107	270108	270109	270110	270111	270112	270113	270114	270115	270116	270117
Number of fetuses examined	10	6	6	10	0[c]	9	10	8	4	8	10	7	7
Number of fetuses within normal limits	3	6	2	10		3	7	0	0	0	6	4	0
Number of fetuses with one or more skeletal variants	7	0	4	0		6	3	8	4	8	4	3	7
Percent of fetuses with one or more skeletal variants (%)	70.00	0.00	66.67	0.00		66.67	30.00	100.00	100.00	100.00	40.00	42.86	100.00
Identification letter(s) of fetuses with:													
Lagging Ossification													
Skull - incomplete closure (<75%)						B		BCEFHIKL	CDFO	BCEFHILM		L	BCFGKLN
- interparietal ossification incomplete/nonossified						B		CE	CDFO	EFHI			BCFGKLN
- parietals ossification irregular													
- supraoccipital ossification incomplete/nonossified						CH		BFHK	CDO	BCEFHILM		L	N
- supraoccipital nonfused								CEILH	F				BCFGKL
- hyoid ossification incomplete/nonossified								E				L	CGKLN
Pectoral girdle - scapula ossification incomplete	CFHILNO		CFI			BEFHK		BCFHIK	CDO	BCEFHIL	BFIN	E	BFGKL
Rib cage - sternebrae bipartite													
- sternebrae 1 and 2 fused													
- sternebrae <3 ossified													
- ribs <13 ossified[b]								CH					CGKN
- ribs >13 ossified (cervical region)													
- ribs 13th pair small[b]													
- ribs ossification irregular													
Vertebral column - sacral arches nonossified								CH	FO	I			CGKN
- thoracic centra ossification incomplete/nonossified			K			K		CH	FO	BI			
- thoracic centra nonfused							BH	K				C	
- caudals <3 ossified								BCEHIK	CDFO	CEFI			BCFGKLN
Pelvic girdle - pubis ossification incomplete/nonossified								CH		I			CFGKLN
- ischium nonossified								C					CN
Forelimbs - <3 ossified								BCEFHKL	CDFO	CEHI			BCFGKLN
Hindlimbs - <4 ossified													
Bone Alignment													
Ribs - angulated	HN							EF	CDFO				N
Sternebrae - checkered							O						

NP = Not Pregnant.
[a] There were no skeletal anomalies noted in any fetuses.
[b] Possible preparation damage at time of evisceration.
[c] All fetuses resorbed and/or dead.

Appendix 8 - Continued
Individual Skeletal Findings in Fetuses[a]
Rat Teratology Study of T-3351
Group 4 - 10 mg/kg

Maternal Number:	27118	27119	27120	27121	27122	27123	27124	27125	27126	27127	27128	27129
Number of fetuses examined	NP	0[c]	FD	10	8	10	10	8	9	FD	NP	NP
Number of fetuses within normal limits				4	5	10	3	0	0			
Number of fetuses with one or more skeletal variants				6	3	0	7	8	9			
Percent of fetuses with one or more skeletal variants (%)				60.00	37.50	0.00	70.00	100.00	100.00			
Identification letter(s) of fetuses with:												
Lagging Ossification												
Skull - incomplete closure (<75%)							B	BCEFHI KL	CDFIJL MO			
- interparietal ossification incomplete/nonossified					B		CJMP	BFIKL	I			
- parietals ossification irregular												
- supraoccipital ossification incomplete/nonossified				CIKO	N		BCMP	HK	CIJLMO			
- supraoccipital nonfused								BCEFIL				
- hyoid ossification incomplete/nonossified				CF				K				
Pectoral girdle - scapula ossification incomplete												
Rib cage - sternebrae bipartite				FIKO	M			BCEFHI L	CDFGIJ LMO			
- sternebrae 1 and 2 fused												
- sternebrae <3 ossified								CKL				
- ribs <13 ossified[b]												
- ribs >13 ossified (cervical region)												
- ribs 13th pair small[b]												
- ribs ossification irregular												
Vertebral column - sacral arches nonossified							B	CKL				
- thoracic centra ossification incomplete/ nonossified				C			BMP	L	IL			
- thoracic centra nonfused				HI								
- caudals <3 ossified							BI	BCEFHI KL	CJL			
Pelvic girdle - pubis ossification incomplete/ nonossified								BCHIKL				
- ischium nonossified								KL				
Forelimbs - <3 ossified								KL				
Hindlimbs - <4 ossified							B	BCEFHI KL	CIJLO			
Bone Alignment												
Ribs - angulated												
Sternebrae - checkered												

NP = Not Pregnant.
FD = Found Dead.
[a] There were no skeletal anomalies noted in any fetuses.
[b] Possible preparation damage at time of evisceration.
[c] All fetuses resorbed and/or dead.

Appendix 9
References
Rat Teratology Study of T-3351

STATISTICAL METHODS/LABORATORY PROCEDURES

Armitage, P., "Tests for Linear Trends in Proportions and Frequencies." Biometrics, 11:375-385, 1955.

Bartlett, M. S., "Some Examples of Statistical Methods of Research in Agriculture and Applied Biology." J. Royal Statist. Soc. Suppl., IV:137-170, 1937.

Cochran, W. G., "Some Methods of Strengthening the Common Chi-Square Tests." Biometrics, 10:417-451, 1954.

Cochran, W. G., and Cox, G. M., Experimental Designs, 2nd Ed., John Wiley and Sons, Inc., New York, 1957.

Draper, N. R., and Hunter, W. G., "Transformations: Some Examples Revisited." Technometrics, 11:23-40, 1969.

Dunnett, C. W., "A Multiple Comparison Procedure for Comparing Several Treatments with a Control." J. Am. Stat. Assoc., 50:1096-1121, 1955.

Dunnett, C. W., "New Tables for Multiple Comparisons with a Control." Biometrics, 20:482-491, 1964.

Fisher, R. A., Statistical Methods for Research Workers, Oliver & Boyd, Edinburgh, 1950.

Fleiss, J. L., Statistical Methods for Rates and Proportions, 2nd Ed., Wiley, New York, Ch. 9, 1981.

Jonckheere, A. R., "A Distribution-free k-sample Test Against Ordered Alteratives." Biometrika 41:133-45, 1954.

Kruskal, W. H. and Wallis, W. A., "Use of Ranks in One-Criterion Variance Analysis." J. Am. Stat. Assoc., 47:583-621, 1952.

Kruskal, W. H. and Wallis, W. A., "Errata to Use of Ranks in One-Criterion Variance Analysis." J. Am. Stat. Assoc., 48:907-911, 1953.

Levene, H., "Robust Tests for Equality of Variances," in I. Olkin edited, Contributions to Probability and Statistics, Stanford University Press, Palo Alto, 1960.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill, New York, 1966, Ch. 6.1.

Appendix 9 - Continued
References
Rat Teratology Study of T-3351

STATISTICAL METHODS/LABORATORY PROCEDURES

Staples, R. E., "Detection of Visceral Alterations in Mammalian Fetuses." Teratology, 9(3):A-37, 1974.

Staples, R. E. and Schnell, V. L., "Refinements in Rapid Clearing Technic in the KOH-Alizarin Red S Method for Fetal Bone." Stain Technology, 39:61-63, 1964.

Terpstra, T. J., "The Asymptotic Normality and Consistency of Kendall's Test Against Trend, When Ties are Present in One Ranking." Indag. Math. 14:327-333, 1952.

Wilson, J. G. and Warkany, J., editors. Teratology: Principles and Techniques, The Univ. of Chicago Press, Chicago, 1965, pp. 251-277.

Winer, B. J., Statistical Principles in Experimental Design, 2nd Ed., McGraw-Hill, N.Y., Chs. 3-9, 1971.

Winer, B. J., Statistical Principles in Experimental Design, 2nd Ed., McGraw-Hill, N.Y., Ch. 10, 1971.

JEFFERSON MEDICAL COLLEGE
of
THOMAS JEFFERSON UNIVERSITY

RECEIVED
1983

Daniel Baugh Institute
Department of Anatomy
Office of the
Director and Chairman

Philadelphia, 19107
(215) 928-7820

December 7, 1983

Mr. William C. McCormick, III
Toxicology Services, Medical Department
220-2E 3M Center
St. Paul, MN 55101

Dear Mr. McCormick:

I have received the draft report of T3351 from Dr. Wetzel and after careful review find it to be a clearly explained study. It is a state-of-the-art safety evaluation for developmental toxicity.

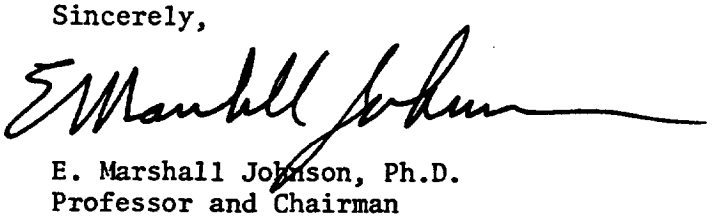
The data demonstrate severe maternal toxicity is evident both during and after treatment at both 5 and 10 mg T3351/kg/da and that the higher dosed group was the more severely affected. Their food intake was reduced and, not only did the dams not gain weight normally, actual maternal weight losses occurred in both these treatment groups. Numerous clinical signs of maternal distress and even hair loss were observed. There were directly related reductions in fetal body weights from these ill dams. However, for such ill mothers and small fetuses, the 5 mg group showed fewer skeletal delays than one might expect in such severely stressed mothers. No signs of either maternal or developmental toxicity occurred in the low dose group. In short, at dose levels severely maternally toxic and even lethal, T3351 exerts only a relative few of the adverse effects on the conceptus one would expect. At a dose level below that producing maternal death, but numerous other signs of maternal toxicity, there were only minor effects on the rate of fetal development and, at the all important maternally nontoxic dose level, embryonic and fetal development were absolutely normal.

This study clearly illustrates the safety of the test substance for the conceptus. The mother appears more vulnerable to T3351 than the conceptus and one would conclude that as long as exposures are kept below adult toxic levels, embryonic development would also not be affected.

This study of T3351 makes a clear statement of a no effect level and fetal safety. You have gone the extra mile and are to be congratulated for your

high motivation. I cannot envision a customer or a regulatory agency being confused by, or having reservations regarding, this data base clearly establishing T3351 to not represent a developmental hazard to the conceptus.

Sincerely,



E. Marshall Johnson, Ph.D.
Professor and Chairman
Director, Daniel Baugh Institute

EMJ:amf
cc Dr. Lawrence T. Wetzel, Ph.D.
Department of Toxicology
Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22180

Oral Teratology Study of FC-95 in Rats

Experiment No.:	0680TR0008
Conducted At:	Safety Evaluation Laboratory Riker Laboratories, Inc. St. Paul, Minnesota
Dosing Period:	July 14, 1980 through July 24, 1980
Study Director:	E. G. Gortner

E. G. Gortner 12/17/80

E. G. Gortner Date
Senior Research Technologist
Animal Reproduction-Teratology
Study Director

Eldon G. Lamprecht 12/17/80

E. G. Lamprecht Date
Research Veterinary Pathologist

Marvin T. Case 12/18/80

M. T. Case, DVM, PhD Date
Manager, Pathology-Toxicology
Safety Evaluation Laboratory

Summary

Oral administration of FC-95 at doses of 10, 5 and 1 mg/kg/day to pregnant Sprague-Dawley rats during days 6 through 15 of gestation (period of organogenesis) resulted in fetuses with teratogenic changes in the lens of the eye. The teratogenic effect was a developmental eye abnormality which appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus, followed by secondary aberrations of the secondary lens fibers of the fetal nucleus. The lens abnormality occurred in all FC-95 dose groups, but the proportion of fetuses with the lens changes was significantly higher than the control group only in the 10 mg/kg/day group.

FC-95 administration was maternally toxic only to the 10 mg/kg/day group. At gestation days 12 through 20 their mean maternal body weights were significantly lower than the controls. FC-95 was not maternally toxic to the 5 and 1 mg/kg/day groups.

FC-95 was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams. The compound did not produce an increase in the number or proportion of abnormal fetal skeleton aberrations.

Introduction

This teratology study[a] of FC-95 in rats was conducted to evaluate the embryotoxic and teratogenic effects of orally administered FC-95. The study was sponsored by 3M Commerical Chemical Division, St. Paul, Minnesota and was conducted by the Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota. The compound administration period was from July 14 through July 24, 1980. The protocol and list of the principal participants and supervisory personnel can be found in Appendices I and II respectively.

All portions of this study were conducted according to the Good Laboratory Practice (GLP) regulations and the Safety Evaluation Laboratory Standard Operating Procedures (see Appendix III for Quality Assurance Unit statement). The storage location for specimens, raw data and a copy of the final report is maintained in the Safety Evaluation Laboratory's record archives.

Methods

Time mated Spraque-Dawley derived rats were obtained from Charles River Breeding Laboratory and assigned cages according to a computer-generated random numbers table. The rats were then divided into four groups of 22 animals weighing 175 to 261 grams. The rats were housed individually in hanging stainless steel cages with wire mesh floors and fronts in a temperature and humidity controlled room. Food[b] and water were available ad libitum. The lights were on a 12 hour light/dark cycle.

The animals were observed daily from day 3 through day 20 of gestation for abnormal clinical signs. Body weights were recorded on days 3, 6, 9, 12, 15 and 20 of gestation and the rats dosed accordingly using a constant dose volume of 5 ml/kg of body weight. The four groups were dosed with FC-95 (Lot 640) suspended daily in corn oil at 0, 10, 5 or 1 mg/kg/day. FC-95 was administered daily by oral intubation with a syringe equipped with a ball-tipped intubation needle to the rats on days 6 through 15 of gestation (day 0 indicated by sperm-positive vaginal smear). FC-95 analytical characterization (see Appendix IV) was provided by 3M Commercial Chemical Division, St. Paul, Minnesota.

All animals were sacrificed on day 20 by cervical dislocation and the ovaries and uterus, including its contents, were examined immediately to determine the following: number of corpora lutea, number of viable fetuses, number of resorption sites, pup weights and sex, and any gross fetal abnormalities. Approximately one-third of the fetuses were fixed in Bouin's solution for subsequent free-hand sectioning by the Wilson technique to determine visceral abnormalities. The remaining fetuses were preserved in alcohol for clearing and staining of the skeleton with alizarin red to detect skeletal abnormalities. Selected free-hand sections were processed for histological evaluation.

[a] Riker Experiment No. 0680TR0008
[b] Purina Laboratory Chow, Ralston Purina Company, St. Louis, MO

Results

FC-95 administered during the period of organogenesis was toxic to the high dose group (10 mg/kg/day) maternal rats. The mean body weights of all dose groups were similar at gestation days three through nine (Table 1, Appendix V). At gestation days 12 through 20 the high dose group rats weighed significantly less than controls (0 mg/kg/day). The mean maternal body weights of mid (5 mg/kg/day) and low (1 mg/kg/day) dose groups were not different from the controls throughout the study. Even though FC-95 was maternally toxic at the high dose level, no compound-related clinical signs were observed in any of the dose groups.

FC-95 was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams. The mean number of male, female, total and dead fetuses; the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights of the three FC-95 dose groups were not significantly different from the controls (Table 2, Appendix VI). The high dose group did have a lower mean number of viable male, female and total fetuses than the other three groups which resulted from a lower number of embryos at the start of the study. Contributing pieces of evidence to the lower number of high dose embryos are the low mean number of implantation sites, corpora lutea, resorption sites and the absence of dead fetuses.

FC-95 did not cause compound-related abnormal gross fetal findings (Table 3), nor did FC-95 treatment produce an increase in the number or proportion of abnormal fetal skeletal aberrations. Fetal skeleton results of the three compound treated groups were not significantly different from the control group (Table 4). The incidence and proportions of sternebrae nonossified and associated changes of sternebrae assymetrical, sternebrae bipartite and one sternebrae missing were unusually high in all dose groups of this study including the control group.

FC-95 was teratogenic in the rat at all dose levels administered in this study. The teratogenic effect was a developmental eye abnormality which appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus, followed by secondary aberrations of the secondary lens fibers of the fetal nucleus. All eye abnormalities were localized to the area of the embryonal lens nucleus although a variety of morphological appearances were present within that location. The range of morphological appearances as observed under the dissecting microscope varied from a slight discoloration of the lens near the anterior margin to a dark colored oval area, often containing a cleft, extending from beneath the lens epithelium to half-way through the lens posteriorly. Histologically the discolorations were due to presence of lens vesicle remnants surrounding the abnormal embryonal lens nucleus. One of the most severly affected eyes had most of the embryonal lens nucleus replaced by sinus spaces containing red blood cells. Also contributing to the discolorations were primary lens fibers which appeared to have not elongated. These lens fibers were tortuous and lacked nuclei in a normal lens bow of nuclei. Secondary lens fiber development progressed normally except immediately surrounding the abnormal embryonal nucleus. Secondary aberrations of secondary lens fibers included the bending of the fibers around the abnormal oval area, the subsequent formation of prominant anterior and posterior Y sutures of the converging fibers and lens vesicle remnants surrounding the embryonal nucleus.

The lens abnormality occurred in all dose groups except the control group. The proportion of fetuses with the lens abnormality in one or both lenses was significantly higher in the high dose group than the control (Table 5). The lens abnormality recorded for one control fetus under the dissecting microscope was an artifact when evaluated by transmission light microscopy. A no-effect dose level for the teratogenic lens abnormality was not established in this study.

Discussion

Optimal visual functional requirements are met during embryonic development by the differentiation of highly specialized populations of cells from undifferentiated precoursers and by the coordinated morphogenesis of the resulting tissues. Both processes are controlled to a remarkable extent by interactions which occur among emerging tissues. Each tissue of the eye is brought to its final state of differentiation, its cell population size and its definitive geometry, not only by intrinsic processes, but also by extrinsic influences exerted by neighboring tissues[1].

The embryonal origin of the lens is undifferentiated ectoderm. The tip of the optic vesicle, presumably the neural retina, plays the final role in inducing lens from overlying ectoderm and in aligning the lens precisely with the rest of the eye. Alternative or sequential action of tissues derived from endoderm (foregut) and mesoderm (heart) on the same target tissue decreases the probability that lens formation would be aborted by accidents during the early phases of induction. While the nature of the inductive influence remains unknown, there are indications that substances may be transferred from the presumptive neural retina to the overlying ectoderm during induction. A prolonged period of inductive interaction not only increases the probabililty that lens induction will occur successfully in the face of interference, but provides a mechanism for continuously adjusting the size, shape, position and orientation of the lens to that of the retina[2].

During the early stages of the inductive process, the ectodermal cells immediately overlying the tip of the optic vesicle elongate prependicularly to the body surface to form a thickened disc (lens placode). The change in cell shape is accomplished without change in cell volume. The number of cells, however, continues to increase during this period. Toward the end of lens placode formation, acidophilic fibrils appear in the apices of the lens placode cells. At about this time, the placode invaginates to form the lens cup. This invagination is independent of the concomitant invagination of the underlying optic vesicle, and is probably due to forces operating within the lens ectoderm. As the lens cup deepens, its opening (lens pore) becomes progressively constricted until its lips meet and fuse, cutting off the lens vesicle internally and re-establishing continuity in the overlying ectoderm. Closure of the lens pore is attended by, and possibly accomplished by, a local and temporaty restricted wave of cell death. Following closure of the lens pore, the cells at the back of the lens vesicle continue to elongate, under the influence of the neural retina, to form the lens fibers. As the fibers grow the cavity of the lens vesicle is obliterated. The lens cells toward the ectoderm, which do not elongate further, form the lens epithelium[2].

The cuboidal lens epithelial cells which face the cornea continue to grow after the lens vesicle forms. As the cells rotate through the equator region, they take their places on the surface of the growing fiber mass. These cells differentiate into secondary lens fibers at the equator and elongate rapidly toward the poles of the lens where they meet with other fibers in planes of junction called sutures. As secondary fibers grow their nuclei become positioned at about the center of the fibers and form a convex lens bow outward. Since the newer fibers are always deposited superficially, the oldest fibers in the lens come to lie centrally and are referred to collectively as the lens nucleus. With time the lens cell nuclei in this region become pycnotic and finally disappear. The cell fibers, however, are not broken down and removed but remain in place. Thus the size and shape of the lens are controlled by factors which control the number, size and shape of lens cells[2].

The teratogenic effect of FC-95 probably occurred during the portion of organogenesis between differentiation of lens tissue from ectoderm and the formation of secondary lens fibers surrounding the embryonal lens nucleus. The exact time of the teratogenic insult and the morphogenesis of the abnormality were not determined in the study. The developmental lens abnormality appears to be unique because it has not been described as a compound-related abnormality[3]. A similar-appearing structural lens abnormality has been reported to occur spontaneously in rat fetuses, but with a very low incidence of 1.2%[4]. The abnormality resembles the Fraser developmental lens abnormality of a mutant mouse strain which results from degenerative primary lens cells[5].

References

1. Coulombre AJ, Coulombre JL: Abnormal Organogenesis of the Eye, in Wilson J,, Fraser FC (eds): Handbook of Teratology:2 Mechanisms and Pathogenesis. New York, Plenum Press, 1977, pp 329-341.

2. Coulombre AJ: The Eye, in DeHaan RL, Ursprung H (eds): Organogenesis. New York, Holt Rinehart and Winston, 1965, pp 227-232.

3. Mann I: Development Abnormalities of the Eye, 2nd ed. Philadelphia, JB Lippincott Co., 1957.

4. Weisse I, Niggeschulze A, Stotzer H: Spontaneous congenital cataracts in rats, mice and rabbits. Archiv Fuer Toxikologie 32: pp 199-207, 1974.

5. Hamai Y, Kuwabara T: Early cytologic changes of Fraser cataract. An electron microscopic study. Investigative Ophthalmology 14 (7): pp 517-527, 1975.

Table 1

Oral Teratology Study of FC-95 in Rats
Mean Maternal Body Weights with Standard Deviations

Dose Group		Gestation Day 3	6	9	12	15	20
0 mg/kg/day	MEAN	200	223	247	272	305	380
	STAN. DEV	16.7	17.6	20.9	20.5	24.4	33.8
10 mg/kg/day	MEAN	199	223	243	257[a]	277[a]	343[a]
	STAN. DEV	11.8	13.8	18.2	16.2	18.6	34.6
5 mg/kg/day	MEAN	205	228	249	268	294	373
	STAN. DEV	20.0	16.4	12.6	13.2	17.8	23.8
1 mg/kg/day	MEAN	205	226	252	272	303	379
	STAN. DEV	18.8	19.1	19.7	19.5	24.6	31.8

[a] Significantly lower than the controls (Dunnett's t test $p < 0.05$)

Table 2

Oral Teratology Study of FC-95 in Rats
Mean Litter Data and Pup Weights with
Standard Deviations[a]

Dose Group	No. of Animals	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPORA LUTEA	MEAN WT. FETUS(G)
0 mg/kg/day	20	5.2	4.9	10.0	0.0	0.7	10.8	11.2	4.3
		1.7	2.1	2.3	0.0	0.9	2.5	2.7	0.4
10 mg/kg/day	17	3.8	3.9	7.7	0.0	0.4	8.1	9.2	4.3
		2.8	2.6	4.3	0.0	0.6	4.3	3.1	0.3
5 mg/kg/day	17	5.0	5.5	10.5	0.0	0.7	11.2	11.1	4.2
		1.9	2.0	2.2	0.0	1.0	2.2	2.0	0.3
1 mg/kg/day	19	4.7	5.4	10.1	0.1	0.4	10.6	10.9	4.2
		1.7	2.1	2.8	0.2	0.8	2.7	2.6	0.4

[a] Treatment groups were not significantly different from controls (Dunnett's t test $p < 0.05$)

Table 3

Oral Teratology Study of FC-95 in Rats
Number of Fetuses with Gross Findings[a]

Finding	0 mg/kg/day	10 mg/kg/day	5 mg/kg/day	1 mg/kg/day
No. of fetuses examined	201	131	178	192
Umbilical hernia	1	---	1	---
Runted	---	1	---	1
Total Normal Fetuses	200	130	177	191
Total Abnormal Fetuses	1	1	1	1

[a] Treatment groups were not significantly different from the control (Chi-square $p < 0.05$)

Table 4

Oral Teratology Study of FC-95 in Rats
Number and Percent of Fetuses with Skeleton Findings[a]

Skeleton Finding	0 mg/kg/day	10 mg/kg/day	5 mg/kg/day	1 mg/kg/day
Fontanelle not closed	10 (7)	10 (11)	7 (6)	5 (4)
Frontal nonossified	4 (3)	1 (1)	---	1 (1)
Parietal nonossified	2 (1)	1 (1)	1 (1)	1 (1)
Interparietal nonossified	3 (2)	---	---	1 (1)
Occipital nonossified	1 (1)	1 (1)	---	---
Sternebrae nonossified	114 (81)	77 (85)	100 (81)	107 (81)
Sternebrae asymmetrical	53 (38)	23 (25)	36 (29)	39 (29)
Sternebrae bipartite	7 (5)	4 (4)	5 (4)	6 (5)
One sternebrae missing	30 (21)	13 (14)	26 (21)	26 (20)
Two sternebrae missing	10 (7)	2 (2)	4 (3)	6 (5)
13 ribs	5 (4)	2 (2)	2 (2)	6 (5)
13 ribs spurred	7 (5)	7 (8)	8 (7)	4 (3)
Wavy ribs	1 (1)	2 (2)	---	1 (1)
Protrusion on ribs	6 (4)	9 (10)	3 (2)	8 (6)
One body of the vertebrae bipartite	32 (23)	21 (23)	25 (20)	32 (24)
Two bodies of the vertebrae bipartite	18 (13)	7 (8)	11 (9)	9 (7)
Three bodies of the vertebrae bipartite	4 (3)	1 (1)	1 (1)	3 (4)
Four bodies of the vertebrae bipartite	---	---	---	1 (1)
Total No. Normal Fetuses	7 (5)	3 (3)	10 (8)	10 (8)
Total No. Abnormal Fetuses	133 (95)	88 (97)	113 (92)	123 (92)
Total No. of Fetuses Examined	140	91	123	133

[a] Treatment groups were not significantly different from the control (Chi-square $p < 0.05$)
( ) = percent of total examined

Table 5

Oral Teratology Study of FC-95 in Rats
Number and Percent of Fetuses with Internal Findings

Internal Finding	0 mg/kg/day	10 mg/kg/day	5 mg/kg/day	1 mg/kg/day
Eye abnormality	1[a] (2)	14[b] (35)[c]	4[b] (7)	2[b] (3)
Thoracic cavity full of blood	---	1 (3)	---	---
Enlarged atria	1 (2)	---	---	---
Enlarged renal pelvis area in the kidney	3 (5)	---	---	3 (5)
Abdominal cavity full of blood	4 (7)	2 (5)	5 (9)	2 (3)
Total No. Normal Fetuses	52 (85)	23 (57)	47 (85)	53 (90)
Total No. Abnormal Fetuses	9 (15)	17 (43)	8 (15)	6 (10)
Total No. of Fetuses Examined	61	40	55	59

[a] Eye abnormality was an artifact and was not considered for statistical evaluations
[b] Eye abnormalities were developmental lens abnormalities with secondary lens aberrations
[c] Significantly higher than the control (Chi-square $p < 0.05$)
( ) = percent of total examined

Appendix I

Oral Teratology Study of FC-95 in Rats
Protocol

Objective

A teratology study will be used to evaluate the embryotoxic and teratogenic effects of orally administered FC-95 to pregnant rats during the period of organogenesis. The procedure complies with the general recommendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study will be conducted according to the 1978 Good Laboratory Practice regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

Sponsor

3M Commercial Chemical Division, St. Paul, Minnesota.

Testing Facility

Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota.

Study Director

E. G. Gortner

Start of Dosing

Mid July, 1980.

Test System

Eighty-eight sexually mature, time mated Sprague-Dawley derived female rats from Charles River Breeding Laboratory will be housed in hanging stainless steel cages with wire mesh floors and fronts in a temperature and humidity controlled room. This strain of rats will be used because of historical control data and time mated females are readily available. Purina Laboratory Chow and water will be available ad libitum. The lights will be on a 12 hour light/dark cycle.

Test System Identification

Each animal will be ear tagged and that number will be indicated on the outside of the cage.

Appendix I (Continued)

Randomization

The animals will be assigned cages according to a computer-generated random numbers table.

Control Article

Corn oil.

Test Article

FC-95.

Analytical Specifications

The test article, composition and purity will be determined by the Sponsor (3M Commercial Chemical group) prior to the start of the study and at the end of dosing.

Dosage Levels and Experiment Design

The test article will be suspended in corn oil daily. The test article suspension and control article will be administered by oral intubation to the rats on days 6 through 15 of gestation according to the following:

Dose Group	Dose Level	Group Size
High	10 mg/kg/day	22 ♀
Mid	5 mg/kg/day	22 ♀
Low	1 mg/kg/day	22 ♀
Control	0 mg/kg/day	22 ♀

The oral route of administration will be used because of metabolism studies showed radiolabeled FC-95 was well absorbed. No dietary contaminants are known to interfere with the test article.

The animals will be observed daily from day 3 through day 20 of gestation for abnormal clinical signs. Body weights will be recorded on days 3, 6, 9, 12, 15 and 20 of pregnancy and the rats dosed accordingly using a constant dose volume of 5 ml/kg of body weight.

The females will be killed on day 20 and the ovaries, uterus and its contents will be examined to determine: number of corpora lutea, number of fetuses (live and dead), number of resorption sites, number of implantation sites, pup weight and gross abnormalities. Approximately one-third of the pups will be fixed in Bouin's solution for subsequent free-hand sectioning by the Wilson technique to determine any visceral abnormalities using a dissecting

Appendix I (Concluded)

microscope. The remaining approximately two-thirds of the pups will be fixed in ethyl alcohol for subsequent skeletal examination after clearing and staining with alizarin red.

Data Analysis and Final Report

The proposed statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities. The proposed date for the final report is 2-3 months after detailed pup examinations have been completed (approximately fourth quarter, 1980).

Appendix II

Oral Teratology Study of FC-95 in Rats
List of Principal Participating Personnel

NAME	FUNCTION
Edwin G. Gortner	Study Director
Elden G. Lamprecht	Veterinary Pathologist
Cathy E. Ludemann	Coordinator-Histology
Gary C. Pecore	Supervisor-Animal Care
Loren O. Wiseth	Technician

STATEMENT OF QUALITY ASSURANCE

STUDY NUMBER: 0680TR0008

TITLE: Oral Teratology Study of FC-95 in Rats

Audits and/or inspections were performed by the Riker Quality Assurance Unit for the above titled study, and reported to the study director and to management as follows:

Date Performed	Date Reported
18 July 1980	21 July 1980
28 July 1980	28 July 1980
15 December 1980	17 December 1980
17 December 1980	17 December 1980

J. E. Orterstrom
Laboratory Quality Assurance
Riker Laboratories, Inc.

December 17, 1980
Date

APPENDIX IV

Test and/or Control Article Characterization

for

FC-95, Lot 640

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of May 8, 1980.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

   yes X no ____

3. The stability of the test and/or control substances have been determined or will be determined as of Completion of Tox Testing If Necessary

The above information and documentation are located in the sponsor's records.

D. Rocker 5/21/80

Sponsor Date

Form 19793 PWO

Appendix V

Oral Teratology Study of FC-95 in Rats
Individual Body Weights (g)

Dose Group and Rat No.	Study Day 3	6	9	12	15	20
0 MG/KG/DAY						
N0R 12996	186	212	235	254	287	357
N0R 12997	224	261	239	306	345	424
N0R 12998	216	238	240	277	315	397
N0R 12999	212	232	271	274	302	373
N0R 13000	224	250	245	307	335	435
N0R 13016	182	207	284	255	284	342
N0R 13018	175	201	259	246	277	354
N0R 13019	193	219	237	277	309	378
N0R 13020	194	221	236	277	319	400
N0R 13036	205	228	222	284	322	408
N0R 13040	186	208	233	261	293	381
N0R 13041	195	219	285	258	289	355
N0R 13043	220	239	253	295	340	426
N0R 13044	207	228	284	273	296	359
N0R 13060	230	248	235	310	349	442
N0R 13061	195	212	213	259	297	366
N0R 13062	210	229	222	272	302	362
N0R 13063	185	208	247	257	289	342
N0R 13064	188	211	250	256	289	368
N0R 13080	179	194	258	238	256	321
MEAN	200	223	247	272	305	380
STAN. DEV	16.7	17.6	20.9	20.5	24.4	33.8
NON PREGNANT ANIMALS						
N0R 13017	186	198	243	217	234	253
N0R 13042	188	209	260	247	255	272

Appendix V (Continued)

Oral Teratology Study of FC-95 in Rats
Individual Body Weights (g)

Dose Group and Rat No.	Study Day 3	6	9	12	15	20
10 MG/KG/DAY						
00R 13001	189	222	239	253	281	347
00R 13002	190	217	230	256	283	356
00R 13003	192	222	224	265	290	381
00R 13004	193	212	218	233	255	319
00R 13005	201	225	260	261	285	369
00R 13021	227	257	247	278	293	360
00R 13022	212	244	243	288	311	402
00R 13023	180	206	258	227	245	285
00R 13025	208	237	251	268	297	382
00R 13037	187	214	229	250	274	357
00R 13045	195	216	228	259	289	361
00R 13048	186	205	226	236	248	311
00R 13065	204	223	274	263	279	304
00R 13066	207	226	275	263	262	358
00R 13067	210	234	222	268	278	322
00R 13069	203	228	262	262	283	338
00R 13081	194	209	238	237	251	281
MEAN	199	223	243	257	277	343
STAN. DEV	11.8	13.8	18.2	16.2	18.6	34.6
NON PREGNANT ANIMALS						
00R 13024	195	217	233	230	242	252
00R 13046	187	209	242	228	232	231
00R 13047	184	201	244	221	233	235
00R 13049	213	237	243	250	251	266
00R 13068	216	232	236	239	250	261

Appendix V (Continued)

Oral Teratology Study of FC-95 in Rats
Individual Body Weights (g)

Dose Group and Rat No.	Study Day 3	6	9	12	15	20
5 MG/KG/DAY						
P0R 13006	192	218	233	258	272	340
P0R 13007	226	249	272	264	304	388
P0R 13008	197	225	262	262	288	394
P0R 13009	188	212	274	254	283	361
P0R 13010	194	220	245	263	282	343
P0R 13027	212	232	251	269	288	369
P0R 13028	215	235	228	274	294	383
P0R 13029	199	229	241	272	288	366
P0R 13030	176	210	260	270	294	379
P0R 13038	198	219	235	263	288	366
P0R 13050	188	204	239	246	265	333
P0R 13051	222	243	256	283	323	407
P0R 13053	235	248	242	291	325	408
P0R 13054	197	224	238	259	279	349
P0R 13070	254	266	245	297	327	410
P0R 13071	200	223	250	274	304	378
P0R 13072	188	211	260	256	292	363
MEAN	205	228	249	268	294	373
STAN. DEV	20.0	16.4	12.6	13.2	17.8	23.8
NON PREGNANT ANIMALS						
P0R 13026	217	235	294	252	252	261
P0R 13052	218	237	252	248	254	262
P0R 13073	206	231	250	250	244	259
P0R 13074	207	234	244	257	272	287
P0R 13082	195	214	240	225	232	240

Appendix V (Concluded)

Oral Teratology Study of FC-95 in Rats
Individual Body Weights (g)

Dose Group and Rat No.	Study Day 3	6	9	12	15	20
1 MG/KG/DAY						
00R 13011	198	224	250	261	288	367
00R 13012	217	235	248	282	310	386
00R 13013	183	204	230	267	300	379
00R 13014	198	221	224	272	301	376
00R 13015	200	228	253	284	326	413
00R 13031	234	258	241	300	332	411
00R 13032	195	220	246	255	276	322
00R 13033	204	230	244	289	320	407
00R 13034	193	226	254	262	286	355
00R 13035	185	201	236	251	271	352
00R 13039	225	252	302	301	334	416
00R 13055	201	220	232	261	303	379
00R 13056	204	223	259	263	286	371
00R 13057	196	211	236	250	268	333
00R 13059	201	224	264	270	301	375
00R 13075	185	206	283	257	291	362
00R 13077	198	215	263	262	296	363
00R 13078	208	226	243	262	297	374
00R 13083	261	279	278	323	368	459
MEAN	205	226	252	272	303	379
STAN. DEV	18.8	19.1	19.7	19.5	24.6	31.8
NON PREGNANT ANIMALS						
00R 13058	183	205	247	226	238	253
00R 13076	192	213	236	254	278	270
00R 13079	196	218	228	244	255	266

Appendix VI

Oral Teratology Study of FC-95 in Rats
Individual Litter Data with Pup Weights

Dose Group and Rat No.	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPRA LUTEA	MEAN FETUS WT (GM) AVG	M	F
0 mg/kg/day										
N0R 12996	5	4	9	0	1	10	9	4.9	5.1	4.7
N0R 12997	4	9	13	0	1	14	16	3.6	3.8	3.5
N0R 12998	7	4	11	0	0	11	12	4.3	4.3	4.2
N0R 12999	7	4	11	0	2	13	13	4.0	4.1	3.9
N0R 13000	7	7	14	0	0	14	17	4.1	4.2	4.0
N0R 13016	4	5	9	0	1	10	9	3.7	3.3	4.0
N0R 13017	NOT PREGNANT									
N0R 13018	7	3	10	0	1	11	11	4.5	4.5	4.3
N0R 13019	6	1	7	0	0	7	6	5.1	5.1	4.9
N0R 13020	4	8	12	0	0	12	12	4.7	4.8	4.7
N0R 13036	5	5	10	0	1	11	8	4.1	4.3	3.9
N0R 13040	6	7	13	0	0	13	12	4.4	4.5	4.2
N0R 13041	6	3	9	0	1	10	12	4.2	4.3	4.1
N0R 13042	NOT PREGNANT									
N0R 13043	4	6	10	0	3	13	15	4.2	4.4	4.1
N0R 13044	5	2	7	0	0	7	11	3.9	3.8	3.9
N0R 13060	8	5	13	0	1	14	12	4.1	4.1	3.9
N0R 13061	4	6	10	0	2	12	11	4.2	4.3	4.1
N0R 13062	3	4	7	0	0	7	9	4.1	4.4	3.8
N0R 13063	1	5	6	0	0	6	9	4.3	4.3	4.3
N0R 13064	5	7	12	0	0	12	12	4.2	4.2	4.2
N0R 13080	6	2	8	0	1	9	9	4.4	4.4	4.2
MEAN	5.2	4.9	10.0	0.0	0.7	10.8	11.2	4.3		
STAN. DEV.	1.7	2.1	2.3	0.0	0.9	2.5	2.7	0.4		

Appendix VI (Continued)

Oral Teratology Study of FC-95 in Rats
Individual Litter Data with Pup Weights

Dose Group and Rat No.	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPRA LUTEA	MEAN FETUS WT(G) AVG	M	F
10 mg/kg/day										
00R 13001	4	6	10	0	1	11	10	4.5	4.6	4.5
00R 13002	3	6	9	0	2	11	11	3.9	4.1	3.8
00R 13003	4	7	11	0	0	11	12	4.2	4.4	4.1
00R 13004	7	2	9	0	0	9	9	4.2	4.2	4.0
00R 13005	5	7	12	0	0	12	12	4.3	4.3	4.2
00R 13021	1	3	4	0	0	4	7	4.4	4.4	4.4
00R 13022	11	2	13	0	0	13	14	4.2	4.2	3.8
00R 13023	2	0	2	0	0	2	5	4.8	4.8	0.0
00R 13024	NOT PREGNANT									
00R 13025	6	6	12	0	0	12	12	4.4	4.5	4.4
00R 13037	5	5	10	0	0	10	12	4.3	4.4	4.2
00R 13045	4	6	10	0	1	11	11	4.5	4.6	4.4
00R 13046	NOT PREGNANT									
00R 13047	NOT PREGNANT									
00R 13048	5	3	8	0	1	9	8	4.2	4.3	3.9
00R 13049	NOT PREGNANT									
00R 13065	0	1	1	0	0	1	6	4.3	0.0	4.3
00R 13066	4	8	12	0	0	12	11	4.0	4.2	3.9
00R 13067	1	1	2	0	1	3	8	4.6	4.8	4.5
00R 13068	NOT PREGNANT									
00R 13069	2	3	5	0	1	6	6	3.5	3.3	3.7
00R 13081	0	1	1	0	0	1	3	4.1	0.0	4.1
MEAN	3.8	3.9	7.7	0.0	0.4	8.1	9.2	4.3		
STAN. DEV.	2.8	2.6	4.3	0.0	0.6	4.3	3.1	0.3		

Appendix VI (Continued)

Oral Teratology Study of FC-95 in Rats
Individual Litter Data with Pup Weights

Dose Group and Rat No.	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPORA LUTEA	MEAN FETUS WT(GM) AVG	M	F
5 mg/kg/day										
P0R 13006	3	3	6	0	3	9	9	4.6	4.8	4.4
P0R 13007	9	3	12	0	0	12	12	4.3	4.4	4.0
P0R 13008	5	7	12	0	0	12	12	3.9	4.0	3.8
P0R 13009	5	4	9	0	0	9	8	3.8	4.1	3.5
P0R 13010	4	7	11	0	0	11	12	3.9	4.0	3.8
P0R 13026	NOT PREGNANT									
P0R 13027	8	3	11	0	2	13	10	4.0	4.1	3.8
P0R 13028	4	8	12	0	0	12	13	4.3	4.4	4.3
P0R 13029	4	3	7	0	0	7	10	4.8	5.2	4.3
P0R 13030	4	9	13	0	1	14	14	4.5	4.5	4.5
P0R 13038	5	5	10	0	0	10	10	4.4	4.7	4.2
P0R 13050	4	5	9	0	1	10	9	4.0	4.2	3.9
P0R 13051	4	7	11	0	2	13	12	4.3	4.4	4.2
P0R 13052	NOT PREGNANT									
P0R 13053	9	5	14	0	0	14	14	3.6	3.7	3.5
P0R 13054	4	6	10	0	0	10	11	4.2	4.3	4.1
P0R 13070	5	8	13	0	2	15	14	4.2	4.2	4.2
P0R 13071	3	6	9	0	1	10	9	4.4	4.7	4.3
P0R 13072	5	4	9	0	0	9	9	4.3	4.4	4.2
P0R 13073	NOT PREGNANT									
P0R 13074	NOT PREGNANT									
P0R 13082	NOT PREGNANT									
MEAN	5.0	5.5	10.5	0.0	0.7	11.2	11.1	4.2		
STAN. DEV.	1.9	2.0	2.2	0.0	1.0	2.2	2.0	0.3		

Appendix VI (Concluded)

Oral Teratology Study of FC-95 in Rats
Individual Litter Data with Pup Weights

Dose Group and Rat No.	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPRA LUTEA	MEAN FETUS WT (G) AVG	M	F
1 mg/kg/day										
Q0R 13011	7	3	10	0	0	10	8	4.4	4.5	4.4
Q0R 13012	5	6	11	0	0	11	12	4.1	4.1	4.0
Q0R 13013	3	6	9	0	0	9	11	4.4	4.4	4.3
Q0R 13014	5	7	12	0	0	12	13	3.4	3.8	3.2
Q0R 13015	4	6	10	0	0	10	9	3.8	3.8	3.8
Q0R 13031	7	6	13	0	0	13	14	4.0	3.9	4.1
Q0R 13032	1	1	2	0	0	2	4	3.8	4.3	3.3
Q0R 13033	4	9	13	0	0	13	14	4.5	4.6	4.5
Q0R 13034	2	4	6	0	3	9	8	5.0	5.1	4.9
Q0R 13035	5	5	10	0	1	11	11	4.6	4.7	4.4
Q0R 13039	6	6	12	0	0	12	12	4.3	4.4	4.2
Q0R 13055	7	4	11	0	1	12	12	4.3	4.2	4.3
Q0R 13056	5	6	11	1	1	13	11	4.1	4.3	4.0
Q0R 13057	4	5	9	0	1	10	12	3.9	3.8	3.9
Q0R 13058	NOT PREGNANT									
Q0R 13059	6	4	10	0	0	10	11	4.1	4.3	3.8
Q0R 13075	6	4	10	0	0	10	11	4.2	4.2	4.1
Q0R 13076	NOT PREGNANT									
Q0R 13077	3	5	8	0	1	9	9	4.4	4.4	4.4
Q0R 13078	5	5	10	0	0	10	10	4.6	4.9	4.4
Q0R 13079	NOT PREGNANT									
Q0R 13083	4	11	15	0	0	15	15	4.1	4.2	4.0
MEAN	4.7	5.4	10.1	0.1	0.4	10.6	10.9	4.2		
STAN. DEV.	1.7	2.1	2.8	0.2	0.8	2.7	2.6	0.4		

DISTRIBUTION LIST

K. L. Ebbens → J. D. Henderson → G. R. Steffen → M. T. Case

E. G. Gortner (original +1)

F. Keller (QA file)

E. G. Lamprecht

W. C. McCormick (2)

R. A. Nelson → E. L. Mutsch → R. E. Ober

cc: M. T. Case
E. G. Gortner (original + 1)
F. Keller (QA File)
E. G. Lamprecht
E. L. Mutsch → R. A. Nelson → R. E. Ober
W. C. McCormick → F. D. Griffith (2)
W. H. Pearlson
G. R. Steffen → J. D. Henderson → K. L. Ebbens

Amendment to the Final Report of the Oral
Teratology Study of FC-95 in Rats

Experiment No.: 0680TR0008
Issued: 12/18/80

Please add the amended summary, the amended table 5, and the amendment to the results and discussion sections to the above report. The study conclusions were changed by this amendment to the report.

E G Gortner 7/26/82
E. G. Gortner Date
Senior Research Technologist
Animal Teratology Reproduction

Eldon G Lamprecht 7-26-82
E. G. Lamprecht, DVM, PhD Date
Research Veterinary Pathologist

Maurice T Case 7/27/82
M. T. Case, DVM, PhD Date
Manager, Pathology-Toxicology
Safety Evaluation Laboratory

Amended Summary (p. 1) to the Oral Teratology Study of FC-95 in Rats
Experiment No. 0680TR0008

Oral administration of FC-95 at doses of 10, 5 and 1 mg/kg/day to pregnant Sprague-Dawley rats during days 6 through 15 of gestation (period of organogenesis) was not teratogenic.

FC-95 administration was maternally toxic only to the 10 mg/kg/day group. At gestation days 12 through 20 the maternal body weights of the high dose females were significantly lower than the controls. FC-95 was not maternally toxic to the 5 and 1 mg/kg/day groups.

FC-95 was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams. The compound did not produce an increase in the number or proportion of fetal skeleton variations.

Amendment to the Results and Discussion Sections (p. 3-5)
of the Oral Teratology Study of FC-95 in Rats

Experiment No. 0680TR0008

(This amendment addresses the last two paragraphs of the results section and the entire discussion section.)

FC-95 was labeled a teratogen of the lens because apparent lens abnormalities were observed at the 10, 5 and 1 mg/kg/day dose levels. Based on subsequent studies, particularly Riker Experiment No. 0681TR0362, the interpretations of these observations have been extensively modified. The lens findings observed under the dissecting microscope are now known to be either freehand sectioning artifacts or a normal area of lens cell degeneration. The fetal rat lens findings were incorrectly interpreted as a teratogenic change in this study.

The gross finding of a lens cleft was an artifact created by freehand sectioning. It represents a separation between the embryonal nucleus lens cells and the lens epithelium. The gross finding of a lens dark streak was a normal observation of the embryonal nucleus. The embryonal nucleus is an area of normal lens cell degeneration in the gestation day 20 fetus.

The gross appearance of the rat lens at day 20 of gestation is determined by the region of the lens which is transected by freehand sectioning. In a subsequent study (Riker Experiment No. 0681TR0362) the compound-related occurence of the lens findings could not be repeated when the fetuses were coded before freehand sectioning and gross evaluation. The range of gross lens observations and the differences among the dose group incidences were due to the manner and frequency in which the lens cleft artifact was created by freehand sectioning and the limitations inherent in visualizing the embryonal nucleus.

In summary, FC-95 <u>in utero</u> exposed fetuses did not have compound-related changes in their lenses.

Amended Table 5 (p. 10)

Oral Teratology Study of FC-95 in Rats
Number and Percent of Fetuses with Internal Findings

Internal Finding	0 mg/kg/day	10 mg/kg/day	5 mg/kg/day	1 mg/kg/day
Lens findings[a]	1 (2)	14 (35)[b]	4 (7)	2 (3)
Thoracic cavity full of blood	---	1 (3)	---	---
Enlarged atria	1 (2)	---	---	---
Enlarged renal pelvis	3 (5)	---	---	3 (5)
Abdominal cavity full of blood	4 (7)	2 (5)	5 (9)	2 (3)
No. of Fetuses Examined	61	40	55	59

[a] The lens findings observed under the dissecting microscope were either freehand sectioning artifacts or a normal area of lens cell degeneration

[b] Significantly higher than the control (chi-square with Yates correction $p < 0.05$)

( ) = percent of total examined

DISTRIBUTION LIST

K. L. Ebbens

E. G. Gortner (original + 1)

F. D. Griffith → F. A. Ubel

F. Keller (QA File)

E. G. Lamprecht

R. E. Ober → R. A. Nelson → E. L. Mutsch

W. H. Pearlson

G. R. Steffen → J. D. Henderson → M. T. Case

W. C. McCormick

Amended Appendix VII

STATEMENT OF QUALITY ASSURANCE

STUDY NUMBER: Amendment to 0680TR0008

TITLE: Amendment to the Final Report of the Oral Teratology Study of FC-95 in Rats

Audits and/or inspections were performed by the Riker Compliance Audit unit for the above titled study, and reported to the study director and to management as follows:

Date Performed	Date Reported
July 16 and 19, 1982	July 21, 1982
July 22, 1982	July 23, 1982

Gab E Van Buskirk
Compliance Audit
Riker Laboratories, Inc.

Date July 23, 1982

DISTRIBUTION LIST

K. L. Ebbens → J. D. Henderson → G. R. Steffen → M. T. Case

E. G. Gortner (original +1)

F. Keller (QA file)

E. G. Lamprecht

[redacted]

R. A. Nelson → E. L. Mutsch → R. E. Ober

JEFFERSON MEDICAL COLLEGE
of
THOMAS JEFFERSON UNIVERSITY

Daniel Baugh Institute of Anatomy
Department of Anatomy
Office of the
Director and Chairman

Philadelphia, 19107
(215) 928-7820

November 12, 1982

Dr. Franklin D. Griffith
Toxicology Services
Medical Department
220-2E-02 3M Center
St. Paul, Minnesota 55101

Dear Dr. Griffith:

I would like to thank you and your associates for the hospitality extended to me during my recent visit. In particular, Drs. Ebbens and Lamprecht were most cooperative under what must have been for them somewhat difficult circumstances. Their willingness and ability to retrieve and make available the raw data and both the histologic and gross anatomical specimens which I requested was commendable and essential to my review.

I had ample opportunity to make a critical spot check and personal examination of the actual microscopic and gross anatomical specimen previously recorded in the raw data sheets as being abnormal. No statistical or score sheet mode of conclusion is possible, but the great majority of the stained and gross preparations of eyes which had been reported as abnormal are actually quite normal term fetal rat eyes. A standard artifact of preservation and a normal developmental sequence were erroneously identified and reported as abnormalities.

During the course of the original studies, two-thirds of the specimens were prepared for evaluation of skeletal morphology. The remaining one-third was used for soft tissue evaluation and from these, some were evidently selected for histologic preparation on the basis of a technician reporting the presence of one of the artifacts. Coded specimens were not employed during either the identification or selection phases of the experiment and one can observe a general date, if you will, on which the "findings" began to become reported as test substance-related adverse effects. Factors such as these preclude development of a fully documented view. However, in addition to clearly establishing that the "effects" reported are normal and expected occurrences, I was able to further come to the perception that the test substance actually poses no unique hazard to the conceptus.

One cannot determine how much negative data is needed to overcome the one misidentified positive. It is necessary in my view, however, to resolve any lingering scientific questions that may exist. This is best achieved by subjecting both FC95 and FM3422 to carefully controlled and executed state-of-the-art safety

evaluations in an independent outside laboratory. I recommend that a segment II-type of safety evaluation be made with each substance in pregnant rats.

I am confident that the compounds will prove to not be hazardous to development. 3M Corporation is to be commended for its excellent response to an apparent substantial risk. The concern was unwarranted and any lingering impugnment of FC95 and FM3422 regarding developmental toxicity needs to be removed.

Sincerely,

E. Marshall Johnson

E. Marshall, Johnson, Ph.D.
Professor and Chairman
Director, Daniel Baugh Institute of Anatomy

EMJ:sp

Summary PFOS Rabbit Teratology

Study Numbers: 3M **T-6295.10**, Argus 418-012

Compound & Lot: PFOS (Perfluorooctanesulfonate) – Lot 217, 98.4% pure (SMD Analytical Request 53030) Analytical Documentation filed along with final report. Note – same lot as used in two-year rat PFOS carcinogenicity study (T-6295, Covance 6329-183).

Study Title: Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits

Report Date: 11 January 1999

Study Monitor Summary: (written by Marvin T. Case) Pregnant rabbit females, 22 per group, were given daily oral (intubation) of PFOS at dose levels of 0, 0.1, 1.0, 2.5, and 3.75 mg/kg/day on days 7 through 20 of gestation. The rabbit fetuses which had been exposed in utero during organogensis were collected at day 29 of gestation. All of the fetuses were examined for soft tissue (visceral) changes using a dissection microscope and then all fetuses were examined for skeletal changes.

A dose related body weight effect occurred in the 1.0, 2.5, and 3.75 mg/kg dose groups. The difference in mean body weights was statistically significant at the 3.75 mg/kg dose. A significant reduction in food consumption was also present at the 2.5 and 3.75 mg/kg doses. No compound related deaths occurred at any dose level.

Abortions occurred in one 2.5 mg/kg doe, and in ten 3.75 mg/kg does. The abortions at the high dose were considered to be compound related. Mean pup weights were decreased in the 2.5 and 3.75 mg/kg groups. The reduced food consumption and adverse body weight effect that occurred at the two highest dose levels were indicative of dam toxicity. The increased number of abortions and reduced pup weights were probably secondary to the dam toxicity.

Increased number of delayed ossification sites were found in the 2.5 and 3.75 mg/kg fetuses which is another reflection of maternal toxicity at these dose levels.

No compound-related soft tissue or skeletal malformations were found at any dose level. Thus, the compound was not found to be teratogenic in the rabbit.

The maternal toxic NOEL was 0.1 mg/kg.

The fetal toxic NOEL was 1.0 mg/kg.

Teratogenic NOEL in the rabbit was > 3.75 mg/kg.

NOTE: Dose preparation samples were collected twice during the study and these samples were sent to 3M Evirornmental Analytical laboratory in Bldg 2. Likewise, samples were collected from extra females assigned as toxicokinetic satellite animals (3/group except low and high dose 5/group). These animals were killed on gestation day 21 (day after last dose) and the following samples were collected: serum and liver from pregnant dams, fetuses and placentas (pooled by litter) from uterus. These samples were frozen and sent to 3M Evirornmental Analytical laboratory in Bldg 2. At time of this summary, March 1999, analysis of these samples had not been done; therefore, these analytical results were not included in the final report nor in this summary.

FINAL REPORT

PROTOCOL 418-012

ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS

SPONSOR'S STUDY NUMBER: 6295.10

FINAL REPORT DATE: 11 JANUARY 1999

PROTOCOL 418-012

ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS

SPONSOR'S STUDY NUMBER: 6295.10

TABLE OF CONTENTS

SUBJECT		PAGE
I.	SUMMARY AND CONCLUSION	I-1
A.	Methods	I-1
B.	Results	I-2
C.	Conclusion	I-3
II.	DESCRIPTION OF TEST PROCEDURES	II-1
A.	Conduct of Study	II-1
A.1.	Sponsor	II-1
A.2.	Testing Facility	II-1
A.3.	Study Number	II-1
A.4.	Sponsor's Study Number	II-1
A.5.	Purpose of the Study	II-1
A.6.	Study Design	II-1

SUBJECT		PAGE
A.7.	Regulatory Compliance	II-1
A.8.	Ownership of the Study	II-2
A.9.	Study Monitor	II-2
A.10.	Alternate Study Monitor	II-2
A.11	Study Director	II-2
A.12.	Technical Performance	II-2
A.13.	Report Preparation	II-2
A.14.	Report Review	II-2
A.15.	Date Protocol Signed	II-2
A.16.	Dates of Technical Performance	II-3
A.17.	Records Maintained	II-3
B.	Test Article Information	II-3
B.1.	Description	II-3
B.2.	Lot Number	II-3
B.3.	Date Received and Storage Conditions	II-3
B.4.	Special Handling Instructions	II-3
B.5.	Analysis of Activity	II-3
C.	Vehicle Information	II-4
C.1.	Description	II-4
C.2.	Lot Number	II-4
C.3.	Date Received and Storage Conditions	II-4
C.4.	Special Handling Instructions	II-4

SUBJECT		PAGE
C.5.	Analysis of Purity	II-4
D.	Test Article Preparation	II-4
D.1.	Sample Information	II-5
D.2.	Analytical Results	II-5
E.	Test System	II-5
E.1.	Species	II-5
E.2.	Strain	II-5
E.3.	Supplier (Source)	II-5
E.4.	Sex	II-5
E.5.	Rationale for Test System	II-6
E.6.	Test System Data	II-6
E.7.	Method of Randomization	II-6
E.8.	System of Identification	II-6
F.	Husbandry	II-7
F.1.	Research Facility Registration	II-7
F.2.	Study Rooms	II-7
F.3.	Housing	II-7
F.4.	Lighting	II-7
F.5.	Sanitization	II-7
F.6.	Feed	II-7
F.7.	Feed Analysis	II-8
F.8.	Water	II-8

SUBJECT		PAGE
F.9.	Water Analysis	II-8
G.	Methods	II-9
G.1.	Dosage Administration	II-9
G.2.	Rationale for Dosage Selection	II-9
G.3.	Route of Administration	II-9
G.4.	Rationale for Route of Administration	II-9
G.5.	Frequency of Administration	II-9
G.6.	Length of Study	II-9
G.7.	Method of Study Performance	II-10
G.8.	Gross Necropsy	II-10
G.9	Statistical Analyses	II-12
III.	RESULTS	III-1
A.	Mortality, Abortions, Clinical and Necropsy Observations	III-1
A.1.	Abortions	III-1
A.2.	Clinical Observations	III-3
A.3.	Necropsy Observations	III-3
B.	Maternal Body Weights and Body Weight Changes	III-3
C.	Maternal Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values	III-4
D.	Caesarean-Sectioning and Litter Observations	III-4
E.	Fetal Alterations	III-5
E.1.	Summary of Fetal Alterations	III-5
E.2.	Fetal Gross External Alterations	III-6

SUBJECT		PAGE
E.3.	Fetal Soft Tissue Alterations	III-6
E.4.	Fetal Skeletal Alterations	III-7
F.	Satellite Rabbits	III-10
REFERENCES		III-12
APPENDIX A - REPORT FIGURE		
Figure 1.	Maternal Body Weights	A-1
APPENDIX B - REPORT TABLES		
Table 1.	Clinical Observations and Necropsy Observations – Summary	B-1
Table 2.	Uterine Contents and Litter Data for Individual Rabbits That Aborted	B-2
Table 3.	Maternal Body Weights - Summary	B-4
Table 4.	Maternal Body Weight Changes - Summary	B-6
Table 5.	Maternal Absolute Feed Consumption Values (g/day) - Summary	B-7
Table 6.	Maternal Relative Feed Consumption Values (g/kg/day) - Summary	B-8
Table 7.	Caesarean-Sectioning Observations - Summary	B-9
Table 8.	Litter Observations (Caesarean-Delivered Fetuses) – Summary	B-10
Table 9.	Fetal Alterations - Summary	B-11
Table 10.	Fetal Gross External Alterations - Summary	B-12
Table 11.	Fetal Soft Tissue Alterations - Summary	B-13
Table 12.	Fetal Skeletal Alterations - Summary	B-14

SUBJECT		PAGE
Table 13.	Fetal Ossification Sites - Caesarean-Delivered Live Fetuses (Day 29 of Gestation) - Summary	B-19
Table 14.	Clinical Observations - Individual Data	B-20
Table 15.	Necropsy Observations - Individual Data	B-26
Table 16.	Maternal Body Weights - Individual Data	B-33
Table 17.	Maternal Feed Consumption Values - Individual Data	B-48
Table 18.	Caesarean-Sectioning Observations - Individual Data	B-63
Table 19.	Litter Observations (Caesarean-Delivered Fetuses) – Individual Data	B-69
Table 20.	Fetal Sex, Vital Status and Body Weight - Individual Data	B-75
Table 21.	Fetal Alterations - Individual Data	B-87
APPENDX C -	PROTOCOL AND AMENDMENT	C-1 to C-32
APPENDIX D -	PILOT REPORT	D-1 to D-94
APPENDIX E -	HISTORICAL CONTROL DATA	E-1 to E-14
APPENDIX F -	STATEMENT OF THE STUDY DIRECTOR	F-1
APPENDIX G -	QUALITY ASSURANCE UNIT FINAL REPORT STATEMENT	G-1 to G-4

TITLE: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS

ARGUS RESEARCH LABORATORIES, INC.
PROTOCOL NUMBER: 418-012
SPONSOR'S STUDY NUMBER: 6295.10

I. SUMMARY AND CONCLUSION

A. Methods[a]

One hundred ten New Zealand White [Hra(NZW)SPF] rabbits were assigned to each of five dosage groups (Groups I through V) for the main portion of the study. Nineteen additional female rabbits were assigned to one of five dosage groups for the satellite study (three, five, three, three and five rabbits assigned to Groups I through V, respectively). The test article, PFOS, or vehicle, 0.5% Tween® 80 in Reverse Osmosis Membrane Processed Deionized Water (R.O. Deionized Water), was administered orally (via stomach tube) once daily to female rabbits on days 7 through 20 of presumed gestation (DGs 7 through 20). Dosages of 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day were administered at a dosage volume of 5 mL/kg, adjusted daily on the basis of individual body weights.

The female rabbits were observed for viability at least twice each day of the study. The rabbits were also examined for clinical observations of effects of the test article, abortions, premature deliveries and deaths before and approximately 60 minutes after each daily intubation during the dosage period, and once daily during the postdosage period. Body weights were recorded on DGs 0 and 6 through 29. Feed consumption values were recorded daily throughout the study.

All surviving rabbits were sacrificed by intravenous administration of Beuthanasia®-D Special euthanasia solution on DG 29 and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number of corpora lutea in each ovary was recorded. The uterus of each rabbit was

---

a. Detailed descriptions of all procedures used in the conduct of this study are provided in the appropriate sections of this report and in APPENDIX C (PROTOCOL AND AMENDMENT).

examined for pregnancy, number and distribution of implantations, live and dead fetuses and early and late resorptions. The fetuses were weighed, examined for gross external alterations and sex. Visceral alterations and cavitated organs were evaluated by dissection. The brains of approximately one half of the fetuses in each litter were free-hand cross-sectioned and examined *in situ*. All fetuses were eviscerated, stained with alizarin red S and evaluated for skeletal alterations.

Rabbits in the satellite study were sacrificed on DG 21. Blood samples were collected and centrifuged. The liver was excised, weighed and sectioned. Fetuses were examined grossly to the extent possible as described for rabbits assigned to the main study. Fetuses and placentae were pooled per litter. After completion of sample collection, serum, liver section, fetal and placental samples were shipped to the Sponsor for analysis.

B. Results

Administration of the 2.5 and 3.75 mg/kg/day dosages of the test article resulted in ten abortions (one in the 2.5 mg/kg/day dosage group and nine in the 3.75 mg/kg/day dosage group). These abortions occurred after the completion of the dosing period (on DGs 22, 24, 25 or 28). All other does survived to scheduled sacrifice.

Increased numbers of does in the 3.75 mg/kg/day dosage group had observations of scant or no feces. Scant feces also occurred in one and three rabbits in the 1.0 and 2.5 mg/kg/day dosage groups, respectively. The 1.0 mg/kg/day dosage group rabbit also had soft or liquid feces on one day. One doe in the 3.75 mg/kg/day dosage group had red substance in the cage pan associated with impending abortion. No other clinical or necropsy observations related to the test article occurred.

Dosage-dependent, significant body weight reductions or body weight losses occurred in the 1.0, 2.5 and 3.75 mg/kg/day dosage groups for the entire dosage period (calculated as DGs 7 to 21). Dosage-dependent reductions in body weight gains occurred in the 2.5 and 3.75 mg/kg/day dosage groups for the entire gestation period (DGs 0 to 29) and for the gestation period after the initiation of dosing (DGs 7 to 29). Average body weights were significantly reduced on DGs 17 through 24 in the 3.75 mg/kg/day dosage group. Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced in the 2.5 and 3.75 mg/kg/day dosage groups for the entire dosage period (DGs 7 to 21), and the entire period after the initiation of dosage (DGs 7 to 29).

Fetal body weights (total, male and female) were significantly reduced in the 2.5 and 3.75 mg/kg/day dosage groups. Delays in fetal ossification associated with

the significantly reduced fetal body weights in the 2.5 and 3.75 mg/kg/day dosage groups were evident as significant reductions in the litter averages for ossified sternal centers in the 2.5 and 3.75 mg/kg/day dosage groups and significant reductions in the litter averages for ossified hyoid and metacarpals and significant reductions in the litter and fetal incidences of incompletely ossified pubes in the 3.75 mg/kg/day dosage group.

C. Conclusion

Based on these data, the maternal no-observable -effect-level (NOEL) for PFOS is 0.1 mg/kg/day. The 1.0 mg/kg/day and higher dosages reduced maternal body weight gain. Dosages of 2.5 and 3.75 mg/kg/day caused abortions and reduced feed consumption. The developmental NOEL is 1.0 mg/kg/day. Dosages of 2.5 and 3.75 mg/kg/day caused reductions in fetal body weights and delays in ossification. Based on these data, PFOS should not be identified as a selective developmental toxicant; the compound was not found to be teratogenic in the rabbit.

11 JAN 99

Mildred S. Christian, Ph.D., Fellow, ATS Date
Executive Director of Research

11 JAN 99

Alan M. Hoberman, Ph.D., DABT Date
Director of Research

11-JAN-99

Raymond G. York, Ph.D., DABT Date
Associate Director of Research and
Study Director

II. DESCRIPTION OF TEST PROCEDURES

A. Conduct of Study:

A.1. Sponsor:

3M Corporate Toxicology, 3M Center Building 220-2E-02
St. Paul, Minnesota 55144-1000

A.2. Testing Facility:

Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297

A.3. Study Number:

418-012

A.4. Sponsor's Study Number:

6295.10

A.5. Purpose of the Study:

The purpose of this study was designed to detect adverse effects of PFOS on New Zealand White [Hra:(NZW)SPF] presumed pregnant female rabbits and development of the embryo and fetus consequent to exposure of the doe from implantation to closure of the hard palate. This study was designed to evaluate ICH Harmonised Tripartite Guideline stages C and D of the reproductive process in a nonrodent species.

A.6. Study Design:

The requirements of the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline[1] were used as the basis for study design.

A.7. Regulatory Compliance:

The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. Food and Drug Administration (FDA)[2], the Japanese Ministry of Health and Welfare (MHW)[3] and the European Economic Community (EEC)[4]. There were no significant deviations from the GLP regulations that affected the quality or integrity of the study. Quality Assurance

Unit findings derived from the inspections during the conduct of this study are documented and have been provided to the Study Director and the Testing Facility management.

A.8. Ownership of the Study:

The Sponsor owns the study. All raw data, analyses, reports and preserved tissues are the property of the Sponsor.

A.9. Study Monitor:

Marvin T. Case, D.V.M., Ph.D.

A.10. Alternate Study Monitor:

Andrew M. Seacat, Ph.D.

A.11. Study Director:

Raymond G. York, Ph.D., DABT (Associate Director of Research)

A.12. Technical Performance:

John F. Barnett, B.S. (Director of Laboratory Operations)
Kristen Iandola Sherer, B.S. (Research Associate/Fetal Evaluation)
Matthew J. Vaneman, B.S. (Laboratory Technician)

A.13. Report Preparation:

Raymond G. York, Ph.D., DABT
Michelle R. Rzaca, B.S. (Study Coordinator)
Susan K. Bradshaw, B.S. (Data Management Specialist)
Karen G. Parker, A.A. (Report Administrator)

A.14. Report Review:

Alan M. Hoberman, Ph.D., DABT (Director of Research)
Mildred S. Christian, Ph.D., Fellow, ATS (Executive Director of Research)

A.15. Date Protocol Signed:

11 August 1998

A.16. Dates of Technical Performance:

Rabbit Arrival Date	21 AUG 98
Dosage Period [Days 7 through 20 of presumed gestation (DGs 7 through 20)]	23 AUG 98 – 09 SEP 98
Toxicokinetic Sample Collection (DG 21)	06 SEP 98 – 10 SEP 98
Caesarean-Sectioning Period (DG 29)	14 SEP 98 – 18 SEP 98

A.17. Records Maintained:

The original report, raw data and reserve samples of the test article and vehicle components are retained in the archives of Argus Research Laboratories, Inc. Any preserved tissues are retained in the archives of the Testing Facility for one year after the mailing of the draft final report, after which time the Sponsor will decide their final disposition. All unused test article suspensions were discarded at the Testing Facility. Unused bulk test article will remain at the Testing Facility until its disposition is decided by the Sponsor.

B. Test Article Information:

B.1. Description:

PFOS – an off-white powder

B.2. Lot Number:

217 (Expiration date: May 2000)

B.3. Date Received and Storage Conditions:

The test article was received on 20 May 1998, and stored at room temperature. Prepared formulations were stored refrigerated.

B.4. Special Handling Instructions:

Standard safety precautions (use of protective clothing, gloves, dust-mist respirator, safety goggles or safety glasses and a face-shield) were taken when handling the bulk test article and prepared suspensions.

B.5. Analysis of Purity:

Information regarding the purity, identity, strength and composition of the test article is on file with the Sponsor.

C. Vehicle Information:

C.1. Description:

0.5% Tween® 80 prepared using 2% Tween® 80, a clear viscous to yellow liquid, in Reverse Osmosis Membrane Processed Deionized Water (R.O. Deionized Water).

C.2. Lot Number:

M03H05

C.3. Date Received and Storage Conditions:

The 2% Tween® 80 was received on 8 July 1998, and stored at room temperature. R.O. deionized water is available from a continuous source at the Testing Facility and is maintained at room temperature.

C.4. Special Handling Instructions:

Standard safety precautions (use of protective clothing, gloves, dust-mist respirator, safety goggles or safety glasses and a face-shield) were taken when handling the vehicle.

C.5. Analysis of Purity:

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to be present in the vehicle that would interfere with the results of this study.

D. Test Article Preparation:

Suspensions of PFOS were prepared daily at concentrations of 0, 0.02, 0.2, 0.5 and 0.75 mg/mL. The test article was considered 100% pure for the purpose of dosage calculations.

D.1. Sample Information:

Sample Type	Components	Size	Date Retained	Storage Conditions	Shipped To	Date Shipped
Concentration (all levels)	N/A	2 mL[a]	23 AUG 98[b] 09 SEP 98[c]	Frozen	Sponsor	23 AUG 98 09 SEP 98
Bulk Test Article Reserve	N/A	1 g	25 AUG 98	Room temperature	Testing Facility Archives	Not available
Vehicle Reserve	Tween® 80	5 mL	25 AUG 98	Room temperature	Testing Facility Archives	Not available
	R.O. deionized water	5 mL	25 AUG 98	Room temperature	Testing Facility Archives	Not available

a. Duplicate samples were taken from each concentration. One set of samples was sent for analysis; the remaining samples were retained at the Testing Facility as backups.
b. First day of preparation.
c. Last day of preparation.

Homogeneity and stability of prepared formulations are on file with the Sponsor.

D.2. Analytical Results:

Concentration samples (2 mL) were taken on the first and last days of preparation for analyses by 3M Environmental Technology and Safety Services. The results of these analyses were not available at the time of this report.

E. Test System:

E.1. Species:

Rabbit

E.2. Strain:

New Zealand White [Hra:(NZW)SPF]

E.3. Supplier (Source):

Covance Research Products, Inc., Denver, Pennsylvania

E.4. Sex:

Timed-pregnant female

E.5. Rationale for Test System:

The New Zealand White [Hra:(NZW)SPF] rabbit was selected as the Test System because: 1) it is one non-rodent mammalian species accepted and widely used throughout the industry for nonclinical studies of developmental toxicity (embryo-fetal toxicity/teratogenicity); 2) this strain of rabbit has been demonstrated to be sensitive to developmental toxins; 3) historical data and experience exist at the Testing Facility[5-7]; and 4) the test article is biologically active in the species and strain.

E.6. Test System Data:

Number of Rabbits	133
Approximate Date of Birth	21 MAR 98
Approximate Age at Arrival	5 months
Weight (kg) on DG 0	2.77 – 4.20
Weight (kg) at Arrival	2.84 – 4.21

E.7. Method of Randomization:

Upon arrival, rabbits were assigned to individual housing on the basis of computer-generated random units. Rabbits were assigned to one of five dosage groups (Groups I through V), 22 rabbits per dosage group, for the main portion of the study. An additional 19 satellite rabbits were assigned for toxicokinetic evaluation; five rabbits were assigned to each of the low and high dosage groups (Groups II and V) and three rabbits were assigned to each of the remaining dosage groups (Groups I, III and IV). Rabbits were assigned to dosage groups using a computer-generated (weight-ordered) randomization procedure based on body weights recorded by and at the Supplier (Covance Research Products, Inc.) on DG 0.

E.8. System of Identification:

Cage tags were marked with the study number, permanent rabbit number, sex, test article identification and dosage level. Each rabbit was individually identified with a Monel® self-piercing ear tag (Gey Band and Tag Co., Inc., No. MSPT 20103) inscribed with the rabbit's designated unique permanent number.

F. Husbandry:

F.1. Research Facility Registration:

USDA Registration No. 23-R-099 under the Animal Welfare Act, 7 U.S.C. 2131 *et seq.*

F.2. Study Rooms:

The study rooms were maintained under conditions of positive airflow relative to a hallway and independently supplied with a minimum of ten changes per hour of 100% fresh air that had been passed through 99.97% HEPA filters (Airo Clean® rooms). Room temperature and humidity were monitored constantly throughout the study. Room temperature was targeted at 61°F to 72°F (16°C to 22°C); relative humidity was targeted at 30% to 70%.

F.3. Housing:

Rabbits were individually housed. All cage sizes and housing conditions are in compliance with the *Guide for the Care and Use of Laboratory Animals*[8].

F.4. Lighting:

An automatically-controlled fluorescent light cycle was maintained at 12-hours light:12-hours dark, with each dark period beginning at 1900 hours EST.

F.5. Sanitization:

Cage pan liners were changed approximately three times each week. Cages were changed approximately every other week.

F.6. Feed:

Approximately 150 g of Certified Rabbit Chow® #5322 (PMI Nutrition International, St. Louis, Missouri) was available to each rabbit each day until the first day of dosage, at which time approximately 180 g of the same certified feed was offered to each rabbit each day. The certified feed was available from individual stainless steel "J-type" feeders attached to each cage.

F.7. Feed Analysis:

Analyses were routinely performed by the feed supplier. No contaminants at levels exceeding the maximum concentration for certified feed or deviations from expected nutritional requirements were detected by these analyses. Copies of the results of the feed analyses are available in the raw data.

Neither the Sponsor nor the Study Director was aware of any agent present in the feed that was known to interfere with the results of this study.

F.8. Water:

Local water that had been processed by passage through a reverse osmosis membrane (R.O. water) was available to the rabbits *ad libitum* from individual water bottles and/or from an automatic watering system (individual sipper tubes). Chlorine was added to the processed water as a bacteriostat.

F.9. Water Analysis:

The processed water is analyzed twice annually for possible chemical contamination (Lancaster Laboratories, Lancaster, Pennsylvania) and monthly for possible bacterial contamination (Analytical Laboratories, Inc., Chalfont, Pennsylvania). Copies of the results of the water analyses are available in the raw data.

Neither the Sponsor nor the Study Director was aware of any agent present in the water that was known to interfere with the results of this study.

G. Methods:

G.1. Dosage Administration:

Dosage Group	Number of Rabbits	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Assigned Rabbit Numbers	
					Main	Satellite[a]
I	22+3[a]	0 (Vehicle)	0	5	8443-8464	8553-8555
II	22+5[a]	0.1	0.02	5	8465-8486	8556-8560
III	22+3[a]	1.0	0.2	5	8487-8508	8561-8563
IV	22+3[a]	2.5	0.5	5	8509-8530	8564-8566
V	22+5[a]	3.75	0.75	5	8531-8552	8567-8571

The test article was considered 100% pure for the purpose of dosage calculations.
a. Rabbits assigned to the toxicokinetic evaluation.

G.2. Rationale for Dosage Selection:

Dosages were selected on the basis of a dosage-range study (Argus Research Laboratories, Inc., Protocol 418-012P).

G.3. Route of Administration:

Oral (stomach tube)

G.4. Rationale for Route of Administration:

The oral (stomach tube) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one of the possible routes of human exposure.

G.5. Frequency of Administration:

Appropriate dosages of the test article were administered orally (via stomach tube) once daily to naturally-bred rabbits on DGs 7 through 20. Dosages of 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day of the test article were administered at a dosage volume of 5 mL/kg, adjusted daily on the basis of the individual body weights recorded before intubation. The rabbits were intubated at approximately the same time each day.

G.6. Length of Study:

Approximately 4 weeks

G.7. Method of Study Performance:

The female rabbits were naturally bred by breeder male rabbits of the same source and strain before shipment to the Testing Facility. The rabbits were mated on five consecutive days and shipped to the Testing Facility on the day after the last day of mating. The day of mating was considered to be DG 0. A computer-generated (weight-ordered) randomization procedure was used to assign the rabbits to five dosage groups based on body weights recorded on DG 0 and supplied by Covance Research Products, Inc.

All rabbits were observed for viability at least twice each day of the study and for general appearance several times during acclimation and on DG 0. Additional examinations for clinical observations of effects of the test article, abortions, premature deliveries and deaths were made at least once during the predosage period, once before each daily intubation and approximately 60 minutes after intubation during the dosage period. These observations were also made once daily during the postdosage period (DGs 21 through 29).

Body weights were recorded on DGs 0 and 6 through 29. Feed consumption values were recorded daily throughout the study.

G.8. Gross Necropsy:

G.8.a. Satellite Rabbits Assigned to Toxicokinetic Sample Collection:

On day 21 of presumed gestation (the day following the last dosage), toxicokinetic samples were collected from the rabbits assigned to the toxicokinetic evaluation. Following anesthesia of pentobarbital, blood samples (approximately 4 mL per rabbit) were collected from the inferior vena cava into serum separator tubes and centrifuged. The resulting serum (approximately 2 mL) was immediately frozen on dry ice and maintained frozen (-70°C) until shipment to the Sponsor for analysis. The liver was excised, weighed, and a sample was taken from the right lateral lobe, frozen and retained at -70°C until shipment to the Sponsor for analysis.

Rabbits were Caesarean-sectioned and fetuses were examined grossly to the furthest extent. Fetuses and placentae were pooled per litter and retained frozen (-70°C) until shipment to the Sponsor for analysis.

After completion of sample collection, serum, liver sections, fetal and placental samples were shipped (frozen on dry ice) to 3M Environmental Technology and Safety Services, St. Paul, Minnesota.

G.8.b. Scheduled Sacrifice:

All surviving rabbits were sacrificed by intravenous administration of Beuthanasia®-D Special euthanasia solution on DG 29. The thoracic, abdominal and pelvic viscera of each rabbit were examined for gross lesions. Gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation (with the exception of parovarian cysts, which are common, spontaneous lesions in rabbits); all other tissues were discarded.

The number of corpora lutea in each ovary was recorded. The uterus was excised and examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead fetuses. Uteri from does that appeared nonpregnant were stained with 10% ammonium sulfide to confirm the absence of implantation sites[9]. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to mechanical stimuli. Nonresponding term fetuses are considered to be dead (there were no dead fetuses). Dead fetuses and late resorptions are differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

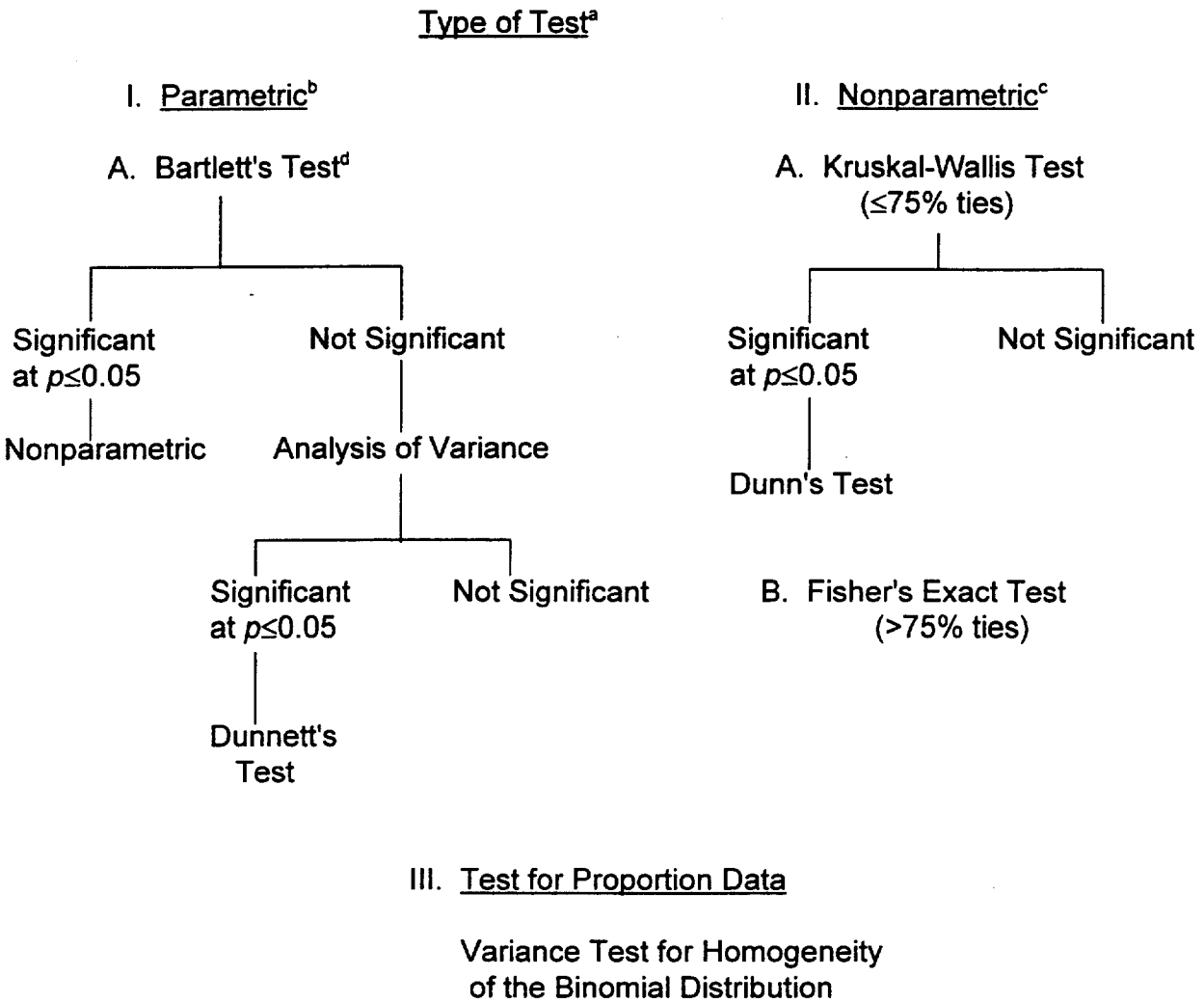
Each Caesarean-delivered fetus was weighed, examined for gross external alterations and individually identified with a tag noting study number, litter number, and uterine distribution. Live fetuses were sacrificed by an intraperitoneal injection of Beuthanasia®-D Special. All fetuses were examined internally to identify sex and visceral alterations; cavitated organs, including the brain, were evaluated by dissection[10]; and the brain was free-hand cross-sectioned (a single cross-section was made between the parietal and the frontal bones) and examined *in situ*. Fetal gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation.

All fetuses were eviscerated, stained with alizarin red S[11] and evaluated for skeletal alterations. All skeletal preparations were stored in 80% glycerin with thymol crystals added to retard fungal growth. Late resorptions were examined to the extent possible. Representative photographs of fetal alterations are available in the raw data.

Rabbits that aborted were examined on the day the observation was made. Pregnancy status and uterine contents were recorded. Aborted fetuses were examined to the extent possible, using the same methods described for fetuses. Uteri of apparently nonpregnant does were stained with 10% ammonium sulfide to confirm the absence of implantation sites[9].

G.9. Statistical Analyses:

The following schematic represents the statistical analyses of the data:



---

a. Statistically significant probabilities are reported as either at $p \leq 0.05$ or $p \leq 0.01$.
b. Used only to analyze data with homogeneity of variance.
c. Proportion data are not included in this category.
d. Test for homogeneity of variance.

Clinical observation and other proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[12].

Continuous data (e.g., maternal body weights, body weight changes, feed consumption values and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, fetal anomaly data and fetal ossification site data) were analyzed using Bartlett's Test of Homogeneity of Variances[13] and the Analysis of Variance[14], when appropriate [i.e., Bartlett's Test was not significant ($p>0.05$)]. If the Analysis of Variance was significant ($p\leq0.05$), Dunnett's Test[15] was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ($p\leq0.05$)], the Kruskal-Wallis Test[16] was used, when less than or equal to 75% ties were present; when more than 75% ties were present, Fisher's Exact Test[17] was used. In cases in which the Kruskal-Wallis Test was statistically significant ($p\leq0.05$), Dunn's Method of Multiple Comparisons[18] was used to identify the statistical significance of the individual groups.

Count data obtained at Caesarean-sectioning were evaluated using the procedures previously described for the Kruskal-Wallis Test[16].

Group I rabbit 8481 had a litter consisting of ten dead fetuses. Because such occurrences can abnormally skew the distribution of the data, statistical analyses were made with and without the values for this rabbit and litter. Data for this litter were excluded from summarization and statistical analyses; values are presented on the individual tables.

III. RESULTS

A. Mortality, Abortions, Clinical and Necropsy Observations (Summary - Table 1; Individual Data - Tables 3, 14 and 15)

A.1 Abortions

A total of ten rabbits aborted; one in the 2.5 mg/kg/day dosage group and nine** in the 3.75 mg/kg/day dosage group. These abortions occurred after the completion of the dosing period [on days 22, 24, 25 or 28 of gestation (DGs 22, 24, 25 or 28)] and were considered related to the test article because they occurred at dosage-dependent incidences in the two highest dosage groups. All other rabbits survived to scheduled sacrifice.

2.5 mg/kg/day

Doe 8517 aborted eight late resorptions on DG 25 and was sacrificed. There were no adverse clinical observations before aborting. This doe lost body weight and feed consumption was severely reduced after DG 13. Necropsy revealed eight implantation sites and all tissues appeared normal. Autolysis precluded further evaluation of the eight late resorptions.

3.75 mg/kg/day

Doe 8534 aborted one dead fetus on DG 22 and was sacrificed. There were no adverse clinical observations before aborting. This doe generally lost weight and had severely reduced feed consumption values throughout the study. Necropsy revealed seven live fetuses in the uterus and all tissues appeared normal. All fetuses appeared normal for their developmental ages at gross external and soft tissue examination. Fetuses 1, 2, 3, 4, 5, 6, 7 and 8 had not ossified pubes at skeletal examination.

Doe 8537 aborted five late resorptions on DG 25 and was sacrificed. The only adverse clinical observation before abortion was absent feces (DG 24). This doe generally lost weight and had severely reduced feed consumption values throughout the study. Necropsy revealed five implantation sites and all tissues appeared normal. Autolysis precluded further evaluation of the five late resorptions.

Doe 8538 aborted eight late resorptions on DG 24 and was sacrificed. There were no adverse clinical observations before aborting. This doe generally lost weight and had severely reduced feed consumption values throughout the study. Necropsy revealed nine implantation sites and all tissues appeared normal. One conceptus was presumed cannibalized and autolysis precluded further evaluation of the eight late resorptions.

** Significantly different from the vehicle control group value ($p \leq 0.01$).

Doe 8539 aborted seven live fetuses on DG 24 and was sacrificed. The only adverse clinical observation before abortion was scant feces (DGs 22 and 23). This doe generally lost weight and had severely reduced feed consumption values throughout the study. Necropsy revealed seven implantation sites and all tissues appeared normal. All fetuses appeared normal for their developmental ages at gross external and soft tissue examination. Fetuses 1, 2, 3, 4, 5, 6 and 7 had not ossified pubes and fetuses 2 and 3 had not ossified sternal centra at skeletal examination.

Doe 8540 aborted four dead fetuses on DG 25 and was sacrificed. There were no adverse clinical observations before aborting. This doe lost weight and feed consumption values were severely reduced after DG 15. Necropsy revealed three live fetuses, one dead fetuses and six late resorptions in the uterus. All tissues appeared normal at necropsy. All fetuses appeared normal for their developmental ages at gross external and soft tissue examination. Autolysis precluded further evaluation of the six late resorptions. Fetuses 1, 2, 3, 5, 7, 8, 9, and 10 had not ossified pubes and fetus 2 had incompletely ossified nasal bones and short maxillae at skeletal examination.

Doe 8542 aborted six dead fetuses on DG 28 and was sacrificed. The only adverse clinical observation before abortion was scant feces (DGs 21 to 23 and 27). This doe lost weight and had severely reduced feed consumption after DG 18. Necropsy revealed eight implantation sites and all tissues appeared normal. Two conceptuses were presumed cannibalized and all six aborted fetuses were partially cannibalized. All fetuses appeared normal at soft tissue examination.

Doe 8544 aborted nine dead fetuses on DG 25 and was sacrificed. The only adverse clinical observation before abortion was scant feces (DGs 19 to 20 and 22 to 24). After DG 12, this doe lost weight and had severely reduced feed consumption until sacrifice. Necropsy revealed nine implantation sites and all tissues appeared normal. All fetuses appeared normal at gross external and soft tissue examination. Fetuses 1, 2, 3, 4, 5, 6, 7, 8 and 9 had not ossified pubes and fetus 3 had not ossified sternal centra at skeletal examination.

Doe 8547 aborted eight late resorptions on DG 22 and was sacrificed. The only adverse clinical observations before abortion were scant feces (DG 18) and a red substance in the cage pan (DG 21). Body weights and feed consumption values were reduced DGs 11 to 21. Necropsy revealed eight implantation sites and all tissues appeared normal. Autolysis precluded evaluation of the eight late resorptions.

Doe 8548 aborted one dead fetus on DG 25 and was sacrificed. The only adverse clinical observation before abortion was scant feces (DGs 21 and 23 to 24). This doe lost weight after DG 15 and feed consumption was severely reduced after DG 17. Necropsy revealed seven live fetuses, one dead fetus and

two late resorptions in the uterus. All tissues appeared normal at necropsy. All fetuses appeared normal for their developmental ages at gross external and soft tissue examination. The late resorptions appeared normal at gross external examination; autolysis precluded further evaluation. Fetuses 1, 2, 3, 4, 5, 6, 7, 8 and 11 had not ossified pubes at skeletal examination.

A.2. Clinical Observations

Increased numbers of does in the 3.75 mg/kg/day dosage group had observations of scant or no feces; the incidence of scant feces was significant ($p \leq 0.01$). Scant feces also occurred in one and three rabbits in the 1.0 and 2.5 mg/kg/day dosage groups respectively. The 1.0 mg/kg/day dosage group rabbit also had soft or liquid feces on one day. One doe in the 3.75 mg/kg/day dosage group had red substance in the cage pan associated with impending abortion.

All other adverse clinical observations, and any statistically significant ($p \leq 0.01$) increases in the incidences of these observations, were considered unrelated to the test article because the incidences were not dosage-dependent. These observations included localized alopecia on the limbs, back and/or underside and ungroomed coat.

A.3. Necropsy Observations

With the exception of persistent adverse clinical observations, no additional gross lesions were identified at necropsy.

B. Maternal Body Weights and Body Weight Changes (Figure 1; Summaries - Tables 3 and 4; Individual Data - Table 16)

Maternal body weight gains were reduced or body weight losses occurred in the 1.0, 2.5 and 3.75 mg/kg/day dosage groups at most tabulated intervals during the dosage period; these reductions or body weight losses were statistically significant ($p \leq 0.05$ or $p \leq 0.01$) in the 3.75 mg/kg/day dosage group on DGs 10 to 13, 13 to 16 and 16 to 19.

During the first three days of the postdosage period (DGs 21 to 24), body weight gains continued to be reduced in the 1.0 and 2.5 mg/kg/day dosage groups; significant ($p \leq 0.05$) body weight loss occurred in the 3.75 mg/kg/day dosage group during this period. As a result of these reductions, dosage-dependent, significant body weight reductions or body weight losses ($p \leq 0.05$ or $p \leq 0.01$) occurred in the 1.0, 2.5 and 3.75 mg/kg/day dosage groups for the entire dosage period (calculated as DGs 7 to 21).

Dosage-dependent reductions in body weight gains occurred in the 2.5 and 3.75 mg/kg/day dosage groups for the entire gestation period (DGs 0 to 29) and for the gestation period after the initiation of dosing (DGs 7 to 29; significant at

$p \leq 0.01$ in the 2.5 mg/kg/day dosage group). Average body weights were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) on DGs 17 through 24 in the 3.75 mg/kg/day dosage group, as compared with the vehicle control group values.

Body weights and body weight gains were unaffected by the 0.1 mg/kg/day dosage of PFOS. The significant reduction ($p \leq 0.05$) in body weight gain in the 0.1 mg/kg/day dosage group on DGs 10 to 13 was not considered treatment-related because it was transient and not dosage-dependent.

C. Maternal Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables 5 and 6; Individual Data - Table 17)

Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced during the dosage period (after DG 10) in the 2.5 and 3.75 mg/kg/day dosage groups; these reductions were statistically significant ($p \leq 0.05$ or $p \leq 0.01$) in one or both of these groups on DGs 13 to 16 (relative only), 16 to 19 and 19 to 21. Absolute and relative feed consumption values continued to be reduced in these two groups during the postdosage period; the reductions were generally statistically significant ($p \leq 0.05$ or $p \leq 0.01$) on DGs 21 to 24.

Reflecting these effects of the test article, absolute and relative feed consumption values were reduced or significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 2.5 and 3.75 mg/kg/day dosage groups for the entire dosage period (DGs 7 to 21), and the entire period after the initiation of dosage (DGs 7 to 29).

Absolute and relative feed consumption values were unaffected by the 1.0 mg/kg/day dosage of PFOS.

D. Caesarean-Sectioning and Litter Observations (Summaries - Tables 7 and 8; Individual Data - Tables 18 through 20)

Pregnancy occurred in 20 (90.9%), 19 (86.4%), 19 (86.4%), 17 (77.3%) and 21 (95.4%) rabbits in each dosage group. Caesarean-sectioning observations on DG 29 were based on 20, 18, 19, 16 and 12 pregnant rabbits in each of the five respective dosage groups. One and nine does aborted in the 2.5 and 3.75 mg/kg/day dosage groups, respectively. Values for one 0.1 mg/kg/day dosage group doe (8481) that had a litter consisting of ten dead fetuses were excluded from data tabulation and statistical analyses; this total litter death was considered to be a non test article related, spontaneous event.

Fetal body weights (total, male and female) were significantly reduced ($p \leq 0.05$ and $p \leq 0.01$, respectively) in the 2.5 and 3.75 mg/kg/day dosage groups, as compared to the vehicle control group values. There were no other biologically important or statistically significant differences in the litter averages for corpora lutea, implantations, live fetuses and early or late resorptions. There were no does with all conceptuses resorbed and all placentae appeared normal.

E. Fetal Alterations (Summaries - Tables 9 through 13; Individual Data - Table 21)

Fetal alterations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); and 2) variations (common findings in this species/strain, and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.

Fetal evaluations were based on 175, 162, 152, 130 and 108 DG 29 Caesarean delivered live fetuses in 20, 18, 19, 16 and 12 litters in the 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day dosage groups, respectively. All fetuses were examined for gross external, soft tissue and skeletal alterations and fetal ossification site averages. It was also possible to examine the ten dead fetuses from litter 8481 in the 2.5 mg/kg/day dosage group for gross external, soft tissue and skeletal alterations. The embryonic sacs of all of these fetuses contained a dark red substance. One fetus had downward flexion of the forelimbs, an observation associated with *in utero* compression (skeletal ossification not affected); another fetus had angulated hyoid alae, and two fetuses had only ten or six caudal vertebrae. The remaining six fetuses appeared normal.

E.1. Summary of Fetal Alterations (Summary - Table 10; Individual Data - Table 22)

Combination of malformations and variations resulted in the following incidences for fetal alterations. In the 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day dosage groups, respectively, 14 (70.0%), 11 (61.1%), 9 (47.3%), 4 (25.0%) and 8 (66.7%) litters had fetuses with one or more alterations observed. In these same respective dosage groups, the total numbers of fetuses with any identified alterations were 25 (14.3%), 25 (15.4%), 14 (9.2%), 5 (3.8%)** and 19 (17.6%). One or more alterations occurred in averages of 14.1%, 17.0%, 9.5%, 3.6% and 17.4% of the fetuses per litter in the five respective dosage groups. The significant reduction ($p \leq 0.05$) in the total number of fetuses with identified alterations in the 2.5 mg/kg/day dosage group was not considered treatment-related because the expected response to a toxicant would be an increase, rather than a decrease, in the number of alterations.

Delays in fetal ossification associated with the significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) fetal body weights in the 2.5 and 3.75 mg/kg/day dosage groups were evident as significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in the litter averages for ossified sternal centers in the 2.5 and 3.75 mg/kg/day dosage groups and significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in the litter averages for ossified hyoid and metacarpals and significant reductions ($p \leq 0.01$) in the litter and fetal incidences of incompletely ossified pubes in the 3.75 mg/kg/day dosage group.

** Significantly different from the vehicle control group value ($p \leq 0.01$).

All other fetal gross external, soft tissue and skeletal alterations (malformations and variations) were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; and/or 2) the incidences were within ranges observed historically at the Testing Facility[a].

E.2. Fetal Gross External Alterations (Summary - Table 10; Individual Data - Table 21)

E.2.a. Malformations

The first digit was absent on both forelimbs for vehicle control group fetus 8448-1. Skeletal examination of this fetus revealed absent 1st medial and distal phalanges and 1st metacarpal on both forelimbs. No additional alterations occurred in this fetus.

E.2.b. Variations

One fetus (8473-6) in a 0.1 mg/kg/day dosage group litter and another fetus (8543-3) in a 3.75 mg/kg/day dosage group litter had downward flexed forepaws, an observation associated with *in utero* compression. Skeletal examination of fetus 8543-3 revealed a variation in skull ossification (hole in the parietal bone). Fetus 8473-6 had no additional alterations.

E.3. Fetal Soft Tissue Alterations (Summary - Table 11; Individual Data - Table 21)

E.3.a. Malformations

No soft tissue malformations were observed.

E.3.b. Variations

E.3.b.1. Eyes

One control dosage group fetus (8449-6) had a circumcorneal hemorrhage of the left eye, a variation generally attributable to trauma during processing. Skeletal evaluation of this fetus revealed variations in ossification of the skull and hyoid (irregular nasal-frontal suture and angulated alae).

---

a. See APPENDIX E (HISTORICAL CONTROL DATA).

E.3.b.2. Lungs

The intermediate lobe of the lungs was absent in 2, 7**, 1, 0 and 1 fetuses from 2, 4, 1, 0 and 1 litters in the 0 (Vehicle), 0.1, 1.0, 2.5 ad 3.75 mg/kg/day dosage groups, respectively. Two of the 0.1 mg/kg/day dosage group fetuses (8476-8, 8483-4) also had fused 3rd and 4th sternal centra.

The significant increase ($p \leq 0.01$) in the fetal incidence of this observation in the 0.1 mg/kg/day dosage group was not considered related to the test article because: 1) this variation in lung development is frequent in this rabbit strain[19], 2) the fetal and litter incidences are within the historical ranges of the Testing Facility, 3) it was not dosage-dependent; and 4) it was not significantly increased for the unit of measurement, the litter.

E.4. Fetal Skeletal Alterations (Summaries - Tables 12 and 13; Individual Data - Table 21)

E.4.a. Malformations

E.4.a.1. Thoracic Vertebrae/Ribs

Interrelated vertebral/rib malformations or malformations of only the ribs occurred in one, three and one fetuses in the 0 (Vehicle), 0.1 and 1.0 mg/kg/day dosage groups, respectively. These types of vertebral/rib malformations are relatively common at maternally toxic dosages in rabbits and generally considered to be secondary to maternal stress[20].

Fetus 8452-1 in the vehicle control group had a left hemivertebra present as the 3rd thoracic vertebra and a split 2nd left rib. This fetus also had asymmetric 1st to 3rd sternal centra and an angulated hyoid ala. Fetus 8476-1 in the 0.1 mg/kg/day dosage group had fused centra of the 11th and 12th thoracic vertebrae and a bifid centrum of the 12th thoracic vertebra, and its littermate, fetus 8476-7, had fused 5th and 6th ribs as the only alteration. Fetus 8485-2 in the 0.1 mg/kg/day dosage group had a small left arch of the 11th thoracic vertebra, fused left centra of the 11th and 12th thoracic vertebrae and proximate bases of the 10th and 11th left ribs. Fetus 8500-3 in the 1.0 mg/kg/day dosage group had split 8th right and 7th left ribs as the only alteration.

E.4.a.2. Lumbar Vertebrae

Fetus 8500-8 had a left hemivertebra present as the 1st lumbar vertebra and a bifid centrum of the 2nd lumbar vertebra.

** Significantly different from the vehicle control group value ($p \leq 0.01$).

E.4.a.3. Metacarpals/Phalanges

The 1st medial and distal phalanges and 1st metacarpal on both forelimbs were absent for vehicle control group fetus 8448-1 that had missing first digits at external examination.

E.4.b. Variations

E.4.b.1. Skull

Common small irregularities in ossification of the skull[19] [the presence of small ossification sites within the sutures or calvaria (nasal, frontal or parietal bones) and/or irregular shaping or fusion of the bones] occurred in 7, 9, 3, 1* and 10** fetuses in 6, 7, 3, 1 and 3 litters in the 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day dosage groups, respectively. Irregular ossification of the nasal bones (irregular nasal-frontal suture, fused, internasal, midline suture displaced and intranasal) were the most common of these small irregularities in skull ossification, occurring in 6, 9, 3, 1 and 1 fetuses in 5, 7, 3, 1 and 1 litters in the five respective dosage groups.

The significant reduction ($p \leq 0.05$) in the fetal incidence of variations in skull ossification in the 2.5 mg/kg/day dosage group was considered unrelated to the test article because the expected effect of a toxicant would be an increase in delayed skull ossification, rather than a decrease. The significant increase ($p \leq 0.01$) in the fetal incidence of variations in skull ossification in the 3.75 mg/kg/day dosage group was considered to reflect the significantly increased ($p \leq 0.01$) fetal incidence of hole(s) in the parietal and unrelated to the test article, as discussed below. The fetal incidence of nasal midline suture displaced was significantly increased ($p \leq 0.01$) in the 0.1 mg/kg/day dosage group but was considered unrelated to the test article because: 1) it was not dosage-dependent; and 2) the litter incidence was not significantly increased.

The fetal incidence of a hole in the parietal was significantly increased ($p \leq 0.01$) in the 3.75 mg/kg/day dosage group. This increase was considered unrelated to the test article because the observation occurred in seven fetuses from only one high dosage group litter (8543-1, -3, -4, -5, -6, -8 and -10), so the litter incidence, the more relevant parameter[1], was not significant. Two of the fetuses in this litter also had not ossified pubes; all other fetuses had no additional alterations

One 0.1 mg/kg/day dosage group fetus (8471-1) had unossified premaxillae as the only alteration.

---

* Significantly different from the vehicle control group value ($p \leq 0.05$).

** Significantly different from the vehicle control group value ($p \leq 0.01$).

E.4.b.2. Hyoid

One or both alae of the hyoid were angulated in 10, 4, 7, 3 and 5 fetuses in 6, 3, 3, 2 and 3 litters in the 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day dosage groups, respectively. One fetus (8452-1) in the vehicle control group also had vertebral/rib malformations and asymmetric 1st to 3rd sternal centra, as previously described.

E.4.b.3. Vertebrae

E.4.b.3.a. Cervical

One 0.1 mg/kg/day dosage group fetus had unilateral ossification of the centrum of the 2nd cervical vertebrae as the only alteration.

E.4.b.3.b. Thoracic

One vehicle control group fetus had unilateral ossification of the centrum of the 5th thoracic vertebrae as the only alteration.

E.4.b.3.c. Lumbar

Bifid centrum of a lumbar vertebra occurred in one fetus in the 1.0 and 3.75 mg/kg/day dosage groups, respectively. Fetus 8500-8 in the 1.0 mg/kg/day dosage group also had a hemivertebra present as a lumbar vertebra, as previously described. The fetus in the 3.75 mg/kg/day dosage group had no additional alterations.

E.4.b.3.d. Caudal

A misaligned 17th caudal vertebra occurred in one vehicle control group fetus as the only alteration.

E.4.b.4. Ribs

Cervical ribs were present at the 7th cervical vertebra for one fetus in each of the 0 (Vehicle) and 2.5 mg/kg/day dosage groups. No additional alterations occurred in these fetuses.

Thickened areas in two or three ribs occurred in one vehicle control group fetus (8456-4) and one 1.0 mg/kg/day dosage group fetus (8493-7). No other alterations occurred in these fetuses.

E.4.b.5. Sternum

Fused 3rd and 4th sternal centra occurred as the only skeletal alteration in 2 and 3 fetuses from different litters in the 0 (Vehicle) and 0.1 mg/kg/day dosage groups, respectively.

Fetus 8452-1 in the vehicle control group had asymmetric 1st to 3rd sternal centra. This fetus also had vertebral/rib malformations and an angulated hyoid ala, as previously described.

E.4.b.6. Pelvis

Four** fetuses from two** 3.75 mg/kg/day dosage group litters had incompletely ossified pubes. Two of these fetuses also had a variation in skull ossification (hole in parietal), as described previously. The significant increase ($p \leq 0.01$) in the fetal and litter incidences of this observation was considered a test article related delay in ossification and to reflect the significantly reduced ($p \leq 0.01$) fetal body weights in this dosage group.

E.4.b.7. Fetal Ossification Site Averages

The average numbers of ossification sites per fetus were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 3.75 mg/kg/day dosage group for hyoid, sternal centers and metacarpals. The average numbers of ossified sternal centers were also significantly reduced ($p \leq 0.01$) in the 2.5 mg/kg/day dosage group, as compared with the vehicle control group value. The values were below the ranges observed historically at the Testing Facility. These delays in ossification were considered effects of the test article associated with the significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) fetal body weights in these dosage groups.

The average numbers of ossification sites in the vertebrae (cervical, thoracic, lumbar, sacral and caudal), ribs, sternum (manubrium and xiphoid), forelimbs (carpals and phalanges) and hindlimbs (tarsals, metatarsals and phalanges) occurred at similar incidences in litters in all dosage groups and did not significantly differ.

F. Satellite Rabbits (Individual Data – Tables 14 through 20)

All rabbits in the satellite dosage groups survived to scheduled sacrifice. There were no adverse clinical observations in these does. Patterns of body weight gain and feed consumption were comparable to the rabbits in the main study at the same dosage levels. Only one rabbit in the 3.75 mg/kg/day dosage group was not pregnant at Caesarean-sectioning on DG 21. Caesarean-sectioning and litter parameters were comparable among the five dosage groups. There was not a reduction in fetal body weights at the 2.5 and 3.75 mg/kg/day dosages as

---

** Significantly different from the vehicle control group value ($p \leq 0.01$).

was observed for the main study rabbits. All does appeared normal at necropsy. Average liver weights for pregnant does were 140.5 ± 29.2, 127.1 ± 15.2, 123.9 ± 33.2, 115.7 ± 5.7 and 90.7 ± 22.2 in the 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day dosage groups, respectively. The data for rabbits assigned to the satellite portion of the study are provided in individual tables only.

REFERENCES:

1. U.S. Food and Drug Administration (1994). International Conference on Harmonisation; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.

2. U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.

3. Japanese Ministry of Health and Welfare (1997). *Good Laboratory Practice Standard for Safety Studies on Drugs*, MHW Ordinance Number 21, March 26, 1997.

4. European Economic Community (1989). *Council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice*. Official Journal of the European Communities: Legislation. 32(No. L 315; 28 October): 1-17.

5. Christian, M.S., Hoberman, A.M. and Smith, T.H.F. (1982). Dosage-range study of the teratogenic potential of suspensions of trinitrofluorenone (TNF) administered orally to New Zealand White rabbits. Toxicologist 2(1):40 (#143).

6. Christian, M.S. (1984). Reproductive toxicity and teratology evaluations of naltrexone (Proceedings of Naltrexone Symposium, New York Academy of Sciences, November 7, 1983), J. Clin. Psychiat. 45(9):7-10.

7. Feussner, E.L., Lightkep, G.E., Hennesy, R.A., Hoberman, A.M. and Christian, M.S. (1992). A decade of rabbit fertility data: Study of historical control animals. Teratology 46(4):349-365.

8. Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, D.C.

9. Salewski, E. (1964). Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Arch. Pathol. Exp. Pharmakol. 247:367.

10. Staples, R.E. (1974). Detection of visceral alterations in mammalian fetuses. Teratology 9(3):A37-38.

11. Staples, R.E. and Schnell, V.L. (1964). Refinement in rapid clearing technique in the KOH-alizarin red S method for fetal bone. Stain Technol. 39:61-63.

12. Snedecor, G.W. and Cochran, W.G. (1967). Variance test for homogeneity of the binomial distribution. *Statistical Methods*, 6th Edition, Iowa State University Press, Ames, pp. 240-241.

13. Sokal, R.R. and Rohlf, F.J. (1969). Bartlett's test of homogeneity of variances. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 370-371.

14. Snedecor, G.W. and Cochran, W.G. (1967). Analysis of Variance. *Statistical Methods*, 6th Edition, Iowa State University Press, Ames, pp. 258-275.

15. Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1129.

16. Sokal, R.R. and Rohlf, F.J. (1969). Kruskal-Wallis Test. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 388-389.

17. Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*, McGraw-Hill, New York, pp. 96-104.

18. Dunn, O.J. (1964). Multiple comparisons using rank sums. Technometrics 6(3):241-252.

19. Christian, M.S., McCarty, R.J., Cox-Sica, D.K. and Cao, C.P. (1987). Recent increases in the incidences of skull, lung and rib alterations in vehicle control New Zealand White rabbits. J. Amer. Col. Toxicol. 6(4):562 (#126).

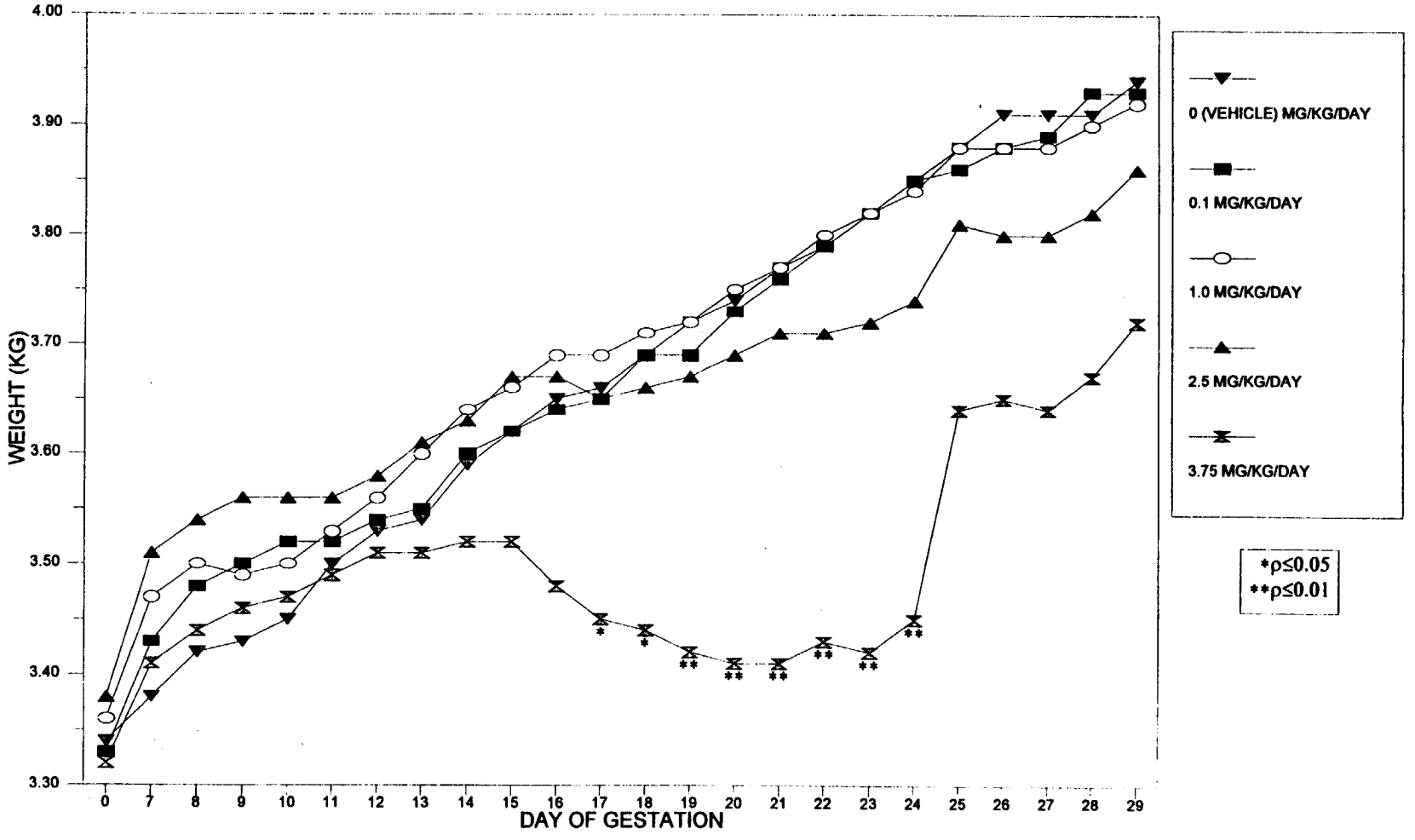
20. Khera, K.S. (1985). Maternal toxicity: A possible etiological factor in embryo-fetal deaths and fetal malformations of rodent-rabbit species. Teratology 31:129-153.

APPENDIX A

REPORT FIGURE

MATERNAL BODY WEIGHTS

Figure 1



APPENDIX B

REPORT TABLES

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 1 (PAGE 1): CLINICAL AND NECROPSY OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	I 0 (VEHICLE)	II 0.1	III 1.0	IV 2.5	V 3.75
MAXIMUM POSSIBLE INCIDENCE	506/ 22	506/ 22	506/ 22	502/ 22	465/ 22
ABORTED	0	0	0	1b	9**c-k
SCANT FECES	0/ 0	0/ 0	2/ 1	5/ 3	24/ 7**f,h-k
NO FECES	0/ 0	0/ 0	0/ 0	0/ 0	2/ 2d
RED SUBSTANCE IN CAGE PAN	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1j
LOCALIZED ALOPECIA: TOTAL	0/ 0	11/ 3**	14/ 5**	4/ 2	0/ 0
LIMBS	0/ 0	8/ 2	7/ 2	3/ 2	0/ 0
BACK	0/ 0	3/ 1	6/ 2	1/ 1	0/ 0
UNDERSIDE	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
UNGROOMED COAT	0/ 0	4/ 2	3/ 1	5/ 1	0/ 0
SOFT OR LIQUID FECES	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0

WITH THE EXCEPTION OF PERSISTENT ADVERSE CLINICAL OBSERVATIONS, NO ADDITIONAL GROSS LESIONS WERE IDENTIFIED AT NECROPSY

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RABBITS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RABBITS)/NUMBER OF RABBITS EXAMINED PER GROUP ON DAYS 7 THROUGH 29 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RABBITS WITH OBSERVATION.

a. Dosage occurred on days 7 through 20 of presumed gestation.
b. Doe 8517 aborted on day 25 of gestation.
c. Doe 8534 aborted on day 22 of gestation.
d. Doe 8537 aborted on day 25 of gestation.
e. Doe 8538 aborted on day 24 of gestation.
f. Doe 8539 aborted on day 24 of gestation.
g. Doe 8540 aborted on day 25 of gestation.
h. Doe 8542 aborted on day 28 of gestation.
i. Doe 8544 aborted on day 25 of gestation.
j. Doe 8547 aborted on day 22 of gestation.
k. Doe 8548 aborted on day 25 of gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 2 (PAGE 1): UTERINE CONTENTS AND LITTER DATA FOR INDIVIDUAL RABBITS THAT ABORTED

DOSAGE GROUP	RABBIT		CORPORA LUTEA			IMPLANTATIONS			FETUSES b				RESORPTIONS c			
DOSAGE (MG/KG/DAY)a	NUMBER	DAY OF DEATH	R	L	T	R	L	T	R	L	A	T	R	L	A	T
I 0 (VEHICLE)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II 0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III 1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV 2.5	8517	ABORTED ON DAY 25 OF GESTATION	6	3	9	5	3	8	0	0	0	0	0	0	8	8

R = RIGHT L = LEFT A = ABORTED T = TOTAL

a. Dosage occurred on days 7 through 20 of gestation.

b. Conceptuses appeared normal for developmental ages.

c. Late resorption unless otherwise noted.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 2 (PAGE 2): UTERINE CONTENTS AND LITTER DATA FOR INDIVIDUAL RABBITS THAT ABORTED

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	RABBIT NUMBER	DAY OF DEATH	CORPORA LUTEA R	CORPORA LUTEA L	CORPORA LUTEA T	IMPLANTATIONS R	IMPLANTATIONS L	IMPLANTATIONS T	FETUSES b R	FETUSES b L	FETUSES b A	FETUSES b T	RESORPTIONS c R	RESORPTIONS c L	RESORPTIONS c A	RESORPTIONS c T
V 3.75	8534	ABORTED ON DAY 22 OF GESTATION	5	4	9	5	3	8	5	2	1	8	0	0	0	0
	8537	ABORTED ON DAY 25 OF GESTATION	4	2	6	3	2	5	0	0	0	0	0	0	5	5
	8538	ABORTED ON DAY 24 OF GESTATION	5	5	10	5	4	9	0	0	0	0	0	0	8	8d
	8539	ABORTED ON DAY 24 OF GESTATION	4	3	7	4	3	7	0	0	7	7	0	0	0	0
	8540	ABORTED ON DAY 25 OF GESTATION	6	8	14	6	8	14	0	4	4	8	2	4	0	6
	8542	ABORTED ON DAY 28 OF GESTATION	4	5	9	4	4	8	0	0	6	6e	0	0	0	0
	8544	ABORTED ON DAY 25 OF GESTATION	4	5	9	4	5	9	0	0	9	9	0	0	0	0
	8547	ABORTED ON DAY 22 OF GESTATION	5	3	8	5	3	8	0	0	0	0	0	0	8	8
	8548	ABORTED ON DAY 25 OF GESTATION	5	7	12	4	7	11	3	5	1	9	0	2	0	2

R = RIGHT L = LEFT A = ABORTED T = TOTAL

a. Dosage occurred on days 7 through 20 of gestation.
b. Conceptuses appeared normal for developmental ages.
c. Late resorptions unless otherwise noted.
d. One aborted conceptus was presumed to have been cannibalized.
e. Two aborted conceptuses were presumed to have been cannibalized.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 3 (PAGE 1): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1.0	IV 2.5	V 3.75
RABBITS TESTED	N	22	22	22	22	22
PREGNANT	N	20	19	19	17	21
INCLUDED IN ANALYSES	N	20	18b	19	17	21
MATERNAL BODY WEIGHT (KG)						
DAY 0	MEAN±S.D.	3.34 ± 0.26	3.33 ± 0.27	3.36 ± 0.27	3.38 ± 0.23	3.32 ± 0.23
DAY 7	MEAN±S.D.	3.38 ± 0.29	3.43 ± 0.22	3.47 ± 0.23	3.51 ± 0.23	3.41 ± 0.20
DAY 8	MEAN±S.D.	3.42 ± 0.30	3.48 ± 0.23	3.50 ± 0.22	3.54 ± 0.25	3.44 ± 0.20
DAY 9	MEAN±S.D.	3.43 ± 0.30	3.50 ± 0.23	3.49 ± 0.22	3.56 ± 0.24	3.46 ± 0.22
DAY 10	MEAN±S.D.	3.45 ± 0.27	3.52 ± 0.23	3.50 ± 0.24	3.56 ± 0.25	3.47 ± 0.23
DAY 11	MEAN±S.D.	3.50 ± 0.27	3.52 ± 0.25	3.53 ± 0.24	3.56 ± 0.24	3.49 ± 0.24
DAY 12	MEAN±S.D.	3.53 ± 0.29	3.54 ± 0.28	3.56 ± 0.24	3.58 ± 0.28	3.51 ± 0.24
DAY 13	MEAN±S.D.	3.54 ± 0.29	3.55 ± 0.25	3.60 ± 0.24	3.61 ± 0.27	3.51 ± 0.24
DAY 14	MEAN±S.D.	3.59 ± 0.30	3.60 ± 0.25	3.64 ± 0.26	3.63 ± 0.26	3.52 ± 0.26
DAY 15	MEAN±S.D.	3.62 ± 0.29	3.62 ± 0.25	3.66 ± 0.26	3.67 ± 0.28	3.52 ± 0.29
DAY 16	MEAN±S.D.	3.65 ± 0.26	3.64 ± 0.27	3.69 ± 0.25	3.67 ± 0.29	3.48 ± 0.30
DAY 17	MEAN±S.D.	3.66 ± 0.27	3.65 ± 0.24	3.69 ± 0.25	3.65 ± 0.28	3.45 ± 0.31*
DAY 18	MEAN±S.D.	3.69 ± 0.28	3.69 ± 0.24	3.71 ± 0.25	3.66 ± 0.29	3.44 ± 0.32*
DAY 19	MEAN±S.D.	3.72 ± 0.28	3.69 ± 0.25	3.72 ± 0.24	3.67 ± 0.29	3.42 ± 0.34**
DAY 20	MEAN±S.D.	3.74 ± 0.28	3.73 ± 0.24	3.75 ± 0.24	3.69 ± 0.31	3.41 ± 0.36**

DAY = DAY OF GESTATION
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for doe 8481, which had a litter that consisted of ten dead fetuses
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 3 (PAGE 2): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
RABBITS TESTED	N	22	22	22	22	22
PREGNANT	N	20	19	19	17	21
INCLUDED IN ANALYSES	N	20	18b	19	17	21
MATERNAL BODY WEIGHT (KG)						
DAY 21	MEAN±S.D.	3.77 ± 0.28	3.76 ± 0.24	3.77 ± 0.24	3.71 ± 0.31	3.41 ± 0.38**
DAY 22	MEAN±S.D.	3.79 ± 0.29	3.79 ± 0.25	3.80 ± 0.25	3.71 ± 0.33	3.43 ± 0.37** [19]c
DAY 23	MEAN±S.D.	3.82 ± 0.30	3.82 ± 0.25	3.82 ± 0.24	3.72 ± 0.33	3.42 ± 0.38** [19]c
DAY 24	MEAN±S.D.	3.85 ± 0.31	3.85 ± 0.28	3.84 ± 0.26	3.74 ± 0.35	3.45 ± 0.41** [18]c
DAY 25	MEAN±S.D.	3.88 ± 0.32	3.86 ± 0.28	3.88 ± 0.26	3.81 ± 0.29 [16]c	3.64 ± 0.20 [13]c
DAY 26	MEAN±S.D.	3.91 ± 0.34	3.88 ± 0.29	3.88 ± 0.26	3.80 ± 0.29 [16]c	3.65 ± 0.23 [13]c
DAY 27	MEAN±S.D.	3.91 ± 0.35	3.89 ± 0.30	3.88 ± 0.25	3.80 ± 0.29 [16]c	3.64 ± 0.22 [13]c
DAY 28	MEAN±S.D.	3.91 ± 0.35	3.93 ± 0.30	3.90 ± 0.26	3.82 ± 0.28 [16]c	3.67 ± 0.25 [12]c
DAY 29	MEAN±S.D.	3.94 ± 0.36	3.93 ± 0.30	3.92 ± 0.26	3.86 ± 0.28 [16]c	3.72 ± 0.26 [12]c

DAY = DAY OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for doe 8481, which had a litter that consisted of ten dead fetuses.
c. Excludes values for rabbits that aborted.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 4 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1.0	IV 2.5	V 3.75
RABBITS TESTED	N	22	22	22	22	22
PREGNANT	N	20	19	19	17	21
INCLUDED IN ANALYSES	N	20	18b	19	17	21
MATERNAL BODY WEIGHT CHANGE (KG)						
DAYS 0 - 7	MEAN±S.D.	+0.04 ± 0.13	+0.10 ± 0.13	+0.11 ± 0.08	+0.12 ± 0.08	+0.09 ± 0.11
DAYS 7 - 10	MEAN±S.D.	+0.07 ± 0.07	+0.08 ± 0.06	+0.03 ± 0.06	+0.05 ± 0.06	+0.06 ± 0.12
DAYS 10 - 13	MEAN±S.D.	+0.09 ± 0.08	+0.03 ± 0.07*	+0.10 ± 0.05	+0.06 ± 0.07	+0.04 ± 0.08*
DAYS 13 - 16	MEAN±S.D.	+0.11 ± 0.09	+0.09 ± 0.07	+0.08 ± 0.05	+0.05 ± 0.09	-0.03 ± 0.11**
DAYS 16 - 19	MEAN±S.D.	+0.06 ± 0.09	+0.05 ± 0.08	+0.03 ± 0.06	+0.00 ± 0.06	-0.07 ± 0.10**
DAYS 19 - 21	MEAN±S.D.	+0.05 ± 0.03	+0.07 ± 0.07	+0.05 ± 0.04	+0.04 ± 0.03	-0.01 ± 0.08
DAYS 21 - 24	MEAN±S.D.	+0.09 ± 0.05	+0.09 ± 0.06	+0.07 ± 0.05	+0.04 ± 0.08	-0.03 ± 0.13* [18]c
DAYS 24 - 29	MEAN±S.D.	+0.08 ± 0.11	+0.08 ± 0.09	+0.08 ± 0.08	+0.06 ± 0.14 [16]c	+0.08 ± 0.10 [12]c
DAYS 7 - 21	MEAN±S.D.	+0.38 ± 0.10	+0.33 ± 0.09	+0.30 ± 0.08*	+0.20 ± 0.17**	-0.01 ± 0.35**
DAYS 21 - 29	MEAN±S.D.	+0.17 ± 0.13	+0.17 ± 0.12	+0.15 ± 0.07	+0.11 ± 0.15 [16]c	+0.11 ± 0.20 [12]c
DAYS 7 - 29	MEAN±S.D.	+0.55 ± 0.18	+0.50 ± 0.16	+0.45 ± 0.12	+0.34 ± 0.16** [16]c	+0.30 ± 0.38 [12]c
DAYS 0 - 29	MEAN±S.D.	+0.59 ± 0.21	+0.60 ± 0.13	+0.56 ± 0.13	+0.46 ± 0.14 [16]c	+0.39 ± 0.38 [12]c

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for doe 8481, which had a litter that consisted of ten dead fetuses.
c. Excludes values for rabbits that aborted.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 5 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1.0	IV 2.5	V 3.75
RABBITS TESTED	N	22	22	22	22	22
PREGNANT	N	20	19	19	17	21
INCLUDED IN ANALYSES	N	20	18b	19	17	21
MATERNAL FEED CONSUMPTION (G/DAY)						
DAYS 7 - 10	MEAN±S.D.	142.6 ± 50.4	171.3 ± 19.1	161.2 ± 22.8 [18]c	161.2 ± 18.9	151.5 ± 41.0
DAYS 10 - 13	MEAN±S.D.	149.7 ± 35.7	142.0 ± 47.8	158.8 ± 24.0	142.6 ± 35.9	129.6 ± 53.7
DAYS 13 - 16	MEAN±S.D.	162.3 ± 24.5	158.1 ± 35.7	162.8 ± 36.6	143.0 ± 45.8	95.8 ± 75.0
DAYS 16 - 19	MEAN±S.D.	177.5 ± 11.8	175.2 ± 12.8 [17]c	163.0 ± 33.7	145.6 ± 43.4**	77.1 ± 76.7**
DAYS 19 - 21	MEAN±S.D.	173.6 ± 19.9	180.1 ± 7.0 [17]c	170.2 ± 20.5	146.1 ± 45.5	69.7 ± 72.9**
DAYS 21 - 24	MEAN±S.D.	160.9 ± 32.5	165.2 ± 20.7	160.2 ± 25.3	130.4 ± 41.0	80.5 ± 72.0** [17]d
DAYS 24 - 29	MEAN±S.D.	124.5 ± 40.5	135.5 ± 38.7	131.3 ± 33.6	112.8 ± 37.2 [16]d	114.6 ± 51.1 [12]d
DAYS 7 - 21	MEAN±S.D.	160.2 ± 20.5	163.3 ± 20.8 [17]c	162.9 ± 20.7	147.8 ± 28.8	107.2 ± 54.6**
DAYS 21 - 29	MEAN±S.D.	138.4 ± 34.8	146.9 ± 29.2	142.1 ± 24.8	120.7 ± 29.3 [16]d	112.7 ± 50.9 [12]d
DAYS 7 - 29	MEAN±S.D.	152.4 ± 21.7	157.8 ± 21.2	155.3 ± 16.6	141.0 ± 20.5 [16]d	127.7 ± 39.0 [12]d

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for doe 8481, which had a litter that consisted of ten dead fetuses.
c. Excludes values that were associated with spillage or wet feed.
d. Excludes values for rabbits that aborted.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 6 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1.0	IV 2.5	V 3.75
RABBITS TESTED	N	22	22	22	22	22
PREGNANT	N	20	19	19	17	21
INCLUDED IN ANALYSES	N	20	18b	19	17	21
MATERNAL FEED CONSUMPTION (G/KG/DAY)						
DAYS 7 - 10	MEAN±S.D.	41.4 ± 14.5	49.1 ± 4.2	46.0 ± 6.5 [18]c	45.5 ± 4.5	43.7 ± 11.9
DAYS 10 - 13	MEAN±S.D.	42.5 ± 9.1	39.9 ± 12.8	44.8 ± 6.9	39.6 ± 8.7	36.7 ± 14.8
DAYS 13 - 16	MEAN±S.D.	45.1 ± 6.5	43.7 ± 9.1	44.5 ± 9.7	39.0 ± 11.8	26.4 ± 20.7*
DAYS 16 - 19	MEAN±S.D.	48.4 ± 3.9	47.8 ± 3.7 [17]c	44.1 ± 9.4	39.3 ± 11.2**	21.2 ± 20.9**
DAYS 19 - 21	MEAN±S.D.	46.6 ± 5.8	48.5 ± 3.4 [17]c	45.5 ± 5.7	39.1 ± 11.6*	19.1 ± 19.6**
DAYS 21 - 24	MEAN±S.D.	42.3 ± 8.0	43.3 ± 4.2	42.1 ± 6.6	34.6 ± 9.3*	22.0 ± 19.4** [17]d
DAYS 24 - 29	MEAN±S.D.	31.7 ± 9.0	34.5 ± 8.6	33.9 ± 8.9	29.5 ± 9.3 [16]d	30.8 ± 13.6 [12]d
DAYS 7 - 21	MEAN±S.D.	44.8 ± 5.1	45.4 ± 4.6 [17]c	45.1 ± 5.8	40.6 ± 6.4	30.4 ± 14.9*
DAYS 21 - 29	MEAN±S.D.	35.7 ± 7.8	37.9 ± 6.0	36.9 ± 6.6	31.7 ± 6.8 [16]d	30.5 ± 13.6 [12]d
DAYS 7 - 29	MEAN±S.D.	41.4 ± 4.9	42.7 ± 4.1	42.0 ± 4.6	38.1 ± 4.0* [16]d	35.4 ± 10.4 [12]d

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for doe 8481, which had a litter that consisted of ten dead fetuses.
c. Excludes values that were associated with spillage or wet feed.
d. Excludes values for rabbits that aborted.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 7 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1.0	IV 2.5	V 3.75
RABBITS TESTED	N	22	22	22	22	22
PREGNANT	N(%)	20(90.9)	19(86.4)	19(86.4)	17(77.3)	21(95.4)
ABORTED	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.9)	9(42.8)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	20	19	19	16	12
INCLUDED IN ANALYSES	N	20	18b	19	16	12
CORPORA LUTEA	MEAN±S.D.	10.8 ± 2.5	10.9 ± 2.5	10.5 ± 2.3	10.4 ± 2.2	11.1 ± 1.7
IMPLANTATIONS	MEAN±S.D.	9.0 ± 2.2	9.1 ± 1.9	8.4 ± 1.8	8.4 ± 1.8	9.2 ± 1.9
LITTER SIZES	MEAN±S.D.	8.8 ± 2.2	9.0 ± 2.0	8.0 ± 1.9	8.1 ± 1.8	9.0 ± 2.0
LIVE FETUSES	N	175	162	152	130	108
	MEAN±S.D.	8.8 ± 2.2	9.0 ± 2.0	8.0 ± 1.9	8.1 ± 1.8	9.0 ± 2.0
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.2 ± 0.4	0.1 ± 0.3	0.4 ± 0.8	0.2 ± 0.4	0.2 ± 0.4
EARLY RESORPTIONS	N	3	2	2	2	1
	MEAN±S.D.	0.2 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3
LATE RESORPTIONS	N	1	0	5	2	1
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.3 ± 0.7	0.1 ± 0.3	0.1 ± 0.3
DOES WITH ANY RESORPTIONS	N(%)	4(20.0)	2(11.1)	5(26.3)	4(25.0)	2(16.7)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0	0	0	0	0
DOES WITH VIABLE FETUSES	N(%)	20(100.0)	18(100.0)	19(100.0)	16(100.0)	12(100.0)
PLACENTAE APPEARED NORMAL	N(%)	20(100.0)	18(100.0)	19(100.0)	16(100.0)	12(100.0)

a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for doe 8481, which had a litter that consisted of ten dead fetuses.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 8 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1.0	IV 2.5	V 3.75
LITTERS WITH ONE OR MORE LIVE FETUSES	N	20	18	19	16	12
IMPLANTATIONS	MEAN±S.D.	9.0 ± 2.2	9.1 ± 1.9	8.4 ± 1.8	8.4 ± 1.8	9.2 ± 1.9
LIVE FETUSES	N	175	162	152	130	108
	MEAN±S.D.	8.8 ± 2.2	9.0 ± 2.0	8.0 ± 1.9	8.1 ± 1.8	9.0 ± 2.0
LIVE MALE FETUSES	N	87	74	71	66	50
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	50.4 ± 15.1	45.8 ± 19.2	45.6 ± 18.0	50.2 ± 14.1	44.1 ± 21.7
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	44.15 ± 4.50	41.67 ± 3.28	42.37 ± 4.34	39.89 ± 4.33*	33.41 ± 7.61**
MALE FETUSES	MEAN±S.D.	44.50 ± 4.90	41.82 ± 2.77	43.12 ± 5.13	40.56 ± 4.52*	34.10 ± 7.36** [11]b
FEMALE FETUSES	MEAN±S.D.	43.86 ± 4.84	41.36 ± 4.09	41.94 ± 4.90	38.98 ± 5.28*	32.31 ± 8.27**
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	2.1 ± 4.4	1.4 ± 4.1	4.4 ± 9.4	3.1 ± 5.7	1.8 ± 4.1

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Litter 8546 had no male fetuses.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 9 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
LITTERS EVALUATED	N	20	19b	19	16	12
FETUSES EVALUATED	N	175	172	152	130	108
LIVE	N	175	162	152	130	108
DEAD	N	0	10b	0	0	0
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	14(70.0)	11(61.1)	9(47.4)	4(25.0)	8(66.7)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	25(14.3)	25(15.4)	14(9.2)	5(3.8)**	19(17.6)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	14.1 ± 12.9	17.0 ± 18.2	9.5 ± 11.7	3.6 ± 7.0	17.4 ± 22.2

a. Dosage occurred on days 7 through 20 of gestation.
b. Litter 8481 consisted of ten dead fetuses; values were excluded from group averages and statistical analyses. Adverse observations for this litter are cited on Table 21.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 10 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
LITTERS EVALUATED	N	20	19b	19	16	12
FETUSES EVALUATED	N	175	162	152	130	108
LIVE	N	175	162	152	130	108
DEAD	N	0	10b	0	0	0
FORELIMBS: FLEXED DOWNWARD						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)	1(8.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	1(0.9)
FORELIMB: POLLEX ABSENT						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

a. Dosage occurred on days 7 through 20 of gestation.
b. Litter 8481 consisted of ten dead fetuses; values were excluded from group averages and statistical analyses. Adverse observations for this litter are cited on Table 21.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 11 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
LITTERS EVALUATED	N	20	19b	19	16	12
FETUSES EVALUATED	N	175	172	152	130	108
LIVE	N	175	162	152	130	108
DEAD	N	0	10b	0	0	0
EYES: CIRCUMCORNEAL HEMORRHAGE						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
LUNGS: INTERMEDIATE LOBE ABSENT						
LITTER INCIDENCE	N(%)	2(10.0)	4(22.2)	1(5.3)	0(0.0)	1(8.3)
FETAL INCIDENCE	N(%)	2(1.1)	7(4.3)**	1(0.6)	0(0.0)	1(0.9)

a. Dosage occurred on days 7 through 20 of gestation.
b. Litter 8481 consisted of ten dead fetuses; values were excluded from group averages and statistical analyses. Adverse observations for this litter are cited on Table 21.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 12 (PAGE 1): FETAL SKELETAL OBSERVATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
LITTERS EVALUATED	N	20	19b	19	16	12
FETUSES EVALUATED	N	175	172	152	130	108
LIVE	N	175	162	152	130	108
DEAD	N	0	10b	0	0	0
SKULL - IRREGULAR OSSIFICATION:c (SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF THE SKULL d; INDIVIDUAL SUBCATEGORIES CITED BELOW)						
LITTER INCIDENCE	N(%)	6(30.0)	7(38.9)	3(15.8)	1(6.2)	3(25.0)
FETAL INCIDENCE	N(%)	7(4.0)	9(5.6)	3(2.0)	1(0.8)*	10(9.2)**
SKULL: NASAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF FUSED; IRREGULAR SUTURE; MIDLINE SUTURE DISPLACED; INTRANASAL; INTERNASAL)						
LITTER INCIDENCE	N(%)	5(25.0)	7(38.9)	3(15.8)	1(6.2)	1(8.3)
FETAL INCIDENCE	N(%)	6(3.4)	9(5.6)	3(2.0)	1(0.8)	1(0.9)
SKULL: NASAL - FRONTAL, IRREGULAR SUTURE						
LITTER INCIDENCE	N(%)	1(5.0)	2(11.1)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)f	2(1.2)	0(0.0)	0(0.0)	0(0.0)
SKULL: NASALS, FUSED						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SKULL: NASALS, CONTAINED AN INTERNASAL						
LITTER INCIDENCE	N(%)	1(5.0)	2(11.1)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	3(1.8)	0(0.0)	1(0.8)	0(0.0)
SKULL: NASALS, MIDLINE SUTURE DISPLACED						
LITTER INCIDENCE	N(%)	0(0.0)	3(16.7)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.5)**	1(0.6)	0(0.0)	0(0.0)
SKULL: NASAL, CONTAINED AN INTRANASAL						
LITTER INCIDENCE	N(%)	3(15.0)	0(0.0)	2(10.5)	0(0.0)	1(8.3)
FETAL INCIDENCE	N(%)	3(1.7)	0(0.0)	2(1.3)	0(0.0)	1(0.9)

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 12 (PAGE 2): FETAL SKELETAL OBSERVATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
LITTERS EVALUATED	N	20	19b	19	16	12
FETUSES EVALUATED	N	175	172	152	130	108
LIVE	N	175	162	152	130	108
DEAD	N	0	10b	0	0	0
SKULL: FRONTALS, CONTAINED AN INTERFRONTAL						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	1(8.3)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)	2(1.8)
SKULL: PARIETAL, CONTAINED A HOLE						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(8.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	7(6.5)k,l**
SKULL - OTHER ALTERATIONS:b						
HYOID: ALA, ANGULATED						
LITTER INCIDENCE	N(%)	6(30.0)	3(16.7)	3(15.8)	2(12.5)	3(25.0)
FETAL INCIDENCE	N(%)	10(5.7)f,g	4(2.5)	7(4.6)	3(2.3)	5(4.6)
SKULL: PREMAXILLAE, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	1(0.8)	0(0.0)
CERVICAL VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRUM, BIFID						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)h	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRA, FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	2(11.1)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)h,i	0(0.0)	0(0.0)	0(0.0)

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 12 (PAGE 3): FETAL SKELETAL OBSERVATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
LITTERS EVALUATED	N	20	19b	19	16	12
FETUSES EVALUATED	N	175	172	152	130	108
LIVE	N	175	162	152	130	108
DEAD	N	0	10b	0	0	0
THORACIC VERTEBRAE: HEMIVERTEBRA						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)g	0(0.0)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: ARCH, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)i	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
LUMBAR VERTEBRAE: CENTRUM, BIFID						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)	1(8.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)j	0(0.0)	1(0.9)
LUMBAR VERTEBRAE: HEMIVERTEBRA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)j	0(0.0)	0(0.0)
CAUDAL VERTEBRAE: MISALIGNED						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RIBS: FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
RIBS: SPLIT						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)g	0(0.0)	1(0.6)	0(0.0)	0(0.0)

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 12 (PAGE 4): FETAL SKELETAL OBSERVATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
LITTERS EVALUATED	N	20	19b	19	16	12
FETUSES EVALUATED	N	175	172	152	130	108
LIVE	N	175	162	152	130	108
DEAD	N	0	10b	0	0	0
RIBS: THICKENED						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.6)	0(0.0)	0(0.0)
RIBS: PROXIMATE						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
STERNAL CENTRA: FUSED						
LITTER INCIDENCE	N(%)	2(10.0)	3(16.7)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.1)	3(1.8)	0(0.0)	0(0.0)	0(0.0)
STERNAL CENTRA: ASYMMETRIC						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)g	0(0.0)	0(0.0)	0(0.0)	0(0.0)
METACARPALS: FORELIMB, 4 METACARPALS PRESENT						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)e	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FOREDIGITS: FORELIMB, 4 DIGITS PRESENT						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)e	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FOREPHALANGES: FORELIMB, 1ST MEDIAL AND DISTAL PHALANGES ABSENT						
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)e	0(0.0)	0(0.0)	0(0.0)	0(0.0)
PELVIS: PUBIS, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(16.7)**
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(3.7)k,l**

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 12 (PAGE 5): FETAL SKELETAL OBSERVATIONS - SUMMARY

FOOTNOTES:

---

a. Dosage occurred on days 7 through 20 of gestation.
b. Litter 8481 consisted of ten dead fetuses; values were excluded from group averages and statistical analyses. Adverse observations for this litter are cited on Table 21.
c. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summary table, except when alterations of other ossification sites were also present.
d. Includes all alterations noted for the skull except hyoid, ala, angulated and skull, premaxillae, not ossified. These categories are excluded because these alterations do not result from irregular ossification.
e. Fetus 8448-1 had other skeletal alterations.
f. Fetus 8449-6 had other skeletal alterations.
g. Fetus 8452-1 had other skeletal alterations.
h. Fetus 8476-1 had other skeletal alterations.
i. Fetus 8485-2 had other skeletal alterations.
j. Fetus 8500-8 had other skeletal alterations.
k. Fetus 8543-4 had other skeletal alterations.
l. Fetus 8543-6 had other skeletal alterations.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 13 (PAGE 1): FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1.0	2.5	3.75
LITTERS EXAMINED	N	20	19b	19	16	12
FETUSES EXAMINED	N	175	172	152	130	108
LIVE	N	175	162	152	130	108
DEAD	N	0	10b	0	0	0
OSSIFICATION SITES PER FETUS PER LITTER						
HYOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.92 ± 0.20**
VERTEBRAE						
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.72 ± 0.23	12.58 ± 0.28	12.70 ± 0.29	12.49 ± 0.33	12.52 ± 0.32
LUMBAR	MEAN±S.D.	6.26 ± 0.23	6.42 ± 0.28	6.29 ± 0.29	6.51 ± 0.33	6.47 ± 0.31
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	16.90 ± 0.24	17.03 ± 0.30	16.95 ± 0.38	16.86 ± 0.28	16.92 ± 0.50
RIBS (PAIRS)	MEAN±S.D.	12.66 ± 0.26	12.50 ± 0.26	12.60 ± 0.29	12.42 ± 0.28	12.47 ± 0.34
STERNUM						
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.98 ± 0.05	3.92 ± 0.11	3.95 ± 0.12	3.81 ± 0.17**	3.82 ± 0.22*
XIPHOID	MEAN±S.D.	0.98 ± 0.04	0.99 ± 0.05	0.97 ± 0.06	0.94 ± 0.10	0.95 ± 0.10
FORELIMB c						
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.98 ± 0.05	4.97 ± 0.06	4.99 ± 0.04	4.97 ± 0.07	4.82 ± 0.31*
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.92 ± 0.12	13.92 ± 0.17	13.90 ± 0.17	13.96 ± 0.07	13.92 ± 0.15
HINDLIMB c						
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	1.92 ± 0.23
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	11.92 ± 0.23

a. Dosage occurred on days 7 through 20 of gestation.
b. Litter 8481 consisted of ten dead fetuses; values excluded from group averages and statistical analyses. Adverse observations for this litter are cited on Table 21.
c. Calculated as average per limb.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 14 (PAGE 1): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RABBIT #	DESCRIPTION
DOSAGE GROUP I	0 (VEHICLE) MG/KG/DAY
8443	NO ADVERSE FINDINGS
8444	NO ADVERSE FINDINGS
8445	NO ADVERSE FINDINGS
8446	NO ADVERSE FINDINGS
8447	NO ADVERSE FINDINGS
8448	NO ADVERSE FINDINGS
8449	NO ADVERSE FINDINGS
8450	NO ADVERSE FINDINGS
8451	NO ADVERSE FINDINGS
8452	NO ADVERSE FINDINGS
8453	NO ADVERSE FINDINGS
8454	NO ADVERSE FINDINGS
8455	NO ADVERSE FINDINGS
8456	NO ADVERSE FINDINGS
8457	NO ADVERSE FINDINGS
8458	NO ADVERSE FINDINGS
8459	NO ADVERSE FINDINGS
8460	NO ADVERSE FINDINGS
8461	NO ADVERSE FINDINGS
8462	NO ADVERSE FINDINGS
8463	NO ADVERSE FINDINGS
8464	NO ADVERSE FINDINGS

DG = DAY OF PRESUMED GESTATION

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 14 (PAGE 2): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RABBIT #		DESCRIPTION
DOSAGE GROUP II		0.1 MG/KG/DAY
8465	DG(28- 29)	LOCALIZED ALOPECIA: LIMBS a
8466		NO ADVERSE FINDINGS
8467		NO ADVERSE FINDINGS
8468		NO ADVERSE FINDINGS
8469		NO ADVERSE FINDINGS
8470		NO ADVERSE FINDINGS
8471		NO ADVERSE FINDINGS
8472		NO ADVERSE FINDINGS
8473	DG(27- 29)	LOCALIZED ALOPECIA: BACK a
8474		NO ADVERSE FINDINGS
8475		NO ADVERSE FINDINGS
8476		NO ADVERSE FINDINGS
8477		NO ADVERSE FINDINGS
8478	DG(28)	UNGROOMED COAT
8479		NO ADVERSE FINDINGS
8480		NO ADVERSE FINDINGS
8481		NO ADVERSE FINDINGS
8482	DG(24- 29)	LOCALIZED ALOPECIA: LIMBS a
8483		NO ADVERSE FINDINGS
8484		NO ADVERSE FINDINGS
8485		NO ADVERSE FINDINGS
8486	DG(27- 29)	UNGROOMED COAT

DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 14 (PAGE 3): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RABBIT #		DESCRIPTION
DOSAGE GROUP III		1.0 MG/KG/DAY
8487		NO ADVERSE FINDINGS
8488		NO ADVERSE FINDINGS
8489	DG(29)	LOCALIZED ALOPECIA: UNDERSIDE a
8490		NO ADVERSE FINDINGS
8491		NO ADVERSE FINDINGS
8492	DG(9)	SOFT OR LIQUID FECES
	DG(9- 11)	UNGROOMED COAT
	DG(10- 11)	SCANT FECES
8493	DG(27- 29)	LOCALIZED ALOPECIA: LIMBS a
8494		NO ADVERSE FINDINGS
8495		NO ADVERSE FINDINGS
8496	DG(26- 29)	LOCALIZED ALOPECIA: BACK a
8497		NO ADVERSE FINDINGS
8498		NO ADVERSE FINDINGS
8499		NO ADVERSE FINDINGS
8500		NO ADVERSE FINDINGS
8501		NO ADVERSE FINDINGS
8502		NO ADVERSE FINDINGS
8503		NO ADVERSE FINDINGS
8504		NO ADVERSE FINDINGS
8505		NO ADVERSE FINDINGS
8506	DG(26- 29)	LOCALIZED ALOPECIA: LIMBS a
8507	DG(28- 29)	LOCALIZED ALOPECIA: BACK a
8508		NO ADVERSE FINDINGS

DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 14 (PAGE 4): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RABBIT #		DESCRIPTION
DOSAGE GROUP IV		2.5 MG/KG/DAY
8509		NO ADVERSE FINDINGS
8510		NO ADVERSE FINDINGS
8511		NO ADVERSE FINDINGS
8512		NO ADVERSE FINDINGS
8513		NO ADVERSE FINDINGS
8514		NO ADVERSE FINDINGS
8515		NO ADVERSE FINDINGS
8516	DG(28- 29)	LOCALIZED ALOPECIA: LIMBS a
8517	DG(25)	ABORTED AND SACRIFICED
8518	DG(28- 29)	SCANT FECES
8519		NO ADVERSE FINDINGS
8520		NO ADVERSE FINDINGS
8521	DG(28)	LOCALIZED ALOPECIA: LIMBS
	DG(29)	ALOPECIA NO LONGER APPARENT
	DG(29)	LOCALIZED ALOPECIA: BACK a
8522		NO ADVERSE FINDINGS
8523		NO ADVERSE FINDINGS
8524	DG(24- 25)	SCANT FECES
8525	DG(26)	SCANT FECES
8526		NO ADVERSE FINDINGS
8527	DG(24- 28)	UNGROOMED COAT
8528		NO ADVERSE FINDINGS
8529		NO ADVERSE FINDINGS
8530		NO ADVERSE FINDINGS

DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 14 (PAGE 5): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RABBIT #		DESCRIPTION
DOSAGE GROUP V		3.75 MG/KG/DAY
8531		NO ADVERSE FINDINGS
8532		NO ADVERSE FINDINGS
8533		NO ADVERSE FINDINGS
8534	DG(22)	ABORTED AND SACRIFICED
8535	DG(26- 27)	SCANT FECES
8536		NO ADVERSE FINDINGS
8537	DG(24)	NO FECES
	DG(25)	ABORTED AND SACRIFICED
8538	DG(24)	ABORTED AND SACRIFICED
8539	DG(22- 23)	SCANT FECES
	DG(24)	ABORTED AND SACRIFICED
8540	DG(25)	ABORTED AND SACRIFICED
8541		NO ADVERSE FINDINGS
8542	DG(21- 23)	SCANT FECES
	DG(27)	SCANT FECES
	DG(28)	ABORTED AND SACRIFICED
8543	DG(19- 20)	SCANT FECES
	DG(22- 23)	SCANT FECES
	DG(24)	NO FECES
	DG(25- 27)	SCANT FECES
8544	DG(19- 20)	SCANT FECES
	DG(22- 24)	SCANT FECES
	DG(25)	ABORTED AND SACRIFICED
8545		NO ADVERSE FINDINGS
8546		NO ADVERSE FINDINGS
8547	DG(18)	SCANT FECES
	DG(21)	RED SUBSTANCE IN CAGE PAN
	DG(22)	ABORTED AND SACRIFICED
8548	DG(21)	SCANT FECES
	DG(23- 24)	SCANT FECES
	DG(25)	ABORTED AND SACRIFICED
8549		NO ADVERSE FINDINGS
8550		NO ADVERSE FINDINGS
8551		NO ADVERSE FINDINGS
8552		NO ADVERSE FINDINGS

DG = DAY OF PRESUMED GESTATION

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 14 (PAGE 6): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RABBIT #	DESCRIPTION
SATELLITE DOSAGE GROUP I	0 (VEHICLE) MG/KG/DAY
8553	NO ADVERSE FINDINGS
8554	NO ADVERSE FINDINGS
8555	NO ADVERSE FINDINGS
SATELLITE DOSAGE GROUP II	0.1 MG/KG/DAY
8556	NO ADVERSE FINDINGS
8557	NO ADVERSE FINDINGS
8558	NO ADVERSE FINDINGS
8559	NO ADVERSE FINDINGS
8560	NO ADVERSE FINDINGS
SATELLITE DOSAGE GROUP III	1.0 MG/KG/DAY
8561	NO ADVERSE FINDINGS
8562	NO ADVERSE FINDINGS
8563	NO ADVERSE FINDINGS
SATELLITE DOSAGE GROUP IV	2.5 MG/KG/DAY
8564	NO ADVERSE FINDINGS
8565	NO ADVERSE FINDINGS
8566	NO ADVERSE FINDINGS
SATELLITE DOSAGE GROUP V	3.75 MG/KG/DAY
8567	NO ADVERSE FINDINGS
8568	NO ADVERSE FINDINGS
8569	NO ADVERSE FINDINGS
8570	NO ADVERSE FINDINGS
8571	NO ADVERSE FINDINGS

DG = DAY OF PRESUMED GESTATION

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 15 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
I					
0 (VEHICLE)	8443	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8444	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8445	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8446	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8447	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8448	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8449	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8450	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8451	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8452	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8453	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8454	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8455	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8456	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8457	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8458	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8459	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8460	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8461	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8462	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8463	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8464	DG 29	P	14	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 15 (PAGE 2): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
II					
0.1	8465	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8466	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8467	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8468	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8469	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8470	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8471	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8472	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8473	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8474	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8475	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8476	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8477	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8478	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8479	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8480	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8481	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8482	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8483	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8484	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8485	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8486	DG 29	P	14	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 15 (PAGE 3): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
III					
1.0	8487	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8488	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8489	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8490	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8491	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8492	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8493	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8494	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8495	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8496	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8497	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8498	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8499	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8500	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8501	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8502	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8503	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8504	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8505	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8506	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8507	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8508	DG 29	P	14	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 15 (PAGE 4): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
IV 2.5	8509	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8510	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8511	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8512	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8513	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8514	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8515	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8516	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8517	DG 25	P	14	ABORTED ON DAY 25 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8518	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8519	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8520	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8521	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8522	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8523	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8524	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8525	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8526	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8527	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8528	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8529	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8530	DG 29	P	14	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 15 (PAGE 5): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
V					
3.75	8531	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8532	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8533	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8534	DG 22	P	14	ABORTED ON DAY 22 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8535	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8536	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8537	DG 25	P	14	ABORTED ON DAY 25 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8538	DG 24	P	14	ABORTED ON DAY 24 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8539	DG 24	P	14	ABORTED ON DAY 24 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8540	DG 25	P	14	ABORTED ON DAY 25 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8541	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8542	DG 28	P	14	ABORTED ON DAY 28 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8543	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8544	DG 25	P	14	ABORTED ON DAY 25 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8545	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8546	DG 29	P	14	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 15 (PAGE 6): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
V 3.75 (cont.)	8547	DG 22	P	14	ABORTED ON DAY 22 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8548	DG 25	P	14	ABORTED ON DAY 25 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8549	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8550	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8551	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8552	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 15 (PAGE 7): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

SATELLITE DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a	LIVER WEIGHT (G)
I						
0 (VEHICLE)	8553	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	154.4
	8554	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	160.1
	8555	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	107.0
II						
0.1	8556	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	138.0
	8557	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	134.9
	8558	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	136.7
	8559	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	124.1
	8560	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	101.6
III						
1.0	8561	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	159.0
	8562	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	119.9
	8563	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	92.9
IV						
2.5	8564	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	116.7
	8565	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	109.6
	8566	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	120.9
V						
3.75	8567	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	77.9
	8568	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	97.7
	8569	DG 21	NP	14	ALL TISSUES APPEARED NORMAL.	77.6
	8570	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	118.6
	8571	DG 21	P	14	ALL TISSUES APPEARED NORMAL.	68.5

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 1): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP I				0 (VEHICLE) MG/KG/DAY								
8443 NP	3.22	3.35	3.42	3.38	3.41	3.36	3.43	3.46	3.53	3.53	3.63	3.55	3.60
8444 P	3.40	3.42	3.51	3.52	3.48	3.48	3.46	3.48	3.52	3.53	3.52	3.53	3.56
8445 NP	3.58	3.76	3.81	3.91	3.89	3.87	3.94	3.94	3.92	3.93	4.01	3.91	3.95
8446 P	3.24	3.38	3.45	3.40	3.29	3.31	3.41	3.50	3.56	3.63	3.68	3.59	3.62
8447 P	3.44	3.79	3.82	3.82	3.85	3.94	3.97	3.96	4.00	4.05	4.01	4.04	4.10
8448 P	3.44	3.44	3.54	3.54	3.62	3.61	3.63	3.67	3.74	3.84	3.78	3.81	3.84
8449 P	3.55	3.50	3.41	3.34	3.40	3.55	3.54	3.66	3.66	3.66	3.66	3.69	3.77
8450 P	3.07	3.24	3.29	3.32	3.37	3.34	3.39	3.36	3.40	3.43	3.50	3.55	3.55
8451 P	3.34	3.41	3.43	3.47	3.50	3.51	3.58	3.65	3.80	3.77	3.74	3.77	3.74
8452 P	3.17	2.86	2.83	2.80	2.97	3.15	3.12	3.11	3.16	3.25	3.31	3.40	3.42
8453 P	3.66	3.63	3.70	3.73	3.72	3.80	3.82	3.82	3.92	3.86	3.90	3.98	3.98
8454 P	3.56	3.58	3.58	3.66	3.64	3.73	3.74	3.77	3.77	3.80	3.75	3.78	3.89
8455 P	3.37	3.44	3.49	3.51	3.56	3.58	3.66	3.67	3.75	3.67	3.72	3.72	3.78
8456 P	3.31	3.20	3.16	3.13	3.24	3.34	3.37	3.36	3.41	3.44	3.54	3.58	3.57
8457 P	3.01	3.13	3.19	3.24	3.23	3.24	3.26	3.22	3.26	3.30	3.32	3.38	3.42
8458 P	3.80	3.89	3.91	3.90	3.85	3.87	3.88	3.98	4.04	4.06	4.08	4.10	4.15
8459 P	3.76	3.82	3.89	3.86	3.90	3.94	4.08	4.03	4.07	4.11	4.11	4.09	4.12
8460 P	3.53	3.64	3.64	3.66	3.66	3.74	3.84	3.77	3.81	3.84	3.86	3.91	3.91
8461 P	2.99	3.06	3.14	3.14	3.17	3.16	3.12	3.15	3.15	3.20	3.55	3.24	3.26
8462 P	3.18	3.22	3.29	3.34	3.34	3.35	3.34	3.35	3.40	3.41	3.45	3.45	3.47
8463 P	3.12	3.12	3.21	3.28	3.28	3.34	3.35	3.30	3.32	3.39	3.45	3.48	3.48
8464 P	2.88	2.93	2.90	2.95	3.01	3.05	3.08	3.10	3.14	3.17	3.16	3.17	3.20

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 2): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 19	20	21	22	23	24	25	26	27	28	29
RABBIT #	DOSAGE GROUP I				0 (VEHICLE) MG/KG/DAY						
8443 NP	3.60	3.64	3.70	3.68	3.73	3.70	3.74	3.75	3.80	3.80	3.84
8444 P	3.61	3.61	3.64	3.65	3.65	3.67	3.68	3.70	3.68	3.71	3.76
8445 NP	4.06	4.14	4.17	4.16	4.13	4.16	4.20	4.23	4.20	4.38	4.27
8446 P	3.67	3.67	3.72	3.77	3.80	3.85	3.84	3.78	3.69	3.70	3.74
8447 P	4.13	4.18	4.18	4.27	4.31	4.36	4.44	4.52	4.52	4.61	4.64
8448 P	3.85	3.90	3.93	3.98	4.00	4.04	4.13	4.17	4.21	4.24	4.25
8449 P	3.79	3.83	3.83	3.92	3.93	3.97	4.06	4.09	4.14	4.12	4.12
8450 P	3.57	3.60	3.63	3.62	3.66	3.67	3.73	3.73	3.81	3.81	3.88
8451 P	3.78	3.81	3.82	3.83	3.87	3.92	3.97	3.93	3.91	3.88	3.89
8452 P	3.46	3.49	3.50	3.50	3.51	3.57	3.60	3.67	3.65	3.64	3.66
8453 P	3.99	4.04	4.07	4.07	4.12	4.15	4.18	4.23	4.29	4.28	4.37
8454 P	3.89	3.92	3.95	4.00	4.06	4.09	4.11	4.16	4.16	4.12	4.15
8455 P	3.82	3.86	3.90	3.91	3.95	3.98	4.00	4.05	4.07	4.05	4.10
8456 P	3.62	3.61	3.66	3.70	3.69	3.72	3.72	3.74	3.71	3.72	3.72
8457 P	3.47	3.50	3.54	3.55	3.61	3.62	3.64	3.66	3.60	3.61	3.58
8458 P	4.10	4.14	4.16	4.17	4.18	4.21	4.23	4.25	4.24	4.16	4.10
8459 P	4.18	4.15	4.18	4.20	4.29	4.33	4.32	4.42	4.39	4.40	4.52
8460 P	3.98	3.96	3.99	3.98	3.99	4.11	4.15	4.23	4.22	4.24	4.26
8461 P	3.26	3.30	3.29	3.30	3.33	3.33	3.32	3.31	3.34	3.34	3.35
8462 P	3.51	3.50	3.49	3.44	3.50	3.49	3.53	3.53	3.55	3.58	3.61
8463 P	3.52	3.53	3.55	3.58	3.57	3.65	3.68	3.68	3.66	3.67	3.66
8464 P	3.23	3.24	3.29	3.29	3.33	3.35	3.37	3.36	3.37	3.37	3.39

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 3): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP II				0.1 MG/KG/DAY								
8465 P	3.58	3.88	3.94	3.91	3.94	3.97	4.02	4.02	4.09	4.07	4.11	4.04	4.08
8466 NP	3.53	3.63	3.63	3.64	3.57	3.64	3.63	3.66	3.63	3.64	3.68	3.72	3.78
8467 NP	3.43	3.63	3.66	3.66	3.68	3.77	3.73	3.77	3.87	3.80	3.92	3.85	3.85
8468 P	3.23	3.53	3.55	3.57	3.52	3.53	3.60	3.64	3.68	3.72	3.73	3.68	3.77
8469 P	3.17	3.39	3.45	3.46	3.49	3.47	3.50	3.56	3.62	3.65	3.84	3.60	3.65
8470 P	3.00	3.17	3.28	3.22	3.17	3.18	3.17	3.16	3.21	3.27	3.24	3.31	3.33
8471 P	3.10	3.30	3.37	3.40	3.39	3.38	3.30	3.34	3.34	3.40	3.40	3.43	3.46
8472 P	3.40	3.40	3.47	3.55	3.64	3.64	3.63	3.66	3.69	3.75	3.68	3.71	3.78
8473 P	2.95	3.15	3.11	3.11	3.25	3.28	3.25	3.30	3.32	3.30	3.27	3.35	3.37
8474 P	3.28	3.34	3.36	3.41	3.40	3.40	3.42	3.44	3.48	3.54	3.59	3.60	3.58
8475 P	3.51	3.47	3.54	3.54	3.49	3.56	3.63	3.67	3.77	3.72	3.77	3.79	3.83
8476 P	3.58	3.46	3.51	3.52	3.64	3.68	3.59	3.61	3.70	3.64	3.64	3.68	3.71
8477 NP	3.47	3.54	3.58	3.64	3.59	3.57	3.64	3.74	3.78	3.73	3.68	3.70	3.67
8478 P	2.95	3.21	3.24	3.25	3.22	3.05	2.96	3.15	3.28	3.26	3.26	3.26	3.32
8479 P	3.11	3.31	3.39	3.39	3.34	3.34	3.34	3.32	3.33	3.27	3.32	3.39	3.46
8480 P	3.74	3.69	3.76	3.78	3.78	3.84	3.96	3.90	3.94	3.99	4.01	4.04	4.03
8481 P a	3.32	3.43	3.45	3.42	3.35	3.37	3.38	3.46	3.52	3.60	3.57	3.53	3.57
8482 P	3.25	3.30	3.41	3.37	3.36	3.39	3.41	3.44	3.50	3.57	3.56	3.57	3.65
8483 P	3.60	3.57	3.61	3.67	3.72	3.71	3.84	3.74	3.80	3.88	3.92	3.93	3.99
8484 P	3.18	3.25	3.24	3.29	3.34	3.39	3.46	3.39	3.48	3.53	3.58	3.58	3.58
8485 P	3.47	3.49	3.54	3.60	3.63	3.64	3.64	3.68	3.72	3.74	3.75	3.76	3.78
8486 P	3.87	3.90	3.96	3.99	3.97	3.96	3.91	3.89	3.89	3.94	3.95	3.98	4.04

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.
a. Doe 8481 had a litter which consisted on 10 dead fetuses; values excluded from group averages and statistical analyses.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 4): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 19	20	21	22	23	24	25	26	27	28	29
RABBIT #	DOSAGE GROUP II				0.1 MG/KG/DAY						
8465 P	4.12	4.15	4.15	4.25	4.23	4.26	4.25	4.32	4.33	4.34	4.34
8466 NP	3.82	3.87	3.89	3.92	3.95	3.94	3.96	4.01	4.03	3.99	4.01
8467 NP	3.90	3.94	3.98	3.99	4.01	3.98	4.05	4.08	4.07	4.13	4.20
8468 P	3.77	3.83	3.89	3.88	3.89	3.92	3.98	3.94	3.97	3.94	3.91
8469 P	3.66	3.69	3.70	3.75	3.83	3.82	3.88	3.87	3.87	3.96	4.03
8470 P	3.37	3.34	3.36	3.32	3.30	3.28	3.31	3.35	3.37	3.38	3.40
8471 P	3.40	3.50	3.58	3.61	3.64	3.67	3.62	3.63	3.66	3.70	3.73
8472 P	3.82	3.84	3.87	3.93	3.95	3.98	4.03	4.05	4.10	4.12	4.12
8473 P	3.36	3.45	3.48	3.50	3.49	3.51	3.48	3.46	3.46	3.42	3.43
8474 P	3.60	3.65	3.64	3.71	3.74	3.75	3.73	3.77	3.79	4.15	3.81
8475 P	3.85	3.85	3.85	3.87	3.90	3.96	3.99	4.03	4.04	4.11	4.13
8476 P	3.74	3.81	3.91	3.91	3.91	3.97	4.01	4.05	4.10	4.13	4.18
8477 NP	3.74	3.78	3.80	3.82	3.82	3.87	3.88	3.93	3.93	3.97	4.06
8478 P	3.34	3.35	3.42	3.44	3.44	3.48	3.48	3.47	3.40	3.55	3.53
8479 P	3.53	3.57	3.59	3.64	3.72	3.74	3.74	3.80	3.78	3.78	3.77
8480 P	4.03	4.06	4.11	4.18	4.20	4.29	4.26	4.25	4.29	4.29	4.30
8481 P a	3.64	3.65	3.72	3.74	3.78	3.86	3.88	3.93	3.92	3.88	3.82
8482 P	3.51	3.65	3.70	3.70	3.77	3.78	3.81	3.87	3.86	3.89	3.91
8483 P	3.99	3.96	4.02	4.02	4.06	4.13	4.15	4.22	4.21	4.22	4.26
8484 P	3.58	3.62	3.64	3.66	3.71	3.67	3.69	3.62	3.63	3.58	3.58
8485 P	3.80	3.79	3.74	3.80	3.80	3.95	3.88	3.97	4.00	4.02	4.13
8486 P	3.99	4.02	4.07	4.11	4.15	4.20	4.25	4.24	4.15	4.16	4.21

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.
a. Doe 8481 had a litter which consisted on 10 dead fetuses; values excluded from group averages and statistical analyses.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 5): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP III				1.0 MG/KG/DAY								
8487 P	3.37	3.47	3.50	3.49	3.50	3.57	3.60	3.64	3.69	3.68	3.72	3.71	3.77
8488 P	3.18	3.28	3.33	3.33	3.32	3.35	3.40	3.43	3.38	3.44	3.48	3.44	3.49
8489 P	3.09	3.23	3.31	3.34	3.36	3.38	3.40	3.43	3.48	3.50	3.58	3.51	3.56
8490 P	3.58	3.69	3.68	3.59	3.63	3.69	3.77	3.79	3.87	3.88	3.90	3.90	3.94
8491 P	3.65	3.61	3.62	3.66	3.72	3.72	3.76	3.83	3.86	3.92	3.82	3.83	3.89
8492 P	3.16	3.37	3.35	3.29	3.28	3.26	3.29	3.27	3.28	3.34	3.36	3.41	3.43
8493 P	3.71	3.72	3.78	3.74	3.74	3.81	3.84	3.90	3.94	4.00	4.01	4.03	4.03
8494 P	3.33	3.38	3.40	3.40	3.39	3.47	3.52	3.57	3.66	3.64	3.64	3.67	3.65
8495 P	3.43	3.57	3.60	3.66	3.67	3.70	3.64	3.74	3.84	3.82	3.83	3.78	3.78
8496 P	3.52	3.55	3.60	3.45	3.57	3.62	3.65	3.71	3.74	3.76	3.78	3.75	3.82
8497 P	3.25	3.40	3.37	3.35	3.37	3.41	3.44	3.45	3.40	3.28	3.49	3.52	3.46
8498 P	2.87	2.99	3.04	3.06	3.04	3.07	3.11	3.16	3.17	3.19	3.22	3.25	3.28
8499 P	3.56	3.60	3.60	3.63	3.67	3.69	3.73	3.78	3.76	3.80	3.85	3.86	3.90
8500 P	3.17	3.30	3.37	3.34	3.30	3.38	3.44	3.46	3.52	3.53	3.56	3.61	3.59
8501 P	2.96	3.23	3.17	3.24	3.16	3.15	3.17	3.25	3.34	3.44	3.44	3.44	3.48
8502 NP	3.02	3.31	3.33	3.31	3.21	3.29	3.28	3.24	3.22	3.20	3.28	3.32	3.40
8503 P	3.91	4.04	4.03	4.08	4.12	4.10	4.09	4.15	4.20	4.26	4.29	4.33	4.31
8504 P	3.38	3.48	3.53	3.57	3.60	3.62	3.60	3.61	3.64	3.61	3.61	3.59	3.58
8505 NP	3.22	3.28	3.37	3.33	3.38	3.42	3.44	3.43	3.49	3.53	3.54	3.59	3.61
8506 P	3.26	3.50	3.58	3.58	3.53	3.56	3.58	3.64	3.69	3.75	3.79	3.80	3.80
8507 P	3.57	3.56	3.62	3.58	3.57	3.60	3.65	3.66	3.69	3.70	3.71	3.75	3.76
8508 NP	3.49	3.58	3.60	3.57	3.63	3.66	3.72	3.71	3.74	3.81	3.84	3.83	3.87

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 6): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

RABBIT #	PREGNANCY STATUS	DAY 19	20	21	22	23	24	25	26	27	28	29
DOSAGE GROUP III						1.0 MG/KG/DAY						
8487	P	3.76	3.80	3.81	3.86	3.89	3.92	3.94	3.92	3.88	3.88	3.90
8488	P	3.56	3.56	3.59	3.64	3.66	3.69	3.70	3.75	3.75	3.72	3.83
8489	P	3.59	3.61	3.64	3.68	3.73	3.73	3.78	3.77	3.82	3.82	3.86
8490	P	3.92	3.97	4.02	4.08	4.06	4.09	4.09	4.12	4.12	4.13	4.15
8491	P	3.89	3.91	3.98	4.02	4.04	4.13	4.15	4.17	4.15	4.18	4.20
8492	P	3.46	3.49	3.54	3.57	3.58	3.61	3.61	3.59	3.62	3.68	3.71
8493	P	3.95	3.96	3.95	3.99	3.88	3.98	4.01	4.03	4.03	4.08	4.13
8494	P	3.67	3.70	3.70	3.72	3.75	3.73	3.77	3.76	3.78	3.78	3.81
8495	P	3.82	3.86	3.90	3.96	3.97	3.96	3.96	3.92	3.93	3.93	3.92
8496	P	3.89	3.86	3.92	3.92	3.90	3.88	3.96	3.96	3.96	4.04	4.08
8497	P	3.40	3.50	3.53	3.51	3.58	3.59	3.68	3.66	3.65	3.67	3.65
8498	P	3.33	3.33	3.31	3.33	3.36	3.34	3.37	3.38	3.36	3.37	3.37
8499	P	3.96	3.97	3.97	4.02	4.02	4.05	4.14	4.16	4.24	4.26	4.26
8500	P	3.52	3.64	3.66	3.72	3.73	3.72	3.80	3.82	3.80	3.80	3.79
8501	P	3.46	3.48	3.48	3.46	3.49	3.49	3.51	3.56	3.60	3.65	3.65
8502	NP	3.46	3.44	3.44	3.47	3.48	3.48	3.45	3.42	3.45	3.49	3.51
8503	P	4.31	4.37	4.34	4.38	4.43	4.46	4.48	4.51	4.42	4.45	4.47
8504	P	3.60	3.61	3.62	3.72	3.73	3.78	3.77	3.80	3.72	3.65	3.69
8505	NP	3.54	3.62	3.64	3.69	3.69	3.74	3.76	3.77	3.78	3.86	3.84
8506	P	3.78	3.81	3.84	3.84	3.89	3.94	3.99	4.01	3.99	4.03	4.08
8507	P	3.76	3.81	3.83	3.88	3.89	3.91	3.93	3.91	3.90	3.91	3.98
8508	NP	3.85	3.86	3.86	3.84	3.90	3.99	4.00	3.99	3.88	3.90	3.97

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 7): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP IV				2.5 MG/KG/DAY								
8509 P	3.00	3.11	3.13	3.13	3.09	3.11	3.13	3.20	3.23	3.24	3.25	3.23	3.25
8510 P	3.40	3.53	3.53	3.58	3.61	3.60	3.68	3.78	3.80	3.81	3.78	3.77	3.76
8511 NP	3.17	3.33	3.47	3.47	3.50	3.54	3.56	3.60	3.55	3.54	3.51	3.53	3.56
8512 P	3.37	3.54	3.51	3.60	3.60	3.60	3.59	3.62	3.68	3.73	3.78	3.71	3.77
8513 NP	3.43	3.56	3.57	3.63	3.61	3.59	3.58	3.66	3.67	3.63	3.64	3.66	3.63
8514 P	3.32	3.57	3.64	3.65	3.60	3.56	3.61	3.68	3.72	3.74	3.72	3.70	3.72
8515 P	3.58	3.59	3.71	3.71	3.74	3.71	3.74	3.84	3.85	3.93	3.87	3.86	3.89
8516 P	3.55	3.63	3.67	3.68	3.64	3.62	3.61	3.59	3.68	3.70	3.69	3.71	3.73
8517 P	3.14	3.44	3.38	3.37	3.41	3.45	3.49	3.49	3.43	3.32	3.24	3.20	3.16
8518 P	3.50	3.70	3.69	3.74	3.74	3.78	3.88	3.82	3.88	3.91	3.91	3.91	3.85
8519 P	3.27	3.32	3.29	3.36	3.41	3.47	3.44	3.46	3.50	3.55	3.56	3.55	3.56
8520 P	3.08	3.14	3.16	3.18	3.14	3.15	3.17	3.20	3.26	3.28	3.32	3.32	3.30
8521 P	3.23	3.44	3.48	3.49	3.47	3.47	3.44	3.45	3.51	3.59	3.57	3.52	3.54
8522 P	3.74	3.81	3.85	3.91	3.93	3.93	3.96	4.11	4.02	4.10	4.18	4.11	4.10
8523 NP	3.62	3.83	3.82	3.86	3.90	3.88	3.96	3.96	3.93	3.94	3.80	3.73	3.66
8524 NP	2.88	3.10	3.14	3.14	3.13	3.10	3.12	3.14	3.20	3.24	3.26	3.26	3.24
8525 P	3.57	3.66	3.73	3.70	3.68	3.68	3.65	3.68	3.56	3.64	3.67	3.68	3.71
8526 NP	2.83	2.98	2.95	2.93	2.96	2.99	3.00	3.01	3.01	3.00	3.01	3.04	3.08
8527 P	3.51	3.53	3.62	3.71	3.69	3.61	3.62	3.64	3.63	3.68	3.61	3.63	3.77
8528 P	3.17	3.29	3.28	3.29	3.29	3.26	3.25	3.25	3.28	3.37	3.39	3.37	3.39
8529 P	3.26	3.33	3.41	3.40	3.41	3.41	3.45	3.50	3.53	3.56	3.55	3.57	3.58
8530 P	3.85	4.01	4.07	4.02	4.00	4.03	4.22	4.11	4.19	4.24	4.24	4.24	4.24

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 8): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

RABBIT #	PREGNANCY STATUS	DAY 19	20	21	22	23	24	25	26	27	28	29
DOSAGE GROUP IV						2.5 MG/KG/DAY						
8509	P	3.24	3.29	3.31	3.32	3.34	3.29	3.33	3.34	3.34	3.39	3.42
8510	P	3.75	3.76	3.81	3.80	3.84	3.83	3.89	3.91	3.92	3.94	3.98
8511	NP	3.58	3.62	3.63	3.64	3.65	3.62	3.63	3.68	3.68	3.73	3.72
8512	P	3.82	3.79	3.83	3.85	3.87	3.91	3.93	3.86	3.88	3.86	3.92
8513	NP	3.65	3.64	3.65	3.68	3.65	3.66	3.70	3.71	3.75	3.77	3.76
8514	P	3.72	3.73	3.77	3.70	3.70	3.80	3.82	3.82	3.83	3.86	3.86
8515	P	3.90	3.94	4.00	4.01	4.03	4.04	4.04	4.07	4.07	4.01	3.95
8516	P	3.70	3.71	3.76	3.77	3.81	3.89	3.86	3.86	3.89	3.90	3.98
8517	P	3.11	3.10	3.09	3.03	3.03	2.98	ABORTED ON DAY 25 OF GESTATION				
8518	P	3.92	3.92	3.94	3.96	3.96	3.93	3.86	3.74	3.62	3.56	3.57
8519	P	3.60	3.61	3.66	3.65	3.68	3.69	3.69	3.66	3.73	3.74	3.78
8520	P	3.33	3.33	3.32	3.32	3.35	3.38	3.39	3.39	3.41	3.43	3.49
8521	P	3.51	3.50	3.54	3.57	3.60	3.63	3.68	3.65	3.64	3.71	3.74
8522	P	4.13	4.19	4.18	4.23	4.27	4.33	4.34	4.37	4.39	4.39	4.46
8523	NP	3.60	3.56	3.50	3.46	3.40	3.36	3.32	3.42	3.51	3.63	3.69
8524	NP	3.20	3.25	3.24	3.28	3.24	3.22	3.18	3.25	3.34	3.33	3.36
8525	P	3.70	3.75	3.71	3.70	3.70	3.82	3.83	3.86	3.88	3.84	3.93
8526	NP	3.11	3.15	3.18	3.23	3.26	3.29	3.26	3.26	3.23	3.24	3.26
8527	P	3.79	3.83	3.84	3.89	3.75	3.72	3.77	3.78	3.75	3.86	3.93
8528	P	3.42	3.41	3.43	3.44	3.44	3.44	3.48	3.46	3.46	3.46	3.51
8529	P	3.58	3.60	3.59	3.60	3.58	3.60	3.64	3.68	3.73	3.80	3.81
8530	P	4.19	4.27	4.28	4.31	4.35	4.40	4.41	4.37	4.34	4.34	4.36

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 9): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP V				3.75 MG/KG/DAY								
8531 P	3.36	3.43	3.47	3.52	3.56	3.61	3.66	3.63	3.68	3.74	3.83	3.70	3.78
8532 P	3.42	3.55	3.54	3.56	3.53	3.55	3.48	3.60	3.58	3.52	3.46	3.43	3.34
8533 P	3.55	3.47	3.54	3.48	3.48	3.46	3.49	3.52	3.55	3.58	3.56	3.57	3.51
8534 P	3.28	3.52	3.47	3.48	3.38	3.33	3.31	3.29	3.30	3.35	3.19	3.22	3.14
8535 P	3.42	3.64	3.66	3.63	3.57	3.51	3.63	3.69	3.80	3.82	3.78	3.79	3.80
8536 P	3.32	3.40	3.33	3.71	3.72	3.77	3.74	3.77	3.74	3.72	3.74	3.68	3.66
8537 P	3.25	3.37	3.36	3.27	3.31	3.31	3.42	3.40	3.32	3.33	3.22	3.25	3.24
8538 P	2.86	2.96	2.92	2.84	2.79	2.72	2.74	2.78	2.74	2.68	2.66	2.58	2.56
8539 P	3.07	3.36	3.43	3.36	3.33	3.29	3.30	3.29	3.23	3.17	3.12	3.06	3.04
8540 P	3.58	3.47	3.52	3.53	3.65	3.64	3.67	3.76	3.84	3.84	3.73	3.60	3.55
8541 P	3.16	3.25	3.31	3.34	3.35	3.38	3.42	3.44	3.52	3.53	3.52	3.53	3.54
8542 P	3.58	3.69	3.72	3.74	3.71	3.70	3.74	3.71	3.74	3.77	3.76	3.77	3.77
8543 P	3.68	3.81	3.82	3.86	3.88	3.86	3.79	3.73	3.67	3.62	3.61	3.52	3.50
8544 P	3.00	3.12	3.18	3.20	3.21	3.27	3.28	3.23	3.14	3.08	3.01	2.94	2.89
8545 P	3.02	3.25	3.30	3.35	3.43	3.40	3.42	3.41	3.47	3.50	3.54	3.54	3.56
8546 P	3.21	3.36	3.38	3.38	3.41	3.46	3.51	3.54	3.56	3.58	3.61	3.62	3.60
8547 P	3.53	3.50	3.60	3.61	3.63	3.65	3.62	3.58	3.54	3.46	3.42	3.34	3.31
8548 P	3.59	3.59	3.59	3.65	3.73	3.80	3.88	3.81	3.85	3.89	3.84	3.81	3.79
8549 P	3.20	3.26	3.32	3.30	3.34	3.40	3.39	3.40	3.43	3.41	3.37	3.32	3.32
8550 P	3.12	3.25	3.31	3.38	3.45	3.51	3.52	3.54	3.54	3.53	3.57	3.56	3.60
8551 P	3.49	3.40	3.46	3.49	3.51	3.60	3.69	3.66	3.70	3.77	3.60	3.72	3.75
8552 NP	3.90	3.98	3.99	4.06	3.94	3.96	3.96	3.93	3.90	3.96	3.90	3.93	3.96

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 10): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

RABBIT #	PREGNANCY STATUS	DAY 19	20	21	22	23	24	25	26	27	28	29
DOSAGE GROUP V						3.75 MG/KG/DAY						
8531	P	3.78	3.84	3.87	3.90	3.97	3.97	3.98	4.08	4.03	4.10	4.13
8532	P	3.30	3.24	3.20	3.36	3.41	3.49	3.49	3.56	3.51	3.60	3.65
8533	P	3.48	3.46	3.42	3.45	3.48	3.51	3.56	3.59	3.56	3.57	3.63
8534	P	3.03	3.05	2.93	ABORTED ON DAY 22 OF GESTATION							
8535	P	3.82	3.80	3.75	3.66	3.61	3.55	3.49	3.43	3.49	3.53	3.63
8536	P	3.68	3.73	3.82	3.83	3.83	3.92	3.90	3.95	3.92	3.95	4.02
8537	P	3.10	3.10	3.12	3.05	3.07	3.02	ABORTED ON DAY 25 OF GESTATION				
8538	P	2.55	2.51	2.49	2.53	2.53	2.32	ABORTED ON DAY 24 OF GESTATION				
8539	P	3.02	2.94	2.96	2.85	2.79	ABORTED ON DAY 24 OF GESTATION					
8540	P	3.52	3.40	3.36	3.32	3.28	3.21	ABORTED ON DAY 25 OF GESTATION				
8541	P	3.57	3.61	3.65	3.66	3.71	3.73	3.73	3.70	3.62	3.64	3.73
8542	P	3.70	3.66	3.66	3.58	3.59	3.54	3.58	3.52	3.57	ABORTED ON DAY 28 OF GESTATION	
8543	P	3.45	3.44	3.40	3.36	3.30	3.25	3.19	3.20	3.16	3.11	3.08
8544	P	2.84	2.88	2.85	2.86	2.80	2.74	ABORTED ON DAY 25 OF GESTATION				
8545	P	3.61	3.64	3.66	3.60	3.59	3.66	3.69	3.66	3.69	3.66	3.70
8546	P	3.63	3.65	3.73	3.67	3.67	3.69	3.70	3.70	3.68	3.73	3.76
8547	P	3.25	3.24	3.19	ABORTED ON DAY 22 OF GESTATION							
8548	P	3.76	3.67	3.70	3.62	3.58	3.58	ABORTED ON DAY 25 OF GESTATION				
8549	P	3.30	3.29	3.33	3.36	3.39	3.46	3.52	3.50	3.56	3.54	3.59
8550	P	3.60	3.62	3.64	3.62	3.68	3.68	3.76	3.76	3.77	3.79	3.73
8551	P	3.76	3.83	3.85	3.82	3.74	3.74	3.80	3.81	3.82	3.87	3.96
8552	NP	3.89	3.88	3.88	3.81	3.92	3.89	3.93	3.89	3.92	3.88	3.93

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 11): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	SATELLITE DOSAGE GROUP I					0 (VEHICLE) MG/KG/DAY							
8553 P	3.95	3.96	4.05	4.06	4.09	4.11	3.87	4.07	4.09	4.01	4.18	4.20	4.21
8554 P	3.36	3.44	3.45	3.52	3.61	3.67	3.60	3.56	3.51	3.68	3.67	3.71	3.74
8555 P	3.09	3.05	3.06	3.12	3.13	3.17	3.13	3.09	3.12	3.18	3.29	3.26	3.28

	DAY 19	20	21
8553 P	4.23	4.25	4.34
8554 P	3.74	3.75	3.84
8555 P	3.33	3.39	3.39

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 12): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	SATELLITE DOSAGE GROUP II					0.1 MG/KG/DAY							
8556 P	3.80	3.92	3.88	3.88	3.88	3.86	3.80	3.88	3.98	4.00	3.95	3.89	3.91
8557 P	3.39	3.41	3.45	3.51	3.46	3.47	3.44	3.46	3.44	3.46	3.51	3.57	3.58
8558 P	3.56	3.51	3.58	3.61	3.70	3.76	3.66	3.58	3.57	3.67	3.69	3.71	3.74
8559 P	3.61	3.50	3.60	3.60	3.64	3.63	3.61	3.68	3.72	3.71	3.75	3.82	3.81
8560 P	3.12	3.22	3.31	3.26	3.28	3.36	3.26	3.34	3.43	3.47	3.52	3.53	3.56

	DAY 19	20	21
8556 P	3.95	3.98	4.03
8557 P	3.63	3.66	3.72
8558 P	3.82	3.83	3.92
8559 P	3.86	3.86	3.88
8560 P	3.50	3.50	3.52

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 13): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	SATELLITE DOSAGE GROUP III					1.0 MG/KG/DAY							
8561 P	3.98	3.87	3.95	3.96	3.97	4.04	3.92	3.96	3.98	3.98	4.01	4.03	4.04
8562 P	3.54	3.55	3.58	3.64	3.63	3.65	3.65	3.70	3.73	3.80	3.81	3.83	3.81
8563 P	2.98	3.16	3.19	3.15	3.20	3.26	3.23	3.24	3.27	3.30	3.32	3.32	3.34

	DAY 19	20	21
8561 P	4.12	4.08	4.06
8562 P	3.84	3.84	3.90
8563 P	3.37	3.36	3.35

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 14): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	SATELLITE DOSAGE GROUP IV					2.5 MG/KG/DAY							
8564 P	3.86	3.88	3.91	3.95	3.98	4.03	3.95	3.95	4.02	4.07	4.04	4.04	4.04
8565 P	3.34	3.36	3.42	3.51	3.49	3.55	3.38	3.37	3.34	3.28	3.21	3.33	3.44
8566 P	3.08	3.09	3.19	3.24	3.30	3.39	3.30	3.32	3.26	3.23	3.24	3.24	3.21

	DAY 19	20	21
8564 P	4.10	4.07	4.18
8565 P	3.49	3.57	3.60
8566 P	3.27	3.35	3.39

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 16 (PAGE 15): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	SATELLITE DOSAGE GROUP V					3.75 MG/KG/DAY							
8567 P	3.91	3.86	3.91	3.94	3.91	3.90	3.97	3.90	3.95	3.96	4.03	3.98	3.94
8568 P	3.37	3.31	3.34	3.36	3.31	3.30	3.23	3.22	3.28	3.31	3.32	3.31	3.31
8569 NP	3.60	3.59	3.58	3.59	3.51	3.53	3.37	3.31	3.26	3.20	3.17	3.13	3.10
8570 P	3.75	3.58	3.59	3.65	3.72	3.78	3.75	3.71	3.62	3.53	3.45	3.40	3.49
8571 P	2.75	2.85	2.84	2.89	2.88	2.87	2.90	2.91	2.88	2.85	2.84	2.87	2.84

	DAY 19	20	21
8567 P	3.85	3.78	3.68
8568 P	3.34	3.34	3.39
8569 NP	3.06	3.02	3.00
8570 P	3.52	3.60	3.65
8571 P	2.84	2.88	2.90

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 1): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #		DOSAGE GROUP I				0 (VEHICLE) MG/KG/DAY								
8443	NP	185.	180.	184.	180.	181.	185.	183.	180.	167.	183.	180.	183.	180.
8444	P	183.	146.	96.	89.	53.	87.	41.	111.	97.	185.	182.	181.	184.
8445	NP	185.	180.	185.	183.	185.	180.	185.	180.	182.	185.	182.	183.	185.
8446	P	151.	94.	92.	124.	171.	185.	185.	184.	157.	182.	181.	182.	185.
8447	P	183.	180.	180.	183.	183.	177.	180.	166.	185.	182.	180.	184.	180.
8448	P	116.	111.	185.	157.	165.	165.	184.	171.	182.	181.	183.	185.	183.
8449	P	80.	12.	81.	169.	151.	184.	150.	127.	127.	152.	180.	185.	185.
8450	P	180.	184.	166.	168.	166.	161.	125.	182.	184.	182.	185.	184.	180.
8451	P	181.	182.	182.	180.	185.	185.	183.	181.	182.	185.	185.	185.	180.
8452	P	16.	26.	27.	107.	90.	65.	86.	143.	185.	181.	185.	185.	180.
8453	P	183.	180.	180.	185.	182.	181.	185.	182.	182.	185.	184.	185.	185.
8454	P	184.	180.	15.	180.	185.	160.	107.	181.	180.	185.	185.	185.	180.
8455	P	185.	180.	180.	169.	180.	185.	180.	180.	184.	181.	181.	180.	181.
8456	P	4.	5.	107.	159.	118.	90.	169.	185.	183.	185.	185.	180.	182.
8457	P	185.	184.	165.	133.	97.	74.	93.	183.	182.	167.	173.	180.	185.
8458	P	183.	94.	98.	115.	110.	139.	183.	151.	154.	181.	184.	124.	111.
8459	P	180.	184.	180.	182.	185.	185.	184.	184.	181.	180.	180.	180.	184.
8460	P	180.	183.	183.	184.	185.	183.	181.	183.	182.	185.	184.	185.	185.
8461	P	171.	165.	150.	131.	81.	119.	123.	146.	148.	153.	154.	101.	104.
8462	P	184.	185.	167.	130.	110.	114.	152.	166.	182.	172.	181.	184.	180.
8463	P	180.	184.	164.	184.	160.	145.	121.	165.	183.	183.	180.	185.	185.
8464	P	90.	134.	164.	185.	148.	181.	185.	165.	165.	150.	158.	184.	159.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 2): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
RABBIT #	DOSAGE GROUP I				0 (VEHICLE) MG/KG/DAY				
8443 NP	182.	180.	180.	181.	180.	180.	180.	180.	184.
8444 P	163.	180.	122.	106.	173.	100.	101.	102.	122.
8445 NP	184.	185.	185.	182.	180.	182.	180.	181.	180.
8446 P	182.	180.	185.	182.	180.	63.	19.	37.	94.
8447 P	180.	185.	184.	182.	182.	185.	167.	185.	181.
8448 P	183.	185.	185.	181.	185.	182.	152.	180.	107.
8449 P	180.	185.	180.	185.	185.	185.	138.	120.	66.
8450 P	175.	172.	174.	147.	172.	158.	180.	153.	137.
8451 P	185.	185.	185.	177.	182.	125.	99.	35.	130.
8452 P	185.	185.	185.	182.	183.	185.	180.	120.	135.
8453 P	185.	185.	184.	180.	182.	181.	180.	138.	134.
8454 P	182.	180.	182.	166.	159.	132.	154.	104.	83.
8455 P	171.	168.	182.	161.	180.	130.	155.	100.	96.
8456 P	177.	181.	166.	180.	135.	142.	84.	99.	73.
8457 P	185.	182.	153.	182.	145.	154.	83.	91.	68.
8458 P	116.	87.	55.	69.	51.	66.	15.	2.	1.
8459 P	184.	181.	184.	180.	183.	183.	180.	96.	184.
8460 P	181.	180.	180.	180.	182.	182.	184.	184.	181.
8461 P	141.	128.	148.	117.	98.	45.	77.	95.	89.
8462 P	143.	53.	121.	102.	128.	90.	86.	115.	86.
8463 P	185.	172.	155.	112.	151.	113.	102.	91.	90.
8464 P	185.	140.	157.	125.	2.	105.	a	109.	85.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 3): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP II				0.1 MG/KG/DAY								
8465 P	185.	184.	183.	184.	182.	180.	185.	184.	183.	181.	184.	180.	180.
8466 NP	164.	180.	184.	165.	143.	129.	70.	118.	121.	181.	183.	185.	185.
8467 NP	185.	185.	183.	184.	180.	135.	185.	181.	185.	184.	183.	185.	181.
8468 P	183.	185.	180.	180.	183.	181.	183.	185.	185.	183.	185.	183.	181.
8469 P	181.	181.	185.	180.	181.	181.	183.	184.	185.	185.	185.	183.	184.
8470 P	180.	139.	79.	55.	97.	51.	95.	116.	182.	183.	182.	180.	183.
8471 P	184.	180.	183.	129.	3.	76.	83.	102.	181.	164.	181.	181.	a
8472 P	184.	183.	183.	181.	148.	173.	183.	185.	184.	180.	185.	181.	185.
8473 P	62.	165.	182.	153.	180.	166.	135.	99.	71.	137.	138.	160.	180.
8474 P	184.	180.	180.	147.	149.	123.	108.	170.	174.	183.	175.	181.	184.
8475 P	184.	181.	180.	159.	185.	185.	185.	180.	185.	182.	185.	183.	180.
8476 P	180.	181.	184.	133.	79.	92.	115.	183.	185.	180.	184.	182.	181.
8477 NP	185.	184.	156.	162.	184.	185.	181.	180.	130.	108.	159.	180.	180.
8478 P	180.	125.	95.	17.	14.	81.	184.	185.	144.	116.	181.	185.	185.
8479 P	185.	180.	117.	127.	118.	102.	80.	67.	89.	128.	155.	181.	185.
8480 P	181.	184.	184.	183.	180.	182.	184.	180.	184.	183.	184.	183.	185.
8481 P b	131.	6.	72.	109.	94.	166.	185.	168.	123.	73.	184.	181.	180.
8482 P	184.	150.	123.	158.	130.	182.	185.	184.	180.	185.	184.	184.	181.
8483 P	184.	180.	182.	184.	183.	184.	181.	183.	182.	180.	183.	181.	184.
8484 P	168.	183.	181.	183.	184.	180.	183.	185.	180.	182.	182.	c	185.
8485 P	182.	184.	182.	185.	138.	181.	185.	181.	180.	180.	180.	185.	185.
8486 P	181.	185.	185.	151.	85.	59.	68.	110.	115.	115.	166.	182.	168.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Wet feed precluded the calculation of this value.
b. Doe 8481 had a litter which consisted of 10 dead fetuses; values excluded from group averages and statistical analyses.
c. Spilled feed precluded the calculation of this value.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 4): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
RABBIT #	DOSAGE GROUP II				0.1 MG/KG/DAY				
8465 P	183.	181.	183.	185.	181.	180.	185.	180.	182.
8466 NP	183.	184.	170.	180.	185.	167.	182.	144.	126.
8467 NP	180.	184.	184.	183.	182.	180.	180.	180.	180.
8468 P	180.	180.	182.	126.	163.	120.	140.	86.	81.
8469 P	181.	180.	185.	85.	183.	180.	182.	180.	183.
8470 P	132.	93.	172.	50.	81.	123.	98.	134.	90.
8471 P	185.	185.	180.	103.	70.	99.	97.	161.	110.
8472 P	181.	185.	181.	183.	180.	181.	181.	181.	155.
8473 P	185.	159.	157.	158.	134.	119.	112.	104.	81.
8474 P	153.	152.	157.	109.	121.	103.	119.	80.	82.
8475 P	182.	185.	184.	181.	180.	183.	185.	184.	160.
8476 P	185.	183.	180.	158.	159.	147.	182.	160.	154.
8477 NP	183.	180.	180.	182.	183.	185.	183.	184.	172.
8478 P	185.	184.	131.	164.	147.	75.	19.	96.	98.
8479 P	180.	184.	180.	181.	133.	181.	113.	100.	67.
8480 P	180.	185.	183.	180.	185.	168.	172.	167.	140.
8481 P a	185.	180.	182.	185.	180.	153.	154.	132.	0.
8482 P	185.	183.	184.	156.	183.	181.	163.	166.	133.
8483 P	182.	181.	181.	180.	181.	180.	157.	143.	131.
8484 P	185.	160.	162.	118.	98.	40.	50.	64.	48.
8485 P	b	181.	180.	182.	180.	161.	180.	140.	181.
8486 P	184.	185.	182.	154.	150.	96.	b	70.	69.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Doe 8481 had a litter which consisted of 10 dead fetuses; values excluded from group averages and statistical analyses.
b. Spilled feed precluded the calculation of this value.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 5): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP III				1.0 MG/KG/DAY								
8487 P	143.	144.	162.	185.	184.	185.	184.	182.	184.	144.	185.	183.	180.
8488 P	185.	150.	133.	163.	170.	152.	110.	150.	144.	183.	185.	183.	185.
8489 P	182.	182.	181.	180.	181.	185.	185.	180.	185.	182.	185.	185.	183.
8490 P	149.	69.	145.	167.	185.	185.	181.	163.	183.	181.	180.	185.	185.
8491 P	182.	183.	180.	170.	182.	176.	184.	183.	185.	184.	184.	180.	180.
8492 P	156.	90.	84.	84.	109.	86.	67.	83.	87.	116.	135.	169.	162.
8493 P	180.	156.	182.	182.	181.	185.	182.	180.	139.	142.	153.	110.	118.
8494 P	185.	180.	171.	184.	185.	153.	185.	182.	184.	184.	180.	180.	185.
8495 P	185.	185.	182.	180.	118.	183.	185.	183.	184.	125.	115.	148.	164.
8496 P	184.	115.	139.	146.	156.	152.	153.	182.	184.	185.	185.	181.	185.
8497 P	132.	138.	138.	158.	140.	92.	31.	29.	105.	124.	26.	97.	183.
8498 P	185.	184.	142.	173.	173.	158.	148.	183.	184.	184.	183.	185.	183.
8499 P	180.	181.	181.	183.	183.	183.	180.	185.	184.	185.	182.	185.	184.
8500 P	180.	126.	123.	145.	155.	143.	183.	184.	183.	183.	182.	137.	102.
8501 P	180.	184.	a	183.	114.	156.	183.	170.	182.	184.	173.	184.	172.
8502 NP	180.	132.	60.	131.	145.	123.	113.	135.	165.	160.	149.	165.	150.
8503 P	180.	184.	183.	116.	118.	158.	180.	181.	183.	181.	180.	180.	185.
8504 P	180.	185.	184.	163.	125.	115.	180.	156.	98.	70.	47.	126.	107.
8505 NP	184.	163.	163.	185.	181.	184.	181.	184.	180.	180.	182.	185.	180.
8506 P	181.	132.	156.	158.	126.	184.	174.	182.	185.	180.	182.	185.	183.
8507 P	183.	170.	154.	166.	168.	181.	170.	157.	180.	184.	181.	184.	184.
8508 NP	180.	182.	182.	185.	185.	183.	180.	183.	182.	185.	183.	185.	184.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 6): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
RABBIT #	DOSAGE GROUP III				1.0 MG/KG/DAY				
8487 P	185.	182.	182.	185.	183.	128.	110.	77.	99.
8488 P	184.	184.	180.	185.	185.	181.	181.	181.	181.
8489 P	185.	182.	180.	182.	185.	183.	185.	168.	173.
8490 P	184.	183.	185.	180.	180.	183.	114.	144.	85.
8491 P	185.	185.	182.	180.	160.	180.	120.	127.	94.
8492 P	170.	178.	173.	182.	133.	157.	138.	184.	120.
8493 P	135.	147.	136.	182.	114.	138.	132.	154.	103.
8494 P	185.	185.	155.	94.	139.	117.	161.	116.	101.
8495 P	162.	181.	162.	109.	83.	56.	90.	66.	62.
8496 P	167.	154.	136.	106.	149.	127.	152.	124.	127.
8497 P	185.	181.	184.	180.	184.	153.	175.	119.	107.
8498 P	164.	162.	165.	109.	183.	88.	101.	73.	50.
8499 P	185.	185.	180.	183.	184.	180.	184.	180.	157.
8500 P	178.	185.	166.	120.	151.	183.	121.	133.	55.
8501 P	107.	74.	75.	130.	129.	182.	161.	169.	125.
8502 NP	154.	184.	130.	180.	80.	114.	112.	134.	46.
8503 P	184.	181.	184.	167.	153.	165.	103.	119.	94.
8504 P	151.	147.	142.	183.	84.	98.	8.	7.	52.
8505 NP	181.	184.	184.	182.	185.	165.	174.	175.	146.
8506 P	180.	180.	2.	180.	184.	140.	136.	132.	120.
8507 P	180.	166.	181.	125.	142.	82.	81.	103.	110.
8508 NP	182.	181.	181.	159.	182.	52.	95.	154.	155.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 7): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP IV				2.5 MG/KG/DAY								
8509 P	146.	130.	72.	102.	85.	140.	125.	50.	108.	130.	180.	133.	158.
8510 P	182.	185.	183.	180.	184.	183.	182.	130.	106.	153.	167.	144.	170.
8511 NP	185.	182.	181.	181.	181.	173.	112.	100.	119.	142.	147.	153.	145.
8512 P	182.	181.	181.	180.	169.	178.	184.	185.	180.	180.	180.	182.	184.
8513 NP	183.	185.	159.	163.	147.	168.	136.	112.	108.	161.	115.	94.	97.
8514 P	184.	134.	80.	76.	108.	184.	185.	133.	122.	182.	180.	182.	153.
8515 P	182.	125.	182.	180.	182.	183.	185.	185.	185.	182.	182.	183.	183.
8516 P	180.	158.	108.	79.	101.	49.	110.	138.	180.	169.	140.	98.	113.
8517 P	112.	180.	184.	165.	161.	130.	52.	6.	18.	1.	3.	0.	5.
8518 P	179.	184.	185.	182.	185.	127.	133.	129.	184.	171.	92.	172.	163.
8519 P	121.	185.	185.	162.	125.	138.	110.	162.	180.	144.	145.	144.	181.
8520 P	181.	173.	125.	147.	121.	149.	133.	176.	153.	148.	127.	130.	142.
8521 P	180.	153.	136.	133.	88.	100.	137.	182.	166.	124.	99.	111.	131.
8522 P	180.	185.	184.	185.	185.	182.	183.	185.	185.	180.	180.	180.	184.
8523 NP	185.	183.	164.	149.	185.	185.	184.	183.	109.	39.	8.	1.	58.
8524 NP	184.	167.	155.	127.	140.	111.	175.	166.	159.	153.	118.	102.	117.
8525 P	185.	133.	124.	154.	164.	151.	181.	184.	183.	180.	180.	183.	183.
8526 NP	162.	147.	133.	106.	155.	143.	159.	136.	143.	152.	148.	163.	166.
8527 P	180.	184.	147.	150.	88.	80.	57.	81.	20.	89.	155.	180.	171.
8528 P	159.	145.	156.	106.	84.	62.	113.	146.	143.	132.	135.	150.	94.
8529 P	185.	135.	152.	151.	136.	184.	169.	160.	160.	143.	143.	109.	127.
8530 P	183.	184.	181.	185.	184.	183.	184.	180.	183.	180.	182.	185.	181.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 8): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
RABBIT #	DOSAGE GROUP IV				2.5 MG/KG/DAY				
8509 P	184.	119.	87.	59.	114.	94.	117.	93.	86.
8510 P	150.	154.	138.	152.	180.	166.	157.	133.	118.
8511 NP	143.	153.	146.	135.	150.	144.	159.	147.	132.
8512 P	183.	185.	185.	176.	146.	92.	76.	98.	90.
8513 NP	139.	128.	122.	135.	144.	122.	154.	143.	116.
8514 P	132.	92.	153.	109.	164.	105.	147.	121.	117.
8515 P	183.	185.	185.	180.	110.	154.	106.	90.	10.
8516 P	122.	125.	154.	167.	130.	109.	97.	180.	123.
8517 P	0.	38.	54.	75.	ABORTED ON DAY 25 OF GESTATION				
8518 P	132.	118.	119.	52.	2.	0.	0.	7.	26.
8519 P	148.	137.	134.	90.	94.	78.	126.	104.	108.
8520 P	91.	133.	144.	125.	140.	116.	134.	107.	106.
8521 P	120.	137.	147.	126.	134.	108.	136.	118.	120.
8522 P	185.	181.	150.	180.	154.	180.	160.	166.	183.
8523 NP	4.	0.	2.	0.	0.	93.	108.	163.	172.
8524 NP	128.	98.	67.	28.	78.	176.	158.	145.	110.
8525 P	185.	a	185.	181.	108.	138.	102.	106.	88.
8526 NP	160.	185.	166.	183.	133.	133.	116.	158.	141.
8527 P	182.	149.	30.	89.	128.	168.	84.	180.	170.
8528 P	140.	101.	105.	73.	62.	74.	66.	79.	80.
8529 P	124.	92.	87.	84.	144.	122.	150.	159.	126.
8530 P	185.	180.	183.	182.	181.	116.	103.	62.	94.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 9): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP V				3.75 MG/KG/DAY								
8531 P	181.	185.	184.	181.	181.	152.	135.	185.	184.	184.	185.	182.	185.
8532 P	184.	146.	117.	93.	64.	183.	131.	21.	5.	10.	0.	10.	3.
8533 P	183.	139.	102.	111.	118.	142.	113.	90.	64.	94.	96.	61.	58.
8534 P	147.	145.	86.	66.	19.	56.	29.	3.	11.	2.	0.	6.	6.
8535 P	167.	93.	65.	105.	185.	185.	185.	150.	181.	180.	152.	150.	115.
8536 P	44.	184.	183.	184.	178.	144.	81.	66.	80.	81.	67.	127.	129.
8537 P	134.	73.	99.	102.	165.	73.	3.	0.	12.	0.	4.	7.	2.
8538 P	8.	44.	18.	2.	46.	23.	5.	0.	2.	0.	10.	16.	5.
8539 P	184.	103.	65.	23.	83.	5.	3.	0.	3.	3.	13.	2.	0.
8540 P	184.	184.	185.	183.	180.	185.	184.	169.	78.	3.	0.	0.	0.
8541 P	180.	183.	180.	180.	171.	174.	165.	182.	181.	184.	185.	180.	180.
8542 P	184.	182.	149.	147.	113.	100.	107.	158.	76.	121.	84.	32.	8.
8543 P	185.	163.	159.	118.	86.	22.	6.	1.	9.	4.	0.	1.	14.
8544 P	185.	180.	184.	182.	143.	84.	10.	2.	2.	24.	12.	0.	27.
8545 P	180.	180.	179.	165.	159.	129.	175.	183.	185.	184.	183.	182.	133.
8546 P	0.	182.	182.	183.	182.	180.	183.	182.	183.	182.	150.	185.	185.
8547 P	181.	181.	151.	90.	28.	22.	4.	1.	7.	3.	5.	3.	0.
8548 P	180.	183.	183.	185.	183.	184.	150.	147.	140.	121.	104.	40.	7.
8549 P	185.	185.	185.	185.	126.	157.	145.	113.	60.	6.	17.	15.	70.
8550 P	183.	185.	182.	185.	160.	171.	183.	184.	184.	173.	152.	148.	160.
8551 P	183.	183.	180.	184.	185.	183.	185.	182.	185.	166.	183.	182.	180.
8552 NP	161.	161.	125.	119.	106.	144.	122.	127.	143.	104.	130.	94.	110.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 10): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
RABBIT #	DOSAGE GROUP V				3.75 MG/KG/DAY				
8531 P	180.	180.	185.	184.	185.	182.	180.	127.	141.
8532 P	0.	89.	124.	137.	183.	180.	182.	185.	182.
8533 P	17.	46.	81.	78.	114.	92.	108.	70.	103.
8534 P	5.	ABORTED ON DAY 22 OF GESTATION							
8535 P	40.	4.	1.	2.	2.	1.	41.	171.	82.
8536 P	166.	167.	180.	185.	164.	182.	124.	183.	121.
8537 P	0.	0.	0.	0.	ABORTED ON DAY 25 OF GESTATION				
8538 P	9.	10.	7.	ABORTED ON DAY 24 OF GESTATION					
8539 P	0.	0.	0.	ABORTED ON DAY 24 OF GESTATION					
8540 P	4.	1.	0.	6.	ABORTED ON DAY 25 OF GESTATION				
8541 P	185.	180.	183.	147.	138.	70.	23.	56.	107.
8542 P	1.	2.	7.	3.	13.	8.	10.	ABORTED ON DAY 28 OF GESTATION	
8543 P	2.	2.	0.	1.	1.	0.	0.	0.	0.
8544 P	1.	4.	4.	0.	ABORTED ON DAY 25 OF GESTATION				
8545 P	118.	182.	181.	180.	157.	183.	119.	11.	71.
8546 P	159.	124.	145.	5.	132.	84.	105.	106.	112.
8547 P	0.	ABORTED ON DAY 22 OF GESTATION							
8548 P	180.	12.	2.	122.	ABORTED ON DAY 25 OF GESTATION				
8549 P	92.	95.	99.	181.	180.	144.	166.	128.	131.
8550 P	175.	111.	184.	111.	181.	131.	136.	127.	65.
8551 P	127.	91.	41.	56.	139.	116.	149.	169.	154.
8552 NP	99.	78.	141.	107.	143.	95.	107.	104.	110.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 11): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	SATELLITE DOSAGE GROUP I					0 (VEHICLE) MG/KG/DAY							
8553 P	184.	185.	169.	184.	7.	180.	185.	185.	180.	180.	184.	185.	184.
8554 P	185.	184.	180.	185.	185.	183.	184.	184.	180.	180.	184.	185.	180.
8555 P	103.	142.	114.	97.	87.	113.	85.	126.	107.	150.	171.	139.	158.

	DAYS 20 - 21
8553 P	180.
8554 P	182.
8555 P	120.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 12): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	SATELLITE DOSAGE GROUP II					0.1 MG/KG/DAY							
8556 P	147.	142.	133.	130.	128.	165.	143.	165.	85.	68.	157.	166.	184.
8557 P	181.	172.	104.	180.	94.	150.	92.	110.	146.	154.	180.	183.	181.
8558 P	185.	183.	184.	182.	151.	132.	30.	118.	159.	180.	183.	179.	180.
8559 P	182.	180.	183.	185.	83.	182.	184.	183.	185.	185.	181.	185.	183.
8560 P	185.	153.	115.	126.	90.	182.	184.	184.	185.	180.	166.	101.	122.

	DAYS 20 - 21
8556 P	182.
8557 P	183.
8558 P	159.
8559 P	184.
8560 P	108.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 13): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	SATELLITE DOSAGE GROUP III					1.0 MG/KG/DAY							
8561 P	184.	180.	185.	138.	74.	155.	184.	180.	180.	184.	184.	185.	184.
8562 P	181.	180.	180.	184.	134.	185.	185.	183.	180.	185.	180.	185.	182.
8563 P	134.	124.	151.	147.	125.	166.	141.	139.	150.	147.	140.	100.	109.
DAYS 20 - 21													
8561 P	180.												
8562 P	173.												
8563 P	95.												

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 14): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	SATELLITE DOSAGE GROUP IV					2.5 MG/KG/DAY							
8564 P	164.	180.	177.	141.	116.	158.	125.	139.	127.	132.	180.	163.	185.
8565 P	183.	163.	185.	151.	38.	30.	2.	1.	1.	154.	185.	185.	185.
8566 P	185.	184.	183.	180.	107.	126.	49.	32.	63.	57.	86.	139.	183.
DAYS 20 - 21													
8564 P	166.												
8565 P	123.												
8566 P	151.												

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 17 (PAGE 15): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	SATELLITE DOSAGE GROUP V					3.75 MG/KG/DAY							
8567 P	182.	183.	114.	116.	153.	65.	182.	143.	129.	34.	109.	4.	3.
8568 P	140.	139.	79.	63.	55.	64.	73.	83.	83.	81.	113.	119.	139.
8569 NP	185.	132.	67.	182.	0.	4.	2.	0.	5.	12.	8.	0.	2.
8570 P	185.	184.	184.	182.	148.	180.	83.	4.	7.	13.	6.	54.	164.
8571 P	119.	118.	115.	184.	72.	79.	19.	25.	48.	66.	107.	97.	105.

	DAYS 20 - 21
8567 P	0.
8568 P	132.
8569 NP	0.
8570 P	174.
8571 P	84.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 18 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

			VIABLE FETUSES			DEAD FETUSES			EARLY RESORPTIONS			LATE RESORPTIONS			IMPLANTATION SITES			CORPORA LUTEA		
	SEX		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT	
RABBIT #	M	F	HORN		TOTAL	HORN		TOTAL	HORN		TOTAL	HORN		TOTAL	HORN		TOTAL	OVARY		TOTAL
DOSAGE GROUP I								0 (VEHICLE) MG/KG/DAY												
8443	NOT PREGNANT																			
8444	5	2	2	5	7	0	0	0	0	0	0	0	0	0	2	5	7	2	6	8
8445	NOT PREGNANT																			
8446	3	4	2	5	7	0	0	0	0	0	0	0	0	0	2	5	7	6	7	13
8447	5	7	5	7	12	0	0	0	0	0	0	0	0	0	5	7	12	7	8	15
8448	6	4	6	4	10	0	0	0	0	0	0	1	0	1	7	4	11	7	5	12
8449	7	2	4	5	9	0	0	0	0	0	0	0	0	0	4	5	9	6	6	12
8450	6	4	6	4	10	0	0	0	0	1	1	0	0	0	6	5	11	7	6	13
8451	5	2	5	2	7	0	0	0	0	0	0	0	0	0	5	2	7	7	2	9
8452	2	4	5	1	6	0	0	0	0	1	1	0	0	0	5	2	7	6	3	9
8453	5	4	5	4	9	0	0	0	0	0	0	0	0	0	5	4	9	6	7	13
8454	3	7	6	4	10	0	0	0	0	0	0	0	0	0	6	4	10	7	5	12
8455	5	6	5	6	11	0	0	0	0	0	0	0	0	0	5	6	11	6	7	13
8456	4	2	3	3	6	0	0	0	0	0	0	0	0	0	3	3	6	3	4	7
8457	4	4	5	3	8	0	0	0	0	0	0	0	0	0	5	3	8	5	3	8
8458	6	8	7	7	14	0	0	0	0	0	0	0	0	0	7	7	14	7	7	14
8459	2	5	6	1	7	0	0	0	0	0	0	0	0	0	6	1	7	6	1	7
8460	4	3	4	3	7	0	0	0	0	0	0	0	0	0	4	3	7	4	4	8
8461	3	3	2	4	6	0	0	0	0	0	0	0	0	0	2	4	6	6	4	10
8462	3	6	5	4	9	0	0	0	0	0	0	0	0	0	5	4	9	6	5	11
8463	6	4	9	1	10	0	0	0	1	0	1	0	0	0	10	1	11	10	3	13
8464	3	7	7	3	10	0	0	0	0	0	0	0	0	0	7	3	10	7	3	10

M = MALE F = FEMALE
PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 18 (PAGE 2): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

			VIABLE FETUSES			DEAD FETUSES			EARLY RESORPTIONS			LATE RESORPTIONS			IMPLANTATION SITES			CORPORA LUTEA		
	SEX		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT	
RABBIT #	M	F	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	OVARY	OVARY	TOTAL
DOSAGE GROUP II							0.1 MG/KG/DAY													
8465	1	5	3	3	6	0	0	0	1	0	1	0	0	0	4	3	7	4	3	7
8466	NOT PREGNANT																			
8467	NOT PREGNANT																			
8468	3	7	4	6	10	0	0	0	0	0	0	0	0	0	4	6	10	8	7	15
8469	2	5	6	1	7	0	0	0	0	0	0	0	0	0	6	1	7	7	2	9
8470	6	1	4	3	7	0	0	0	0	0	0	0	0	0	4	3	7	4	5	9
8471	3	6	3	6	9	0	0	0	0	0	0	0	0	0	3	6	9	5	8	13
8472	3	6	6	3	9	0	0	0	0	0	0	0	0	0	6	3	9	6	4	10
8473	4	4	5	3	8	0	0	0	1	0	1	0	0	0	6	3	9	6	5	11
8474	4	5	4	5	9	0	0	0	0	0	0	0	0	0	4	5	9	5	5	10
8475	2	5	3	4	7	0	0	0	0	0	0	0	0	0	3	4	7	4	4	8
8476	6	4	6	4	10	0	0	0	0	0	0	0	0	0	6	4	10	6	6	12
8477	NOT PREGNANT																			
8478	6	4	6	4	10	0	0	0	0	0	0	0	0	0	6	4	10	7	5	12
8479	6	9	6	9	15	0	0	0	0	0	0	0	0	0	6	9	15	8	9	17
8480	6	3	6	3	9	0	0	0	0	0	0	0	0	0	6	3	9	6	3	9
8481a	0	0	0	0	0	3	7	10	0	0	0	0	0	0	3	7	10	3	8	11
8482	4	5	3	6	9	0	0	0	0	0	0	0	0	0	3	6	9	3	7	10
8483	6	2	5	3	8	0	0	0	0	0	0	0	0	0	5	3	8	7	4	11
8484	6	3	6	3	9	0	0	0	0	0	0	0	0	0	6	3	9	6	3	9
8485	3	6	6	3	9	0	0	0	0	0	0	0	0	0	6	3	9	7	4	11
8486	3	8	5	6	11	0	0	0	0	0	0	0	0	0	5	6	11	7	6	13

M = MALE F = FEMALE

PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

a. Doe 8481 had a litter which consisted of 10 dead fetuses; values excluded from group averages and statistical analyses.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 18 (PAGE 3): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

			VIABLE FETUSES			DEAD FETUSES			EARLY RESORPTIONS			LATE RESORPTIONS			IMPLANTATION SITES			CORPORA LUTEA		
	SEX		RIGHT HORN	LEFT HORN	TOTAL	RIGHT HORN	LEFT HORN	TOTAL	RIGHT HORN	LEFT HORN	TOTAL	RIGHT HORN	LEFT HORN	TOTAL	RIGHT HORN	LEFT HORN	TOTAL	RIGHT OVARY	LEFT OVARY	TOTAL
RABBIT #	M	F																		
DOSAGE GROUP III							1.0 MG/KG/DAY													
8487	4	2	3	3	6	0	0	0	0	0	0	0	0	0	3	3	6	3	4	7
8488	3	4	2	5	7	0	0	0	1	0	1	0	0	0	3	5	8	5	6	11
8489	2	4	2	4	6	0	0	0	1	0	1	0	0	0	3	4	7	3	5	8
8490	1	4	0	5	5	0	0	0	0	0	0	0	0	0	0	5	5	2	5	7
8491	5	4	3	6	9	0	0	0	0	0	0	0	1	1	3	7	10	5	8	13
8492	2	7	6	3	9	0	0	0	0	0	0	0	0	0	6	3	9	6	3	9
8493	2	3	1	4	5	0	0	0	0	0	0	0	3	3	1	7	8	2	10	12
8494	2	4	2	4	6	0	0	0	0	0	0	0	0	0	2	4	6	5	6	11
8495	7	3	7	3	10	0	0	0	0	0	0	0	0	0	7	3	10	9	4	13
8496	1	8	7	2	9	0	0	0	0	0	0	0	0	0	7	2	9	8	5	13
8497	4	3	3	4	7	0	0	0	0	0	0	0	0	0	3	4	7	3	5	8
8498	3	6	1	8	9	0	0	0	0	0	0	0	0	0	1	8	9	1	8	9
8499	6	2	3	5	8	0	0	0	0	0	0	0	0	0	3	5	8	3	5	8
8500	5	5	2	8	10	0	0	0	0	0	0	0	1	1	2	9	11	3	9	12
8501	8	3	6	5	11	0	0	0	0	0	0	0	0	0	6	5	11	8	6	14
8502	NOT PREGNANT																			
8503	5	5	8	2	10	0	0	0	0	0	0	0	0	0	8	2	10	9	4	13
8504	4	6	3	7	10	0	0	0	0	0	0	0	0	0	3	7	10	5	7	12
8505	NOT PREGNANT																			
8506	4	5	7	2	9	0	0	0	0	0	0	0	0	0	7	2	9	8	3	11
8507	3	3	0	6	6	0	0	0	0	0	0	0	0	0	0	6	6	3	6	9
8508	NOT PREGNANT																			

M = MALE F = FEMALE
PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 18 (PAGE 4): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

			VIABLE FETUSES			DEAD FETUSES			EARLY RESORPTIONS			LATE RESORPTIONS			IMPLANTATION SITES			CORPORA LUTEA		
	SEX		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT	
RABBIT #	M	F	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	OVARY	OVARY	TOTAL
DOSAGE GROUP IV							2.5 MG/KG/DAY													
8509	2	5	3	4	7	0	0	0	1	0	1	0	0	0	4	4	8	4	7	11
8510	4	4	5	3	8	0	0	0	0	0	0	0	0	0	5	3	8	7	5	12
8511	NOT PREGNANT																			
8512	3	4	5	2	7	0	0	0	0	0	0	0	0	0	5	2	7	7	4	11
8513	NOT PREGNANT																			
8514	3	4	3	4	7	0	0	0	0	0	0	0	0	0	3	4	7	6	7	13
8515	4	5	3	6	9	0	0	0	0	0	0	0	0	0	3	6	9	4	6	10
8516	4	3	2	5	7	0	0	0	0	0	0	0	0	0	2	5	7	3	6	9
8517	ABORTED ON DAY 25 OF GESTATION																			
8518	7	2	4	5	9	0	0	0	0	0	0	1	0	1	5	5	10	7	5	12
8519	6	2	3	5	8	0	0	0	0	0	0	0	0	0	3	5	8	3	5	8
8520	3	2	2	3	5	0	0	0	0	0	0	0	0	0	2	3	5	2	4	6
8521	6	4	6	4	10	0	0	0	0	0	0	0	0	0	6	4	10	7	5	12
8522	3	6	5	4	9	0	0	0	0	0	0	0	1	1	5	5	10	6	7	13
8523	NOT PREGNANT																			
8524	NOT PREGNANT																			
8525	5	6	5	6	11	0	0	0	0	0	0	0	0	0	5	6	11	6	7	13
8526	NOT PREGNANT																			
8527	7	4	6	5	11	0	0	0	0	0	0	0	0	0	6	5	11	6	5	11
8528	3	4	5	2	7	0	0	0	0	0	0	0	0	0	5	2	7	6	3	9
8529	2	3	1	4	5	0	0	0	1	0	1	0	0	0	2	4	6	3	4	7
8530	4	6	7	3	10	0	0	0	0	0	0	0	0	0	7	3	10	7	3	10

M = MALE F = FEMALE
PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 18 (PAGE 5): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

			VIABLE FETUSES			DEAD FETUSES		EARLY RESORPTIONS		LATE RESORPTIONS		IMPLANTATION SITES			CORPORA LUTEA		
	SEX		RIGHT	LEFT		RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT		RIGHT	LEFT	
RABBIT #	M	F	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	TOTAL	HORN	TOTAL	HORN	TOTAL	OVARY	OVARY	TOTAL
DOSAGE GROUP V							3.75 MG/KG/DAY										
8531	6	3	4	5	9	0	0	0	0	0	0	0	0	0	4	5	9	4	7	11
8532	4	4	5	3	8	0	0	0	0	0	0	0	0	0	5	3	8	6	6	12
8533	2	4	3	3	6	0	0	0	0	0	0	0	0	0	3	3	6	7	5	12
8534	ABORTED ON DAY 22 OF GESTATION																			
8535	8	3	8	3	11	0	0	0	0	0	0	0	0	0	8	3	11	8	3	11
8536	3	5	2	6	8	0	0	0	1	0	1	0	0	0	3	6	9	3	8	11
8537	ABORTED ON DAY 25 OF GESTATION																			
8538	ABORTED ON DAY 24 OF GESTATION																			
8539	ABORTED ON DAY 24 OF GESTATION																			
8540	ABORTED ON DAY 25 OF GESTATION																			
8541	2	5	5	2	7	0	0	0	0	0	0	0	0	0	5	2	7	5	3	8
8542	ABORTED ON DAY 28 OF GESTATION																			
8543	2	7	6	3	9	0	0	0	0	0	0	0	1	1	6	4	10	7	5	12
8544	ABORTED ON DAY 25 OF GESTATION																			
8545	5	8	6	7	13	0	0	0	0	0	0	0	0	0	6	7	13	6	7	13
8546	0	7	2	5	7	0	0	0	0	0	0	0	0	0	2	5	7	3	5	8
8547	ABORTED ON DAY 22 OF GESTATION																			
8548	ABORTED ON DAY 25 OF GESTATION																			
8549	6	4	5	5	10	0	0	0	0	0	0	0	0	0	5	5	10	7	5	12
8550	7	3	5	5	10	0	0	0	0	0	0	0	0	0	5	5	10	7	6	13
8551	5	5	5	5	10	0	0	0	0	0	0	0	0	0	5	5	10	5	5	10
8552	NOT PREGNANT																			

M = MALE F = FEMALE
PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 18 (PAGE 6): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

RABBIT #	VIABLE FETUSES RIGHT HORN	VIABLE FETUSES LEFT HORN	VIABLE FETUSES TOTAL	DEAD FETUSES RIGHT HORN	DEAD FETUSES LEFT HORN	DEAD FETUSES TOTAL	EARLY RESORPTIONS RIGHT HORN	EARLY RESORPTIONS LEFT HORN	EARLY RESORPTIONS TOTAL	LATE RESORPTIONS RIGHT HORN	LATE RESORPTIONS LEFT HORN	LATE RESORPTIONS TOTAL	IMPLANTATION SITES RIGHT HORN	IMPLANTATION SITES LEFT HORN	IMPLANTATION SITES TOTAL	CORPORA LUTEA RIGHT OVARY	CORPORA LUTEA LEFT OVARY	CORPORA LUTEA TOTAL
SATELLITE DOSAGE GROUP I						0 (VEHICLE) MG/KG/DAY												
8553	4	9	13	0	0	0	0	0	0	0	0	0	4	9	13	4	9	13
8554	5	5	10	0	0	0	0	0	0	0	0	0	5	5	10	5	5	10
8555	3	5	8	0	0	0	0	0	0	0	0	0	3	5	8	3	5	8
SATELLITE DOSAGE GROUP II						0.1 MG/KG/DAY												
8556	4	4	8	0	0	0	0	0	0	0	0	0	4	4	8	4	4	8
8557	3	4	7	0	0	0	0	0	0	1	0	1	4	4	8	4	4	8
8558	5	5	10	0	0	0	0	0	0	0	0	0	5	5	10	5	5	10
8559	7	4	11	0	0	0	0	0	0	0	0	0	7	4	11	7	4	11
8560	4	7	11	0	0	0	0	0	0	0	0	0	4	7	11	4	7	11
SATELLITE DOSAGE GROUP III						1.0 MG/KG/DAY												
8561	0	2	2	0	0	0	0	0	0	0	0	0	0	2	2	0	3	3
8562	5	4	9	0	0	0	0	0	0	0	0	0	5	4	9	5	4	9
8563	1	6	7	0	0	0	0	0	0	0	0	0	1	6	7	2	6	8
SATELLITE DOSAGE GROUP IV						2.5 MG/KG/DAY												
8564	5	5	10	0	0	0	0	0	0	0	0	0	5	5	10	5	5	10
8565	7	3	10	0	0	0	0	0	0	0	0	0	7	3	10	7	3	10
8566	4	4	8	0	0	0	0	0	0	0	0	0	4	4	8	4	5	9
SATELLITE DOSAGE GROUP V						3.75 MG/KG/DAY												
8567	3	7	10	0	0	0	0	0	0	0	0	0	3	7	10	3	7	10
8568	5	2	7	0	0	0	0	0	0	0	0	0	5	2	7	5	3	8
8569 NOT PREGNANT																		
8570	4	5	9	0	0	0	0	0	0	0	0	0	4	5	9	6	6	12
8571	0	2	2	0	0	0	1	0	1	0	0	0	1	2	3	2	3	5

PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 19 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - INDIVIDUAL DATA

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			---- CONCEPTUSES ------ DEAD OR RESORBED		
RABBIT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP I				0 (VEHICLE) MG/KG/DAY					
8443	NOT PREGNANT								
8444	5	2	7	51.30	49.86	50.89	7	0	0.0
8445	NOT PREGNANT								
8446	3	4	7	51.60	46.62	48.75	7	0	0.0
8447	5	7	12	44.66	42.94	43.66	12	0	0.0
8448	6	4	10	47.24	44.60	46.18	11	1	9.1
8449	7	2	9	44.62	47.62	45.29	9	0	0.0
8450	6	4	10	44.86	42.48	43.91	11	1	9.1
8451	5	2	7	47.34	49.38	47.92	7	0	0.0
8452	2	4	6	47.98	50.26	49.50	7	1	14.3
8453	5	4	9	50.65	45.67	48.44	9	0	0.0
8454	3	7	10	44.11	39.29	40.74	10	0	0.0
8455	5	6	11	46.24	42.17	44.02	11	0	0.0
8456	4	2	6	46.98	51.80	48.59	6	0	0.0
8457	4	4	8	44.81	41.70	43.26	8	0	0.0
8458	6	8	14	37.79	36.06	36.80	14	0	0.0
8459	2	5	7	39.94	43.44	42.44	7	0	0.0
8460	4	3	7	44.86	44.63	44.76	7	0	0.0
8461	3	3	6	44.44	47.33	45.88	6	0	0.0
8462	3	6	9	38.30	38.28	38.29	9	0	0.0
8463	6	4	10	40.69	38.04	39.63	11	1	9.1
8464	3	7	10	31.60	34.98	33.97	10	0	0.0

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 19 (PAGE 2): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - INDIVIDUAL DATA

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			---- CONCEPTUSES ------ DEAD OR RESORBED		
RABBIT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP II				0.1 MG/KG/DAY					
8465	1	5	6	45.60	47.09	46.84	7	1	14.3
8466	NOT PREGNANT								
8467	NOT PREGNANT								
8468	3	7	10	43.49	39.60	40.77	10	0	0.0
8469	2	5	7	43.00	47.51	46.22	7	0	0.0
8470	6	1	7	41.04	36.76	40.43	7	0	0.0
8471	3	6	9	41.56	41.22	41.33	9	0	0.0
8472	3	6	9	44.55	40.00	41.52	9	0	0.0
8473	4	4	8	38.61	39.84	39.23	9	1	11.1
8474	4	5	9	44.18	43.07	43.57	9	0	0.0
8475	2	5	7	41.22	39.68	40.12	7	0	0.0
8476	6	4	10	41.29	36.39	39.33	10	0	0.0
8477	NOT PREGNANT								
8478	6	4	10	38.49	38.54	38.51	10	0	0.0
8479	6	9	15	37.45	34.32	35.57	15	0	0.0
8480	6	3	9	43.58	44.16	43.78	9	0	0.0
8481b	0	0	0	----	----	----	10	10	100.0
8482	4	5	9	37.77	36.95	37.31	9	0	0.0
8483	6	2	8	47.47	48.04	47.61	8	0	0.0
8484	6	3	9	41.16	43.89	42.07	9	0	0.0
8485	3	6	9	42.66	45.71	44.70	9	0	0.0
8486	3	8	11	39.65	41.71	41.14	11	0	0.0

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.
b. Doe 8481 had a litter which consisted of 10 dead fetuses; values excluded from group averages and statistical analyses.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 19 (PAGE 3): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - INDIVIDUAL DATA

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			---- CONCEPTUSES ------ DEAD OR RESORBED		
RABBIT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP III				1.0 MG/KG/DAY					
8487	4	2	6	45.08	47.92	46.03	6	0	0.0
8488	3	4	7	44.95	48.43	46.94	8	1	12.5
8489	2	4	6	47.74	45.64	46.34	7	1	14.3
8490	1	4	5	52.43	49.38	49.99	5	0	0.0
8491	5	4	9	43.39	38.25	41.11	10	1	10.0
8492	2	7	9	39.84	39.32	39.44	9	0	0.0
8493	2	3	5	45.12	40.46	42.33	8	3	37.5
8494	2	4	6	40.86	41.00	40.95	6	0	0.0
8495	7	3	10	39.82	37.36	39.09	10	0	0.0
8496	1	8	9	50.85	43.20	44.05	9	0	0.0
8497	4	3	7	44.44	46.43	45.30	7	0	0.0
8498	3	6	9	32.90	35.34	34.52	9	0	0.0
8499	6	2	8	38.95	39.98	39.21	8	0	0.0
8500	5	5	10	40.43	37.51	38.97	11	1	9.1
8501	8	3	11	40.83	35.95	39.50	11	0	0.0
8502	NOT PREGNANT								
8503	5	5	10	40.58	37.54	39.06	10	0	0.0
8504	4	6	10	34.81	37.76	36.58	10	0	0.0
8505	NOT PREGNANT								
8506	4	5	9	49.77	45.23	47.25	9	0	0.0
8507	3	3	6	29.49	50.22	39.85	6	0	0.0
8508	NOT PREGNANT								

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 19 (PAGE 4): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - INDIVIDUAL DATA

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			---- CONCEPTUSES ------ DEAD OR RESORBED		
RABBIT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP IV				2.5 MG/KG/DAY					
8509	2	5	7	42.72	44.49	43.98	8	1	12.5
8510	4	4	8	46.61	34.32	40.46	8	0	0.0
8511	NOT PREGNANT								
8512	3	4	7	46.69	46.17	46.39	7	0	0.0
8513	NOT PREGNANT								
8514	3	4	7	44.41	40.65	42.26	7	0	0.0
8515	4	5	9	36.02	35.39	35.67	9	0	0.0
8516	4	3	7	44.57	41.71	43.34	7	0	0.0
8517	ABORTED ON DAY 25 OF GESTATION								
8518	7	2	9	33.81	29.02	32.74	10	1	10.0
8519	6	2	8	37.80	36.28	37.42	8	0	0.0
8520	3	2	5	38.96	44.59	41.21	5	0	0.0
8521	6	4	10	41.46	36.27	39.39	10	0	0.0
8522	3	6	9	43.88	44.50	44.29	10	1	10.0
8523	NOT PREGNANT								
8524	NOT PREGNANT								
8525	5	6	11	35.57	31.32	33.25	11	0	0.0
8526	NOT PREGNANT								
8527	7	4	11	32.91	33.76	33.22	11	0	0.0
8528	3	4	7	45.15	43.71	44.32	7	0	0.0
8529	2	3	5	38.80	41.40	40.36	6	1	16.7
8530	4	6	10	39.56	40.17	39.93	10	0	0.0

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 19 (PAGE 5): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - INDIVIDUAL DATA

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			---- CONCEPTUSES ------ DEAD OR RESORBED		
RABBIT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP V				3.75 MG/KG/DAY					
8531	6	3	9	40.47	39.78	40.24	9	0	0.0
8532	4	4	8	34.20	34.17	34.18	8	0	0.0
8533	2	4	6	40.65	37.75	38.72	6	0	0.0
8534	ABORTED ON DAY 22 OF GESTATION								
8535	8	3	11	32.41	22.67	29.75	11	0	0.0
8536	3	5	8	36.22	36.17	36.19	9	1	11.1
8537	ABORTED ON DAY 25 OF GESTATION								
8538	ABORTED ON DAY 24 OF GESTATION								
8539	ABORTED ON DAY 24 OF GESTATION								
8540	ABORTED ON DAY 25 OF GESTATION								
8541	2	5	7	43.72	39.86	40.96	7	0	0.0
8542	ABORTED ON DAY 28 OF GESTATION								
8543	2	7	9	17.69	13.84	14.70	10	1	10.0
8544	ABORTED ON DAY 25 OF GESTATION								
8545	5	8	13	27.58	25.53	26.32	13	0	0.0
8546	0	7	7	----	38.59	38.59	7	0	0.0
8547	ABORTED ON DAY 22 OF GESTATION								
8548	ABORTED ON DAY 25 OF GESTATION								
8549	6	4	10	28.54	26.52	27.73	10	0	0.0
8550	7	3	10	36.26	34.96	35.87	10	0	0.0
8551	5	5	10	37.36	37.93	37.64	10	0	0.0
8552	NOT PREGNANT								

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 19 (PAGE 6): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - INDIVIDUAL DATA

	NUMBER OF LIVE FETUSES	AVERAGE FETAL BODY WEIGHT (G)	---- CONCEPTUSES ------ DEAD OR RESORBED		
RABBIT #	TOTAL	TOTAL a	N	N	%
SATELLITE DOSAGE GROUP I		0 (VEHICLE) MG/KG/DAY			
8553	13	4.96	13	0	0.0
8554	10	4.59	10	0	0.0
8555	8	5.14	8	0	0.0
SATELLITE DOSAGE GROUP II		0.1 MG/KG/DAY			
8556	8	5.17	8	0	0.0
8557	7	4.69	8	1	12.5
8558	10	4.56	10	0	0.0
8559	11	5.21	11	0	0.0
8560	11	4.96	11	0	0.0
SATELLITE DOSAGE GROUP III		1.0 MG/KG/DAY			
8561	2	5.37	2	0	0.0
8562	9	5.12	9	0	0.0
8563	7	5.06	7	0	0.0
SATELLITE DOSAGE GROUP IV		2.5 MG/KG/DAY			
8564	10	4.89	10	0	0.0
8565	10	5.49	10	0	0.0
8566	8	4.14	8	0	0.0
SATELLITE DOSAGE GROUP V		3.75 MG/KG/DAY			
8567	10	4.30	10	0	0.0
8568	7	4.61	7	0	0.0
8569	NOT PREGNANT				
8570	9	5.18	9	0	0.0
8571	2	5.78	3	1	33.3

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 1): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP I						0 (VEHICLE) MG/KG/DAY													
RABBIT # CLs																			
8443	NOT PREGNANT																		
8444 2/ 6	MA	FA /	MA	MA	FA	MA	MA												
	56.21	51.03	53.74	53.50	48.70	49.27	43.80												
8445	NOT PREGNANT																		
8446 6/ 7	MA	MA /	MA	FA	FA	FA	FA												
	52.73	45.56	56.50	52.59	46.91	43.50	43.48												
8447 7/ 8	FA	FA	MA	FA	MA /	MA	MA	FA	FA	FA	FA	MA							
	52.34	43.91	43.11	44.81	44.16	44.38	43.14	46.06	44.06	32.77	36.63	48.49							
8448 7/ 5	MA	FA	MA	MA	L	MA	FA /	FA	MA	FA	MA								
	44.73	43.94	50.24	48.10		45.20	42.35	48.09	50.37	44.00	44.83								
8449 6/ 6	FA	FA	MA	MA /	MA	MA	MA	MA	MA										
	46.84	48.40	42.94	48.11	49.00	46.13	42.21	39.98	44.01										
8450 7/ 6	MA	MA	FA	FA	MA	FA /	MA	E	FA	MA	MA								
	50.79	47.41	45.63	41.80	37.55	40.11	50.49		42.39	37.99	44.95								
8451 7/ 2	MA	MA	MA	FA	MA /	FA	MA												
	47.90	49.72	44.85	45.48	49.43	53.27	44.79												
8452 6/ 3	FA	FA	FA	MA	MA /	E	FA												
	45.63	55.44	52.04	48.77	47.20		47.92												
8453 6/ 7	MA	FA	MA	MA	FA /	FA	MA	MA	FA										
	62.37	51.25	52.51	50.58	36.80	48.85	39.38	48.41	45.78										
8454 7/ 5	FA	FA	FA	FA	FA	FA /	FA	MA	MA	MA									
	41.88	38.46	37.67	41.55	36.23	39.41	39.85	42.97	45.40	43.97									
8455 6/ 7	MA	FA	MA	FA	FA /	MA	FA	MA	FA	FA	MA								
	46.20	43.72	42.71	44.40	46.73	48.40	45.18	45.99	36.63	36.36	47.92								

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 2): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP I						0 (VEHICLE) MG/KG/DAY													
RABBIT # CLs																			
8456 3/ 4	FA	MA	MA /	FA	MA	MA													
	50.69	48.39	46.64	52.91	47.19	45.70													
8457 5/ 3	MA	MA	FA	FA	MA /	FA	MA	FA											
	49.24	40.91	45.36	41.77	39.57	43.53	49.51	36.15											
8458 7/ 7	MA	FA	FA	FA	MA	FA	MA /	FA	FA	MA	MA	FA	FA	MA					
	48.33	34.86	37.64	41.01	30.01	32.55	38.35	41.70	37.67	40.03	35.05	33.42	29.59	34.98					
8459 6/ 1	FA	FA	MA	MA	FA	FA /	FA												
	50.26	46.54	43.89	35.98	37.66	40.16	42.57												
8460 4/ 4	FA	MA	FA	MA /	MA	FA	MA												
	47.79	45.01	47.77	40.17	49.24	38.33	45.04												
8461 6/ 4	MA	FA /	FA	FA	MA	MA													
	43.96	43.13	48.41	50.44	42.14	47.23													
8462 6/ 5	MA	FA	FA	MA	FA /	FA	FA	FA	MA										
	40.36	38.51	38.20	41.28	40.99	39.45	38.36	34.18	33.26										
8463 10/ 3	E	FA	MA	FA	MA	MA	FA	FA	MA	MA /	MA								
		41.92	43.20	44.40	38.14	34.30	37.73	28.09	34.76	42.28	51.47								
8464 7/ 3	MA	FA	FA	FA	FA	MA	FA /	FA	MA	FA									
	34.27	30.24	35.00	32.69	30.71	26.55	36.96	40.65	33.98	38.63									

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 3): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP II							0.1 MG/KG/DAY													
RABBIT #	CLs																			
8465	4/ 3	MA	FA	FA	E /	FA	FA	FA												
		45.60	43.81	48.51		48.37	48.03	46.75												
8466		NOT PREGNANT																		
8467		NOT PREGNANT																		
8468	8/ 7	MA	MA	MA	FA /	FA	FA	FA	FA	FA	FA									
		47.27	40.38	42.81	36.38	44.10	44.88	39.45	35.81	37.69	38.89									
8469	7/ 2	FA	FA	FA	FA	MA	FA /	MA												
		53.28	51.18	45.06	44.83	39.47	43.21	46.53												
8470	4/ 5	MA	FA	MA	MA /	MA	MA	MA												
		42.48	36.76	36.81	42.03	46.43	41.31	37.18												
8471	5/ 8	MA	MA	FA /	FA	FA	MA	FA	FA	FA										
		33.93	45.03	40.91	38.12	40.87	45.72	42.20	41.34	43.88										
8472	6/ 4	FA	FA	MA	FA	FA	FA /	MA	FA	MA										
		44.77	42.76	40.73	41.27	32.00	33.58	52.07	45.61	40.86										
8473	6/ 5	MA	FA	E	MA	MA	FA /	FA	FA	MA										
		49.18	38.09		34.07	25.96	29.61	47.33	44.35	45.23										
8474	5/ 5	MA	MA	MA	FA /	FA	FA	FA	MA	FA										
		43.52	44.47	43.89	38.06	47.20	45.16	43.96	44.86	40.99										
8475	4/ 4	FA	MA	FA /	MA	FA	FA	FA												
		33.93	42.02	41.30	40.42	38.71	43.59	40.89												
8476	6/ 6	FA	MA	FA	FA	FA	MA /	MA	MA	MA	MA									
		44.27	44.37	38.82	30.39	32.09	37.95	47.12	42.34	35.65	40.30									
8477		NOT PREGNANT																		

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 4): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP II						0.1 MG/KG/DAY													
RABBIT # CLs																			
8478 7/ 5	MA	FA	MA	MA	MA	FA /	MA	FA	MA	FA									
	46.49	39.16	38.92	33.12	35.57	41.39	44.32	35.54	32.50	38.05									
8479 8/ 9	FA	FA	FA	FA	FA	MA /	MA	MA	MA	MA	FA	MA	FA	FA	FA				
	22.79	35.71	35.64	38.48	43.11	35.88	34.26	42.60	41.00	43.87	45.69	27.08	25.39	32.10	29.98				
8480 6/ 3	MA	FA	FA	MA	MA	MA /	FA	MA	MA										
	46.60	41.98	40.91	46.54	39.30	42.05	49.60	45.06	41.95										
8481a 3/ 8	FD	FD	FD /	FD	MD	FD	FD	FD	FD	FD									
	45.86	46.91	45.99	51.11	47.50	45.01	38.98	41.14	38.31	41.88									
8482 3/ 7	MA	FA	FA /	MA	MA	FA	MA	FA	FA										
	43.89	45.85	37.85	39.97	35.69	31.76	31.53	30.39	38.88										
8483 7/ 4	MA	FA	FA	MA	MA /	MA	MA	MA											
	55.20	51.95	44.14	38.19	45.14	45.41	47.60	53.27											
8484 6/ 3	FA	MA	MA	MA	FA	MA /	MA	FA	MA										
	48.76	48.13	42.50	46.08	39.88	25.38	42.43	43.02	42.47										
8485 7/ 4	FA	MA	FA	FA	MA	FA /	FA	FA	MA										
	49.61	48.36	43.09	44.08	38.45	43.78	52.45	41.27	41.17										
8486 7/ 6	FA	MA	FA	FA	FA /	FA	FA	MA	FA	MA	FA								
	45.21	44.96	44.27	37.15	39.12	44.51	45.63	40.85	36.11	33.13	41.65								

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).
a. Doe 8481 had a litter which consisted of 10 dead fetuses; values excluded from group averages and statistical analyses.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 5): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP III						1.0 MG/KG/DAY													
RABBIT # CLs																			
8487 3/ 4	FA	MA	MA /	FA	MA	MA													
	46.05	48.00	43.38	49.80	45.05	43.88													
8488 5/ 6	E	FA	FA /	FA	FA	MA	MA	MA											
		46.18	49.04	50.97	47.52	43.30	42.11	49.43											
8489 3/ 5	MA	FA	E /	FA	MA	FA	FA												
	47.90	46.58		44.56	47.57	43.24	48.17												
8490 2/ 5 /	MA	FA	FA	FA	FA														
	52.43	50.12	43.68	48.93	54.79														
8491 5/ 8	MA	FA	MA /	FA	MA	L	MA	MA	FA	FA									
	46.90	38.03	43.41	45.04	48.94	1.26	41.01	36.71	35.45	34.47									
8492 6/ 3	MA	FA	FA	MA	FA	FA /	FA	FA	FA										
	42.28	38.88	39.50	37.41	33.81	36.29	44.06	40.60	42.09										
8493 2/10	FA /	MA	L	L	L	MA	FA	FA											
	45.19	45.57	5.95	0.79	0.98	44.67	37.65	38.55											
8494 5/ 6	MA	MA /	FA	FA	FA	FA													
	41.15	40.58	43.50	40.57	38.44	41.47													
8495 9/ 4	MA	MA	FA	MA	FA	MA	MA /	MA	FA	MA									
	43.56	42.88	35.41	31.44	32.83	28.21	45.91	45.94	43.85	40.84									
8496 8/ 5	FA	FA	FA	FA	FA	FA	FA /	FA	MA										
	53.70	48.23	45.40	35.33	41.06	34.07	35.52	52.26	50.85										
8497 3/ 5	MA	FA	FA /	FA	MA	MA	MA												
	47.84	47.91	44.18	47.21	47.13	39.11	43.69												
8498 1/ 8	MA /	FA	FA	FA	MA	FA	FA	MA	FA										
	38.14	38.40	36.49	35.23	32.16	30.78	32.92	28.40	38.21										
8499 3/ 5	MA	MA	MA /	FA	FA	MA	MA	MA											
	42.91	40.01	39.98	41.98	37.98	34.30	37.94	38.55											

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 6): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP III						1.0 MG/KG/DAY													
RABBIT # CLs																			
8500 3/ 9	FA	MA /	FA	MA	FA	MA	L	FA	FA	MA	MA								
	43.88	42.23	35.01	43.85	35.53	36.84	1.33	38.59	34.52	42.42	36.79								
8501 8/ 6	MA	MA	MA	MA	MA	MA /	MA	FA	FA	MA	FA								
	43.17	36.70	41.88	42.12	40.72	40.73	48.19	39.68	33.34	33.14	34.84								
8502	NOT PREGNANT																		
8503 9/ 4	MA	MA	MA	MA	FA	FA	MA	FA /	FA	FA									
	49.13	46.49	39.69	39.73	37.16	28.48	27.87	30.10	47.22	44.76									
8504 5/ 7	FA	FA	FA /	MA	FA	MA	MA	FA	MA	FA									
	43.92	40.39	34.65	39.94	38.17	32.76	35.21	36.00	31.32	33.46									
8505	NOT PREGNANT																		
8506 8/ 3	FA	FA	MA	FA	FA	FA	MA /	MA	MA										
	48.96	49.18	52.74	49.93	43.75	34.33	47.69	50.65	48.01										
8507 3/ 6 /	MA	FA	FA	FA	MA	MA													
	51.22	52.40	48.86	49.39	47.99	40.47													
8508	NOT PREGNANT																		

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 7): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP IV							2.5 MG/KG/DAY													
RABBIT #	CLs																			
8509	4/ 7	MA	E	FA	FA /	FA	FA	FA	MA											
		48.53		41.94	46.99	44.40	40.30	48.82	36.91											
8510	7/ 5	MA	MA	FA	FA	FA /	MA	FA	MA											
		52.02	46.80	33.61	35.29	30.02	51.25	38.34	36.38											
8511		NOT PREGNANT																		
8512	7/ 4	MA	FA	MA	FA	MA /	FA	FA												
		52.12	47.92	42.50	43.65	45.46	49.14	43.96												
8513		NOT PREGNANT																		
8514	6/ 7	FA	FA	FA /	MA	MA	FA	MA												
		44.25	42.18	37.29	44.61	45.67	38.88	42.96												
8515	4/ 6	FA	FA	MA /	FA	MA	FA	MA	FA	MA										
		39.20	36.77	37.54	34.18	37.55	34.51	33.67	32.27	35.34										
8516	3/ 6	MA	FA /	MA	MA	FA	MA	FA												
		45.09	41.03	48.93	42.06	42.55	42.20	41.54												
8517		ABORTED ON DAY 25 OF GESTATION																		
8518	7/ 5	MA	MA	MA	MA	L /	MA	FA	MA	MA	FA									
		35.13	37.26	33.35	31.55	0.58	37.57	34.95	30.47	31.32	23.08									
8519	3/ 5	MA	MA	FA /	MA	FA	MA	MA	MA											
		40.52	35.27	34.34	37.48	38.23	37.67	36.38	39.47											
8520	2/ 4	FA	MA /	MA	MA	FA														
		44.00	43.14	35.80	37.94	45.18														
8521	7/ 5	MA	MA	MA	MA	FA	FA /	FA	MA	FA	MA									
		45.57	45.12	37.02	37.95	35.74	36.05	38.45	39.43	34.83	43.70									

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 8): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP IV						2.5 MG/KG/DAY													
RABBIT # CLs																			
8522 6/ 7	FA	FA	FA	MA	MA /	MA	FA	FA	FL	FA									
	46.76	45.76	45.05	44.61	38.81	48.21	43.39	43.87	24.30	42.16									
8523	NOT PREGNANT																		
8524	NOT PREGNANT																		
8525 6/ 7	MA	FA	MA	FA	FA /	FA	MA	MA	MA	FA	FA								
	40.08	38.03	34.97	27.49	29.49	36.56	38.99	34.21	29.61	26.10	30.27								
8526	NOT PREGNANT																		
8527 6/ 5	MA	MA	MA	MA	MA	MA /	FA	FA	FA	MA	FA								
	41.16	41.97	38.11	30.51	21.05	18.80	35.72	30.23	34.01	38.80	35.06								
8528 6/ 3	FA	MA	FA	FA	MA /	FA	MA												
	47.70	45.44	41.92	43.95	42.15	41.26	47.86												
8529 3/ 4	E	MA /	FA	FA	MA	FA													
		42.11	42.69	43.75	35.48	37.75													
8530 7/ 3	FA	MA	MA	FA	FA	MA	FA /	FA	FA	MA									
	41.58	41.84	34.18	38.16	38.01	38.42	40.79	43.07	39.43	43.79									

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 9): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP V						3.75 MG/KG/DAY													
RABBIT # CLs																			
8531 4/ 7	FA	FA	FA	MA /	MA	MA	MA	MA	MA										
	40.31	40.21	38.81	31.50	49.16	42.77	39.52	38.03	41.82										
8532 6/ 6	MA	FA	FA	FA	MA /	FA	MA	MA											
	38.54	34.70	33.13	29.40	30.58	39.44	33.18	34.49											
8533 7/ 5	FA	MA	FA /	MA	FA	FA													
	41.03	39.95	37.99	41.35	36.50	35.49													
8534	ABORTED ON DAY 22 OF GESTATION																		
8535 8/ 3	MA	MA	MA	FA	MA	FA	MA	MA /	FA	MA	MA								
	38.06	35.58	37.84	14.27	23.13	21.76	24.00	27.76	31.98	34.42	38.48								
8536 3/ 8	MA	MA	E /	FA	FA	FA	FA	MA	FA										
	39.65	44.51		46.24	41.41	33.54	25.63	24.51	34.03										
8537	ABORTED ON DAY 25 OF GESTATION																		
8538	ABORTED ON DAY 24 OF GESTATION																		
8539	ABORTED ON DAY 24 OF GESTATION																		
8540	ABORTED ON DAY 25 OF GESTATION																		
8541 5/ 3	MA	FA	MA	FA	FA /	FA	FA												
	47.60	42.99	39.85	31.84	38.05	44.51	41.90												
8542	ABORTED ON DAY 28 OF GESTATION																		
8543 7/ 5	FA	MA	MA	FA	FA	FA /	L	FA	FA	FA									
	18.98	21.05	14.33	9.61	12.62	11.15	5.01	13.45	17.13	13.97									

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 10): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP V							3.75 MG/KG/DAY													
RABBIT #	CLs																			
8544		ABORTED ON DAY 25 OF GESTATION																		
8545	6/ 7	FA	FA	MA	FA	FA	FA /	FA	FA	MA	MA	MA	FA	MA						
		33.44	23.74	31.73	30.19	15.63	14.05	34.34	29.81	34.38	26.13	20.57	23.06	25.11						
8546	3/ 5	FA	FA /	FA	FA	FA	FA	FA												
		39.89	43.53	39.73	37.76	40.56	34.02	34.62												
8547		ABORTED ON DAY 22 OF GESTATION																		
8548		ABORTED ON DAY 25 OF GESTATION																		
8549	7/ 5	FA	FA	MA	MA	MA /	MA	FA	MA	MA	FA									
		32.68	19.63	27.15	26.92	31.62	32.57	25.28	30.14	22.82	28.47									
8550	7/ 6	FA	MA	FA	MA	MA /	MA	MA	FA	MA	MA									
		37.27	36.52	39.52	34.53	34.56	41.67	39.86	28.09	32.32	34.34									
8551	5/ 5	FA	MA	MA	MA	FA /	FA	FA	MA	MA	FA									
		41.03	37.47	42.30	33.52	36.51	45.27	36.10	37.49	36.02	30.74									
8552		NOT PREGNANT																		

M = MALE F = FEMALE A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 11): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
SATELLITE DOSAGE GROUP I						0 (VEHICLE) MG/KG/DAY													
RABBIT # CLs																			
8553 4/ 9	A	A	A	A /	A	A	A	A	A	A	A	A	A						
	4.78	4.78	4.99	5.18	5.59	4.45	5.56	4.67	4.97	5.12	4.87	4.57	4.89						
8554 5/ 5	A	A	A	A	A /	A	A	A	A	A									
	4.80	3.99	5.19	4.34	4.69	4.63	4.56	4.41	4.25	5.05									
8555 3/ 5	A	A	A /	A	A	A	A	A											
	5.37	5.03	5.58	5.27	5.61	4.18	5.09	5.01											
SATELLITE DOSAGE GROUP II						0.1 MG/KG/DAY													
RABBIT # CLs																			
8556 4/ 4	A	A	A	A /	A	A	A	A											
	5.44	5.41	5.49	4.02	4.99	5.31	5.62	5.10											
8557 4/ 4	A	A	A	L /	A	A	A	A											
	4.05	4.89	5.05		4.81	4.70	4.87	4.45											
8558 5/ 5	A	A	A	A	A /	A	A	A	A	A									
	3.74	4.78	5.08	4.67	4.46	4.09	4.77	4.53	4.93	4.57									
8559 7/ 4	A	A	A	A	A	A	A /	A	A	A	A								
	5.32	5.54	4.57	5.05	5.54	5.41	4.99	5.33	5.32	5.11	5.10								
8560 4/ 7	A	A	A	A /	A	A	A	A	A	A	A								
	4.37	5.19	5.20	4.59	5.43	4.70	4.63	5.77	4.99	4.55	5.20								
SATELLITE DOSAGE GROUP III						1.0 MG/KG/DAY													
RABBIT # CLs																			
8561 0/ 3 /	A	A																	
	5.38	5.36																	
8562 5/ 4	A	A	A	A	A /	A	A	A	A										
	5.24	4.76	5.55	5.27	5.34	4.77	5.48	5.00	4.67										
8563 2/ 6	A /	A	A	A	A	A	A												
	5.24	5.06	4.40	5.13	5.17	4.82	5.64												

A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 20 (PAGE 12): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
SATELLITE DOSAGE GROUP IV						2.5 MG/KG/DAY													
RABBIT # CLs																			
8564 5/ 5	A	A	A	A	A /	A	A	A	A	A									
	4.71	5.07	4.76	4.89	5.06	4.72	4.96	4.84	4.81	5.05									
8565 7/ 3	A	A	A	A	A	A	A /	A	A	A									
	6.03	5.78	5.32	5.82	5.76	5.34	4.77	6.15	5.14	4.78									
8566 4/ 5	A	A	A	A /	A	A	A	A											
	4.33	4.06	3.71	3.82	4.42	4.35	4.37	4.10											
SATELLITE DOSAGE GROUP V						3.75 MG/KG/DAY													
RABBIT # CLs																			
8567 3/ 7	A	A	A /	A	A	A	A	A	A	A									
	4.82	4.53	4.08	4.65	4.49	4.44	4.18	4.28	3.62	3.95									
8568 5/ 3	A	A	A	A	A /	A	A												
	4.64	4.69	4.61	4.46	4.41	4.63	4.82												
8569	NOT PREGNANT																		
8570 6/ 6	A	A	A	A /	A	A	A	A	A										
	5.60	5.44	5.43	4.85	5.51	5.34	4.79	4.85	4.86										
8571 2/ 3	E /	A	A																
		5.93	5.62																

A = ALIVE D = DEAD E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 1): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP I 0 (VEHICLE) MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8443	NOT PREGNANT						
8444	1(14.3)	0/ 7		0/ 7		1/ 7	FETUS 4 HYOID: ALA, ANGULATED, bilateral
8445	NOT PREGNANT						
8446	0(0.0)	0/ 7		0/ 7		0/ 7	
8447	5(41.7)	0/12		0/12		5/12	FETUS 4 HYOID: ALA, ANGULATED, bilateral FETUS 7 HYOID: ALA, ANGULATED, left FETUS 8 CAUDAL VERTEBRAE: MISALIGNED, 17th FETUS 10 THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION, left 5th FETUS 11 HYOID: ALA, ANGULATED, bilateral

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 2): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP I 0 (VEHICLE) MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8448	1(10.0)	1/10	FETUS 1 FORELIMB: POLLEX ABSENT, bilateral FETUS 5 LATE RESORPTION, autolysis precluded further evaluation	0/10		1/10	FETUS 1 FORELIMB: 4 METACARPALS PRESENT, bilateral, 1st metacarpal absent; 4 DIGITS PRESENT, bilateral, 1st digit absent; FOREPHALANGES, 1st MEDIAL AND DISTAL PHALANGES ABSENT, bilateral
8449	3(33.3)	0/ 9		1/ 9	FETUS 6 EYES: CIRCUMCORNEAL HEMORRHAGE, left eye	3/ 9	FETUS 2 HYOID: ALA, ANGULATED, bilateral FETUS 6 SKULL: NASAL - FRONTAL, IRREGULAR SUTURE, bilateral HYOID: ALA, ANGULATED, bilateral FETUS 7 HYOID: ALA, ANGULATED, bilateral

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 3): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP I 0 (VEHICLE) MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	DESCRIPTION	SOFT TISSUE EXAMINATION N/N	DESCRIPTION	SKELETAL EXAMINATION N/N	DESCRIPTION
8450	3(30.0)	0/10		1/10	FETUS 7 LUNGS: INTERMEDIATE LOBE ABSENT	2/10	FETUS 2 SKULL: NASAL, CONTAINED AN INTRANASAL, right, 2.0 mm x 3.0 mm, left, 2.5 mm x 4.0 mm FETUS 3 SKULL: NASALS, CONTAINED AN INTERNASAL, 1.) 0.5 mm x 2.5 mm 2.) 0.5 mm x 2.0 mm
8451	1(14.3)	0/ 7		0/ 7		1/ 7	FETUS 1 SKULL: NASAL, CONTAINED AN INTRANASAL, left, 3.0 mm x 5.0 mm
8452	1(16.7)	0/ 6		0/ 6		1/ 6	FETUS 1 HYOID: ALA, ANGULATED, left THORACIC VERTEBRAE: HEMIVERTEBRA, left 3rd, arch and centrum RIBS: SPLIT, left 2nd, medial - distal STERNAL CENTRA: ASYMMETRIC, 1st - 3rd
8453	0(0.0)	0/ 9		0/ 9		0/ 9	
8454	1(10.0)	0/10		0/10		1/10	FETUS 3 SKULL: NASALS, FUSED, partially, 4.0 mm

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 4): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP I 0 (VEHICLE) MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8455	0(0.0)	0/11		0/11		0/11	
8456	1(16.7)	0/ 6		0/ 6		1/ 6	FETUS 4 RIBS: THICKENED, left 8th and 9th, medially
8457	0(0.0)	0/ 8		0/ 8		0/ 8	
8458	1(7.1)	0/14		0/14		1/14	FETUS 4 STERNAL CENTRA: FUSED, 3rd and 4th
8459	2(28.6)	0/ 7		0/ 7		2/ 7	FETUS 4 STERNAL CENTRA: FUSED, 3rd and 4th FETUS 6 SKULL: NASAL, CONTAINED AN INTRANASAL, right, 1.0 mm x 2.0 mm, left, 1.5 mm x 2.0 mm
8460	2(28.6)	0/ 7		0/ 7		2/ 7	FETUS 5 CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA, bilateral FETUS 6 SKULL: FRONTALS, CONTAINED AN INTERFRONTAL, 0.4 mm x 2.0 mm

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 5): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP I 0 (VEHICLE) MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	DESCRIPTION	SOFT TISSUE EXAMINATION N/N	DESCRIPTION	SKELETAL EXAMINATION N/N	DESCRIPTION
8461	0(0.0)	0/ 6		0/ 6		0/ 6	
8462	0(0.0)	0/ 9		0/ 9		0/ 9	
8463	1(10.0)	0/10		0/10		1/10	FETUS 8 HYOID: ALA, ANGULATED, left
8464	2(20.0)	0/10		1/10	FETUS 1 LUNGS: INTERMEDIATE LOBE ABSENT	1/10	FETUS 7 HYOID: ALA, ANGULATED, right

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 6): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP II 0.1 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8465	2(33.3)	0/ 6		0/ 6		2/ 6	FETUS 6 SKULL: NASALS, MIDLINE SUTURE DISPLACED, right FETUS 7 HYOID: ALA, ANGULATED, bilateral
8466	NOT PREGNANT						
8467	NOT PREGNANT						
8468	0(0.0)	0/10		0/10		0/10	
8469	2(28.6)	0/ 7		0/ 7		2/ 7	FETUS 5 SKULL: NASALS, CONTAINED AN INTERNASAL, 1.5 mm x 2.5 mm FETUS 6 SKULL: NASALS, CONTAINED AN INTERNASAL, 0.5 mm x 1.0 mm HYOID: ALA, ANGULATED, right
8470	2(28.6)	0/ 7		0/ 7		2/ 7	FETUS 1 SKULL: NASALS, MIDLINE SUTURE DISPLACED, right FETUS 3 SKULL: NASALS, MIDLINE SUTURE DISPLACED, left

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 7): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP II 0.1 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8471	1(11.1)	0/ 9		0/ 9		1/ 9	FETUS 1 SKULL: PREMAXILLAE, NOT OSSIFIED, bilateral
8472	0(0.0)	0/ 9		0/ 9		0/ 9	
8473	2(25.0)	1/ 8	FETUS 6 FORELIMBS FLEXED DOWNWARD, forepaws bilateral	0/ 8		1/ 8	FETUS 4 STERNAL CENTRA: FUSED, 3rd and 4th
8474	1(11.1)	0/ 9		0/ 9		1/ 9	FETUS 7 SKULL: NASAL - FRONTAL, IRREGULAR SUTURE, bilateral
8475	4(57.1)	0/ 7		1/ 7	FETUS 2 LUNGS: INTERMEDIATE LOBE ABSENT	3/ 7	FETUS 5 SKULL: NASAL - FRONTAL, IRREGULAR SUTURE, bilateral FETUS 6 SKULL: NASALS, CONTAINED AN INTERNASAL, 0.5 mm x 1.5 mm HYOID: ALA, ANGULATED, left FETUS 7 HYOID: ALA, ANGULATED, bilateral

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 8): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP II 0.1 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8476	3(30.0)	0/10		1/10	FETUS 8 LUNGS: INTERMEDIATE LOBE ABSENT	3/10	FETUS 1 THORACIC VERTEBRAE: CENTRUM, BIFID, 12th; CENTRA, FUSED, 11th to left 12th FETUS 7 RIBS: FUSED, left 5th and 6th, medial - distal FETUS 8 STERNAL CENTRA: FUSED, 3rd and 4th
8477	NOT PREGNANT						
8478	0(0.0)	0/10		0/10		0/10	
8479	3(20.0)	0/15		2/15	FETUS 4 LUNGS: INTERMEDIATE LOBE ABSENT FETUS 12 LUNGS: INTERMEDIATE LOBE ABSENT	1/15	FETUS 10 SKULL: NASALS, MIDLINE SUTURE DISPLACED, left
8480	0(0.0)	0/ 9		0/ 9		0/ 9	
8481a	4(40.0)	1/10	FETUS 1 (DEAD) FORELIMBS: FLEXED DOWNWARD	0/10		3/10	FETUS 5 (DEAD) HYOID: ALA, ANGULATED bilateral

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

a. Litter 8481 consisted of ten dead fetuses; all embryonic sacs contained a dark red substance. Values were excluded from group averages and statistical analyses.

Dosage Levels, Concentrations, Volumes and Injection Rates:

Dosage Group	Number of Rabbits	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Argus Batch Number
I	22+3[a]	0 (Vehicle)	0	5	B-418-012-A(Day.Month.Year)
II	22+5[a]	0.1	0.02	5	B-418-012-B(Day.Month.Year)
III	22+3[a]	1.0	0.2	5	B-418-012-C(Day.Month.Year)
IV	22+3[a]	2.5	0.5	5	B-418-012-D(Day.Month.Year)
V	22+5[a]	3.75	0.75	5	B-418-012-E(Day.Month.Year)

a. Rabbits assigned to toxicokinetic evaluation.
The test article will be considered 100% pure for the purpose of dosage calculations.

TESTS, ANALYSES AND MEASUREMENTS:

Viability:

All Periods: At least twice daily.

Clinical Observations and/or General Appearance:

Predosage Period: At least once.

Dosage Period: Twice daily. Prior to dosage administration and once approximately one hour postdosage.

Postdosage Period: Once daily.

Clinical observations may be recorded more frequently than cited above, if deemed appropriate by the Study Director and/or Study Monitor.

Body Weights:

Predosage Period: Day 0 of presumed gestation and on the day of arrival at the Testing Facility.

Dosage Period: Daily.

Postdosage Period: Daily.

Feed Consumption Values:

Predosage Period: Recorded daily after arrival at the Testing Facility (values not tabulated).

Dosage Period: Recorded daily.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 9): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP II 0.1 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	DESCRIPTION	SOFT TISSUE EXAMINATION N/N	DESCRIPTION	SKELETAL EXAMINATION N/N	DESCRIPTION
8481a (Cont.)							FETUS 7 (DEAD) CAUDAL VERTEBRAE: 10 PRESENT FETUS 9 (DEAD) CAUDAL VERTEBRAE: 6 PRESENT
8482	0(0.0)	0/ 9		0/ 9		0/ 9	
8483	4(50.0)	0/ 8		3/ 8	FETUS 3 LUNGS: INTERMEDIATE LOBE ABSENT FETUS 4 LUNGS: INTERMEDIATE LOBE ABSENT FETUS 7 LUNGS: INTERMEDIATE LOBE ABSENT	2/ 8	FETUS 1 CERVICAL VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION, left 2nd FETUS 4 STERNAL CENTRA: FUSED, 3rd and 4th
8484	0(0.0)	0/ 9		0/ 9		0/ 9	
8485	1(11.1)	0/ 9		0/ 9		1/ 9	FETUS 2 THORACIC VERTEBRAE: ARCH, SMALL, left 11th; CENTRA, FUSED, left 11th and 12th RIBS: PROXIMATE, left 10th and 11th, bases
8486	0(0.0)	0/11		0/11		0/11	

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

a. Litter 8481 consisted of ten dead fetuses; all embryonic sacs contained a dark red substance. Values were excluded from group averages and statistical analyses.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 10): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP III 1.0 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8487	0(0.0)	0/ 6		0/ 6		0/ 6	
8488	0(0.0)	0/ 7		0/ 7		0/ 7	
8489	1(16.7)	0/ 6		1/ 6	FETUS 6 LUNGS: INTERMEDIATE LOBE ABSENT	0/ 6	
8490	0(0.0)	0/ 5		0/ 5		0/ 5	
8491	1(11.1)	0/ 9	FETUS 6 LATE RESORPTION, autolysis precluded further evaluation	0/ 9		1/ 9	FETUS 4 SKULL: NASAL, CONTAINED AN INTRANASAL, left, 1.0 mm x 2.0 mm
8492	0(0.0)	0/ 9		0/ 9		0/ 9	
8493	1(20.0)	0/ 5	FETUS 3 LATE RESORPTION, autolysis precluded further evaluation FETUS 4 LATE RESORPTION, autolysis precluded further evaluation FETUS 5 LATE RESORPTION, autolysis precluded further evaluation	0/ 5		1/ 5	FETUS 7 RIBS: THICKENED, left 6th - 8th, medially

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 11): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP III 1.0 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8494	2(33.3)	0/ 6		0/ 6		2/ 6	FETUS 3 HYOID: ALA, ANGULATED, bilateral FETUS 6 HYOID: ALA, ANGULATED, bilateral
8495	1(10.0)	0/10		0/10		1/10	FETUS 3 SKULL: NASALS, MIDLINE SUTURE DISPLACED, right
8496	3(33.3)	0/ 9		0/ 9		3/ 9	FETUS 4 HYOID: ALA, ANGULATED, right FETUS 7 HYOID: ALA, ANGULATED, right FETUS 9 HYOID: ALA, ANGULATED, bilateral
8497	0(0.0)	0/ 7		0/ 7		0/ 7	
8498	0(0.0)	0/ 9		0/ 9		0/ 9	
8499	0(0.0)	0/ 8		0/ 8		0/ 8	

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 12): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP III 1.0 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8500	2(20.0)	0/10	FETUS 7 LATE RESORPTION, autolysis precluded further evaluation	0/10		2/10	FETUS 3 RIBS: SPLIT, right 8th, proximal - distal, left 7th, proximal - distal FETUS 8 LUMBAR VERTEBRAE: HEMIVERTEBRA, left 1st, arch and centrum; CENTRUM, BIFID, 2nd
8501	0(0.0)	0/11		0/11		0/11	
8502	NOT PREGNANT						
8503	2(20.0)	0/10		0/10		2/10	FETUS 1 HYOID: ALA, ANGULATED, bilateral FETUS 9 HYOID: ALA, ANGULATED, right
8504	0(0.0)	0/10		0/10		0/10	
8505	NOT PREGNANT						
8506	0(0.0)	0/ 9		0/ 9		0/ 9	
8507	1(16.7)	0/ 6		0/ 6		1/ 6	FETUS 3 SKULL: NASAL, CONTAINED AN INTRANASAL, left, 1.0 mm x 2.0 mm
8508	NOT PREGNANT						

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 13): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP IV 2.5 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8509	0(0.0)	0/ 7		0/ 7		0/ 7	
8510	0(0.0)	0/ 8		0/ 8		0/ 8	
8511	NOT PREGNANT						
8512	0(0.0)	0/ 7		0/ 7		0/ 7	
8513	NOT PREGNANT						
8514	0(0.0)	0/ 7		0/ 7		0/ 7	
8515	0(0.0)	0/ 9		0/ 9		0/ 9	
8516	1(14.3)	0/ 7		0/ 7		1/ 7	FETUS 1 SKULL: NASALS, CONTAINED AN INTERNASAL, 2.0 mm x 3.0 mm
8517	ABORTED ON DAY 25 OF GESTATION a						
8518	0(0.0)	0/ 9	FETUS 5 LATE RESORPTION, autolysis precluded further evaluation	0/ 9		0/ 9	
8519	1(12.5)	0/ 8		0/ 8		1/ 8	FETUS 2 CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA, bilateral

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

a. Doe 8517 aborted eight late resorptions on day 25 of gestation; autolysis precluded further evaluation.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 14): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP IV 2.5 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8520	0(0.0)	0/ 5		0/ 5		0/ 5	
8521	0(0.0)	0/10		0/10		0/10	
8522	2(22.2)	0/ 9	FETUS 9 LATE RESORPTION, autolysis precluded further evaluation	0/ 9		2/ 9	FETUS 4 HYOID: ALA, ANGULATED, bilateral FETUS 5 HYOID: ALA, ANGULATED, bilateral
8523	NOT PREGNANT						
8524	NOT PREGNANT						
8525	1(9.1)	0/11		0/11		1/11	FETUS 9 HYOID: ALA, ANGULATED, right
8526	NOT PREGNANT						
8527	0(0.0)	0/11		0/11		0/11	
8528	0(0.0)	0/ 7		0/ 7		0/ 7	
8529	0(0.0)	0/ 5		0/ 5		0/ 5	
8530	0(0.0)	0/10		0/10		0/10	

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 15): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP V 3.75 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8531	2(22.2)	0/ 9		0/ 9		2/ 9	FETUS 3 SKULL: FRONTALS, CONTAINED AN INTERFRONTAL, 2.0 mm x 3.0 mm FETUS 8 SKULL: FRONTALS, CONTAINED AN INTERFRONTAL, 2.5 mm x 4.0 mm
8532	1(12.5)	0/ 8		1/ 8	FETUS 5 LUNGS: INTERMEDIATE LOBE ABSENT	0/ 8	
8533	0(0.0)	0/ 6		0/ 6		0/ 6	
8534	ABORTED ON DAY 22 OF GESTATION a						
8535	1(9.1)	0/11		0/11		1/11	FETUS 6 LUMBAR VERTEBRAE: CENTRUM, BIFID, 1st
8536	3(37.5)	0/ 8		0/ 8		3/ 8	FETUS 5 SKULL: NASAL, CONTAINED AN INTRANASAL, left, 2.0 mm x 3.0 mm FETUS 6 HYOID: ALA, ANGULATED, bilateral

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

a. Doe 8534 aborted one dead fetus and had seven live fetuses in utero on day 22 of gestation. All fetuses appeared normal at gross external and soft tissue examination. Fetuses 1, 2, 3, 4, 5, 6, 7, and 8 had pelvis, pubis not ossified (bilateral) at skeletal examination.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 16): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP V 3.75 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8536 (Cont.)							FETUS 7 HYOID: ALA, ANGULATED, bilateral
8537	ABORTED ON DAY 25 OF GESTATION a						
8538	ABORTED ON DAY 24 OF GESTATION b						
8539	ABORTED ON DAY 24 OF GESTATION c						
8540	ABORTED ON DAY 25 OF GESTATION d						
8541	1(14.3)	0/ 7		0/ 7		1/ 7	FETUS 1 HYOID: ALA, ANGULATED, bilateral
8542	ABORTED ON DAY 28 OF GESTATION e						
8543	7(77.8)	1/ 9	FETUS 3 FORELIMBS FLEXED DOWNWARD, bilateral forepaws	0/ 9		7/ 9	FETUS 1 SKULL: PARIETAL, CONTAINED A HOLE, right, 2.5 mm x 3.5 mm - large, left, 2.0 mm x 4.0 mm - large

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

a. Doe 8537 aborted five late resorptions on day 25 of gestation; autolysis precluded further evaluation.

b. Doe 8538 aborted eight late resorptions on day 24 of gestation; autolysis precluded further evaluation. One additional fetus was presumed cannibalized.

c. Doe 8539 aborted seven live fetuses on day 24 of gestation. All fetuses appeared normal at gross external and soft tissue examination. Fetuses 1, 2, 3, 4, 5, 6 and 7 had pelvis, pubis not ossified (bilateral) and fetuses 2 and 3 had sternal centra (first) not ossified at skeletal examination.

d. Doe 8540 aborted four dead fetuses and had three live fetuses, one dead fetus and six late resorptions in utero on day 25 of gestation. Autolysis precluded further evaluation of the late resorptions. All remaining fetuses appeared normal at gross external and soft tissue examination. Fetuses 1, 2, 3, 5, 7, 8, 9 and 10 had pelvis, pubis not ossified (bilateral) and fetus 2 had skull, nasals incompletely ossified and maxillae, short at skeletal examination.

e. Doe 8542 aborted six dead fetuses on day 28 of gestation. All fetuses were partially cannibalized. All pups appeared normal at soft tissue examination.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 17): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP V 3.75 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8543 (Cont.)			FETUS 7 LATE RESORPTION, autolysis precluded further evaluation				FETUS 3 SKULL: PARIETAL, CONTAINED A HOLE, right, 6.0 mm x 8.0 mm - large, left, 6.0 mm x 8.5 mm - large FETUS 4 SKULL: PARIETAL, CONTAINED A HOLE, right, 2.0 mm x 41.0 mm - large, left, 2.5 mm x 5.0 mm - large PELVIS: PUBIS, NOT OSSIFIED, bilateral FETUS 5 SKULL: PARIETAL, CONTAINED A HOLE, right, 2.5 mm x 3.5 mm - large, left, 4.0 mm x 4.5 mm - large FETUS 6 SKULL: PARIETAL, CONTAINED A HOLE, right, 3.0 mm x 5.0 mm - large, left, 4.0 mm x 5.5 mm - large PELVIS: PUBIS, NOT OSSIFIED, bilateral

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 18): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP V 3.75 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	GROSS EXTERNAL EXAMINATION DESCRIPTION	SOFT TISSUE EXAMINATION N/N	SOFT TISSUE EXAMINATION DESCRIPTION	SKELETAL EXAMINATION N/N	SKELETAL EXAMINATION DESCRIPTION
8543 (Cont.)							FETUS 8 SKULL: PARIETAL, CONTAINED A HOLE, right, 1.0 mm x 1.5 mm - small, left, 2.0 mm x 2.5 mm - large FETUS 10 SKULL: PARIETAL, CONTAINED A HOLE, right, 5.0 mm x 8.0 mm - large, left, 5.0 mm x 8.5 mm - large
8544	ABORTED ON DAY 25 OF GESTATION a						
8545	2(15.4)	0/13		0/13		2/13	FETUS 5 PELVIS: PUBIS, NOT OSSIFIED, bilateral FETUS 6 PELVIS: PUBIS, NOT OSSIFIED, bilateral
8546	0(0.0)	0/ 7		0/ 7		0/ 7	
8547	ABORTED ON DAY 22 OF GESTATION b						
8548	ABORTED ON DAY 25 OF GESTATION c						

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

a. Doe 8544 aborted nine dead fetuses on day 25 of gestation. All fetuses appeared normal at gross external and soft tissues examinations. Fetuses 1, 2, 3, 4, 5, 6, 7, 8 and 9 had pelvis, pubis not ossified (bilateral) and fetus 3 had sternal centra (first) not ossified at skeletal examination.

b. Doe 8547 aborted eight late resorptions on day 22 of gestation; autolysis precluded further evaluation.

c. Doe 8548 aborted one dead fetus and had seven live fetuses, one dead fetus and two late resorption in utero on day 25 of gestation. The late resorptions appeared normal at gross external examination; autolysis precluded further evaluation. All fetuses appeared normal at gross external and soft tissue examinations. Fetuses 1, 2, 3, 4, 5, 6, 7, 8 and 11 had pelvis, pubis not ossified (bilateral) at skeletal examination.

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS (SPONSOR'S STUDY NUMBER: 6295.10)

TABLE 21 (PAGE 19): FETAL ALTERATIONS - INDIVIDUAL DATA

DOSAGE GROUP V 3.75 MG/KG/DAY

RABBIT NUMBER	SPECIMENS WITH ANY ALTERATIONS N(%)	GROSS EXTERNAL EXAMINATION N/N	DESCRIPTION	SOFT TISSUE EXAMINATION N/N	DESCRIPTION	SKELETAL EXAMINATION N/N	DESCRIPTION
8549	0(0.0)	0/10		0/10		0/10	
8550	2(20.0)	0/10		0/10		2/10	FETUS 9 HYOID: ALA, ANGULATED, left FETUS 10 HYOID: ALA, ANGULATED, right
8551	0(0.0)	0/10		0/10		0/10	
8552	NOT PREGNANT						

N/N = NUMBER OF SPECIMENS WITH ALTERATIONS/NUMBER OF SPECIMENS EXAMINED

APPENDIX C

PROTOCOL AND AMENDMENT

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

PROTOCOL 418-012

SPONSOR'S STUDY NUMBER: 6295.10

STUDY TITLE: Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits

PURPOSE: The purpose of this study is to detect adverse effects of PFOS on New Zealand White [Hra:(NZW)SPF] presumed pregnant female rabbits and development of the embryo and fetus consequent to exposure of the doe from implantation to closure of the hard palate. This study evaluates ICH Harmonised Tripartite Guideline stages C and D of the reproductive process in a nonrodent species.

TESTING FACILITY: Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1297
Telephone: (215) 443-8710
Telefax: (215) 443-8587

STUDY DIRECTOR: Raymond G. York, Ph.D., DABT
Associate Director of Research

SPONSOR: 3M Toxicology Services
3M Center Building 220-2E-02
St. Paul, Minnesota 55144-1000

STUDY MONITOR: Marvin T. Case, D.V.M., Ph.D.
Telephone: (651) 733-5180
Telefax: (651) 733-1773

ALTERNATE STUDY MONITOR: Andrew M. Seacat, Ph. D.
Telephone: (651) 575-3161
Telefax: (651) 733-1773

REGULATORY CITATIONS:

U.S. Food and Drug Administration (1994). International Conference on Harmonisation; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.

U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.

Japanese Ministry of Health and Welfare (1997). *Good Laboratory Practice Standard for Safety Studies on Drugs*, MHW Ordinance Number 21, March 26, 1997.

European Economic Community (1989). *Council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice*. Official Journal of the European Communities: Legislation. 32(No. L 315; 28 October): 1-17.

REGULATORY COMPLIANCE:

This study will be conducted in compliance with the Good Laboratory Practice (GLP) regulations cited above.

All changes or revisions of this protocol shall be documented, signed by the Study Director and the Sponsor, dated and maintained with the protocol.

The Quality Assurance Unit (QAU) will audit the protocol, the raw data and the report, and will inspect critical phases of the study in accordance with the Standard Operating Procedures of Argus Research Laboratories, Inc.

The final report will include a statement signed by the Study Director that the report accurately reflects the raw data obtained during the performance of the study and that all applicable GLP regulations were followed in the conduct of the study. Should significant deviations from GLP regulations occur, each will be described in detail, together with how the deviation might affect the quality or integrity of the study.

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE:

See ATTACHMENT 1 to the protocol.

TEST ARTICLE AND VEHICLE:

Identification:

Test Article:

Name:	PFOS.
Physical Description:	Light-colored powder.
Lot/Batch Number:	217.
Specific Gravity:	~0.6.
Purity:	98.9%.
Expiration Date:	May 2000.

Information on the identity, composition, strength and purity of the test article is on file with the Sponsor.

Vehicle:

0.5% Tween® 80 in Reverse Osmosis Membrane Processed Deionized Water (R.O. Deionized Water). Supplier and lot identification of Tween® 80 to be documented in the raw data.

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the vehicle that would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

Safety Precautions:

Gloves, mask, appropriate eye protection and a uniform/lab coat are to be worn during formulation preparation and administration. The Material Safety Data Sheet (MSDS) is attached to the protocol (see ATTACHMENT 2).

Storage:

Bulk Test Article:	Room temperature.
Vehicle Components:	Room temperature.
Prepared Vehicle:	Room temperature.
Prepared Formulations:	Room temperature (samples to be frozen).

All test article shipments to the Testing Facility should be addressed to the attention of Julian Gulbinski, Manager of Formulations, at the previously cited address and telephone number.

Shipments should include information concerning storage conditions and shipping cartons should be labeled appropriately. The recipient should be notified in advance of shipment.

FORMULATION:

Frequency of Preparation:

Formulations (suspensions) will be prepared daily at the Testing Facility.

Detailed preparation procedures are attached to this protocol (ATTACHMENT 3).

Adjustment for Purity:

The test article will be considered 100% pure for the purpose of dosage calculations.

Testing Facility Reserve Samples:

The Sponsor will reserve a sample (1 g) of each lot of the bulk test article used during the course of this study. The Testing Facility will reserve a sample (5 mL) of each lot of the vehicle components used during the course of this study. Samples will be stored under the previously cited conditions.

ANALYSES:

Samples additional to those described below may be taken if deemed necessary during the course of the study.

Bulk Test Article Sampling:

No analyses of the bulk test article will be conducted during the course of this study. Information on the stability of the bulk test article is on file with the Sponsor.

Analyses of Prepared Formulations:

Homogeneity and stability of prepared formulations is on file with the Sponsor. However, records will be maintained to document how the test article formulations were prepared.

Concentration of Test Article Formulations:

Concentration of the prepared formulations will be verified during the course of this study. Duplicate samples (2 mL each) will be taken from the first and last preparation on the day prepared. One sample of each set will be shipped for analysis; the remaining samples will be retained at the Testing Facility as backup samples. Backup samples will be stored under the previously cited conditions and discarded at the Testing Facility upon request of the Sponsor.

Shipping Instructions:

Samples to be analyzed will be shipped (frozen on dry ice) to:

Kris J. Hansen, Ph.D.
3M Environmental Technology and Safety Services
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55133-3331
Telephone: (612) 778-6018
Telefax: (612) 778-6176

The recipient will be notified in advance of sample shipment.

DISPOSITION:

Prepared formulations will be discarded at the Testing Facility. All remaining bulk test article will be returned to the Study Monitor at the previously cited address upon completion of all work with the test article.

TEST SYSTEM:

Species/Strain and Reason for Selection:

The New Zealand White [Hra:(NZW)SPF] rabbit was selected as the Test System because: 1) it is one non-rodent mammalian species accepted and widely used throughout the industry for nonclinical studies of developmental toxicity (embryo-fetal toxicity/teratogenicity); 2) this strain of rabbit has been demonstrated to be sensitive to developmental toxins; 3) historical data and experience exist at the Testing Facility[1-3]; and 4) the test article is pharmacologically active in the species and strain.

Number and Sex:

Population evaluated:	110 timed-pregnant female rabbits (22 per dosage group).
Population selected for toxicokinetic evaluation:	19 satellite female rabbits (five at the low and high dose levels plus three at the other dose levels).

Body Weight and Age:

The individual body weights of the female rabbits will range from 2.5 kg to 5.5 kg; the rabbits will be approximately five to seven months of age at the time of study assignment. Actual body weights recorded at receipt and at study assignment will be documented in the raw data, and the weight range will be included in the final report.

Source:

Covance Research Products, Inc.
Swampbridge Road, Box 7200
Denver, Pennsylvania 17517

The rabbits will be shipped in filtered cartons by truck from Covance Research Products, Inc., Denver, Pennsylvania, to the Testing Facility.

Identification:

Rabbits are permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20103). Female rabbits are given unique permanent identification numbers when assigned to the study on the basis of day 0 of presumed gestation body weights.

ANIMAL HUSBANDRY:

All cage sizes are in compliance with the *Guide for the Care and Use of Laboratory Animals*[4].

Housing:

The rabbits will be individually housed in units of six to eight stainless steel cages. No nesting materials will be supplied because the female rabbits will be sacrificed before parturition is expected.

Room Air, Temperature and Humidity:

The animal room is independently supplied with at least ten changes per hour of 100% fresh air that has been passed through 99.97% HEPA filters. Room temperature will be maintained at 61°F (16°C) to 72°F (22°C) and monitored constantly. Room humidity will also be monitored constantly and maintained at 30% to 70%.

Light:

An automatically controlled 12-hour light:12-hour dark fluorescent light cycle will be maintained. Each dark period will begin at 1900 hours EST.

Diet:

Approximately 150 g of Certified Rabbit Chow® #5322 (PMI Nutrition International) will be available to each rabbit each day until the first day of dosage, at which time approximately 180 g of the same certified feed will be offered to each rabbit each day. The certified feed will be available from individual stainless steel "J-type" feeders attached to each cage.

Water:

Water will be available *ad libitum* from individual bottles attached to the cages or from an automatic watering access system. All water will be from a local source and passed through a reverse osmosis membrane before use. Chlorine will be added to the processed water as a bacteriostat; processed water is expected to contain no more than 1.2 ppm chlorine at the time of analysis. Water is analyzed monthly for possible bacterial contamination and twice annually for possible chemical contamination.

Contaminants:

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the certified diet or in the drinking water at levels that would interfere with the results of this study. Therefore, no analyses other than those routinely performed by the feed supplier or those mentioned in this protocol will be conducted.

MATING AND RANDOMIZATION:

The female rabbits will be naturally bred at the Supplier, by breeder male rabbits of the same source and strain, before shipment to the Testing Facility. The day mating occurs will be designated day 0 of presumed gestation. The rabbits will be mated on five consecutive days and shipped to the Testing Facility after the last mating day to arrive on day 1, 2, 3, 4 or 5 of presumed gestation. Before shipment of the rabbits, the Supplier will forward breeding records and day 0 of presumed gestation body weights. A computer-generated (weight-ordered) randomization procedure will be used to assign the rabbits to dosage groups based on this information.

ADMINISTRATION:

Route and Reason for Choice:

The oral (stomach tube) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one of the possible routes of human exposure.

Method and Frequency:

Female rabbits will be given the test article once daily on days 7 through 20 of presumed gestation. Dosages will be adjusted daily for body weight changes and given at approximately the same time each day.

Rationale for Dosage Selection:

Dosages were selected on the basis of a dosage-range study (Argus Research Laboratories, Inc., Protocol 418-012P).

Dosage Levels, Concentrations, Volumes and Injection Rates:

Dosage Group	Number of Rabbits	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Argus Batch Number
I	22+3[a]	0 (Vehicle)	0	5	B-418-012-A(Day.Month.Year)
II	22+5[a]	0.1	0.02	5	B-418-012-B(Day.Month.Year)
III	22+3[a]	1.0	0.2	5	B-418-012-C(Day.Month.Year)
IV	22+3[a]	2.5	0.5	5	B-418-012-D(Day.Month.Year)
V	22+5[a]	3.75	0.75	5	B-418-012-E(Day.Month.Year)

a. Rabbits assigned to toxicokinetic evaluation.
The test article will be considered 100% pure for the purpose of dosage calculations.

TESTS, ANALYSES AND MEASUREMENTS:

Viability:

All Periods: At least twice daily.

Clinical Observations and/or General Appearance:

Predosage Period: At least once.

Dosage Period: Twice daily. Prior to dosage administration and once approximately one hour postdosage.

Postdosage Period: Once daily.

Clinical observations may be recorded more frequently than cited above, if deemed appropriate by the Study Director and/or Study Monitor.

Body Weights:

Predosage Period: Day 0 of presumed gestation and on the day of arrival at the Testing Facility.

Dosage Period: Daily.

Postdosage Period: Daily.

Feed Consumption Values:

Predosage Period: Recorded daily after arrival at the Testing Facility (values not tabulated).

Dosage Period: Recorded daily.

Postdosage Period: Recorded daily.

Feed consumption values during the dosing period will be tabulated for the same intervals as body weight evaluations.

Caesarean-Sectioning Observations:

Rabbits will be Caesarean-sectioned on day 29 of presumed gestation. The fetuses will be removed from the uterus and placed in individual containers. The rabbits will be examined for number and distribution of:

Corpora Lutea.

Implantation Sites.
[Placentae that appear abnormal (size, color or shape) will be noted in the raw data].

Live and Dead Fetuses.
(A live fetus is defined as one that responds to stimuli; a dead fetus is defined as a term fetus that does not respond to stimuli and that is not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis are considered to be late resorptions.)

Early and Late Resorptions.
(A conceptus is defined as a late resorption if it is grossly evident that organogenesis has occurred; if this is not the case, the conceptus is identified as an early resorption.)

Fetal Observations:

Body Weights and Identification:

The body weight of each fetus will be recorded. Only body weights of live fetuses will be used to determine litter fetal body weight averages. Fetuses will be tagged with identification noting study number, litter number and uterine distribution.

Gross External Alterations:

All fetuses will be examined for gross external alterations. Late resorptions and dead fetuses also will be examined for gross external alterations to the extent possible but such observations will not be included in either data summarization or statistical analyses.

Soft Tissue Examination and Sex:

All fetuses will be examined internally to determine sex. Cavitated organs will be evaluated in all fetuses by dissection[5]. A single cross-section will be made between the parietal and frontal bones, and the brain will be examined *in situ*.

Skeletal Examination:

All fetuses will be examined for skeletal alterations after staining with alizarin red S[6]. Skeletal preparations will be retained in glycerin with thymol added as a preservative.

Representative photographs of fetal gross, soft tissue and skeletal alterations will be taken.

METHOD OF SACRIFICE:

Beuthanasia®-D Special (manufactured by Schering-Plough Animal Health) will be used to sacrifice rabbits (via intravenous injection) and live fetuses (via intraperitoneal injection).

NECROPSY:

Gross lesions will be retained in neutral buffered 10% formalin for possible future evaluation (corresponding tissues will be retained from rabbits in the vehicle control group at the discretion of the Study Director). (Exception: Parovarian cysts will be discarded; these are common, spontaneous lesions in rabbits.) Unless specifically cited below, all other tissues will be discarded.

Satellite Rabbits Assigned to Toxicokinetic Sample Collection:

On day 21 of presumed gestation (the day following the last dosage), toxicokinetic samples will be collected from the rabbits assigned to the toxicokinetic evaluation. Following anesthesia of pentobarbital, blood samples (approximately 4 mL per rabbit) will be collected from the inferior vena cava into serum separator tubes and centrifuged. The resulting serum (approximately 2 mL) will be immediately frozen on dry ice and maintained frozen (-70°C) until shipment to the Sponsor for analysis. The liver will be excised, weighed, and a sample will be taken from the right lateral lobe, frozen and retained at -70°C until shipment to the Sponsor for analysis.

Rabbits will be Caesarean-sectioned and fetuses will be examined grossly to the extent possible as described above for rabbits assigned to the main study. Fetuses and placentae will be pooled per litter and retained frozen (-70°C) until shipment to the Sponsor for analysis.

After completion of sample collection, serum, liver sections, fetal and placental samples will be shipped (frozen on dry ice) to Kris J. Hansen, Ph.D., at the previously cited

address for analysis. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

Scheduled Sacrifice:

On day 29 of presumed gestation, female rabbits will be Caesarean-sectioned, and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. Uteri of apparently nonpregnant does will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[7].

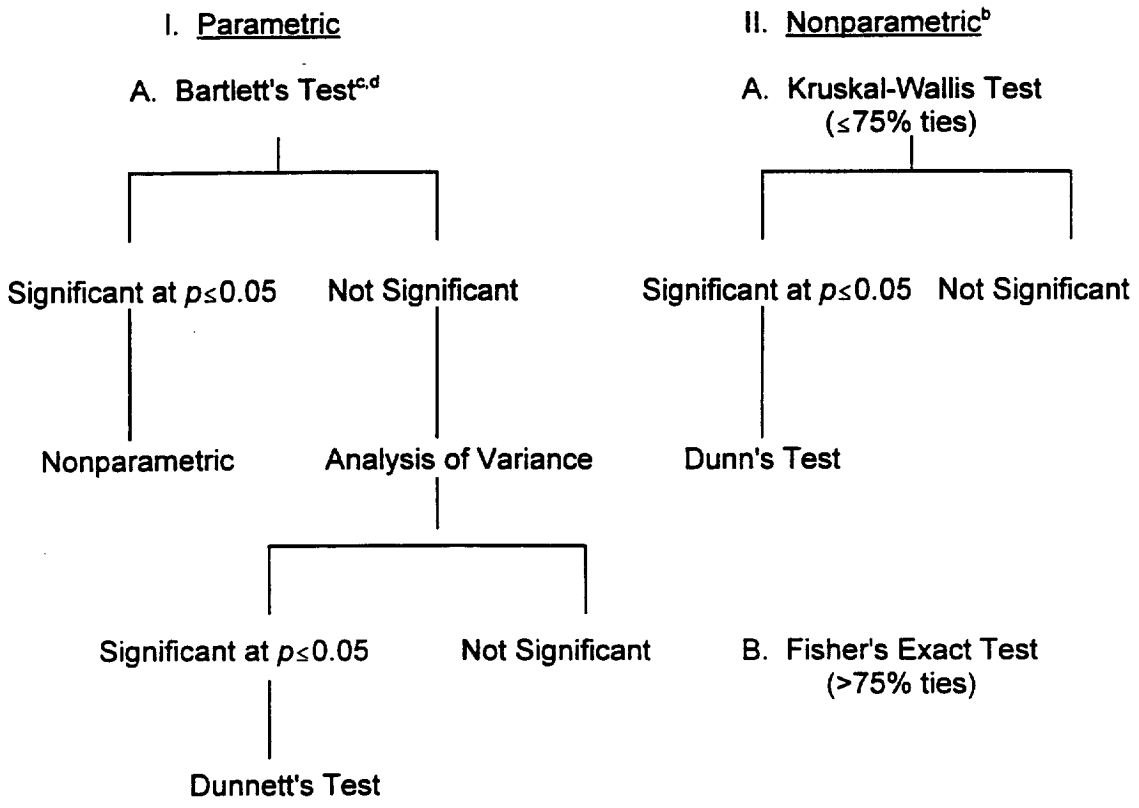
Rabbits Found Dead or Moribund:

Rabbits that die or are sacrificed because of moribund condition, abortion or premature delivery will be examined for the cause of death or moribund condition on the day the observation is made. Pregnancy status and uterine contents will be recorded. Aborted fetuses and/or delivered pups will be examined to the extent possible, using the same methods described for fetuses. Uteri of apparently nonpregnant does will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[7].

PROPOSED STATISTICAL METHODS[8-14]:

Averages and percentages will be calculated. Litter values will be used where appropriate. Additional procedures and/or analyses may be performed, if appropriate.

Type of Test[a]



III. Test for Proportion Data

Variance Test for Homogeneity
of the Binomial Distribution

---

a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
b. Proportion data are not included in this category.
c. Used only to analyze data with homogeneity of variance.
d. Test for homogeneity of variance.

DATA ACQUISITION, VERIFICATION AND STORAGE:

Data will be hand- and/or computer-recorded. Records will be reviewed by the Study Director and/or appropriate management personnel within 21 days after generation. All original records will be stored in the archives of the Testing Facility. All original data will be bound and indexed. A copy of all raw data will be supplied to the Sponsor upon request. Preserved tissues will be stored at the Testing Facility at no charge for one year after mailing of the draft final report, after which time the Sponsor will be contacted to determine the disposition of these materials.

RECORDS TO BE MAINTAINED:

Protocol and Amendments.
Test Article Vehicle and/or Reagent Receipt, Preparation and Use.
Animal Acquisition.
Randomization Schedules.
Veterinarian Examination.
Mating History.
Treatment (if prescribed by Staff Veterinarian).
General Comments.
Clinical Observations and/or General Appearance.
Blood Sample Collection, Processing and Shipment (if required).
Body Weights.
Feed Consumption Values.
Caesarean-Sectioning and Fetal Observations.
Gross Necropsy Observations.
Organ Weights (if required).
Photographs (if required).
Study Maintenance (room and environmental records).
Feed and Water Analyses.
Packing and/or Shipment Lists.

KEY PERSONNEL:

Executive Director of Research: Mildred S. Christian, Ph.D., Fellow, ATS
Director of Research: Alan M. Hoberman, Ph.D., DABT
Associate Director of Research and Study Director: Raymond G. York, Ph.D., DABT
Director of Laboratory Operations: John F. Barnett, B.S.
Manager of Study Coordination: Valerie A. Sharper, M.S.
Manager of Animal Operations and Member, Institutional Animal Care and Use Committee: Dena C. Lebo, V.M.D.
Manager of Regulatory Compliance: Kathleen A. Moran, M.S.
Consultant, Veterinary Pathology: W. Ray Brown, D.V.M., Ph.D., ACVP

FINAL REPORT:

A comprehensive draft final report will be prepared on completion of the study and will be finalized following consultation with the Sponsor. The report will include the following:

Summary and Conclusion.
Experimental Design and Method.
Evaluation of Test Results.
Appendices: Figures, Summary and Individual Tables Summarizing the Above Data, Protocol and Associated Amendments and Deviations, Study Director's GLP Compliance Statement, Reports of Supporting Data (if appropriate) and QAU Statement.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE STATEMENT:

The procedures described in this protocol have been reviewed by the Testing Facility's Institutional Animal Care and Use Committee. All procedures described in this protocol that involve study animals will be conducted in a manner to avoid or minimize discomfort, distress or pain to the animals.

The Sponsor's signature below documents the fact that information concerning the necessity for conducting this study and the fact that this is not an unnecessarily duplicative study may be obtained from the Sponsor. No alternative (*in vitro*) procedures were available for meeting the stated purposes of the study.

REFERENCES:

1. Christian, M.S., Hoberman, A.M. and Smith, T.H.F. (1982). Dosage-range study of the teratogenic potential of suspensions of trinitrofluorenone (TNF) administered orally to New Zealand White rabbits. Toxicologist 2(1):40 (#143).

2. Christian, M.S. (1984). Reproductive toxicity and teratology evaluations of naltrexone (Proceedings of Naltrexone Symposium, New York Academy of Sciences, November 7, 1983), J. Clin. Psychiat. 45(9):7-10.

3. Feussner, E.L., Lightkep, G.E., Hennesy, R.A., Hoberman, A.M. and Christian, M.S. (1992). A decade of rabbit fertility data: Study of historical control animals. Teratology 46(4):349-365.

4. Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, D.C.

5. Staples, R.E. (1974). Detection of visceral alterations in mammalian fetuses. Teratology 9(3):A37-38.

6. Staples, R.E. and Schnell, V.L. (1964). Refinement in rapid clearing technique in the KOH-alizarin red S method for fetal bone. Stain Technol. 39:61-63.

7. Salewski, E. (1964). Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Arch. Pathol. Exp. Pharmakol. 247:367.

8. Snedecor, G.W. and Cochran, W.G. (1967). Variance test for homogeneity of the binomial distribution. *Statistical Methods*, 6th Edition, Iowa State University Press, Ames, pp. 240-241.

9. Sokal, R.R. and Rohlf, F.J. (1969). Bartlett's test of homogeneity of variances. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 370-371.

10. Snedecor, G.W. and Cochran, W.G. (1967). Analysis of Variance. *Statistical Methods*, 6th Edition, Iowa State University Press, Ames, pp. 258-275.

11. Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1129.

12. Sokal, R.R. and Rohlf, F.J. (1969). Kruskal-Wallis Test. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 388-389.

13. Dunn, O.J. (1964). Multiple comparisons using rank sums. Technometrics 6(3):241-252.

14. Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*, McGraw-Hill, New York, pp. 96-104.

PROTOCOL APPROVAL:

FOR THE TESTING FACILITY

George E. Dearlove, Ph.D., DABT
Associate Director of Research

11-AUG-98
Date

Raymond G. York, Ph.D., DABT
Associate Director of Research
Study Director

11-AUG-98
Date

Dena C. Lebo, V.M.D.
Member, Institutional Animal Care and Use Committee

11 Aug 98
Date

FOR THE SPONSOR

Marvin T. Case, D.V.M., Ph.D.
Study Monitor

12 Aug 98
Date

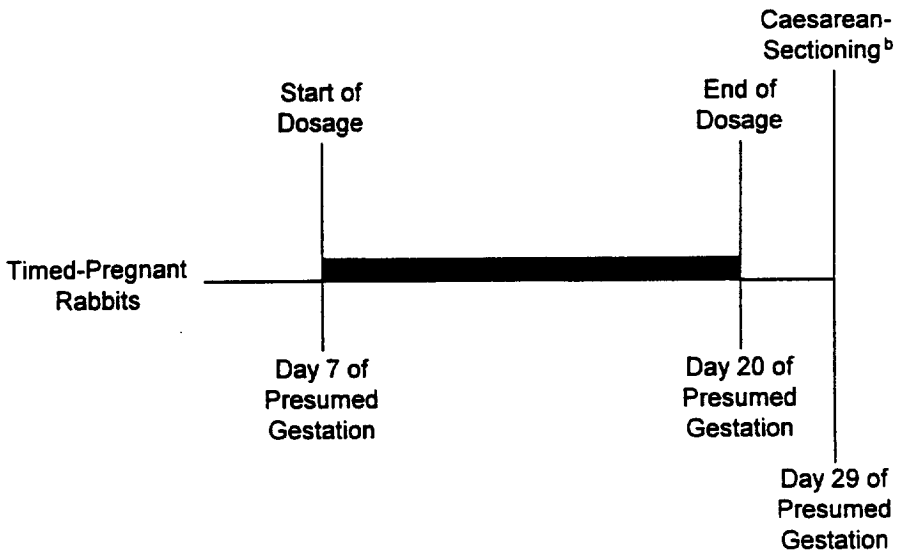
ATTACHMENT 1

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE

ATTACHMENT 1

STUDY SCHEMATIC

RABBIT DEVELOPMENTAL TOXICITY STUDY[a]



= Dosage Period

a = For additional details see "Tests, Analyses and Measurements" section of the protocol.

b = Fetal evaluations (external, soft tissue and skeletal).

SCHEDULE[a]

21 AUG 98	Animals Arrive - Acclimation Begins.
23 AUG 98 - 09 SEP 98	Dosage Period (Days 7 through 20 of presumed gestation).
06 SEP 98 - 10 SEP 98	Toxicokinetic Sample Collection (Day 21 of presumed gestation).
14 SEP 98 - 18 SEP 98	Caesarean-Sectioning Period (Day 29 of presumed gestation).
17 DEC 98	Draft Final Report.

---

a. The study initiation date is the date the Study Director signs the protocol.

ATTACHMENT 2

MATERIAL SAFETY DATA SHEET

MATERIAL SAFETY DATA SHEET

3M
3M Center
St. Paul, Minnesota
55144-1000
1-800-364-3577 or (612) 737-6501 (24 hours)

Copyright, 1998, Minnesota Mining and Manufacturing Company. All rights reserved. Copying and/or downloading of this information for the purpose of properly utilizing 3M products is allowed provided that:
1) the information is copied in full with no changes unless prior agreement is obtained from 3M, and
2) neither the copy nor the original is resold or otherwise distributed with the intention of earning a profit thereon.

DIVISION: 3M CHEMICALS
TRADE NAME:
FC-95 FLUORAD Brand Fluorochemical Surfactant
ID NUMBER/U.P.C.:
98-0207-0103-7 00-51135-09054-1 98-0207-0104-5 00-51135-09055-8
98-0211-0888-5 00-51135-09362-7 98-0211-3916-1 00-51135-02311-2
ZF-0002-1044-1 - - -
ISSUED: January 29, 1998
SUPERSEDES: November 05, 1997
DOCUMENT: 10-3796-9

1. INGREDIENT

INGREDIENT	C.A.S. NO.	PERCENT
POTASSIUM PERFLUOROALKYL SULFONATE.....	2795-39-3	82 - 86
POTASSIUM PERFLUOROALKYL SULFONATE.....	3871-99-6	3 - 8
POTASSIUM PERFLUOROALKYL SULFONATE.....	29420-49-3	3 - 7
POTASSIUM PERFLUOROALKYL SULFONATE.....	60270-55-5	2 - 6
POTASSIUM PERFLUOROALKYL SULFONATE.....	3872-25-1	1 - 3

2. PHYSICAL DATA

BOILING POINT:..... N/A
VAPOR PRESSURE:..... N/A
VAPOR DENSITY:..... N/A
EVAPORATION RATE:..... N/A
SOLUBILITY IN WATER:..... slight
SPECIFIC GRAVITY:..... ca. 0.6 Water=1 (Bulk)
PERCENT VOLATILE:..... 0 %
pH:..... 7 - 8 (0.1% Aqueous)
VISCOSITY:..... N/D
MELTING POINT:..... N/D

APPEARANCE AND ODOR:
Light colored, free flowing powder.

Abbreviations: N/D - Not Determined N/A - Not Applicable CA - Approximately

3. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT:..... None
FLAMMABLE LIMITS - LEL:..... N/A
FLAMMABLE LIMITS - UEL:..... N/A
AUTOIGNITION TEMPERATURE:..... N/A

EXTINGUISHING MEDIA:
Water, Carbon dioxide, Dry chemical, Foam

SPECIAL FIRE FIGHTING PROCEDURES:
Wear full protective clothing, including helmet, self-contained, positive pressure or pressure demand breathing apparatus, bunker coat and pants, bands around arms, waist and legs, face mask, and protective covering for exposed areas of the head.

UNUSUAL FIRE AND EXPLOSION HAZARDS:
See Hazardous Decomposition section for products of combustion.

4. REACTIVITY DATA

STABILITY: Stable

INCOMPATIBILITY - MATERIALS/CONDITIONS TO AVOID:
Not applicable.

HAZARDOUS POLYMERIZATION: Hazardous polymerization will not occur.

HAZARDOUS DECOMPOSITION PRODUCTS:
Carbon Monoxide and Carbon Dioxide, Oxides of Sulfur, Hydrogen Fluoride, Toxic Vapors, Gases or Particulates.

5. ENVIRONMENTAL INFORMATION

SPILL RESPONSE:
Observe precautions from other sections. Vacuum, use wet sweeping compound or water to avoid dusting. CAUTION! A vacuum cleaner could be an ignition source. Clean up residue with water. Place in an approved metal container. Seal the container.

RECOMMENDED DISPOSAL:
Do not release to waterways or sewer. Do not use in products or processes that could result in aquatic concentrations greater than 1/10 of the lowest EC50 or LC50 concentration. Incinerate in an industrial or commercial facility in the presence of a combustible material. Combustion products will include HF. Disposal alternative: Dispose of waste product in a facility permitted to

5. ENVIRONMENTAL INFORMATION (continued)

accept chemical waste.

ENVIRONMENTAL DATA:
96-Hr. Aquatic Fish LC50, Fathead Minnow(Pimephales promelas)=38 mg/l, Bluegill Sunfish(Lepomis macrochirus)=68 mg/l, Rainbow Trout(Salmo gairdneri)=11 mg/l; 48-Hr. EC50, Daphnia Magna = 50 mg/l; COD=.004 g/g; BOD20 = Nil.

REGULATORY INFORMATION:
Volatile Organic Compounds: N/A.
VOC Less H2O & Exempt Solvents: N/A.

Since regulations vary, consult applicable regulations or authorities before disposal. U.S. EPA Hazardous Waste Number = None (Not U.S. EPA Hazardous).

This product complies with the chemical registration requirements of TSCA, EINECS, CDSL, AICS, MITI and Korea.

EPCRA HAZARD CLASS:
FIRE HAZARD: No PRESSURE: No REACTIVITY: No ACUTE: Yes CHRONIC: Yes

6. SUGGESTED FIRST AID

EYE CONTACT:
Immediately flush eyes with large amounts of water for at least 15 minutes. Get immediate medical attention.

SKIN CONTACT:
Immediately flush skin with large amounts of water. Remove contaminated clothing. If irritation persists, call a physician. Wash contaminated clothing before reuse.

INHALATION:
If signs/symptoms occur, remove person to fresh air. If signs/symptoms continue, call a physician.

IF SWALLOWED:
Drink two glasses of water. Call a physician.

7. PRECAUTIONARY INFORMATION

EYE PROTECTION:
Avoid eye contact. Wear vented goggles.

7. PRECAUTIONARY INFORMATION (continued)

SKIN PROTECTION:
Avoid skin contact. Wear appropriate gloves when handling this material. A pair of gloves made from the following material(s) are recommended: butyl rubber. Use one or more of the following personal protection items as necessary to prevent skin contact: head covering, coveralls. Protective garments (other than gloves) should be made of either of the following materials: polyethylene/polyvinylidene chloride (Saranex).

RECOMMENDED VENTILATION:
Use with appropriate local exhaust ventilation. Use in a well-ventilated area. Provide sufficient ventilation to maintain emissions below recommended exposure limits. If exhaust ventilation is not adequate, use appropriate respiratory protection.

RESPIRATORY PROTECTION:
Avoid breathing of dust. Select one of the following NIOSH approved respirators based on airborne concentration of contaminants and in accordance with OSHA regulations: half-mask dust and mist respirator, half-mask supplied air respirator, full-face dust and mist respirator, full-face supplied air respirator.

PREVENTION OF ACCIDENTAL INGESTION:
Do not eat, drink or smoke when using this product. Wash exposed areas thoroughly with soap and water. Wash hands after handling and before eating.

RECOMMENDED STORAGE:
Keep container dry. Keep container closed when not in use.

FIRE AND EXPLOSION AVOIDANCE:
Nonflammable.

OTHER PRECAUTIONARY INFORMATION:
No smoking: Smoking while using this product can result in contamination of the tobacco and/or smoke and lead to the formation of the hazardous decomposition products mentioned in section 4 of this MSDS.

HMIS HAZARD RATINGS: HEALTH: 2 FLAMMABILITY: 0 REACTIVITY: 0
PERSONAL PROTECTION: X (See precautions, section 7.)

EXPOSURE LIMITS

INGREDIENT	VALUE	UNIT	TYPE	AUTH	SKIN*
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y

Abbreviations: N/D - Not Determined N/A - Not Applicable CA - Approximately

EXPOSURE LIMITS (continued)

INGREDIENT	VALUE	UNIT	TYPE	AUTH	SKIN*
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y

* SKIN NOTATION: Listed substances indicated with 'Y' under SKIN refer to the potential contribution to the overall exposure by the cutaneous route including mucous membrane and eye, either by airborne or, more particularly, by direct contact with the substance. Vehicles can alter skin absorption.

SOURCE OF EXPOSURE LIMIT DATA:
- 3M: 3M Recommended Exposure Guidelines

8. HEALTH HAZARD DATA

EYE CONTACT:
Mild Eye Irritation: signs/symptoms can include redness, swelling, pain, and tearing.

SKIN CONTACT:
Mild Skin Irritation (after prolonged or repeated contact): signs/symptoms can include redness, swelling, and itching.

May be absorbed through the skin and persist in the body for an extended time.

INHALATION:
May be harmful if inhaled.

May be absorbed by inhalation and persist in the body for an extended time.

Single overexposure, above recommended guidelines, may cause:

Irritation (upper respiratory): signs/symptoms can include soreness of the nose and throat, coughing and sneezing.

IF SWALLOWED:
Ingestion is not a likely route of exposure to this product.

Illness may result from a single swallowing of a moderate quantity of this material.

May be harmful if swallowed.

MUTAGENICITY:
Mutagenicity assays indicate the product is not mutagenic.

8. HEALTH HAZARD DATA (continued)

REPRODUCTIVE/DEVELOPMENTAL TOXINS:
Not teratogenic in the rat at oral doses below maternally toxic levels.

OTHER HEALTH HAZARD INFORMATION:
This product is not known to contain any substances regulated under California Proposition 65.

A Product Toxicity Summary Sheet is available.

SECTION CHANGE DATES

HEADING	SECTION CHANGED SINCE November 05, 1997	ISSUE

Abbreviations: N/D - Not Determined N/A - Not Applicable CA - Approximately

The information in this Material Safety Data Sheet (MSDS) is believed to be correct as of the date issued. 3M MAKES NO WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR COURSE OF PERFORMANCE OR USAGE OF TRADE. User is responsible for determining whether the 3M product is fit for a particular purpose and suitable for user's method of use or application. Given the variety of factors that can affect the use and application of a 3M product, some of which are uniquely within the user's knowledge and control, it is essential that the user evaluate the 3M product to determine whether it is fit for a particular purpose and suitable for user's method of use or application.

3M provides information in electronic form as a service to its customers. Due to the remote possibility that electronic transfer may have resulted in errors, omissions or alterations in this information, 3M makes no representations as to its completeness or accuracy. In addition, information obtained from a database may not be as current as the information in the MSDS available directly from 3M.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

ATTACHMENT 3

TEST ARTICLE AND VEHICLE PREPARATION PROCEDURE

Test Article: PFOS

Vehicle: 0.5% Tween® 80 in R.O. Deionized Water

A. Purpose: The purpose of this procedure is to provide a method for the preparation of dosage suspensions of PFOS and the vehicle for oral administration to rabbits on Argus Study 418-012.

B. General Information:

1. All suspension containers will be labeled and color coded. Each label will specify the protocol number, test article identification, Argus batch number, concentration, dosage level, preparation date, expiration date and storage conditions.

2a. Suspensions will be prepared:
_X_ Daily ___ Weekly ___ For____ days of use

2b. Vehicle will be prepared:
___ Daily _X_ Weekly ___ For____ days of use

3. Suspensions will be prepared at a final dosage volume of 5 mL/kg.

4. Safety:
_X_ Gloves, lab coat, goggles or safety glasses and faceshield
_X_ Dust-Mist Respirator
___ Half-Face Respirator
___ Full-Face Respirator/Positive Pressure Hood
___ Tyvek Suit/Apron

5. Dosage suspensions adjusted for Free base and % Purity.
___ Yes _X_ No (Calculations based on 100%)
___ Free Base ___ Purity

6. Sampling requirements: Cited in protocol.

7. Storage: Cited in protocol.

TEST ARTICLE AND VEHICLE PREPARATION PROCEDURE

NOTE: Test article will be prepared as a serial dilution from the high dosage to the low dosage. Once the final volumes are achieved, stir bars are to be added to the containers; mixing should occur during sampling and/or administration.

C. Preparation of Vehicle

1. Add the required amount of R.O. deionized water to an appropriately labeled container. Heat the water to 50°C, ±5°C, add the required amount of Tween® 80 and mix until uniform (See TEST ARTICLE CALCULATIONS).

D. Test Article Suspension Preparation:

1. To prepare the 0.75 mg/mL, Group V suspension, add the required amount of test article (See TEST ARTICLE CALCULATIONS) into an appropriately sized, labeled container. QS ad to the required amount with vehicle and heat the mixture to 80°C ±5°C for approximately 30 minutes or until the TA/S dissolves.

2. Once the test article has dissolved; spin while the suspension cools. (Be sure there is a visible vortex, this will achieve the desired emulsion. This may be prepared the day before use.)

3. To prepare the 0.5 mg/mL, Group IV suspension, remove the required amount of stock suspension (Group V) (See TEST ARTICLE CALCULATIONS), QS ad with the vehicle and mix.

4. To prepare the 0.2 mg/mL, Group III suspension, remove the required amount of stock suspension (Group IV) (See TEST ARTICLE CALCULATIONS), QS ad with the vehicle and mix.

5. To prepare the 0.02 mg/mL, Group II suspension, remove the required amount of stock suspension (Group III) (See TEST ARTICLE CALCULATIONS), QS ad with the vehicle and mix.

TEST ARTICLE AND VEHICLE PREPARATION PROCEDURE

6. To prepare the 0 mg/mL, Group I suspension, add required amount of vehicle to an appropriately sized, labeled container (See TEST ARTICLE CALCULATIONS) and mix.

Written by: Mark A. Cohen

Approved by: [illegible] Date: 11-AUG-98

Clarification: X No ___ Yes (See attached clarification form.)

Initials/Date : [illegible] 29 SEP 98

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

PROTOCOL 418-012

ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY
OF PFOS IN RABBITS

SPONSOR'S STUDY NUMBER: 6295.10

Amendment 1 - 11 December 1998

---

1. Sponsor (page 1 of the protocol):

   The Sponsor is 3M Corporate Toxicology, rather than 3M Toxicology Services.

   Reason for Change:

   This change was made at the request of the Sponsor.

2. Species/Strain and Reason for Selection (page 5 of the protocol):

   The test article is biologically active, rather than pharmacologically active in this strain.

   Reason for Change:

   This change was made at the request of the Sponsor.

3. Route and Reason for Choice (page 7 of the protocol):

   The oral (gavage) route is a possible route of human exposure, rather than the one proposed for clinical use.

Reason for Change:

This change was made at the request of the Sponsor.

11-DEC-98	11-DEC-98
Alan M. Hoberman, Ph.D., DABT Date Director of Research	Raymond G. York, Ph.D., DABT Date Associate Director of Research and Study Director
11-DEC-98	14 Dec 98
Dena C. Lebo, V.M.D. Date Chairperson, Institutional Animal Care and Use Committee	Marvin T. Case, D.V.M., Ph.D. Date Study Monitor

APPENDIX D

PILOT REPORT

FINAL PILOT REPORT

Study Title

Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of PFOS in Rabbits

SPONSOR'S STUDY NUMBER: T-6295.10

Author

Raymond G. York Ph.D., DABT
(Study Director)

Study Completed On

6 January 1999
(Final Pilot Report)

Performing Laboratory

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1297

Laboratory Project ID

Argus Research Laboratories, Inc., Protocol Number: 418-012P

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS

SPONSOR'S STUDY NUMBER: T-6295.10

TABLE OF CONTENTS

SUBJECT		PAGE
ABSTRACT		4
I.	Purpose	6
II.	Methods	6
III.	Results	7
IV.	Conclusion	12
Figure 1.	Maternal Body Weights	13
Table 1.	Clinical Observations - Summary	14
Table 2.	Necropsy Observations - Summary	16
Table 3.	Uterine Contents for the Individual Rabbits that were Found Dead or Aborted	20
Table 4.	Maternal Body Weights - Summary	22
Table 5.	Maternal Body Weight Changes - Summary	26
Table 6.	Maternal Absolute Feed Consumption Values (g/day) - Summary	28
Table 7.	Maternal Relative Feed Consumption Values (g/kg/day) - Summary	30

SUBJECT		PAGE
Table 8.	Caesarean-Sectioning Observations - Summary	32
Table 9.	Litter Observations (Caesarean-Delivered Fetuses) - Summary	34
Table 10.	Fetal Gross External Alterations - Summary	36
Table 11.	Clinical Observations - Individual Data	38
Table 12.	Necropsy Observations - Individual Data	42
Table 13.	Maternal Body Weights - Individual Data	45
Table 14.	Maternal Feed Consumption Values - Individual Data	52
Table 15.	Caesarean-Sectioning Observations - Individual Data	59
Table 16.	Litter Observations (Caesarean-Delivered Fetuses) - Individual Data	61
Table 17.	Fetal Sex, Vital Status and Body Weight - Individual Data	63
ATTACHMENT 1 - PROTOCOL		66

TITLE: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS

ARGUS RESEARCH LABORATORIES, INC.
PROTOCOL NUMBER: 418-012P
SPONSOR'S STUDY NUMBER: T-6295.10

ABSTRACT

Thirty-five presumed pregnant New Zealand White [Hra:(NZW)SPF] rabbits were randomly assigned to seven dosage groups, five per group. Suspensions of PFOS were administered orally once daily on days 7 through 20 of presumed gestation (DGs 7 to 20) at dosages of 0 (Vehicle), 0.1, 1, 2.5, 5, 10 and 20 mg/kg/day. The vehicle was 0.5% Tween®80 in reverse osmosis membrane processed deionized water. The dosage volume was 10 mL/kg, adjusted daily on the basis of the individual body weights.

Checks for viability were made twice daily. Clinical observations were recorded twice daily during the dosage period (once prior to dosage administration and once approximately one hour after dosage administration) and once daily during the postdosage period. Body weights and feed consumption values were recorded daily during the dosage and postdosage periods.

All surviving rabbits were sacrificed on DG 29 and examined for the number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Fetuses were weighed and examined for gross external alterations and sex.

Two does in the 5 mg/kg/day dosage group and four in the 10 mg/kg/day dosage group aborted and were sacrificed. Additionally, four does in the 20 mg/kg/day dosage group were found dead and one doe in the same dosage group aborted and was sacrificed. Dosage-dependant increases in adverse clinical observations (scant or no feces, ungroomed coat and excess salivation) occurred in the 2.5, 5, 10 and 20 mg/kg/day dosage groups.

Rabbits in the 10 and 20 mg/kg/day dosage groups had body weight losses and severely reduced absolute and relative feed consumption values for all recorded

intervals after initiation of dosage; there were no surviving pregnant rabbits in these groups after DGs 26 and 19, respectively. The 2.5 and 5 mg/kg/day dosage groups had decreased body weight gains or body weight losses for the entire dosage and gestation periods. Absolute and relative feed consumption values for the entire dosage period were reduced in the 2.5 and 5 mg/kg/day dosage groups. Feed consumption values were reduced in the 2.5 and 5 mg/kg/day dosage groups for the entire gestation period after the initiation of dosing.

Fetal body weights were severely reduced and the number of early and late resorptions and the percentage of conceptuses per litter were increased in the 5 mg/kg/day dosage group, however, only two of the four pregnant does in this dosage group survived to Caesarean-sectioning. Fetal body weights were reduced in the 2.5 mg/kg/day dosage group as compared to the concurrent controls but were at the lower end of the range observed historically at the Testing Facility. All other Caesarean-sectioning or litter parameters were unaffected by administration of the test article at dosages as high as 5 mg/kg/day.

One fetus in the 2.5 mg/kg/day dosage group had a cleft snout and absent incisors. Two fetuses in the 0.1 mg/kg/day dosage group had fetal gross malformations. One fetus had a short snout, displaced and small nares and the other fetus had abdominal distention.

Based on these data, dosages of 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day PFOS were recommended for the developmental toxicity study in rabbits (Argus Protocol 418-012).

I. Purpose:

The purpose of this study was to provide information for the selection of dosages to be used in the developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of PFOS administered orally via stomach tube to New Zealand White [Hra:(NZW)SPF] presumed pregnant female rabbits.

II. Methods[a]:

The test article, PFOS (Lot 217), an off white powder, was received on 20 May 1998, and was stored at room temperature. The vehicle, 0.5% Tween®80 (lot M03H05), a clear viscous fluid was received on 1 July 1998, and was stored at room temperature. The vehicle diluent, reverse osmosis membrane processed deionized water (R.O. Deionized Water) was available from a continuous source at the Testing Facility and was maintained at room temperature.

Thirty-five presumed pregnant New Zealand White [Hra:(NZW)SPF] rabbits were randomly assigned to seven dosage groups [five per group (Groups I through VII)]. Suspensions of PFOS were administered orally (stomach tube) once daily on days 7 through 20 of presumed gestation (DGs 7 to 20) at dosages of 0 (Vehicle), 0.1, 1, 2.5, 5, 10 and 20 mg/kg/day. The dosage volume was 10 mL/kg, adjusted daily on the basis of the individual body weights recorded immediately before intubation of the test article.

Checks for viability were made twice daily. Clinical observations were recorded twice daily during the dosage period (once prior to dosage administration and once approximately one hour after dosage administration) and once daily during the postdosage period. Body weights and feed consumption values were recorded daily during the dosage and postdosage periods.

All surviving rabbits were sacrificed on DG 29 and examined for the number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Fetuses were weighed and examined for gross external alterations and sex.

---

a. Detailed descriptions of all procedures used in the conduct of this study are provided in the attached protocol and amendments. Deviations from the Protocol and Standard Operating Procedures of the Testing Facility are available in the raw data.

III. Results:

A. Mortality, Clinical and Necropsy Observations (Summaries - Tables 1 and 2; Individual Data - Tables 3, 11 and 12)

A.1. Mortality, Abortions and Uterine Contents of Aborted Rabbits

Two does (8257 and 8259) in the 5 mg/kg/day dosage group and four does (8261, 8262, 8364 and 8265) in the 10 mg/kg/day dosage group aborted and were sacrificed. Additionally, four does (8266, 8267, 8268 and 8270) in the 20 mg/kg/day dosage group were found dead and one doe (8269) in the same dosage group aborted and was sacrificed. The death of doe 8266 was due to an intubation accident. These does are described below. All other does survived to scheduled sacrifice.

5 mg/kg/day dosage

Doe 8257 in the 5 mg/kg/day dosage group aborted and was sacrificed on DG 23 after 14 daily dosages. The only adverse clinical observation was scant feces (DGs 13 to 22). No gross lesions were revealed by necropsy and five late resorptions were aborted and one late resorption was *in utero*. This doe lost body weight and feed consumption values were reduced throughout the dosage period.

Doe 8259 in the 5 mg/kg/day dosage group aborted and was sacrificed on DG 22 after 14 daily dosages. The only adverse clinical observation was scant feces (DGs 14 and 16 to 21). No gross lesions were revealed by necropsy. This doe aborted seven dead fetuses and two late resorptions; no conceptuses remained *in utero*. This doe lost body weight and feed consumption values were reduced after DG 11.

10 mg/kg/day dosage

Doe 8261 in the 10 mg/kg/day dosage group aborted and was sacrificed on DG 26 after 14 daily dosages. Adverse clinical observations included scant feces (DGs 12, 16 to 20, 23 and 24), no feces (DG 13 to 15, 21, 22, 25 and 26) and an ungroomed coat (DGs 24 to 26). All lobes of the liver were mottled red and tan and the mucosal surface of the stomach had numerous black areas at necropsy. This doe aborted seven dead fetuses and two dead fetuses were *in utero*. The fetuses that were in utero had edematous heads and necks; all other fetuses appeared normal for their developmental ages. This doe generally lost weight and had reduced feed consumption values after DG 9.

Doe 8262 in the 10 mg/kg/day dosage group aborted and was sacrificed on DG 22 after 14 daily dosages. Adverse clinical observations included a mass on the nose (DGs 7 to 21), scant feces (DGs 11 to 12, 14 to 21) and no feces (DG 13). At necropsy, the cut surface of the small mass on the nose revealed a smooth pink material. Nine late resorptions were aborted and no conceptuses remained *in utero*. This doe lost body weight and feed consumption values were reduced after DG 9.

Doe 8264 in the 10 mg/kg/day dosage group aborted and was sacrificed on DG 23 after 14 daily dosages. Adverse clinical observations included scant feces (DGs 12, 14, 15, 19 and 20), no feces (DGs 13, 16 to 18, 21 and 22) and a red substance in the cage pan (DG 22). All tissues appeared normal at necropsy. Nine late resorptions were aborted and no conceptuses remained *in utero*. This doe lost body weight after DG 8 and feed consumption values were reduced after DG 9.

Doe 8265 in the 10 mg/kg/day dosage group aborted and was sacrificed on DG 25 after 14 daily dosages. Adverse clinical observations included scant feces (DGs 9 to 20, 23 and 24), no feces (DGs 21 and 22) and a red substance in the cage pan (DG 25). All tissue s appeared normal at necropsy. This doe aborted one dead fetus and five live fetuses and one early resorption were *in utero*. All fetuses appeared normal for their developmental ages. Body weight losses occurred after DG 8 and feed consumption values were reduced throughout the dosage period.

20 mg/kg/day dosage

Doe 8266 in the 20 mg/kg/day dosage group was found dead on DG 17 after 11 daily dosages. Adverse clinical observations prior to death included scant feces (DGs 12 to 15), a red substance in cage pan, no feces, ungroomed coat, a red perivaginal substance (DGs 16 and 17) and excess salivation (DG 17). Necropsy revealed a white frothy material in the esophagus and inflated lungs. Necropsy also revealed tears in the left diaphragmatic lobe and in the left lateral lobe of the lungs (approximately 2.0 cm in length) and two eroded areas in the fundic mucosa of the stomach (1.5 x 0.5 cm and 1.5 x 0.5 cm). Ten early resorptions were *in utero*. All fetuses appeared normal for their developmental ages. Body weight loss occurred after DG 8 and its feed consumption values were reduced throughout the dosage period. The death of this doe was considered an intubation accident because of the tear in the left lung lobe; however, this doe had adverse clinical and necropsy observations that indicated the doe was moribund.

Doe 8267 in the 20 mg/kg/day dosage group was found dead on DG 20 after 13 daily dosages. Adverse clinical observations included scant feces (DGs 9, 10 and 16 to 19), no feces (DGs 11 to 15) and excess salivation (DG 19). No gross lesions were observed by necropsy and thirteen dead fetuses were *in utero*. All fetuses appeared normal for their developmental ages. Body weight losses occurred after DG 8 and feed consumption values were reduced throughout the dosage period.

Doe 8268 in the 20 mg/kg/day dosage group was found dead on DG 17 after 10 daily dosages. Adverse clinical observations included scant feces (DGs 9 to 15), soft or liquid feces (DGs 12 and 13), a tan perivaginal substance (DG 16) and no feces (DG 16). Necropsy revealed approximately 18 mL of cloudy green fluid in the abdominal cavity, a friable stomach and a ruptured gall bladder. Nine dead fetuses and one early resorption were *in utero*. All fetuses appeared normal for their developmental ages. Body weight losses occurred after DG 9 and feed consumption values were reduced throughout the dosage period.

Doe 8269 in the 20 mg/kg/day dosage group aborted and was sacrificed on DG 19 after 13 daily dosages. Adverse clinical observations included scant feces (DGs 11 to 19), excess salivation (DGs 18 and 19) and a red substance in the cage pan (DG 19). Other than an observation of ungroomed coat and confirmation of persistent adverse clinical observations, no gross lesions were observed by necropsy. This doe aborted three dead fetuses and five live fetuses were *in utero*; all fetuses appeared normal for their developmental ages. Body weight losses occurred and feed consumption values were reduced after DG 8.

Doe 8270 in the 20 mg/kg/day dosage group was found dead on DG 16 after nine daily dosages. The only adverse clinical observation prior to death was scant feces (DGs 9 to 15). Necropsy revealed the non-glandular mucosal surface of the stomach to be red and diffuse. Five fetuses and one early resorption were *in utero*. All fetuses appeared normal for their developmental ages. Body weight losses occurred after DG 7 and feed consumption values were reduced throughout the dosage period.

A.2. Clinical Observations

Dosage-dependant increases in adverse clinical observations occurred in the 2.5, 5, 10 and 20 mg/kg/day dosage groups. One rabbit in the 2.5 dosage group and all rabbits in the 5, 10 and 20 mg/kg/day dosage groups had scant feces (first observed on DGs 14, 13, 9 and 9, respectively). One, five and three rabbits in the 5, 10 and 20 mg/kg/day dosage groups had no feces (first observed on

DG 18, 12 and 11, respectively). Three rabbits in the 20 mg/kg/day dosage group had excess salivation (first observed on DG 17). Two rabbits that aborted or were found dead in each of the 10 and 20 mg/kg/day dosage groups had a red substance in the cage pan (first observed on DGs 22 and 16, respectively). Two rabbits in the 20 mg/kg/day dosage group had a red or tan perivaginal substance (first observed on DG 16). One rabbit in each of the 2.5, 5, 10 and 20 mg/kg/day dosage groups had an ungroomed coat (first observed on DG 18, 23, 24, 24 and 16, respectively).

All other clinical observations were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; or 2) the observations occurred in only one rat. These clinical observations included localized alopecia (underside, head, back and/or limbs) for 2, 1, 1, 0, 2, 0 and 0 does in the seven respective dosage groups, a mass on the nose of one 10 mg/kg/day dosage group doe that aborted, one rabbit in each of the 0.1 and 20 mg/kg/day dosage groups that had soft or liquid feces (first observed on DG 12) and scant feces in one 0.1 mg/kg/day dosage group rabbit on one day of the study.

A.3. Necropsy Observations

There were no necropsy observations for the rabbits that survived until scheduled sacrifice. All necropsy observations in rabbits that died or aborted were described previously.

B. Maternal Body Weights and Body Weight Changes (Figure 1; Summaries - Tables 4 and 5; Individual Data - Table 13)

Rabbits in the 10 and 20 mg/kg/day dosage groups had body weight losses for all recorded intervals after initiation of dosage; there were no surviving pregnant rabbits in these groups after DGs 26 and 19, respectively. The 2.5 and 5 mg/kg/day dosage groups had decreased body weight gains or body weight losses for the entire dosage period (calculated as DGs 7 to 21). During the postdosage period (DGs 21 to 29), body weight gains were increased in the 2.5 and 5 mg/kg/day dosage groups, a rebound phenomenon that commonly occurs in these types of studies. Body weight gains for the entire gestation period (DGs 0 to 29) and the entire gestation period after the initiation of dosing (DGs 7 to 29) were reduced in the 2.5 and 5 mg/kg/day dosage groups.

Body weights and body weight gains were unaffected by the 1 mg/kg/day dosage of the test article.

C. Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables 6 and 7; Individual Data - Table 14)

Rabbits in the 10 and 20 mg/kg/day dosage groups had severely reduced absolute and relative feed consumption values for all recorded intervals after initiation of dosage; there were no surviving pregnant rabbits in these groups after DGs 26 and 19, respectively. Absolute and relative feed consumption values for the entire dosage period (calculated as DGs 7 to 21) were reduced in the 2.5 and 5 mg/kg/day dosage groups. During the postdosage period (DGs 21 to 29), feed consumption values were generally comparable among the five surviving groups, although the 5 mg/kg/day dosage group had slightly increased feed consumption values during this period, a rebound phenomenon that commonly occurs in these types of studies. Feed consumption values were reduced in the 2.5 and 5 mg/kg/day dosage groups for the entire gestation period after the initiation of dosing (DGs 7 to 29).

Absolute and relative feed consumption values were unaffected by the 1 mg/kg/day dosage of the test article.

D. Caesarean-Sectioning and Litter Observations (Summaries - Tables 8 and 9; Individual Data - Tables 15 through 17)

Caesarean-sectioning observations were based on 5, 4, 4, 5 and 2 pregnant rabbits with one or more live fetuses in the 0, 0.1, 1, 2.5 and 5 mg/kg/day dosage groups, respectively. There were no pregnant rabbits that survived to DG 29 Caesarean-sectioning in the 10 and 20 mg/kg/day dosage groups. Fetal body weights were severely reduced and the number of early and late resorptions and the percent resorbed conceptuses per litter were increased (along with the concomitant decrease in litter size) in the 5 mg/kg/day dosage group. Only two of the four pregnant does in this dosage group survived to Caesarean-sectioning. Fetal body weights were also reduced in the 2.5 mg/kg/day dosage group as compared to the concurrent controls but were at the lower end of the range observed historically at the Testing Facility.

All other Caesarean-sectioning or litter parameters were unaffected by administration of the test article at dosages as high as 5 mg/kg/day. The litter averages for corpora lutea, implantations and percent live male fetuses were comparable among the five surviving dosage groups. No does had a litter consisting of only resorbed conceptuses, there were no dead fetuses and all placentae appeared normal.

E. Fetal Gross Observations (Summary - Table 10)

Totals of 42, 37, 34, 42 and 11 fetuses were examined externally for gross alterations in the five respective dosages groups with live litters. Three fetuses had gross external alterations. One fetus (8251-10) in the 2.5 mg/kg/day dosage group had a cleft snout and absent incisors. Two fetuses (8243-8 and 8244-7) in the 0.1 mg/kg/day dosage group had fetal gross malformations; fetus 8243-8 had a short snout, displaced and small nares and fetus 8244-7 had abdominal distention.

IV. Recommendation:

Based on these data, dosages of 0 (Vehicle), 0.1, 1.0, 2.5 and 3.75 mg/kg/day PFOS were recommended for the developmental toxicity study in rabbits (Argus Protocol 418-012). The 0.1 mg/kg/day dosage is expected to be a no-observable-effect-level (NOEL) for both maternal and embryo-fetal toxicity, and the 3.75 mg/kg/day dosage is expected to produce minimal maternal toxicity and little or no developmental toxicity.

06 JAN 99

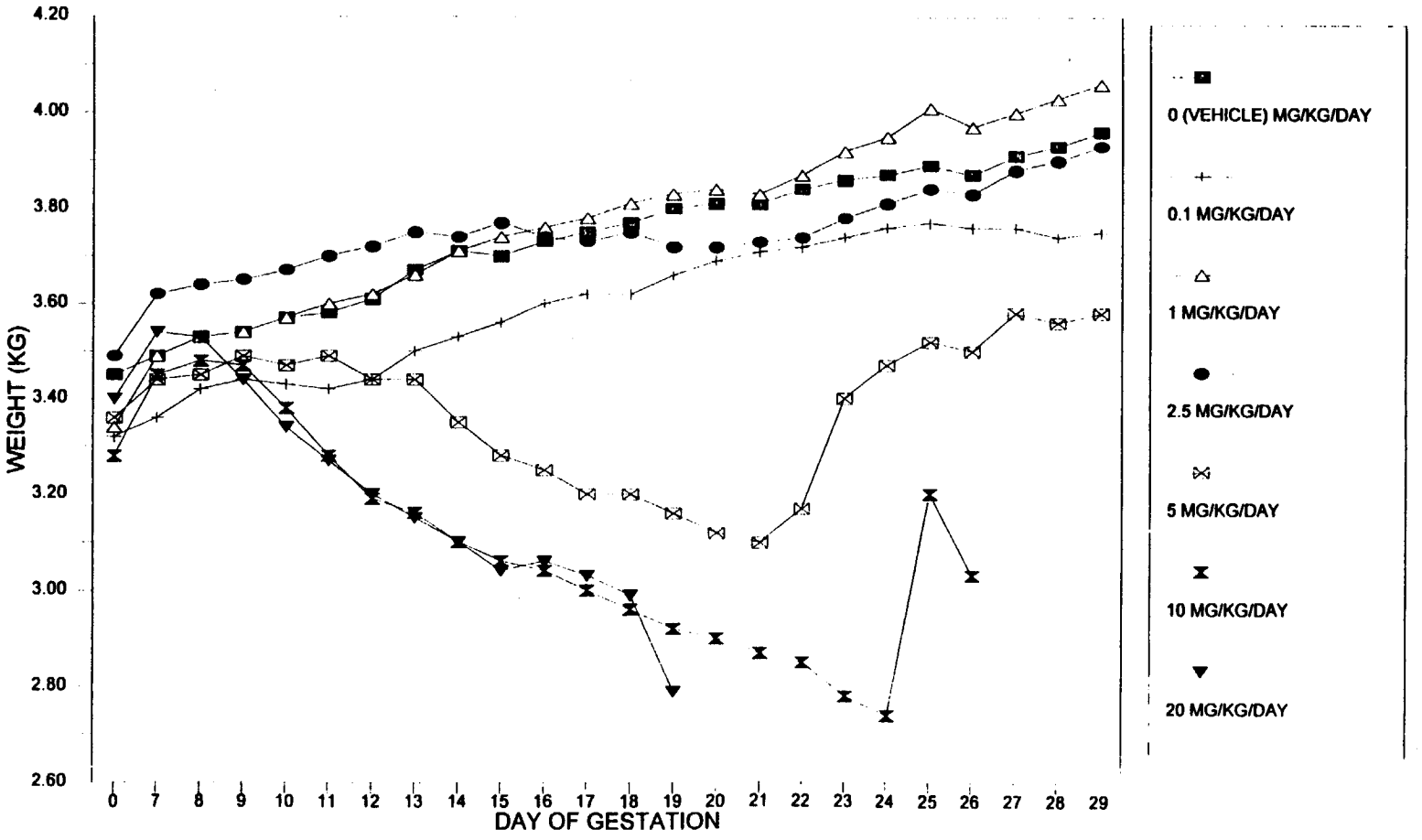
Alan M. Hoberman, Ph.D., DABT Date
Director of Research

06-JAN-99

Raymond G. York, Ph.D., DABT Date
Associate Director of Research
and Study Director

MATERNAL BODY WEIGHTS

Figure 1



PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1	IV 2.5
MAXIMUM POSSIBLE INCIDENCE		115/ 5	115/ 5	115/ 5	115/ 5
MORTALITY		0	0	0	0
FOUND DEAD		0	0	0	0
ABORTED AND SACRIFICED		0	0	0	0
SCANT FECES		0/ 0	1/ 1	0/ 0	8/ 1
NO FECES		0/ 0	0/ 0	0/ 0	0/ 0
EXCESS SALIVATION		0/ 0	0/ 0	0/ 0	0/ 0
RED SUBSTANCE IN CAGE PAN		0/ 0	0/ 0	0/ 0	0/ 0
RED OR TAN PERIVAGINAL SUBSTANCE		0/ 0	0/ 0	0/ 0	0/ 0
SOFT OR LIQUID FECES		0/ 0	2/ 1	0/ 0	0/ 0
UNGROOMED COAT		0/ 0	0/ 0	0/ 0	12/ 1
NOSE: MASS		0/ 0	0/ 0	0/ 0	0/ 0
LOCALIZED ALOPECIA:	TOTAL	15/ 2	3/ 1	2/ 1	0/ 0
	UNDERSIDE	2/ 1	0/ 0	0/ 0	0/ 0
	HEAD	0/ 0	0/ 0	2/ 1	0/ 0
	BACK	0/ 0	3/ 1	0/ 0	0/ 0
	LIMBS	13/ 1	0/ 0	0/ 0	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RABBITS)/NUMBER OF RABBITS EXAMINED PER GROUP ON DAYS 7 THROUGH 29 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RABBITS WITH OBSERVATION.
a. Dosage occurred on days 7 through 20 of presumed gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY

DOSAGE GROUP	V	VI	VII
DOSAGE (MG/KG/DAY)a	5	10	20
MAXIMUM POSSIBLE INCIDENCE	102/ 5	95/ 5	59/ 5
MORTALITY	2	4	5
FOUND DEAD	0	0	4b-e
ABORTED AND SACRIFICED	2f,g	4h-k	1 l
SCANT FECES	50/ 5	49/ 5	33/ 5
NO FECES	2/ 1	23/ 5	8/ 3b-d
EXCESS SALIVATION	0/ 0	0/ 0	4/ 3b,c,l
RED SUBSTANCE IN CAGE PAN	0/ 0	2/ 2j,k	3/ 2b,l
RED OR TAN PERIVAGINAL SUBSTANCE	0/ 0	0/ 0	3/ 2b,d
SOFT OR LIQUID FECES	0/ 0	0/ 0	2/ 1d
UNGROOMED COAT	2/ 1	3/ 1h	2/ 1b
NOSE: MASS	0/ 0	15/ 1i	0/ 0
LOCALIZED ALOPECIA: TOTAL	16/ 2	0/ 0	0/ 0
UNDERSIDE	16/ 2	0/ 0	0/ 0
HEAD	0/ 0	0/ 0	0/ 0
BACK	0/ 0	0/ 0	0/ 0
LIMBS	0/ 0	0/ 0	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RABBITS)/NUMBER OF RABBITS EXAMINED PER GROUP ON DAYS 7 THROUGH 29 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RABBITS WITH OBSERVATION.

a. Dosage occurred on days 7 through 20 of presumed gestation.
b. Doe 8266 was found dead on day 17 of gestation.
c. Doe 8267 was found dead on day 20 of gestation.
d. Doe 8268 was found dead on day 17 of gestation.
e. Doe 8270 was found dead on day 16 of gestation.
f. Doe 8257 aborted on day 23 of gestation.
g. Doe 8259 aborted on day 22 of gestation.
h. Doe 8261 aborted on day 26 of gestation.
i. Doe 8262 aborted on day 22 of gestation.
j. Doe 8264 aborted on day 23 of gestation.
k. Doe 8265 aborted on day 25 of gestation.
l. Doe 8269 aborted on day 19 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1	2.5
RABBITS EXAMINED b	N	5	5	5	5
MORTALITY	N	0	0	0	0
FOUND DEAD	N	0	0	0	0
ABORTED	N	0	0	0	0
APPEARED NORMAL	N	5	5	5	5
ESOPHAGUS:					
WHITE FROTHY MATERIAL	N	0	0	0	0
LUNGS:					
APPEARED INFLATED AND LEFT DIAPHRAGMATIC LOBE, TEAR	N	0	0	0	0
ABDOMINAL CAVITY:					
CLOUDY GREEN FLUID	N	0	0	0	0
GALLBLADDER:					
RUPTURED	N	0	0	0	0

a. Dosage occurred on days 7 through 20 of presumed gestation.
b. Refer to the individual clinical observations table (Table 11) for external observations confirmed at necropsy.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 2 (PAGE 2): NECROPSY OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1	IV 2.5
RABBITS EXAMINED b	N	5	5	5	5
STOMACH:					
FUNDIC MUCOSA, TWO ERODED AREAS	N	0	0	0	0
NON-GLANDULAR MUCOSA, RED AND DIFFUSE	N	0	0	0	0
FRIABLE	N	0	0	0	0
MUCOSAL SURFACE, NUMEROUS BLACK AREAS	N	0	0	0	0
LIVER:					
LEFT LATERAL LOBE, TEAR	N	0	0	0	0
MOTTLED RED AND TAN	N	0	0	0	0
EXTERNAL OBSERVATIONS:					
UNGROOMED COAT	N	0	0	0	0
NOSE, MASS, CUT SURFACE REVEALED SMOOTH PINK MATERIAL	N	0	0	0	0

a. Dosage occurred on days 7 through 20 of presumed gestation.
b. Refer to the individual clinical observations table (Table 11) for external observations confirmed at necropsy.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 2 (PAGE 3): NECROPSY OBSERVATIONS - SUMMARY

DOSAGE GROUP		V	VI	VII
DOSAGE (MG/KG/DAY)a		5	10	20
RABBITS EXAMINED b	N	5	5	5
MORTALITY	N	2	4	5
FOUND DEAD	N	0	0	4c-f
ABORTED	N	2g,h	4i-l	1m
APPEARED NORMAL	N	5g,h	3k,l	2d,m
ESOPHAGUS:				
WHITE FROTHY MATERIAL	N	0	0	1c
LUNGS:				
APPEARED INFLATED AND LEFT DIAPHRAGMATIC LOBE, TEAR	N	0	0	1c
ABDOMINAL CAVITY:				
CLOUDY GREEN FLUID	N	0	0	1e
GALLBLADDER:				
RUPTURED	N	0	0	1e

a. Dosage occurred on days 7 through 20 of presumed gestation.
b. Refer to the individual clinical observations table (Table 11) for external observations confirmed at necropsy.
c. Doe 8266 was found dead on day 17 of gestation.
d. Doe 8267 was found dead on day 20 of gestation.
e. Doe 8268 was found dead on day 17 of gestation.
f. Doe 8270 was found dead on day 16 of gestation.
g. Doe 8257 aborted on day 23 of gestation.
h. Doe 8259 aborted on day 22 of gestation.
i. Doe 8261 aborted on day 26 of gestation.
j. Doe 8262 aborted on day 22 of gestation.
k. Doe 8264 aborted on day 23 of gestation.
l. Doe 8265 aborted on day 25 of gestation.
m. Doe 8269 aborted on day 19 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 2 (PAGE 4): NECROPSY OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		V 5	VI 10	VII 20
RABBITS EXAMINED b	N	5	5	5
MORTALITY	N	2	4	5
FOUND DEAD	N	0	0	4c-f
ABORTED	N	2g,h	4i-l	1m
STOMACH:				
FUNDIC MUCOSA, TWO ERODED AREAS	N	0	0	1c
NON-GLANDULAR MUCOSA, RED AND DIFFUSE	N	0	0	1f
FRIABLE	N	0	0	1e
MUCOSAL SURFACE, NUMEROUS BLACK AREAS	N	0	1i	0
LIVER:				
LEFT LATERAL LOBE, TEAR	N	0	0	1c
MOTTLED RED AND TAN	N	0	1i	0
EXTERNAL OBSERVATIONS:				
UNGROOMED COAT	N	0	0	1m
NOSE, MASS, CUT SURFACE REVEALED SMOOTH PINK MATERIAL	N	0	1j	0

a. Dosage occurred on days 7 through 20 of presumed gestation.
b. Refer to the individual clinical observations table (Table 11) for external observations confirmed at necropsy.
c. Doe 8266 was found dead on day 17 of gestation.
d. Doe 8267 was found dead on day 20 of gestation.
e. Doe 8268 was found dead on day 17 of gestation.
f. Doe 8270 was found dead on day 16 of gestation.
g. Doe 8257 aborted on day 23 of gestation.
h. Doe 8259 aborted on day 22 of gestation.
i. Doe 8261 aborted on day 26 of gestation.
j. Doe 8262 aborted on day 22 of gestation.
k. Doe 8264 aborted on day 23 of gestation.
l. Doe 8265 aborted on day 25 of gestation.
m. Doe 8269 aborted on day 19 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 3 (PAGE 1): UTERINE CONTENTS AND LITTER DATA FOR RABBITS THAT WERE FOUND DEAD OR ABORTED

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	RABBIT NUMBER	DAY OF DEATH	CORPORA LUTEA R	L	T	IMPLANTATIONS R	L	T	EMBRYOS/FETUSES b R	L	A	T	RESORPTIONS c R	L	A	T
I 0 (VEHICLE)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II 0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV 2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V 5	8257	ABORTED ON DAY 23 OF GESTATION	2	4	6	2	4	6	0	0	0	0	0	1	5d (5LR)	6
	8259	ABORTED ON DAY 22 OF GESTATION	4	7	11	3	6	9	0	0	7e	7	0	0	2d (2LR)	2
VI 10	8261	ABORTED ON DAY 26 OF GESTATION	4	7	11	2	7	9	2f	0	7e	9	0	0	0	0
	8262	ABORTED ON DAY 22 OF GESTATION	5	5	10	4	5	9	0	0	0	0	0	0	9d (9LR)	9
	8264	ABORTED ON DAY 23 OF GESTATION	4	5	9	4	5	9	0	0	0	0	0	0	9d (9LR)	4
	8265	ABORTED ON DAY 25 OF GESTATION	4	3	7	4	3	7	2	3	1e	6	1	0	0	1

R = RIGHT L = LEFT T = TOTAL A = ABORTED
a. Dosage occurred on days 7 through 20 of gestation.
b. Conceptuses appeared normal for developmental ages.
c. Early resorptions, unless noted otherwise.
d. Autolysis precluded further evaluation.
e. Dead fetuses.
f. Fetus 8261-1 and 8261-2 had edema on the dorsal head and ventral neck.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 3 (PAGE 2): UTERINE CONTENTS AND LITTER DATA FOR RABBITS THAT WERE FOUND DEAD OR ABORTED

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	RABBIT NUMBER	DAY OF DEATH	CORPORA LUTEA R	L	T	IMPLANTATIONS R	L	T	EMBRYOS/FETUSES b R	L	A	T	RESORPTIONS c R	L	A	T
VII 20	8266	FOUND DEAD ON DAY 17 OF GESTATION	5	5	10	5	5	10	0	0	0	0	5	5	0	10
	8267	FOUND DEAD ON DAY 20 OF GESTATION	9	6	15	7	6	13	7	6	0	13d	0	0	0	0
	8268	FOUND DEAD ON DAY 17 OF GESTATION	6	4	10	6	4	10	6	3	0	9d	0	1	0	1
	8269	ABORTED ON DAY 19 OF GESTATION	7	3	10	5	3	8	3	2	3e	8	0	0	0	0
	8270	FOUND DEAD ON DAY 16 OF GESTATION	5	2	7	5	1	6	4	1	0	5d	1	0	0	1

R = RIGHT L = LEFT T = TOTAL A = ABORTED
a. Dosage occurred on days 7 through 20 of gestation.
b. Conceptuses appeared normal for developmental ages.
c. Early resorptions, unless noted otherwise.
d. Unable to determine viability of conceptuses because of death of doe.
e. Dead fetuses.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 4 (PAGE 1): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1	IV 2.5
RABBITS TESTED	N	5	5	5	5
PREGNANT	N	5	4	4	5
MATERNAL BODY WEIGHT (KG)					
DAY 0	MEAN±S.D.	3.45 ± 0.22	3.32 ± 0.30	3.34 ± 0.18	3.49 ± 0.39
DAY 7	MEAN±S.D.	3.49 ± 0.18	3.36 ± 0.17	3.49 ± 0.23	3.62 ± 0.37
DAY 8	MEAN±S.D.	3.53 ± 0.17	3.42 ± 0.21	3.53 ± 0.24	3.64 ± 0.39
DAY 9	MEAN±S.D.	3.54 ± 0.17	3.44 ± 0.22	3.54 ± 0.22	3.65 ± 0.38
DAY 10	MEAN±S.D.	3.57 ± 0.19	3.43 ± 0.21	3.57 ± 0.22	3.67 ± 0.38
DAY 11	MEAN±S.D.	3.58 ± 0.19	3.42 ± 0.24	3.60 ± 0.19	3.70 ± 0.37
DAY 12	MEAN±S.D.	3.61 ± 0.20	3.44 ± 0.22	3.62 ± 0.25	3.72 ± 0.38
DAY 13	MEAN±S.D.	3.67 ± 0.20	3.50 ± 0.24	3.66 ± 0.27	3.75 ± 0.43
DAY 14	MEAN±S.D.	3.71 ± 0.23	3.53 ± 0.25	3.71 ± 0.28	3.74 ± 0.43
DAY 15	MEAN±S.D.	3.70 ± 0.20	3.56 ± 0.29	3.74 ± 0.28	3.77 ± 0.42
DAY 16	MEAN±S.D.	3.73 ± 0.20	3.60 ± 0.31	3.76 ± 0.28	3.74 ± 0.45
DAY 17	MEAN±S.D.	3.75 ± 0.22	3.62 ± 0.28	3.78 ± 0.30	3.73 ± 0.45
DAY 18	MEAN±S.D.	3.77 ± 0.22	3.62 ± 0.28	3.81 ± 0.28	3.75 ± 0.42
DAY 19	MEAN±S.D.	3.80 ± 0.22	3.66 ± 0.28	3.83 ± 0.30	3.72 ± 0.46
DAY 20	MEAN±S.D.	3.81 ± 0.21	3.69 ± 0.28	3.84 ± 0.28	3.72 ± 0.48
DAY 21	MEAN±S.D.	3.81 ± 0.20	3.71 ± 0.25	3.83 ± 0.31	3.73 ± 0.45

DAY = DAY OF GESTATION
a. Dosage occurred on days 7 through 20 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 4 (PAGE 2): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1	IV 2.5
RABBITS TESTED	N	5	5	5	5
PREGNANT	N	5	4	4	5
MATERNAL BODY WEIGHT (KG)					
DAY 22	MEAN±S.D.	3.84 ± 0.21	3.72 ± 0.26	3.87 ± 0.29	3.74 ± 0.47
DAY 23	MEAN±S.D.	3.86 ± 0.19	3.74 ± 0.26	3.92 ± 0.27	3.78 ± 0.44
DAY 24	MEAN±S.D.	3.87 ± 0.20	3.76 ± 0.23	3.95 ± 0.27	3.81 ± 0.41
DAY 25	MEAN±S.D.	3.89 ± 0.21	3.77 ± 0.20	4.01 ± 0.28	3.84 ± 0.42
DAY 26	MEAN±S.D.	3.87 ± 0.23	3.76 ± 0.20	3.97 ± 0.30	3.83 ± 0.42
DAY 27	MEAN±S.D.	3.91 ± 0.23	3.76 ± 0.18	4.00 ± 0.29	3.88 ± 0.41
DAY 28	MEAN±S.D.	3.93 ± 0.24	3.74 ± 0.14	4.03 ± 0.30	3.90 ± 0.45
DAY 29	MEAN±S.D.	3.96 ± 0.22	3.75 ± 0.16	4.06 ± 0.32	3.93 ± 0.42

DAY = DAY OF GESTATION
a. Dosage occurred on days 7 through 20 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 4 (PAGE 3): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP		V	VI	VII
DOSAGE (MG/KG/DAY)a		5	10	20
RABBITS TESTED	N	5	5	5
PREGNANT	N	4	4	5
MATERNAL BODY WEIGHT (KG)				
DAY 0	MEAN±S.D.	3.36 ± 0.40	3.28 ± 0.41	3.40 ± 0.18
DAY 7	MEAN±S.D.	3.44 ± 0.33	3.45 ± 0.39	3.54 ± 0.22
DAY 8	MEAN±S.D.	3.45 ± 0.35	3.48 ± 0.38	3.53 ± 0.27
DAY 9	MEAN±S.D.	3.49 ± 0.36	3.47 ± 0.42	3.44 ± 0.25
DAY 10	MEAN±S.D.	3.47 ± 0.39	3.38 ± 0.42	3.34 ± 0.24
DAY 11	MEAN±S.D.	3.49 ± 0.37	3.28 ± 0.39	3.27 ± 0.24
DAY 12	MEAN±S.D.	3.44 ± 0.37	3.19 ± 0.40	3.20 ± 0.24
DAY 13	MEAN±S.D.	3.44 ± 0.39	3.16 ± 0.43	3.15 ± 0.24
DAY 14	MEAN±S.D.	3.35 ± 0.38	3.10 ± 0.42	3.10 ± 0.25
DAY 15	MEAN±S.D.	3.28 ± 0.37	3.06 ± 0.42	3.04 ± 0.26
DAY 16	MEAN±S.D.	3.25 ± 0.36	3.04 ± 0.39	3.06 ± 0.16 [4]b
DAY 17	MEAN±S.D.	3.20 ± 0.35	3.00 ± 0.38	3.03 ± 0.15 [3]b
DAY 18	MEAN±S.D.	3.20 ± 0.35	2.96 ± 0.44	2.99 ± 0.18 [2]b
DAY 19	MEAN±S.D.	3.16 ± 0.34	2.92 ± 0.42	2.79 ± 0.00 [1]b
DAY 20	MEAN±S.D.	3.12 ± 0.35	2.90 ± 0.44	b
DAY 21	MEAN±S.D.	3.10 ± 0.38	2.87 ± 0.43	

DAY = DAY OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for rabbits that died or aborted.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 4 (PAGE 4): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP		V	VI	VII
DOSAGE (MG/KG/DAY)a		5	10	20
RABBITS TESTED	N	5	5	5
PREGNANT	N	4	4	5
INCLUDED IN ANALYSES	N	3b	3b	0b
MATERNAL BODY WEIGHT (KG)				
DAY 22	MEAN±S.D.	3.17 ± 0.48	2.85 ± 0.52	
DAY 23	MEAN±S.D.	3.40 ± 0.66 [2]b	2.78 ± 0.71 [2]b	
DAY 24	MEAN±S.D.	3.47 ± 0.66 [2]b	2.74 ± 0.70 [2]b	
DAY 25	MEAN±S.D.	3.52 ± 0.60 [2]b	3.20 ± 0.00 [1]b	
DAY 26	MEAN±S.D.	3.50 ± 0.59 [2]b	3.03 ± 0.00 [1]b	
DAY 27	MEAN±S.D.	3.58 ± 0.56 [2]b	b	
DAY 28	MEAN±S.D.	3.56 ± 0.64 [2]b		
DAY 29	MEAN±S.D.	3.58 ± 0.66 [2]b		

DAY = DAY OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for rabbits that died or aborted.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1	IV 2.5
RABBITS TESTED	N	5	5	5	5
PREGNANT	N	5	4	4	5
MATERNAL BODY WEIGHT CHANGE (KG)					
DAYS 0 - 7	MEAN±S.D.	+0.04 ± 0.09	+0.04 ± 0.14	+0.14 ± 0.10	+0.13 ± 0.08
DAYS 7 - 10	MEAN±S.D.	+0.09 ± 0.04	+0.07 ± 0.05	+0.08 ± 0.02	+0.05 ± 0.02
DAYS 10 - 13	MEAN±S.D.	+0.10 ± 0.04	+0.06 ± 0.05	+0.10 ± 0.05	+0.08 ± 0.10
DAYS 13 - 16	MEAN±S.D.	+0.05 ± 0.01	+0.10 ± 0.08	+0.09 ± 0.06	-0.01 ± 0.11
DAYS 16 - 19	MEAN±S.D.	+0.07 ± 0.02	+0.07 ± 0.04	+0.08 ± 0.05	-0.02 ± 0.11
DAYS 19 - 21	MEAN±S.D.	+0.01 ± 0.04	+0.04 ± 0.03	+0.00 ± 0.05	+0.01 ± 0.05
DAYS 21 - 25	MEAN±S.D.	+0.09 ± 0.06	+0.07 ± 0.08	+0.18 ± 0.05	+0.11 ± 0.06
DAYS 25 - 29	MEAN±S.D.	+0.06 ± 0.04	-0.02 ± 0.12	+0.06 ± 0.09	+0.09 ± 0.06
DAYS 7 - 21	MEAN±S.D.	+0.32 ± 0.04	+0.34 ± 0.10	+0.34 ± 0.09	+0.11 ± 0.28
DAYS 21 - 29	MEAN±S.D.	+0.15 ± 0.08	+0.04 ± 0.16	+0.23 ± 0.09	+0.20 ± 0.06
DAYS 7 - 29	MEAN±S.D.	+0.47 ± 0.11	+0.38 ± 0.07	+0.57 ± 0.12	+0.31 ± 0.25
DAYS 0 - 29	MEAN±S.D.	+0.51 ± 0.18	+0.43 ± 0.21	+0.72 ± 0.20	+0.44 ± 0.31

DAYS = DAYS OF GESTATION
a. Dosage occurred on days 7 through 20 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 5 (PAGE 2): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		V 5	VI 10	VII 20
RABBITS TESTED	N	5	5	5
PREGNANT	N	4	4	5
MATERNAL BODY WEIGHT CHANGE (KG)				
DAYS 0 - 7	MEAN±S.D.	+0.08 ± 0.08	+0.17 ± 0.11	+0.15 ± 0.10
DAYS 7 - 10	MEAN±S.D.	+0.02 ± 0.10	-0.08 ± 0.05	-0.20 ± 0.11
DAYS 10 - 13	MEAN±S.D.	-0.03 ± 0.04	-0.22 ± 0.02	-0.20 ± 0.08
DAYS 13 - 16	MEAN±S.D.	-0.19 ± 0.04	-0.12 ± 0.06	-0.18 ± 0.02 [4]b
DAYS 16 - 19	MEAN±S.D.	-0.08 ± 0.07	-0.12 ± 0.03	-0.21 ± 0.00 [1]b
DAYS 19 - 21	MEAN±S.D.	-0.06 ± 0.06	-0.05 ± 0.02	b
DAYS 21 - 25	MEAN±S.D.	+0.29 ± 0.01 [2]b	-0.16 ± 0.00 [1]b	
DAYS 25 - 29	MEAN±S.D.	+0.05 ± 0.06 [2]b	b	
DAYS 7 - 21	MEAN±S.D.	-0.34 ± 0.09	-0.58 ± 0.06	
DAYS 21 - 29	MEAN±S.D.	+0.35 ± 0.04 [2]b	b	
DAYS 7 - 29	MEAN±S.D.	+0.06 ± 0.11 [2]b		
DAYS 0 - 29	MEAN±S.D.	+0.10 ± 0.01 [2]b		

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for rabbits that died or aborted.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 6 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1	IV 2.5
RABBITS TESTED	N	5	5	5	5
PREGNANT	N	5	4	4	5
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 7 - 10	MEAN±S.D.	181.0 ± 3.2	163.2 ± 21.9	181.8 ± 1.3	181.4 ± 1.6
DAYS 10 - 13	MEAN±S.D.	172.5 ± 22.9	144.2 ± 41.8	169.5 ± 21.3	162.8 ± 33.4
DAYS 13 - 16	MEAN±S.D.	167.6 ± 32.4	152.1 ± 51.5 [3]b	173.9 ± 14.0	135.3 ± 72.7
DAYS 16 - 19	MEAN±S.D.	176.4 ± 12.0	178.8 ± 8.4	175.3 ± 11.8	122.0 ± 80.3
DAYS 19 - 21	MEAN±S.D.	172.3 ± 17.5	182.5 ± 1.8	181.0 ± 4.1	114.4 ± 83.9
DAYS 21 - 25	MEAN±S.D.	153.6 ± 40.4	158.0 ± 37.2	169.6 ± 10.8	125.0 ± 56.2
DAYS 25 - 29	MEAN±S.D.	138.9 ± 37.1	89.0 ± 56.2	144.2 ± 35.8	143.2 ± 27.7
DAYS 7 - 21	MEAN±S.D.	174.1 ± 17.5	163.0 ± 23.6	176.0 ± 10.8	145.2 ± 49.4
DAYS 21 - 29	MEAN±S.D.	146.2 ± 37.4	121.7 ± 45.1	156.9 ± 23.0	134.1 ± 35.8
DAYS 7 - 29	MEAN±S.D.	164.0 ± 22.0	148.4 ± 10.6	169.0 ± 13.9	141.2 ± 42.9

DAYS = DAYS OF GESTATION
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes a value that was associated with spillage.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 6 (PAGE 2): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		V 5	VI 10	VII 20
RABBITS TESTED	N	5	5	5
PREGNANT	N	4	4	5
MATERNAL FEED CONSUMPTION (G/DAY)				
DAYS 7 - 10	MEAN±S.D.	168.4 ± 25.3	111.8 ± 42.6	46.2 ± 24.0
DAYS 10 - 13	MEAN±S.D.	88.4 ± 37.4	3.3 ± 3.6	0.7 ± 0.4
DAYS 13 - 16	MEAN±S.D.	3.7 ± 5.7	0.7 ± 0.5	1.2 ± 0.2 [4]b
DAYS 16 - 19	MEAN±S.D.	11.2 ± 11.3	1.2 ± 0.9	0.7 ± 0.0 [1]b
DAYS 19 - 21	MEAN±S.D.	17.8 ± 27.9	0.4 ± 0.8	b
DAYS 21 - 25	MEAN±S.D.	135.2 ± 50.1 [2]b	1.8 ± 0.0 [1]b	
DAYS 25 - 29	MEAN±S.D.	182.6 ± 1.6 [2]b	b	
DAYS 7 - 21	MEAN±S.D.	60.8 ± 17.6	25.1 ± 9.3	
DAYS 21 - 29	MEAN±S.D.	158.9 ± 25.9 [2]b	b	
DAYS 7 - 29	MEAN±S.D.	103.2 ± 21.7 [2]b		

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for rabbits that died or aborted.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 7 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1	IV 2.5
RABBITS TESTED	N	5	5	5	5
PREGNANT	N	5	4	4	5
MATERNAL FEED CONSUMPTION (G/KG/DAY)					
DAYS 7 - 10	MEAN±S.D.	51.3 ± 2.0	47.8 ± 6.0	51.6 ± 3.2	50.2 ± 5.2
DAYS 10 - 13	MEAN±S.D.	47.7 ± 4.8	41.6 ± 11.0	46.8 ± 3.2	43.9 ± 8.3
DAYS 13 - 16	MEAN±S.D.	45.0 ± 7.2	42.8 ± 12.0 [3]b	46.8 ± 0.7	35.8 ± 19.8
DAYS 16 - 19	MEAN±S.D.	46.9 ± 1.7	49.4 ± 1.8	46.2 ± 0.5	32.4 ± 22.1
DAYS 19 - 21	MEAN±S.D.	45.3 ± 3.2	49.6 ± 3.2	47.4 ± 2.8	30.4 ± 22.8
DAYS 21 - 25	MEAN±S.D.	39.8 ± 9.8	42.7 ± 11.4	43.4 ± 3.4	33.3 ± 14.4
DAYS 25 - 29	MEAN±S.D.	35.4 ± 9.1	24.0 ± 15.6	35.7 ± 7.2	36.8 ± 4.9
DAYS 7 - 21	MEAN±S.D.	47.4 ± 3.2	46.0 ± 5.0	47.8 ± 0.5	39.2 ± 13.8
DAYS 21 - 29	MEAN±S.D.	37.6 ± 9.1	32.9 ± 13.1	39.6 ± 4.4	35.1 ± 8.6
DAYS 7 - 29	MEAN±S.D.	43.7 ± 4.9	41.3 ± 3.8	44.6 ± 1.7	37.6 ± 11.5

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes a value that was associated with spillage.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 7 (PAGE 2): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		V 5	VI 10	VII 20
RABBITS TESTED	N	5	5	5
PREGNANT	N	4	4	5
MATERNAL FEED CONSUMPTION (G/KG/DAY)				
DAYS 7 - 10	MEAN±S.D.	48.9 ± 8.3	31.7 ± 9.9	13.1 ± 6.0
DAYS 10 - 13	MEAN±S.D.	25.0 ± 7.5	1.0 ± 1.1	0.2 ± 0.1
DAYS 13 - 16	MEAN±S.D.	1.0 ± 1.5	0.2 ± 0.2	0.4 ± 0.0 [4]b
DAYS 16 - 19	MEAN±S.D.	3.5 ± 3.6	0.4 ± 0.4	0.2 ± 0.0 [1]b
DAYS 19 - 21	MEAN±S.D.	5.0 ± 7.5	0.1 ± 0.2	b
DAYS 21 - 25	MEAN±S.D.	39.3 ± 7.5 [2]b	0.5 ± 0.0 [1]b	
DAYS 25 - 29	MEAN±S.D.	52.2 ± 8.6 [2]b	b	
DAYS 7 - 21	MEAN±S.D.	18.1 ± 3.6	7.8 ± 2.2	
DAYS 21 - 29	MEAN±S.D.	46.0 ± 0.8 [2]b	b	
DAYS 7 - 29	MEAN±S.D.	29.8 ± 1.1 [2]b		

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 20 of gestation.
b. Excludes values for rabbits that died or aborted.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 8 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1	2.5
RABBITS TESTED	N	5	5	5	5
PREGNANT	N(%)	5(100.0)	4(80.0)	4(80.0)	5(100.0)
FOUND DEAD	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
ABORTED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	5	4	4	5
CORPORA LUTEA	MEAN±S.D.	10.2 ± 1.6	11.8 ± 2.9	10.0 ± 0.8	11.0 ± 1.4
IMPLANTATIONS	MEAN±S.D.	8.8 ± 1.6	9.5 ± 1.7	8.5 ± 1.3	8.8 ± 2.0
LITTER SIZES	MEAN±S.D.	8.4 ± 1.1	9.2 ± 1.5	8.5 ± 1.3	8.4 ± 1.5
LIVE FETUSES	N	42	37	34	42
	MEAN±S.D.	8.4 ± 1.1	9.2 ± 1.5	8.5 ± 1.3	8.4 ± 1.5
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.4 ± 0.5	0.2 ± 0.5	0.0 ± 0.0	0.4 ± 0.5
EARLY RESORPTIONS	N	0	0	0	1
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4
LATE RESORPTIONS	N	2	1	0	1
	MEAN±S.D.	0.4 ± 0.5	0.2 ± 0.5	0.0 ± 0.0	0.2 ± 0.4
DOES WITH ANY RESORPTIONS	N(%)	2(40.0)	1(25.0)	0(0.0)	2(40.0)
DOES WITH ALL CONCEPTUSES RESORBED	N	0	0	0	0
DOES WITH VIABLE FETUSES	N(%)	5(100.0)	4(100.0)	4(100.0)	5(100.0)
PLACENTAE APPEARED NORMAL	N(%)	5(100.0)	4(100.0)	4(100.0)	5(100.0)

a. Dosage occurred on days 7 through 20 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 8 (PAGE 2): CAESAREAN SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP		V	VI	VII
DOSAGE (MG/KG/DAY)a		5	10	20
RABBITS TESTED	N	5	5	5
PREGNANT	N(%)	4(80.0)	4(80.0)	5(100.0)
FOUND DEAD	N(%)	0(0.0)	0(0.0)	4(80.0)
ABORTED	N(%)	2(50.0)	4(100.0)	1(20.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	2	0	0
CORPORA LUTEA	MEAN±S.D.	10.5 ± 0.7		
IMPLANTATIONS	MEAN±S.D.	9.5 ± 0.7		
LITTER SIZES	MEAN±S.D.	5.5 ± 2.1		
LIVE FETUSES	N	11		
	MEAN±S.D.	5.5 ± 2.1		
DEAD FETUSES	N	0		
RESORPTIONS	MEAN±S.D.	4.0 ± 1.4		
EARLY RESORPTIONS	N	5		
	MEAN±S.D.	2.5 ± 3.5		
LATE RESORPTIONS	N	3		
	MEAN±S.D.	1.5 ± 2.1		
DOES WITH ANY RESORPTIONS	N(%)	2(100.0)		
DOES WITH ALL CONCEPTUSES RESORBED	N	0		
DOES WITH VIABLE FETUSES	N(%)	2(100.0)		
PLACENTAE APPEARED NORMAL	N(%)	2(100.0)		

a. Dosage occurred on days 7 through 20 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 9 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1	2.5
LITTERS WITH ONE OR MORE LIVE FETUSES	N	5	4	4	5
IMPLANTATIONS	MEAN±S.D.	8.8 ± 1.6	9.5 ± 1.7	8.5 ± 1.3	8.8 ± 2.0
LIVE FETUSES	N	42	37	34	42
	MEAN±S.D.	8.4 ± 1.1	9.2 ± 1.5	8.5 ± 1.3	8.4 ± 1.5
LIVE MALE FETUSES	N	23	14	13	20
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	53.9 ± 9.4	37.2 ± 5.7	38.0 ± 8.9	48.5 ± 17.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	43.77 ± 5.95	40.76 ± 7.53	44.05 ± 2.70	38.18 ± 5.65
MALE FETUSES	MEAN±S.D.	44.06 ± 5.55	41.31 ± 7.46	45.67 ± 2.75	39.44 ± 5.84
FEMALE FETUSES	MEAN±S.D.	43.37 ± 7.09	40.40 ± 7.66	42.82 ± 3.12	38.04 ± 7.04
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	3.8 ± 5.2	2.3 ± 4.6	0.0 ± 0.0	3.6 ± 5.0

a. Dosage occurred on days 7 through 20 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 9 (PAGE 2): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP		V	VI	VII
DOSAGE (MG/KG/DAY)a		5	10	20
LITTERS WITH ONE OR MORE LIVE FETUSES	N	2	0	0
IMPLANTATIONS	MEAN±S.D.	9.5 ± 0.7		
LIVE FETUSES	N	11		
	MEAN±S.D.	5.5 ± 2.1		
LIVE MALE FETUSES	N	5		
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	46.4 ± 5.1		
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	26.05 ± 5.40		
MALE FETUSES	MEAN±S.D.	28.52 ± 11.60		
FEMALE FETUSES	MEAN±S.D.	23.35 ± 0.47		
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	42.8 ± 18.1		

a. Dosage occurred on days 7 through 20 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 10 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1	2.5
LITTERS EVALUATED	N	5	4	4	5
FETUSES EVALUATED	N	42	37	34	42
LIVE	N	42	37	34	42
SNOUT: CLEFT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(20.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(2.4)b
INCISORS: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(20.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(2.4)b
SNOUT: SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	1(25.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(2.7)c	0(0.0)	0(0.0)
NARES: DISPLACED					
LITTER INCIDENCE	N(%)	0(0.0)	1(25.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(2.7)c	0(0.0)	0(0.0)
NARES: SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(25.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(2.7)c	0(0.0)	0(0.0)
BODY: ABDOMINAL DISTENTION					
LITTER INCIDENCE	N(%)	0(0.0)	1(25.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(2.7)	0(0.0)	0(0.0)

a. Dosage occurred on days 7 through 20 of gestation.
b. Fetus 8251-10 had other gross external alterations.
c. Fetus 8243-8 had other gross external alterations.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 10 (PAGE 2): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		V	VI	VII
DOSAGE (MG/KG/DAY)a		5	10	20
LITTERS EVALUATED	N	2	0	0
FETUSES EVALUATED	N	11	0	0
LIVE	N	11	0	0
SNOUT: CLEFT				
LITTER INCIDENCE	N(%)	0(0.0)		
FETAL INCIDENCE	N(%)	0(0.0)		
INCISORS: ABSENT				
LITTER INCIDENCE	N(%)	0(0.0)		
FETAL INCIDENCE	N(%)	0(0.0)		
SNOUT: SHORT				
LITTER INCIDENCE	N(%)	0(0.0)		
FETAL INCIDENCE	N(%)	0(0.0)		
NARES: DISPLACED				
LITTER INCIDENCE	N(%)	0(0.0)		
FETAL INCIDENCE	N(%)	0(0.0)		
NARES: SMALL				
LITTER INCIDENCE	N(%)	0(0.0)		
FETAL INCIDENCE	N(%)	0(0.0)		
BODY: ABDOMINAL DISTENTION				
LITTER INCIDENCE	N(%)	0(0.0)		
FETAL INCIDENCE	N(%)	0(0.0)		

a. Dosage occurred on days 7 through 20 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 11 (PAGE 1): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RABBIT #		DESCRIPTION
DOSAGE GROUP I		0 (VEHICLE) MG/KG/DAY
8236	DG(17- 29)	LOCALIZED ALOPECIA: LIMBS a
8237	DG(28- 29)	LOCALIZED ALOPECIA: UNDERSIDE a
8238		NO ADVERSE FINDINGS
8239		NO ADVERSE FINDINGS
8240		NO ADVERSE FINDINGS
DOSAGE GROUP II		0.1 MG/KG/DAY
8241	DG(28)	SCANT FECES
	DG(28- 29)	SOFT OR LIQUID FECES
8242		NO ADVERSE FINDINGS
8243		NO ADVERSE FINDINGS
8244	DG(27- 29)	LOCALIZED ALOPECIA: BACK a
8245		NO ADVERSE FINDINGS
DOSAGE GROUP III		1 MG/KG/DAY
8246		NO ADVERSE FINDINGS
8247	DG(28- 29)	LOCALIZED ALOPECIA: HEAD a
8248		NO ADVERSE FINDINGS
8249		NO ADVERSE FINDINGS
8250		NO ADVERSE FINDINGS
DOSAGE GROUP IV		2.5 MG/KG/DAY
8251		NO ADVERSE FINDINGS
8252		NO ADVERSE FINDINGS
7650		NO ADVERSE FINDINGS
8254	DG(14)	SCANT FECES
	DG(16- 22)	SCANT FECES
	DG(18- 29)	UNGROOMED COAT
8255		NO ADVERSE FINDINGS

DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 11 (PAGE 2): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP V		5 MG/KG/DAY
RABBIT #		DESCRIPTION
8256	DG(14)	SCANT FECES
	DG(16- 21)	SCANT FECES
	DG(19- 29)	LOCALIZED ALOPECIA: UNDERSIDE a
8257	DG(13- 22)	SCANT FECES
	DG(23)	ABORTED AND SACRIFICED
8258	DG(13- 27)	SCANT FECES
	DG(25- 29)	LOCALIZED ALOPECIA: UNDERSIDE
	DG(29)	SCANT FECES
8259	DG(14)	SCANT FECES
	DG(16- 21)	SCANT FECES
	DG(22)	ABORTED AND SACRIFICED
8260	DG(13- 17)	SCANT FECES
	DG(18- 19)	NO FECES
	DG(20- 24)	SCANT FECES
	DG(23- 24)	UNGROOMED COAT

DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 11 (PAGE 3): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP VI		10 MG/KG/DAY
RABBIT #		DESCRIPTION
8261	DG(12)	SCANT FECES
	DG(13- 15)	NO FECES
	DG(16- 20)	SCANT FECES
	DG(21- 22)	NO FECES
	DG(23- 24)	SCANT FECES
	DG(24- 26)	UNGROOMED COAT
	DG(25- 26)	NO FECES
	DG(26)	ABORTED AND SACRIFICED
8262	DG(7- 21)	NOSE: MASS (0.3 CM IN DIAMETER)a
	DG(11- 12)	SCANT FECES
	DG(13)	NO FECES
	DG(14- 21)	SCANT FECES
	DG(22)	ABORTED AND SACRIFICED
8263	DG(11)	SCANT FECES
	DG(12- 13)	NO FECES
	DG(14- 20)	SCANT FECES
	DG(21- 23)	NO FECES
	DG(24- 27)	SCANT FECES
	DG(28- 29)	NO FECES
8264	DG(12)	SCANT FECES
	DG(13)	NO FECES
	DG(14- 15)	SCANT FECES
	DG(16- 18)	NO FECES
	DG(19- 20)	SCANT FECES
	DG(21- 22)	NO FECES
	DG(22)	RED SUBSTANCE IN CAGE PAN
	DG(23)	ABORTED AND SACRIFICED
8265	DG(9- 20)	SCANT FECES
	DG(21- 22)	NO FECES
	DG(23- 24)	SCANT FECES
	DG(25)	RED SUBSTANCE IN CAGE PAN
	DG(25)	ABORTED AND SACRIFICED

DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 11 (PAGE 4): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP VII		20 MG/KG/DAY
RABBIT #		DESCRIPTION
8266	DG(12- 15)	SCANT FECES
	DG(16- 17)	RED SUBSTANCE IN CAGE PAN
	DG(16- 17)	RED PERIVAGINAL SUBSTANCE a
	DG(16- 17)	UNGROOMED COAT
	DG(16- 17)	NO FECES
	DG(17)	EXCESS SALIVATION
	DG(17)	FOUND DEAD
8267	DG(9- 10)	SCANT FECES
	DG(11- 15)	NO FECES
	DG(16- 19)	SCANT FECES
	DG(19)	EXCESS SALIVATION
	DG(20)	FOUND DEAD
8268	DG(9- 15)	SCANT FECES
	DG(12- 13)	SOFT OR LIQUID FECES
	DG(16)	TAN PERIVAGINAL SUBSTANCE a
	DG(16)	NO FECES
	DG(17)	FOUND DEAD
8269	DG(11- 19)	SCANT FECES
	DG(18- 19)	EXCESS SALIVATION
	DG(19)	RED SUBSTANCE IN CAGE PAN
	DG(19)	ABORTED AND SACRIFICED
8270	DG(9- 15)	SCANT FECES
	DG(16)	FOUND DEAD

DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 12 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
I 0 (VEHICLE)	8236	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8237	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8238	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8239	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8240	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
II 0.1	8241	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8242	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8243	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8244	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8245	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
III 1	8246	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8247	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8248	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8249	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8250	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
IV 2.5	8251	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8252	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	7650	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8254	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8255	DG 29	P	14	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 11) for external observations confirmed at necropsy.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 12 (PAGE 2): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
V 5	8256	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
	8257	DG 23	P	14	ABORTED ON DAY 23 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8258	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8259	DG 22	P	14	ABORTED ON DAY 22 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8260	DG 29	P	14	ALL TISSUES APPEARED NORMAL.
VI 10	8261	DG 26	P	14	ABORTED ON DAY 26 OF GESTATION. LIVER: MOTTLED RED AND TAN. STOMACH: MUCOSAL SURFACE, NUMEROUS BLACK AREAS (PINPOINT TO 0.2 CM IN DIAMETER). ALL OTHER TISSUES APPEARED NORMAL.
	8262	DG 22	P	14	ABORTED ON DAY 22 OF GESTATION. EXTERNAL OBSERVATIONS: NOSE, MASS (1.0 CM X 1.0 CM X 0.3 CM), CUT SURFACE REVEALED SMOOTH PINK MATERIAL.b ALL OTHER TISSUES APPEARED NORMAL.
	8263	DG 29	NP	14	ALL TISSUES APPEARED NORMAL.
	8264	DG 23	P	14	ABORTED ON DAY 23 OF GESTATION. ALL TISSUES APPEARED NORMAL.
	8265	DG 25	P	14	ABORTED ON DAY 25 OF GESTATION. ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 11) for external observations confirmed at necropsy.
b. Confirms a clinical observation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 12 (PAGE 3): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RABBIT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
VII					
20	8266	DG 17	P	11	FOUND DEAD ON DAY 17 OF GESTATION (11 MINUTES AFTER DOSAGE). ESOPHAGUS: WHITE FROTHY MATERIAL. LUNGS: APPEARED INFLATED AND LEFT DIAPHRAGMATIC LOBE, TEAR (2.0 CM IN LENGTH). LIVER: LEFT LATERAL LOBE, TEAR (2.0 CM IN LENGTH). STOMACH: FUNDIC MUCOSA, TWO ERODED AREAS (1.5 CM X 0.5 CM AND 1.5 CM X 0.5 CM). ALL OTHER TISSUES APPEARED NORMAL.
	8267	DG 20	P	13	FOUND DEAD ON DAY 20 OF GESTATION (DEATH OCCURRED OVERNIGHT). ALL TISSUES APPEARED NORMAL.
	8268	DG 17	P	10	FOUND DEAD ON DAY 17 OF GESTATION (22 HOURS AND 27 MINUTES AFTER DOSAGE). ABDOMINAL CAVITY: CLOUDY GREEN FLUID (APPROXIMATELY 18 ML). STOMACH: FRIABLE. GALLBLADDER: RUPTURED. ALL OTHER TISSUES APPEARED NORMAL.
	8269	DG 19	P	13	ABORTED ON DAY 19 OF GESTATION. EXTERNAL OBSERVATIONS: UNGROOMED COAT. ALL OTHER TISSUES APPEARED NORMAL.
	8270	DG 16	P	9	FOUND DEAD ON DAY 16 OF GESTATION (DEATH OCCURRED OVERNIGHT). STOMACH: NON-GLANDULAR MUCOSA, RED AND DIFFUSE. ALL OTHER TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 11) for external observations confirmed at necropsy.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 13 (PAGE 1): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP I				0 (VEHICLE) MG/KG/DAY								
8236 P	3.76	3.64	3.71	3.74	3.75	3.74	3.74	3.80	3.86	3.82	3.86	3.87	3.91
8237 P	3.38	3.47	3.50	3.54	3.57	3.59	3.62	3.68	3.70	3.72	3.72	3.75	3.76
8238 P	3.44	3.49	3.47	3.48	3.50	3.53	3.51	3.61	3.63	3.62	3.66	3.71	3.73
8239 P	3.52	3.64	3.67	3.66	3.76	3.76	3.84	3.91	3.99	3.94	3.96	4.01	4.01
8240 P	3.14	3.20	3.28	3.30	3.29	3.30	3.32	3.37	3.38	3.41	3.43	3.43	3.42

	DAY 19	20	21	22	23	24	25	26	27	28	29
8236 P	3.93	3.94	3.98	4.01	3.97	3.95	3.98	3.96	4.00	4.01	4.04
8237 P	3.82	3.81	3.84	3.85	3.91	3.96	3.98	4.00	4.01	4.03	4.05
8238 P	3.74	3.78	3.75	3.80	3.82	3.78	3.83	3.77	3.74	3.83	3.84
8239 P	4.03	4.03	3.97	4.02	4.04	4.08	4.12	4.12	4.18	4.21	4.23
8240 P	3.46	3.48	3.50	3.50	3.54	3.56	3.56	3.52	3.61	3.58	3.64

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 13 (PAGE 2): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP II				0.1 MG/KG/DAY								
8241 P	3.68	3.53	3.62	3.62	3.66	3.70	3.71	3.75	3.77	3.85	3.89	3.88	3.89
8242 NP	3.36	3.49	3.60	3.56	3.57	3.64	3.62	3.68	3.70	3.71	3.81	3.78	3.77
8243 P	3.42	3.49	3.58	3.63	3.54	3.51	3.49	3.58	3.64	3.70	3.74	3.73	3.77
8244 P	3.20	3.27	3.30	3.32	3.35	3.34	3.39	3.47	3.53	3.53	3.58	3.62	3.59
8245 P	2.98	3.17	3.18	3.19	3.18	3.13	3.19	3.18	3.19	3.17	3.17	3.23	3.24

	DAY 19	20	21	22	23	24	25	26	27	28	29
8241 P	3.95	3.96	3.99	4.01	4.00	4.00	3.94	3.88	3.82	3.78	3.82
8242 NP	3.82	3.88	3.88	3.88	3.91	3.92	3.91	3.90	3.96	4.03	4.02
8243 P	3.78	3.84	3.79	3.78	3.86	3.85	3.88	3.90	3.92	3.90	3.94
8244 P	3.63	3.63	3.66	3.68	3.72	3.75	3.79	3.77	3.78	3.70	3.65
8245 P	3.30	3.33	3.40	3.39	3.40	3.45	3.48	3.47	3.50	3.56	3.59

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 13 (PAGE 3): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP III				1 MG/KG/DAY								
8246 NP	3.57	3.63	3.76	3.76	3.79	3.80	3.84	3.86	3.87	3.86	3.94	3.94	3.97
8247 P	3.29	3.57	3.63	3.62	3.65	3.63	3.74	3.76	3.79	3.80	3.82	3.88	3.92
8248 P	3.46	3.63	3.64	3.63	3.68	3.68	3.67	3.76	3.87	3.94	3.94	3.94	3.93
8249 P	3.52	3.61	3.69	3.69	3.71	3.75	3.81	3.87	3.88	3.90	3.92	3.96	3.99
8250 P	3.11	3.15	3.17	3.22	3.25	3.32	3.26	3.27	3.30	3.34	3.34	3.34	3.39

	DAY 19	20	21	22	23	24	25	26	27	28	29
8246 NP	4.02	4.05	4.03	4.06	4.05	4.09	4.11	4.11	4.16	4.19	4.21
8247 P	3.93	3.94	3.90	3.93	3.96	3.98	4.03	4.01	4.06	4.12	4.16
8248 P	3.97	3.99	4.04	4.06	4.10	4.14	4.23	4.21	4.24	4.29	4.35
8249 P	4.04	4.00	4.02	4.05	4.09	4.12	4.17	4.11	4.12	4.12	4.13
8250 P	3.38	3.41	3.37	3.44	3.53	3.55	3.60	3.54	3.58	3.59	3.60

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 13 (PAGE 4): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP IV				2.5 MG/KG/DAY								
8251 P	3.62	3.80	3.80	3.81	3.86	3.88	3.94	4.01	4.05	4.08	4.11	4.09	4.11
8252 P	3.31	3.55	3.56	3.56	3.60	3.64	3.67	3.73	3.64	3.75	3.72	3.76	3.79
7650 P	4.05	4.12	4.18	4.18	4.16	4.20	4.23	4.31	4.29	4.30	4.27	4.25	4.20
8254 P	3.47	3.52	3.56	3.55	3.59	3.58	3.54	3.50	3.46	3.39	3.33	3.26	3.36
8255 P	3.00	3.12	3.12	3.14	3.14	3.20	3.23	3.20	3.25	3.34	3.28	3.29	3.29

	DAY 19	20	21	22	23	24	25	26	27	28	29
8251 P	4.13	4.19	4.20	4.24	4.27	4.29	4.32	4.31	4.34	4.40	4.41
8252 P	3.84	3.83	3.82	3.89	3.88	3.93	3.94	3.92	3.94	3.93	3.94
7650 P	4.16	4.14	4.11	4.08	4.09	4.07	4.14	4.15	4.21	4.26	4.28
8254 P	3.17	3.13	3.19	3.16	3.30	3.37	3.40	3.40	3.46	3.47	3.48
8255 P	3.29	3.29	3.33	3.35	3.35	3.40	3.41	3.39	3.47	3.42	3.54

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 13 (PAGE 5): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP V				5 MG/KG/DAY								
8256 P	3.93	3.90	3.94	4.01	4.04	4.02	3.97	4.01	3.91	3.82	3.77	3.71	3.71
8257 P	3.27	3.44	3.40	3.40	3.34	3.33	3.30	3.25	3.23	3.14	3.12	3.15	3.10
8258 NP	3.40	3.44	3.50	3.48	3.46	3.43	3.42	3.35	3.30	3.24	3.20	3.16	3.13
8259 P	3.22	3.29	3.33	3.35	3.34	3.42	3.37	3.35	3.21	3.17	3.15	3.06	3.06
8260 P	3.02	3.14	3.12	3.19	3.15	3.18	3.12	3.14	3.05	2.97	2.95	2.90	2.92

	DAY 19	20	21	22	23	24	25	26	27	28	29
8256 P	3.66	3.64	3.66	3.72	3.87	3.94	3.95	3.92	3.98	4.01	4.04
8257 P	3.08	3.03	2.99	2.94	ABORTED ON DAY 23 OF GESTATION						
8258 NP	3.12	2.98	2.93	2.88	2.83	2.80	2.76	2.69	2.69	2.67	2.62
8259 P	2.98	2.98	2.95	ABORTED ON DAY 22 OF GESTATION							
8260 P	2.93	2.84	2.80	2.85	2.93	3.00	3.10	3.08	3.18	3.10	3.11

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 13 (PAGE 6): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP VI				10 MG/KG/DAY								
8261 P	3.77	3.94	3.92	3.95	3.87	3.73	3.64	3.68	3.60	3.54	3.48	3.44	3.46
8262 P	3.33	3.35	3.40	3.41	3.28	3.18	3.10	3.04	2.98	2.97	2.97	2.96	2.90
8263 NP	3.42	3.45	3.51	3.52	3.41	3.29	3.24	3.17	3.15	3.08	3.00	2.92	2.90
8264 P	3.26	3.52	3.60	3.57	3.50	3.42	3.33	3.27	3.22	3.17	3.15	3.10	3.08
8265 P	2.77	3.00	3.02	2.94	2.86	2.81	2.69	2.64	2.59	2.54	2.54	2.51	2.41

	DAY 19	20	21	22	23	24	25	26	27	28	29
8261 P	3.39	3.39	3.36	3.34	3.28	3.24	3.20	3.03	ABORTED ON DAY 26 OF GESTATION		
8262 P	2.82	2.80	2.74	ABORTED ON DAY 22 OF GESTATION							
8263 NP	2.91	2.88	2.83	2.77	2.73	2.68	2.64	2.56	2.57	2.56	2.53
8264 P	3.06	3.04	3.03	2.90	ABORTED ON DAY 23 OF GESTATION						
8265 P	2.40	2.35	2.34	2.30	2.27	2.25	ABORTED ON DAY 25 OF GESTATION				

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 13 (PAGE 7): MATERNAL BODY WEIGHTS - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 0	7	8	9	10	11	12	13	14	15	16	17	18
RABBIT #	DOSAGE GROUP VII				20 MG/KG/DAY								
8266 P	3.59	3.70	3.74	3.61	3.51	3.42	3.34	3.29	3.24	3.16	3.10	2.98	a
8267 P	3.37	3.37	3.37	3.28	3.21	3.18	3.15	3.14	3.13	3.09	3.00	2.92	2.86
8268 P	3.43	3.64	3.48	3.36	3.27	3.19	3.12	3.06	3.00	2.94	2.88	b	
8269 P	3.49	3.75	3.87	3.77	3.67	3.59	3.51	3.45	3.39	3.34	3.26	3.20	3.12
8270 P	3.10	3.26	3.21	3.16	3.06	2.98	2.88	2.80	2.72	2.65	c		

	DAY 19	20	21	22	23	24	25	26	27	28	29
8266 P	FOUND DEAD ON DAY 17 OF GESTATION										
8267 P	2.79	FOUND DEAD ON DAY 20 OF GESTATION									
8268 P	FOUND DEAD ON DAY 17 OF GESTATION										
8269 P	ABORTED ON DAY 19 OF GESTATION										
8270 P	FOUND DEAD ON DAY 16 OF GESTATION										

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G), ROUNDED TO THREE SIGNIFICANT DIGITS AND REPORTED IN KILOGRAMS (KG).
ALL CALCULATIONS EXCEPT BODY WEIGHT AVERAGES ARE PERFORMED WITH THE UNROUNDED GRAM (G) VALUE.
BODY WEIGHT AVERAGES ARE CALCULATED WITH THE ROUNDED KILOGRAM (KG) VALUE.

a. Doe 8266 was found dead on day 17 of gestation.
b. Doe 8268 was found dead on day 17 of gestation.
c. Doe 8270 was found dead on day 16 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 14 (PAGE 1): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP I				0 (VEHICLE) MG/KG/DAY								
8236 P	181.	183.	183.	185.	180.	183.	184.	185.	181.	182.	182.	183.	157.
8237 P	180.	185.	181.	183.	181.	183.	181.	182.	180.	181.	184.	181.	185.
8238 P	184.	185.	181.	185.	184.	185.	180.	183.	182.	181.	182.	180.	182.
8239 P	182.	182.	182.	181.	181.	182.	184.	183.	180.	180.	184.	181.	183.
8240 P	178.	166.	182.	159.	127.	109.	102.	122.	105.	180.	135.	150.	152.

DAYS	20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
8236 P	184.	151.	106.	75.	82.	92.	96.	108.	121.
8237 P	184.	185.	185.	184.	182.	170.	119.	124.	125.
8238 P	180.	182.	180.	185.	184.	182.	180.	184.	185.
8239 P	183.	182.	180.	182.	184.	182.	170.	151.	180.
8240 P	133.	120.	111.	114.	118.	97.	116.	71.	125.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 14 (PAGE 2): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP II				0.1 MG/KG/DAY								
8241 P	181.	184.	182.	182.	183.	167.	182.	181.	182.	183.	184.	184.	183.
8242 NP	185.	184.	182.	181.	184.	181.	181.	180.	181.	184.	182.	180.	183.
8243 P	181.	165.	98.	126.	102.	123.	125.	185.	a	184.	183.	185.	182.
8244 P	180.	182.	183.	181.	182.	184.	180.	184.	182.	181.	182.	180.	185.
8245 P	138.	143.	141.	93.	118.	89.	124.	93.	61.	136.	182.	181.	178.

	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
8241 P	185.	160.	127.	78.	45.	15.	4.	8.	41.
8242 NP	181.	180.	180.	182.	185.	181.	185.	185.	185.
8243 P	185.	181.	182.	181.	184.	152.	124.	83.	132.
8244 P	180.	a	180.	182.	163.	104.	123.	58.	8.
8245 P	182.	153.	173.	183.	182.	145.	140.	135.	152.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 14 (PAGE 3): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP III				1 MG/KG/DAY								
8246 NP	181.	182.	183.	181.	183.	185.	185.	181.	180.	180.	182.	184.	185.
8247 P	181.	182.	181.	182.	181.	168.	182.	181.	180.	180.	181.	180.	180.
8248 P	182.	181.	182.	181.	181.	182.	185.	183.	169.	180.	180.	184.	184.
8249 P	185.	181.	185.	184.	180.	182.	185.	183.	180.	181.	184.	181.	185.
8250 P	180.	180.	182.	182.	107.	124.	131.	183.	145.	145.	176.	152.	168.

	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
8246 NP	185.	182.	183.	182.	184.	183.	184.	183.	181.
8247 P	183.	182.	183.	183.	181.	173.	181.	174.	183.
8248 P	184.	182.	176.	175.	167.	166.	181.	152.	181.
8249 P	182.	182.	167.	146.	143.	150.	115.	115.	126.
8250 P	182.	181.	175.	153.	138.	114.	113.	93.	90.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 14 (PAGE 4): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS DAYS	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP IV				2.5 MG/KG/DAY								
8251 P	183.	180.	180.	182.	184.	184.	183.	185.	182.	180.	182.	184.	181.
8252 P	184.	181.	183.	183.	182.	180.	126.	184.	180.	183.	181.	183.	183.
7650 P	185.	182.	182.	184.	183.	184.	167.	181.	110.	102.	76.	58.	47.
8254 P	180.	181.	176.	153.	122.	43.	17.	1.	2.	0.	2.	0.	0.
8255 P	182.	179.	183.	166.	164.	148.	162.	180.	169.	166.	165.	168.	172.

	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
8251 P	168.	180.	182.	182.	181.	181.	163.	160.	183.
8252 P	183.	152.	152.	148.	161.	148.	148.	141.	181.
7650 P	37.	30.	30.	10.	94.	123.	161.	155.	169.
8254 P	11.	38.	100.	130.	117.	84.	102.	93.	113.
8255 P	162.	158.	134.	156.	166.	153.	142.	115.	149.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 14 (PAGE 5): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP V				5 MG/KG/DAY								
8256 P	185.	183.	182.	168.	137.	123.	36.	0.	1.	0.	45.	13.	40.
8257 P	117.	144.	131.	115.	66.	9.	1.	0.	3.	1.	3.	0.	3.
8258 NP	182.	182.	169.	134.	84.	13.	1.	0.	0.	0.	2.	0.	1.
8259 P	184.	180.	182.	173.	56.	21.	0.	0.	0.	2.	2.	1.	0.
8260 P	182.	166.	185.	142.	44.	7.	0.	0.	4.	5.	6.	57.	11.

	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
8256 P	79.	133.	181.	184.	185.	184.	185.	181.	185.
8257 P	7.	7.	14.	ABORTED ON DAY 23 OF GESTATION					
8258 NP	2.	3.	1.	0.	0.	1.	4.	1.	1.
8259 P	2.	ABORTED ON DAY 22 OF GESTATION							
8260 P	0.	55.	62.	113.	169.	180.	181.	182.	183.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 14 (PAGE 6): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP VI			10 MG/KG/DAY									
8261 P	181.	171.	86.	2.	0.	3.	0.	0.	0.	1.	0.	1.	2.
8262 P	180.	145.	28.	1.	0.	2.	0.	2.	0.	0.	0.	1.	0.
8263 NP	180.	164.	28.	2.	1.	2.	0.	0.	0.	1.	0.	1.	0.
8264 P	179.	128.	93.	0.	24.	2.	0.	4.	0.	4.	0.	0.	0.
8265 P	121.	30.	0.	3.	0.	2.	0.	1.	1.	2.	5.	0.	0.

	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
8261 P	1.	5.	0.	1.	1.	ABORTED ON DAY 26 OF GESTATION			
8262 P	0.	ABORTED ON DAY 22 OF GESTATION							
8263 NP	0.	2.	1.	0.	2.	4.	3.	0.	0.
8264 P	0.	2.	1.	ABORTED ON DAY 23 OF GESTATION					
8265 P	0.	1.	0.	3.	1.	ABORTED ON DAY 25 OF GESTATION			

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 14 (PAGE 7): MATERNAL FEED CONSUMPTION VALUES - INDIVIDUAL DATA

PREGNANCY STATUS	DAYS 7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20
RABBIT #	DOSAGE GROUP VII				20 MG/KG/DAY								
8266 P	143.	12.	0.	1.	2.	0.	0.	2.	2.	0.	a		
8267 P	124.	5.	3.	0.	0.	1.	0.	2.	1.	0.	1.	1.	b
8268 P	71.	4.	0.	2.	0.	0.	1.	2.	1.	c			
8269 P	181.	69.	2.	2.	0.	1.	0.	2.	1.	3.	2.	d	
8270 P	52.	27.	0.	1.	0.	0.	0.	2.	e				

	DAYS 20 - 21	21 - 22	22 - 23	23 - 24	24 - 25	25 - 26	26 - 27	27 - 28	28 - 29
8266 P	FOUND DEAD ON DAY 17 OF GESTATION								
8267 P	FOUND DEAD ON DAY 20 OF GESTATION								
8268 P	FOUND DEAD ON DAY 17 OF GESTATION								
8269 P	ABORTED ON DAY 19 OF GESTATION								
8270 P	FOUND DEAD ON DAY 16 OF GESTATION								

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Doe 8266 was found dead on day 17 of gestation.
b. Doe 8267 was found dead on day 20 of gestation.
c. Doe 8268 was found dead on day 17 of gestation.
d. Doe 8269 aborted on day 19 of gestation.
e. Doe 8270 was found dead on day 16 of gestation.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 15 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

			VIABLE FETUSES			DEAD FETUSES			EARLY RESORPTIONS			LATE RESORPTIONS			IMPLANTATION SITES			CORPORA LUTEA		
	SEX		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT	
RABBIT #	M	F	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	HORN	HORN	TOTAL	OVARY	OVARY	TOTAL
DOSAGE GROUP I					0 (VEHICLE) MG/KG/DAY															
8236	6	4	3	7	10	0	0	0	0	0	0	0	1	1	3	8	11	4	8	12
8237	3	4	4	3	7	0	0	0	0	0	0	0	0	0	4	3	7	4	4	8
8238	6	3	4	5	9	0	0	0	0	0	0	1	0	1	5	5	10	5	6	11
8239	4	4	5	3	8	0	0	0	0	0	0	0	0	0	5	3	8	7	4	11
8240	4	4	5	3	8	0	0	0	0	0	0	0	0	0	5	3	8	6	3	9
DOSAGE GROUP II					0.1 MG/KG/DAY															
8241	4	6	6	4	10	0	0	0	0	0	0	0	0	0	6	4	10	7	5	12
8242	NOT PREGNANT																			
8243	4	6	6	4	10	0	0	0	0	0	0	0	0	0	6	4	10	8	4	12
8244	4	6	5	5	10	0	0	0	0	0	0	0	1	1	5	6	11	6	9	15
8245	2	5	5	2	7	0	0	0	0	0	0	0	0	0	5	2	7	5	3	8
DOSAGE GROUP III					1 MG/KG/DAY															
8246	NOT PREGNANT																			
8247	2	6	2	6	8	0	0	0	0	0	0	0	0	0	2	6	8	3	7	10
8248	4	6	5	5	10	0	0	0	0	0	0	0	0	0	5	5	10	5	6	11
8249	3	4	3	4	7	0	0	0	0	0	0	0	0	0	3	4	7	5	5	10
8250	4	5	5	4	9	0	0	0	0	0	0	0	0	0	5	4	9	5	4	9
DOSAGE GROUP IV					2.5 MG/KG/DAY															
8251	4	6	7	3	10	0	0	0	0	0	0	1	0	1	8	3	11	9	3	12
8252	4	6	2	8	10	0	0	0	0	1	1	0	0	0	2	9	11	4	9	13
7650	5	2	4	3	7	0	0	0	0	0	0	0	0	0	4	3	7	5	5	10
8254	5	3	4	4	8	0	0	0	0	0	0	0	0	0	4	4	8	6	4	10
8255	2	5	6	1	7	0	0	0	0	0	0	0	0	0	6	1	7	7	3	10

M = MALE F = FEMALE
PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 15 (PAGE 2): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

			VIABLE FETUSES		DEAD FETUSES		EARLY RESORPTIONS		LATE RESORPTIONS		IMPLANTATION SITES		CORPORA LUTEA	
	SEX		RIGHT LEFT		RIGHT LEFT		RIGHT LEFT		RIGHT LEFT		RIGHT LEFT		RIGHT LEFT	
RABBIT #	M	F	HORN	TOTAL	HORN	TOTAL	HORN	TOTAL	HORN	TOTAL	HORN	TOTAL	OVARY	TOTAL
DOSAGE GROUP V				5 MG/KG/DAY										
8256	2	2	2 2	4	0 0	0	0 0	5	5 0	0	0 2	7	9 2	8 10
8257	ABORTED ON DAY 23 OF GESTATION													
8258	NOT PREGNANT													
8259	ABORTED ON DAY 22 OF GESTATION													
8260	3	4	2 5	7	0 0	0	0 0	0	0 2	1	3 4	6	10 4	7 11
DOSAGE GROUP VI				10 MG/KG/DAY										
8261	ABORTED ON DAY 26 OF GESTATION													
8262	ABORTED ON DAY 22 OF GESTATION													
8263	NOT PREGNANT													
8264	ABORTED ON DAY 23 OF GESTATION													
8265	ABORTED ON DAY 25 OF GESTATION													
DOSAGE GROUP VII				20 MG/KG/DAY										
8266	FOUND DEAD ON DAY 17 OF GESTATION													
8267	FOUND DEAD ON DAY 20 OF GESTATION													
8268	FOUND DEAD ON DAY 17 OF GESTATION													
8269	ABORTED ON DAY 19 OF GESTATION													
8270	FOUND DEAD ON DAY 16 OF GESTATION													

M = MALE F = FEMALE
PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 16 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - INDIVIDUAL DATA

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			CONCEPTUSES RESORBED		
RABBIT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP I			0 (VEHICLE) MG/KG/DAY						
8236	6	4	10	41.51	44.35	42.64	11	1	9.1
8237	3	4	7	49.42	48.82	49.08	7	0	0.0
8238	6	3	9	35.68	32.03	34.46	10	1	10.0
8239	4	4	8	47.94	49.68	48.80	8	0	0.0
8240	4	4	8	45.74	41.99	43.87	8	0	0.0
DOSAGE GROUP II			0.1 MG/KG/DAY						
8241	4	6	10	31.86	32.38	32.17	10	0	0.0
8242	NOT PREGNANT								
8243	4	6	10	40.24	36.62	38.06	10	0	0.0
8244	4	6	10	43.30	42.58	42.86	11	1	9.1
8245	2	5	7	49.83	50.02	49.97	7	0	0.0
DOSAGE GROUP III			1 MG/KG/DAY						
8246	NOT PREGNANT								
8247	2	6	8	43.46	44.07	43.92	8	0	0.0
8248	4	6	10	45.87	40.83	42.84	10	0	0.0
8249	3	4	7	49.48	46.60	47.84	7	0	0.0
8250	4	5	9	43.88	39.77	41.59	9	0	0.0
DOSAGE GROUP IV			2.5 MG/KG/DAY						
8251	4	6	10	37.63	33.19	34.97	11	1	9.1
8252	4	6	10	36.26	33.94	34.86	11	1	9.1
7650	5	2	7	43.25	48.32	44.70	7	0	0.0
8254	5	3	8	32.69	32.30	32.54	8	0	0.0
8255	2	5	7	47.36	42.44	43.85	7	0	0.0

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 16 (PAGE 2): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - INDIVIDUAL DATA

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			---- CONCEPTUSES ------ RESORBED		
RABBIT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP V			5 MG/KG/DAY						
8256	2	2	4	36.72	23.02	29.87	9	5	55.6
8257	ABORTED ON DAY 23 OF GESTATION								
8258	NOT PREGNANT								
8259	ABORTED ON DAY 22 OF GESTATION								
8260	3	4	7	20.31	23.68	22.23	10	3	30.0
DOSAGE GROUP VI			10 MG/KG/DAY						
8261	ABORTED ON DAY 26 OF GESTATION								
8262	ABORTED ON DAY 22 OF GESTATION								
8263	NOT PREGNANT								
8264	ABORTED ON DAY 23 OF GESTATION								
8265	ABORTED ON DAY 25 OF GESTATION								
DOSAGE GROUP VII			20 MG/KG/DAY						
8266	FOUND DEAD ON DAY 17 OF GESTATION								
8267	FOUND DEAD ON DAY 20 OF GESTATION								
8268	FOUND DEAD ON DAY 17 OF GESTATION								
8269	ABORTED ON DAY 19 OF GESTATION								
8270	FOUND DEAD ON DAY 16 OF GESTATION								

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 17 (PAGE 1): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP I					0 (VEHICLE) MG/KG/DAY														
RABBIT # CLs																			
8236 4/ 8	MA	MA	FA /	FA	MA	MA	MA	MA	FA	FA	L								
	49.46	45.09	46.98	50.29	40.84	41.52	34.50	37.65	40.06	40.06	1.95								
8237 4/ 4	FA	FA	MA	FA /	MA	FA	MA												
	51.77	48.72	51.46	50.21	47.56	44.56	49.25												
8238 5/ 6	FA	MA	MA	MA	FL /	MA	FA	MA	MA	FA									
	28.71	39.23	34.06	29.22	3.43	37.90	34.83	36.81	36.85	32.56									
8239 7/ 4	FA	FA	MA	MA	MA /	FA	MA	FA											
	51.85	48.42	44.55	46.27	53.37	48.22	47.55	50.21											
8240 6/ 3	MA	FA	MA	FA	FA /	MA	MA	FA											
	48.74	46.03	42.81	39.01	41.58	44.95	46.48	41.33											
DOSAGE GROUP II					0.1 MG/KG/DAY														
8241 7/ 5	FA	FA	FA	MA	MA	FA /	FA	FA	MA	MA									
	35.65	35.91	34.09	28.52	29.38	27.65	29.03	31.97	34.81	34.72									
8242	NOT PREGNANT																		
8243a 8/ 4	FA	FA	FA	FA	MA	MA /	MA	FA	FA	MA									
	36.00	44.04	41.86	31.80	35.20	36.12	44.09	27.20	38.79	45.53									
8244b 6/ 9	MA	FA	FA	FA	FA /	MA	FA	MA	MA	FL	FA								
	47.16	48.85	44.45	39.22	32.31	39.16	52.80	51.48	35.38	7.32	37.84								
8245 5/ 3	FA	FA	FA	FA	FA /	MA	MA												
	52.45	48.54	52.22	46.73	50.17	52.15	47.51												

M = MALE F = FEMALE A = ALIVE E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).
a. Fetus 8243-8 had a short snout, and small and displaced nares.
b. Fetus 8244-7 had abdominal distention.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 17 (PAGE 2): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP III					1 MG/KG/DAY														
RABBIT # CLs																			
8246	NOT PREGNANT																		
8247 3/ 7	MA	FA /	FA	FA	FA	FA	FA	MA											
	48.57	46.46	45.73	42.90	45.12	46.63	37.60	38.34											
8248 5/ 6	MA	FA	FA	MA	FA /	MA	FA	FA	FA	MA									
	51.27	39.44	44.56	37.77	37.44	54.03	44.11	42.15	37.26	40.42									
8249 5/ 5	MA	FA	FA /	MA	MA	FA	FA												
	52.58	47.34	42.63	50.08	45.79	49.87	46.58												
8250 5/ 4	MA	MA	FA	MA	FA /	MA	FA	FA	FA										
	46.56	43.22	42.44	40.21	33.62	45.52	38.82	43.04	40.91										
DOSAGE GROUP IV					2.5 MG/KG/DAY														
8251a 9/ 3	MA	L	FA	FA	MA	FA	FA	MA /	FA	MA	FA								
	47.06	1.11	31.11	31.19	31.49	28.19	29.34	40.49	40.44	31.48	38.87								
8252 4/ 9	FA	FA /	MA	FA	MA	FA	MA	FA	E	MA	FA								
	40.31	37.60	39.80	30.92	36.40	30.75	36.62	32.98		32.20	31.05								
7650 5/ 5	MA	FA	MA	MA /	FA	MA	MA												
	55.61	50.54	45.68	49.64	46.11	34.14	31.18												
8254 6/ 4	FA	MA	MA	FA /	MA	FA	MA	MA											
	38.36	37.08	32.34	32.57	38.10	25.98	28.90	27.03											
8255 7/ 3	FA	MA	FA	FA	FA	FA /	MA												
	47.02	46.53	41.50	40.14	38.39	45.17	48.19												

M = MALE F = FEMALE A = ALIVE E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).
a. Fetus 8251-10 had a cleft snout and no incisors.

PROTOCOL 418-012P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
(SPONSOR'S STUDY NUMBER: T-6295.10)

TABLE 17 (PAGE 3): FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DOSAGE GROUP V				5 MG/KG/DAY															
RABBIT # CLs																			
8256 2/ 8	MA	MA /	FA	E	E	E	E	E	FA										
	40.97	32.48	31.20						14.84										
8257	ABORTED ON DAY 23 OF GESTATION																		
8258	NOT PREGNANT																		
8259	ABORTED ON DAY 22 OF GESTATION																		
8260 4/ 7	FA	L	FA	L /	MA	MA	FA	MA	L	FA									
	26.90	4.03	28.15	4.33	19.67	20.02	21.85	21.23	3.13	17.82									
DOSAGE GROUP VI				10 MG/KG/DAY															
8261	ABORTED ON DAY 26 OF GESTATION																		
8262	ABORTED ON DAY 22 OF GESTATION																		
8263	NOT PREGNANT																		
8264	ABORTED ON DAY 23 OF GESTATION																		
8265	ABORTED ON DAY 25 OF GESTATION																		
DOSAGE GROUP VII				20 MG/KG/DAY															
RABBIT # CLs																			
8266	FOUND DEAD ON DAY 17 OF GESTATION																		
8267	FOUND DEAD ON DAY 20 OF GESTATION																		
8268	FOUND DEAD ON DAY 17 OF GESTATION																		
8269	ABORTED ON DAY 19 OF GESTATION																		
8270	FOUND DEAD ON DAY 16 OF GESTATION																		

M = MALE F = FEMALE A = ALIVE E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY FETAL BODY WEIGHTS WERE RECORDED IN GRAMS (G).

ATTACHMENT 1

PROTOCOL

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044
T: (215) 443-8710 F: (215) 443-8587

PROTOCOL 418-012P

SPONSOR'S STUDY NUMBER: T-6295.10

STUDY TITLE: Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of PFOS in Rabbits

PURPOSE: The purpose of this study is to provide information for the selection of dosages to be used in the developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of PFOS administered orally via stomach tube to New Zealand White [Hra:(NZW)SPF] presumed pregnant female rabbits.

TESTING FACILITY: Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1297
Telephone: (215) 443-8710
Telefax: (215) 443-8587

STUDY DIRECTOR: Raymond G. York, Ph.D., DABT
Associate Director of Research

SPONSOR: 3M Toxicology Services
3M Center, Building 220-2E-02
St. Paul, Minnesota 55144-1000

STUDY MONITOR: Marvin T. Case, D.V.M., Ph.D.
Telephone: (612) 733-5180
Telefax: (612) 733-1773

ALTERNATE STUDY MONITOR: Andrew M. Seacat, Ph.D.
Telephone: (612) 575-3161
Telefax: (612) 733-1773

REGULATORY CITATIONS:

U.S. Food and Drug Administration (1994). International Conference on Harmonisation; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.

U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.

Japanese Ministry of Health and Welfare (1997). *Good Laboratory Practice Standard for Safety Studies on Drugs*, MHW Ordinance Number 21, March 26, 1997.

European Economic Community (1989). *Council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice*. Official Journal of the European Communities: Legislation. 32 (No. L 315; 28 October): 1-17.

REGULATORY COMPLIANCE:

This study will be conducted in the spirit of the Good Laboratory Practice (GLP) regulations cited above in that the Testing Facility personnel will adhere to the Standard Operating Procedures for laboratory operations and data collection. The Testing Facility Quality Assurance Unit (QAU) will not audit the protocol, the raw data, the reports or the critical phases of the study.

All changes or revisions of this protocol shall be documented, signed by the Study Director and the Sponsor, dated and maintained with the protocol.

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE:

See ATTACHMENT 1 to the protocol.

TEST ARTICLE AND VEHICLE:

Identification:

Test Article:

Name:	PFOS.
Physical Description:	Light-colored powder.
Lot/Batch Number:	217.
Specific Gravity:	~0.6.
Purity:	98.9%.
Expiration Date:	May 2000.

Information on the identity, composition, strength and purity of the test article is on file with the Sponsor.

Vehicle:

0.5% Tween® 80 in Reversed Osmosis Membrane Processed Deionized Water (R.O. Deionized Water). Supplier and lot identification of Tween® 80 to be documented in the raw data.

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the vehicle that would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

Safety Precautions:

Gloves, mask, appropriate eye protection and a uniform/lab coat are to be worn during formulation preparation and dosage administration. The Material Safety Data Sheet (MSDS) is attached to the protocol (ATTACHMENT 2).

Storage:

Bulk Test Article:	Room temperature.
Vehicle Components:	Room temperature.
Prepared Vehicle:	Room temperature.
Prepared Formulations:	Frozen (-20°C).

All test article shipments to the Testing Facility should be addressed to the attention of Julian Gulbinski, Manager of Formulations, at the previously cited address and telephone number.

Shipments should include information concerning storage conditions and shipping cartons should be labeled appropriately. The recipient should be notified in advance of shipment.

FORMULATION:

Frequency of Preparation:

Formulations (suspensions) will be prepared daily at the Testing Facility. Vehicle will be prepared weekly at the Testing Facility.

Detailed preparation procedures are attached to this protocol (ATTACHMENT 3).

Adjustment for Purity:

The test article will be considered 100% pure for the purpose of dosage calculations.

Testing Facility Reserve Samples:

The Sponsor will reserve a sample (1 g) of each lot of the bulk test article used during the course of this study. The Testing Facility will reserve a sample (5 mL) of each lot of the vehicle components used during the course of this study. Samples will be stored under the previously cited conditions.

ANALYSES:

Samples additional to those described below may be taken if deemed necessary during the course of the study.

Bulk Test Article Sampling:

No analyses of the bulk test article will be conducted during the course of this study. Information on the stability of the bulk test article is on file with the Sponsor.

Analyses of Prepared Formulations:

At the request of the Sponsor, no analyses of prepared test article formulations will be conducted during the course of the study. However, records will be maintained to document how the test article formulations were prepared.

DISPOSITION:

Prepared formulations will be discarded at the Testing Facility. All remaining bulk test article will be returned to the Study Monitor at the previously cited address.

TEST SYSTEM:

Species/Strain and Reason for Selection:

The New Zealand White [Hra:(NZW)SPF] rabbit was selected as the Test System because: 1) it is one non-rodent mammalian species accepted and widely used throughout the industry for nonclinical studies of developmental toxicity (embryo-fetal toxicity/teratogenicity); 2) this strain of rabbit has been demonstrated to be sensitive to developmental toxins; 3) historical data and experience exist at the Testing Facility[1-3]; and 4) the test article is pharmacologically active in the species and strain.

Number and Sex:

Population evaluated: 35 timed-pregnant female rabbits (5 per dosage group).

Body Weight and Age:

The individual body weights of the female rabbits will range from 2.5 kg to 5.5 kg; the rabbits will be approximately five to seven months of age at the time of study assignment. Actual body weights recorded at receipt and at study assignment will be documented in the raw data.

Source:

Covance Research Products, Inc.
Swampbridge Road, Box 7200
Denver, Pennsylvania 17517

The rabbits will be shipped in filtered cartons by truck from Covance Research Products, Inc., Denver, Pennsylvania, to the Testing Facility.

Identification:

Rabbits are permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20103). Female rabbits are given unique permanent identification numbers when assigned to the study on the basis of day 0 of presumed gestation body weights.

ANIMAL HUSBANDRY:

All cage sizes are in compliance with the *Guide for the Care and Use of Laboratory Animals*[4].

Housing:

The rabbits will be individually housed in units of six to eight stainless steel cages. No nesting materials will be supplied because the female rabbits will be sacrificed before parturition is expected.

Room Air, Temperature and Humidity:

The animal room is independently supplied with at least ten changes per hour of 100% fresh air that has been passed through 99.97% HEPA filters. Room temperature will be maintained at 61°F (16°C) to 72°F (22°C) and monitored constantly. Room humidity will also be monitored constantly and maintained at 30% to 70%.

Light:

An automatically controlled 12-hour light:12-hour dark fluorescent light cycle will be maintained. Each dark period will begin at 1900 hours EST.

Diet:

Approximately 150 g of Certified Rabbit Chow® #5322 (PMI Nutrition International) will be available to each rabbit each day until the first day of dosage, at which time approximately 180 g of the same certified feed will be offered to each rabbit each day. The certified feed will be available from individual, stainless steel, "J-type" feeders attached to each cage.

Water:

Water will be available *ad libitum* from individual bottles attached to the cages or from an automatic watering access system. All water will be from a local source and passed through a reverse osmosis membrane before use. Chlorine will be added to the processed water as a bacteriostat; processed water is expected to contain no more than 1.2 ppm chlorine at the time of analysis. Water is analyzed monthly for possible bacterial contamination and twice annually for possible chemical contamination.

Contaminants:

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the certified diet or in the drinking water at levels that would interfere with the results of this study. Therefore, no analyses other than those routinely performed by the feed supplier or those mentioned in this protocol will be conducted.

MATING AND RANDOMIZATION:

The female rabbits will be naturally bred at the Supplier by breeder male rabbits of the same source and strain before shipment to the Testing Facility. The day mating occurs will be designated day 0 of presumed gestation. The rabbits will be shipped to the Testing Facility after mating, to arrive on day 1 of presumed gestation. Before shipment of the rabbits, the Supplier will forward breeding records and day 0 of presumed gestation body weights. A computer-generated (weight-ordered) randomization procedure will be used to assign the rabbits to dosage groups based on this information.

ADMINISTRATION:

Route and Reason for Choice:

The oral (stomach tube) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one of the possible routes of human exposure.

Method and Frequency:

Female rabbits will be given the test article once daily on days 7 through 20 of presumed gestation. Dosages will be adjusted daily for body weight changes and given at approximately the same time each day.

Rationale for Dosage Selection:

Dosages will be selected by the Sponsor on the basis of previous studies conducted with the test article.

Dosage Levels, Concentrations and Volumes:

Dosage Group	Number of Animals	Dosage (mg/kg/day)	Concentration (mg/mL)	Volume (mL/kg)	Argus Batch Number
I	5	0 (Vehicle)	0	10	B-418-012P-A(Day.Month.Year)
II	5	0.1	0.01	10	B-418-012P-B(Day.Month.Year)
III	5	1	0.1	10	B-418-012P-C(Day.Month.Year)
IV	5	2.5	0.25	10	B-418-012P-D(Day.Month.Year)
V	5	5	0.5	10	B-418-012P-E(Day.Month.Year)
VI	5	10	1	10	B-418-012P-F(Day.Month.Year)
VII	5	20	2	10	B-418-012P-G(Day.Month.Year)

The test article will be considered 100% pure for the purpose of dosage calculations.

TESTS, ANALYSES AND MEASUREMENTS:

Viability:

All Periods: At least twice daily.

Clinical Observations and/or General Appearance:

Predosage Period: At least once.

Dosage Period: Twice daily. Prior to dosage administration and once approximately one hour postdosage.

Postdosage Period: Once daily.

Clinical observations may be recorded more frequently than cited above, if deemed appropriate by the Study Director and/or Study Monitor.

Body Weights:

Predosage Period: Day 0 of presumed gestation and on the day of arrival at the Testing Facility.

Dosage Period: Daily.

Postdosage Period: Daily.

Feed Consumption Values:

Predosage Period:	Recorded daily after arrival at the Testing Facility (values not tabulated).
Dosage Period:	Recorded daily.
Postdosage Period:	Recorded daily.

Feed consumption values during the dosage period will be tabulated for the same intervals as body weight evaluations.

Caesarean-Sectioning Observations:

Rabbits will be Caesarean-sectioned on day 29 of presumed gestation. The fetuses will be removed from the uterus and placed in individual containers. The rabbits will be examined for number and distribution of:

Corpora Lutea.

Implantation Sites.
[Placentae that appear abnormal (size, color or shape) will be noted in the raw data.]

Live and Dead Fetuses.
(A live fetus is defined as one that responds to stimuli; a dead fetus is defined as a term fetus that does not respond to stimuli and that is not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis are considered to be late resorptions.)

Early and Late Resorptions.
(A conceptus is defined as a late resorption if it is grossly evident that organogenesis has occurred; if this is not the case, the conceptus is identified as an early resorption.)

Fetal Observations:

Body Weights:

The body weight of each fetus will be recorded. Only body weights of live fetuses will be used to determine litter fetal body weight averages.

Gross External Alterations:

All fetuses will be examined for gross external alterations. Late resorptions and dead fetuses also will be examined for gross external alterations to the extent possible but such observations will not be included in either data summarization or statistical analyses. Fetuses with gross external alterations will be preserved in neutral buffered 10% formalin. All other fetuses will be discarded.

Representative photographs of fetal gross alterations will be taken.

Sex:

All fetuses will be examined internally to determine sex.

METHOD OF SACRIFICE:

Beuthanasia®-D Special (manufactured by Schering-Plough Animal Health) will be used to sacrifice rabbits (via intravenous injection) and live fetuses (via intraperitoneal injection).

NECROPSY:

Gross lesions will be retained in neutral buffered 10% formalin for possible future evaluation (corresponding tissues will be retained from rabbits in the vehicle control group at the discretion of the Study Director). (Exception: Parovarian cysts will be discarded; these are common, spontaneous lesions in rabbits.) Unless specifically cited below, all other tissues will be discarded.

Scheduled Sacrifice:

On day 29 of presumed gestation, female rabbits will be Caesarean-sectioned, and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. Uteri of apparently nonpregnant does will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5].

Rabbits Found Dead or Moribund:

Rabbits that die or are sacrificed because of moribund condition, abortion or premature delivery will be examined for the cause of death or moribund condition on the day the observation is made. Pregnancy status and uterine contents will be recorded. Aborted fetuses and/or delivered pups will be examined to the extent possible, using the same methods described for fetuses. Uteri of apparently nonpregnant does will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5].

STATISTICAL EVALUATION:

Averages and percentages will be calculated. Litter values will be used where appropriate. Additional procedures and/or analyses may be performed if deemed appropriate.

DATA ACQUISITION, VERIFICATION AND STORAGE:

Data will be hand- and/or computer-recorded. Records will be reviewed by the Study Director and/or appropriate management personnel within 21 days after generation. All original records will be stored in the archives of the Testing Facility. All original data will be bound and indexed. A copy of all raw data will be supplied to the Sponsor upon request. Preserved tissues will be stored at the Testing Facility at no charge for one year after mailing of the draft final report, after which time the Sponsor will be contacted to determine the disposition of these materials.

RECORDS TO BE MAINTAINED:

Protocol and Amendments.
Test Article, Vehicle and/or Reagent Receipt, Preparation and Use.
Animal Acquisition.
Randomization Schedules.
Veterinarian Examination.
Mating History.
Treatment (if prescribed by Staff Veterinarian).
General Comments.
Clinical Observations and/or General Appearance.
Body Weights.
Feed Consumption Values.
Caesarean-Sectioning and Fetal Observations.
Gross Necropsy Observations.
Organ Weights (if required).
Photographs (if required).
Study Maintenance (room and environmental records).
Feed and Water Analyses.
Packing and/or Shipment Lists.

KEY PERSONNEL:

Executive Director of Research: Mildred S. Christian, Ph.D., ATS
Director of Research: Alan M. Hoberman, Ph.D., DABT
Associate Director of Research and Study Director: Raymond G. York, Ph.D., DABT
Director of Laboratory Operations: John F. Barnett, B.S.
Manager of Study Coordination: Valerie A. Sharper, M.S.
Manager of Animal Operations and Member, Institutional Animal Care and Use Committee: Dena C. Lebo, V.M.D.
Manager of Regulatory Compliance: Kathleen A. Moran, M.S.
Consultant, Veterinary Pathology: W. Ray Brown, D.V.M., Ph.D., ACVP

REPORT:

A letter report for the purpose of dosage selection for the full study will be prepared immediately following completion of the in-life phase.

A summary report will be prepared including: abstract, summaries of the methods, results and conclusion; table of contents; copy of the protocol; amendments; summary and individual tables; and reports of supporting data (if appropriate). The report will be included as an appendix to the full study report. The Sponsor will receive one copy of the draft report and two copies of the final report.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE STATEMENT:

The procedures described in this protocol have been reviewed by the Testing Facility's Institutional Animal Care and Use Committee. All procedures described in this protocol that involve study animals will be conducted in a manner to avoid or minimize discomfort, distress or pain to the animals.

The Sponsor's signature below documents the fact that information concerning the necessity for conducting this study and the fact that this is not an unnecessarily duplicative study may be obtained from the Sponsor. No alternative (*in vitro*) procedures were available for meeting the stated purposes of the study.

REFERENCES:

1. Christian, M.S., Hoberman, A.M. and Smith, T.H.F. (1982). Dosage-range study of the teratogenic potential of suspensions of trinitrofluorenone (TNF) administered orally to New Zealand White rabbits. Toxicologist 2(1):40 (#143).

2. Christian, M.S. (1984). Reproductive toxicity and teratology evaluations of naltrexone (Proceedings of Naltrexone Symposium, New York Academy of Sciences, November 7, 1983), J. Clin. Psychiat. 45(9):7-10.

3. Feussner, E.L., Lightkep, G.E., Hennesy, R.A., Hoberman, A.M. and Christian, M.S. (1992). A decade of rabbit fertility data: Study of historical control animals. Teratology 46(4):349-365.

4. Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, D.C.

5. Salewski, E. (1964). Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Arch. Pathol. Exp. Pharmakol. 247:367.

PROTOCOL APPROVAL:

FOR THE TESTING FACILITY

George E. Dearlove — 17-JUN-98

George E. Dearlove, Ph.D., DABT
Associate Director of Research
Date

Raymond G. York — 17-JUN-98

Raymond G. York, Ph.D., DABT
Associate Director of Research
Study Director
Date

Dena C. Lebo — 17 Jun 98

Dena C. Lebo, V.M.D.
Member, Institutional Animal Care and Use Committee
Date

FOR THE SPONSOR

Marvin T. Case — 19 Jan 1998

Marvin T. Case, D.V.M., Ph.D.
Study Monitor
Date

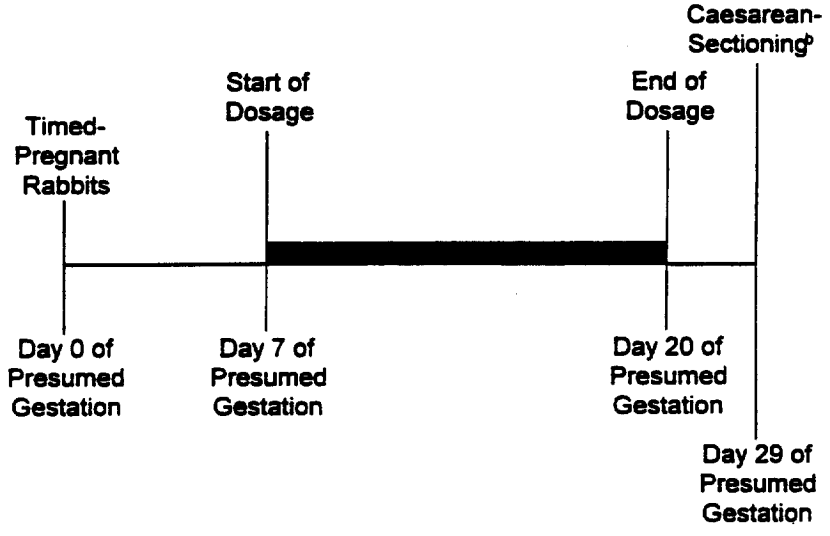
ATTACHMENT 1

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE

ATTACHMENT 1

STUDY SCHEMATIC

DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY[a]



Dosage Period.

a. For additional details see "Tests, Analyses and Measurements" section of the protocol.

b. Fetal evaluations (all fetuses - external examinations).

SCHEDULE[a]

26 JUN 98	Animals Arrive - Acclimation Begins.
02 JUL 98 - 15 JUL 98	Dosage Period (Days 7 through 20 of presumed gestation).
24 JUL 98	Caesarean-Sectioning Period (Day 29 of presumed gestation).
31 JUL 98	Letter Report.
08 OCT 98	Summary Report.

---

a. The study initiation date is the date the Study Director signs the protocol.

ATTACHMENT 2

MATERIAL SAFETY DATA SHEET

PFOS

MATERIAL SAFETY DATA SHEET

3M
3M Center
St. Paul, Minnesota
55144-1000
1-800-364-3577 or (612) 737-6501 (24 hours)

Copyright, 1998, Minnesota Mining and Manufacturing Company.
All rights reserved. Copying and/or downloading of this information for the purpose of properly utilizing 3M products is allowed provided that:
1) the information is copied in full with no changes unless prior agreement is obtained from 3M, and
2) neither the copy nor the original is resold or otherwise distributed with the intention of earning a profit thereon.

DIVISION: 3M CHEMICALS
TRADE NAME:
FC-95 FLUORAD Brand Fluorochemical Surfactant
ID NUMBER/U.P.C.:
98-0207-0103-7 00-51135-09054-1 98-0207-0104-5 00-51135-09055-8
98-0211-0888-5 00-51135-09362-7 98-0211-3916-1 00-51135-02311-2
ZF-0002-1044-1 - - -
ISSUED: January 29, 1998
SUPERSEDES: November 05, 1997
DOCUMENT: 10-3796-9

1. INGREDIENT

INGREDIENT	C.A.S. NO.	PERCENT
POTASSIUM PERFLUOROALKYL SULFONATE.....	2795-39-3	82 - 86
POTASSIUM PERFLUOROALKYL SULFONATE.....	3871-99-6	3 - 8
POTASSIUM PERFLUOROALKYL SULFONATE.....	29420-49-3	3 - 7
POTASSIUM PERFLUOROALKYL SULFONATE.....	60270-55-5	2 - 6
POTASSIUM PERFLUOROALKYL SULFONATE.....	3872-25-1	1 - 3

2. PHYSICAL DATA

BOILING POINT:..... N/A
VAPOR PRESSURE:..... N/A
VAPOR DENSITY:..... N/A
EVAPORATION RATE:..... N/A
SOLUBILITY IN WATER:..... slight
SPECIFIC GRAVITY:..... ca. 0.6 Water=1
(Bulk)
PERCENT VOLATILE:..... 0 %
pH:..... 7 - 8
(0.1% Aqueous)
VISCOSITY:..... N/D
MELTING POINT:..... N/D

APPEARANCE AND ODOR:
Light colored, free flowing powder.

Abbreviations: N/D - Not Determined N/A - Not Applicable CA - Approximately

3. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT:..... None
FLAMMABLE LIMITS - LEL:..... N/A
FLAMMABLE LIMITS - UEL:..... N/A
AUTOIGNITION TEMPERATURE:..... N/A

EXTINGUISHING MEDIA:
Water, Carbon dioxide, Dry chemical, Foam

SPECIAL FIRE FIGHTING PROCEDURES:
Wear full protective clothing, including helmet, self-contained, positive pressure or pressure demand breathing apparatus, bunker coat and pants, bands around arms, waist and legs, face mask, and protective covering for exposed areas of the head.

UNUSUAL FIRE AND EXPLOSION HAZARDS:
See Hazardous Decomposition section for products of combustion.

4. REACTIVITY DATA

STABILITY: Stable

INCOMPATIBILITY - MATERIALS/CONDITIONS TO AVOID:
Not applicable.

HAZARDOUS POLYMERIZATION: Hazardous polymerization will not occur.

HAZARDOUS DECOMPOSITION PRODUCTS:
Carbon Monoxide and Carbon Dioxide, Oxides of Sulfur, Hydrogen Fluoride, Toxic Vapors, Gases or Particulates.

5. ENVIRONMENTAL INFORMATION

SPILL RESPONSE:
Observe precautions from other sections. Vacuum, use wet sweeping compound or water to avoid dusting. CAUTION! A vacuum cleaner could be an ignition source. Clean up residue with water. Place in an approved metal container. Seal the container.

RECOMMENDED DISPOSAL:
Do not release to waterways or sewer. Do not use in products or processes that could result in aquatic concentrations greater than 1/10 of the lowest EC50 or LC50 concentration. Incinerate in an industrial or commercial facility in the presence of a combustible material. Combustion products will include HF. Disposal alternative: Dispose of waste product in a facility permitted to

5. ENVIRONMENTAL INFORMATION (continued)

accept chemical waste.

ENVIRONMENTAL DATA:
96-Hr. Aquatic Fish LC50, Fathead Minnow(Pimephales promelas)=38 mg/l, Bluegill Sunfish(Lepomis macrochirus)=68 mg/l, Rainbow Trout(Salmo gairdneri)=11 mg/l; 48-Hr. EC50, Daphnia Magna = 50 mg/l; COD=.004 g/g; BOD20 = Nil.

REGULATORY INFORMATION:
Volatile Organic Compounds: N/A.
VOC Less H2O & Exempt Solvents: N/A.

Since regulations vary, consult applicable regulations or authorities before disposal. U.S. EPA Hazardous Waste Number = None (Not U.S. EPA Hazardous).

This product complies with the chemical registration requirements of TSCA, EINECS, CDSL, AICS, MITI and Korea.

EPCRA HAZARD CLASS:
FIRE HAZARD: No PRESSURE: No REACTIVITY: No ACUTE: Yes CHRONIC: Yes

6. SUGGESTED FIRST AID

EYE CONTACT:
Immediately flush eyes with large amounts of water for at least 15 minutes. Get immediate medical attention.

SKIN CONTACT:
Immediately flush skin with large amounts of water. Remove contaminated clothing. If irritation persists, call a physician. Wash contaminated clothing before reuse.

INHALATION:
If signs/symptoms occur, remove person to fresh air. If signs/symptoms continue, call a physician.

IF SWALLOWED:
Drink two glasses of water. Call a physician.

7. PRECAUTIONARY INFORMATION

EYE PROTECTION:
Avoid eye contact. Wear vented goggles.

7. PRECAUTIONARY INFORMATION (continued)

SKIN PROTECTION:
Avoid skin contact. Wear appropriate gloves when handling this material. A pair of gloves made from the following material(s) are recommended: butyl rubber. Use one or more of the following personal protection items as necessary to prevent skin contact: head covering, coveralls. Protective garments (other than gloves) should be made of either of the following materials: polyethylene/polyvinylidene chloride (Saranex).

RECOMMENDED VENTILATION:
Use with appropriate local exhaust ventilation. Use in a well-ventilated area. Provide sufficient ventilation to maintain emissions below recommended exposure limits. If exhaust ventilation is not adequate, use appropriate respiratory protection.

RESPIRATORY PROTECTION:
Avoid breathing of dust. Select one of the following NIOSH approved respirators based on airborne concentration of contaminants and in accordance with OSHA regulations: half-mask dust and mist respirator, half-mask supplied air respirator, full-face dust and mist respirator, full-face supplied air respirator.

PREVENTION OF ACCIDENTAL INGESTION:
Do not eat, drink or smoke when using this product. Wash exposed areas thoroughly with soap and water. Wash hands after handling and before eating.

RECOMMENDED STORAGE:
Keep container dry. Keep container closed when not in use.

FIRE AND EXPLOSION AVOIDANCE:
Nonflammable.

OTHER PRECAUTIONARY INFORMATION:
No smoking: Smoking while using this product can result in contamination of the tobacco and/or smoke and lead to the formation of the hazardous decomposition products mentioned in section 4 of this MSDS.

HMIS HAZARD RATINGS: HEALTH: 2 FLAMMABILITY: 0 REACTIVITY: 0
PERSONAL PROTECTION: X (See precautions, section 7.)

EXPOSURE LIMITS

INGREDIENT	VALUE	UNIT	TYPE	AUTH	SKIN*
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y

Abbreviations: N/D - Not Determined N/A - Not Applicable CA - Approximately

EXPOSURE LIMITS (continued)

INGREDIENT	VALUE	UNIT	TYPE	AUTH	SKIN*
POTASSIUM PERFLUOROALKYL SULFONATE...	0.1	MG/M3	TWA	3M	Y

* SKIN NOTATION: Listed substances indicated with 'Y' under SKIN refer to the potential contribution to the overall exposure by the cutaneous route including mucous membrane and eye, either by airborne or, more particularly, by direct contact with the substance. Vehicles can alter skin absorption.

SOURCE OF EXPOSURE LIMIT DATA:
- 3M: 3M Recommended Exposure Guidelines

8. HEALTH HAZARD DATA

EYE CONTACT:
Mild Eye Irritation: signs/symptoms can include redness, swelling, pain, and tearing.

SKIN CONTACT:
Mild Skin Irritation (after prolonged or repeated contact): signs/symptoms can include redness, swelling, and itching.

May be absorbed through the skin and persist in the body for an extended time.

INHALATION:
May be harmful if inhaled.

May be absorbed by inhalation and persist in the body for an extended time.

Single overexposure, above recommended guidelines, may cause:

Irritation (upper respiratory): signs/symptoms can include soreness of the nose and throat, coughing and sneezing.

IF SWALLOWED:
Ingestion is not a likely route of exposure to this product.

Illness may result from a single swallowing of a moderate quantity of this material.

May be harmful if swallowed.

MUTAGENICITY:
Mutagenicity assays indicate the product is not mutagenic.

8. HEALTH HAZARD DATA (continued)

REPRODUCTIVE/DEVELOPMENTAL TOXINS:
Not teratogenic in the rat at oral doses below maternally toxic levels.

OTHER HEALTH HAZARD INFORMATION:
This product is not known to contain any substances regulated under California Proposition 65.

A Product Toxicity Summary Sheet is available.

SECTION CHANGE DATES

HEADING	SECTION CHANGED SINCE November 05, 1997 ISSUE

Abbreviations: N/D - Not Determined N/A - Not Applicable CA - Approximately

The information in this Material Safety Data Sheet (MSDS) is believed to be correct as of the date issued. 3M MAKES NO WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR COURSE OF PERFORMANCE OR USAGE OF TRADE. User is responsible for determining whether the 3M product is fit for a particular purpose and suitable for user's method of use or application. Given the variety of factors that can affect the use and application of a 3M product, some of which are uniquely within the user's knowledge and control, it is essential that the user evaluate the 3M product to determine whether it is fit for a particular purpose and suitable for user's method of use or application.

3M provides information in electronic form as a service to its customers. Due to the remote possibility that electronic transfer may have resulted in errors, omissions or alterations in this information, 3M makes no representations as to its completeness or accuracy. In addition, information obtained from a database may not be as current as the information in the MSDS available directly from 3M.

ATTACHMENT 3

TEST ARTICLE AND VEHICLE PREPARATION PROCEDURE

ATTACHMENT 3

TEST ARTICLE AND VEHICLE PREPARATION PROCEDURE

Test Article: PFOS

Vehicle: 0.5% Tween® 80, in R.O. Water

A. Purpose: The purpose of this procedure is to provide a method for the preparation of dosage suspensions of PFOS and the vehicle for oral administration to rabbits on Argus Study 418-012P.

B. General Information:

1. All suspension containers will be labeled and color coded. Each label will specify the protocol number, test article identification, Argus batch number, concentration, dosage level, preparation date, expiration date and storage conditions.

2a. Suspensions will be prepared:
_X_ Daily ___ Weekly ___ For____ days of use

2b. Vehicle will be prepared:
___ Daily _X_ Weekly ___ For____ days of use

3. Suspensions will be prepared at a final dosage volume of 10 mL/kg.

4. Safety:
_X_ Gloves, lab coat, goggles or safety glasses and faceshield
_X_ Dust-Mist Respirator
___ Half-Face Respirator
___ Full-Face Respirator/Positive Pressure Hood
___ Tyvek Suit/Apron

5. Dosage solutions adjusted for Free base and % Purity.
___ Yes _X_ No (Calculations based on 100%)
___ Free Base ___ Purity

6. Sampling requirements: Cited in protocol.

7. Storage: Cited in protocol.

TEST ARTICLE AND VEHICLE PREPARATION PROCEDURE

NOTE: Test article will be prepared as a serial dilution from the high dosage to the low dosage. Once the final volumes are achieved, stir bars are to be added to the containers; mixing should occur during sampling and/or administration.

C. Preparation of Vehicle

1. Add the required amount of R.O. deionized water to an appropriately labeled container. Heat the water to 50°C, ±5°C, add the required amount of Tween® 80 and mix until uniform (See TEST ARTICLE CALCULATIONS).

D. Test Article Suspension Preparation:

1. To prepare the 2-mg/mL, Group VII suspension, add the required amount of test article (See TEST ARTICLE CALCULATIONS) into an appropriately sized, labeled container. Add the required amount of vehicle and heat the mixture to 80°C, ±5°C for approximately 30 minutes.

2. Once the test article has dissolved; spin while the solution cools. (Be sure there is a visible vortex, this will achieve the desired emulsion. This may be prepared the day before use.)

3. To prepare the 1-mg/mL, Group VI suspension, remove the required amount of stock suspension (Group VII) (See TEST ARTICLE CALCULATIONS), add the required amount of vehicle and mix.

4. To prepare the .5-mg/mL, Group V suspension, remove the required amount of stock suspension (Group VI) (See TEST ARTICLE CALCULATIONS), add the required amount of vehicle and mix.

5. To prepare the .25-mg/mL, Group IV suspension, remove the required amount of stock suspension (Group V) (See TEST ARTICLE CALCULATIONS), add the required amount of vehicle and mix.

ATTACHMENT 3

TEST ARTICLE AND VEHICLE PREPARATION PROCEDURE

6. To prepare the 0.1-mg/mL, Group III suspension, remove the required amount of stock suspension (Group IV) (See TEST ARTICLE CALCULATIONS), add the required amount of vehicle and mix.

7. To prepare the 0.01-mg/mL, Group II suspension, remove the required amount of stock suspension (Group III) (See TEST ARTICLE CALCULATIONS), add the required amount of vehicle and mix.

Written by: _____

Approved by: _____ Date: 12-JUN-98

Clarification: ✓ No ___ Yes (See attached clarification form.)

Initials/Date : _____

APPENDIX E

HISTORICAL CONTROL DATA

SUMMARY OF REPRODUCTIVE INDICES
NZW RABBIT

PERIOD: JANUARY 1996 - JANUARY 1998

NUMBER OF STUDIES:	66
NUMBER OF RABBITS:	
TESTED	901
PREGNANT	861
FOUND DEAD	6*
ABORTED	22
DELIVERED PREMATURELY	4
NUMBER OF RABBITS PREGNANT AT CAESAREAN-SECTIONING ON DAY 29 OF GESTATION:	825
NUMBER OF RABBITS WITH SINGLE CONCEPTUS LITTER:	
LIVE	1
RESORBED	1
ABORTED	2

	MEAN or %	RANGE/STUDY MEAN or %
% PREGNANT	96.0	(75.0-100)
AVERAGE # CORPORA LUTEA	9.7	(7.8-11.7)
AVERAGE # IMPLANTATIONS	8.9	(3.8-10.6)
AVERAGE LITTER SIZE		
AVERAGE # LIVE FETUSES	8.4	(3.2-10.4)
AVERAGE # DEAD FETUSES	0.0	(0-0.1)
AVERAGE # RESORPTIONS	0.4	(0-3.2)
AVERAGE # EARLY RESORPTIONS	0.3	(0-2.8)
AVERAGE # LATE RESORPTIONS	0.1	(0-1.2)

* Three were moribund sacrifices, one was attributed to an intubation accident

SUMMARY OF REPRODUCTIVE INDICES
NZW RABBIT

	MEAN or %	RANGE/STUDY MEAN or %
AVERAGE % DOES WITH ANY RESORPTIONS	26.8	(0-100)
AVERAGE % DOES WITH ALL CONCEPTUSES RESORBED	0.6	(0-20.0)
AVERAGE % DOES WITH ONE OR MORE LIVE FETUSES	99.4	(80.0-100)
AVERAGE SEX RATIO, (% MALES/LITTER)	51.2	(31.4-61.0)
AVERAGE FETAL BODY WEIGHT (G)	43.74	(31.85-55.74)
AVERAGE FOR MALES (G)	44.26	(29.55-56.97)
AVERAGE FOR FEMALES (G)	43.08	(32.25-53.76)
AVERAGE % DEAD OR RESORBED CONCEPTUSES/LITTER	4.4	(0-18.8)

SUMMARY OF MATERNAL NECROPSY OBSERVATIONS
NZW RABBITS

PERIOD	JANUARY 1996 - JANUARY 1998	
TOTAL # STUDIES	67	
TOTAL # DOES	921	
# PREGNANT	876	% OF PREGNANT
# DIED	6*	0.6
# ABORTED	22	2.4
# DELIVERED PREMATURELY	4	0.4
# DOES WITH 100% RESORPTIONS	5	0.5

			RANGE / STUDY	
EXTERNAL OBSERVATIONS	N	%	N	%
Fecal material in perianal region	1	0.11	0-1	(0-5.0)
Localized alopecia	2	0.22	0-1	(0-5.0)
Left ear, torn	1	0.11	0-1	(0-5.0)
GROSS LESIONS				
THYMUS				
Small	1	0.11	0-1	(0-5.0)
LUNGS				
Discolored	6	0.65	0-4	(0-20.0)
Tear in right diaphragmatic lobe	1	0.11	0-1	(0-4.0)
Multiple lesions	1	0.11	0-1	(0-4.2)
THORACIC CAVITY				
Contained red fluid	1	0.11	0-1	(0-4.0)
LIVER				
Pale and/or discolored	7	0.76	0-4	(0-16.0)
Accentuated lobular pattern on lobe(s)	1	0.11	0-1	(0-5.0)
BACK				
Break present in lumbar region of spine	1	0.11	0-1	(0-5.0)

* Three were moribund sacrifices, one was attributed to an intubation accident

SUMMARY OF MATERNAL NECROPSY OBSERVATIONS
NZW RABBITS

GROSS LESIONS		N	%	RANGE / STUDY N	%
BACK (CONT.)					
	Dorsal muscles, three hemorrhagic areas in lumbar region	1	0.11	0-1	(0-12.5)
STOMACH					
	Trichobezoar	2	0.22	0-1	(0-4.3)
	Mucosa, eroded in areas	1	0.11	0-1	(0-4.2)
SPLEEN					
	Small	3	0.32	0-1	(0-20.0)
	Large	1	0.11	0-1	(0-4.0)
KIDNEY(S)					
	Small	1	0.11	0-1	(0-20.0)
	Right, displaced caudally	1	0.11	0-1	(0-5.0)
ADRENAL GLAND					
	Right, absent	1	0.11	0-1	(0-5.0)
UTERUS					
	Right horn contained dark brown fluid	1	0.11	0-1	(0-16.7)
	Horns contained a viscous, green-brown substance	1	0.11	0-1	(0-4.0)
	Vascularization	1	0.11	0-1	(0-4.3)
	Placentae surrounded by a thick, yellow substance	1	0.11	0-1	(0-5.0)
OVARIES					
	Parovarian cyst(s)	30	3.26	0-5	(0-25.0)

SUMMARY OF FETAL GROSS EXTERNAL ALTERATIONS
NZW RABBITS

PERIOD	JANUARY 1996 - JANUARY 1998
# OF STUDIES	65
# LITTERS EXAMINED	816
# LIVE FETUSES EXAMINED (DAY 29)	6929

	ALTERATION		N	%	RANGE / STUDY N	RANGE / STUDY %
SKIN						
	Absent area	L	1	0.12	0-1	(0-5.3)
		F	1	0.01	0-1	(0-0.6)
HEAD						
	Meningocele	L	2	0.24	0-1	(0-16.7)
		F	3	0.04	0-2	(0-3.7)
	Cyclops	L	1	0.12	0-1	(0-5.9)
		F	1	0.01	0-1	(0-0.7)
	Upper jaw in two segments	L	1	0.12	0-1	(0-5.9)
		F	1	0.01	0-1	(0-0.7)
	Nares absent	L	1	0.12	0-1	(0-5.3)
		F	1	0.01	0-1	(0-0.6)
	Fleshy protrusion	L	1	0.12	0-1	(0-5.3)
		F	1	0.01	0-1	(0-0.6)
EYES						
	Bulge depressed	L	3	0.37	0-1	(0-5.3)
		F	3	0.04	0-1	(0-0.6)
	Eyelids open	L	1	0.12	0-1	(0-5.9)
		F	1	0.01	0-1	(0-0.7)
SNOUT						
	Short	L	2	0.24	0-1	(0-5.9)
		F	2	0.03	0-1	(0-0.7)
TONGUE						
	Protrudes	L	1	0.12	0-1	(0-5.3)
		F	1	0.01	0-1	(0-0.6)
BODY						
	Umbilical Hernia	L	9	1.10	0-2	(0-50.0)
		F	10	0.14	0-3	(0-12.0)
	Edema	L	1	0.12	0-1	(0-5.3)
		F	1	0.01	0-1	(0-0.6)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL GROSS EXTERNAL ALTERATIONS
NZW RABBITS

ALTERATION		N	%	RANGE / STUDY N	RANGE / STUDY %
BODY (CONT.)					
Skin discolored purple	L	1	0.12	0-1	(0-5.3)
	F	3	0.04	0-3	(0-1.9)
Spina bifida	L	2	0.24	0-1	(0-20.0)
	F	2	0.03	0-1	(0-1.9)
Hemorrhagic area(s)	L	2	0.24	0-1	(0-5.0)
	F	2	0.03	0-1	(0-0.6)
Meningocele	L	2	0.24	0-1	(0-16.7)
	F	2	0.03	0-1	(0-2.0)
Hematoma	L	1	0.12	0-1	(0-5.9)
	F	1	0.01	0-1	(0-0.8)
Dark red areas	L	1	0.12	0-1	(0-5.6)
	F	1	0.01	0-1	(0-0.6)
FORELIMBS AND/OR HINDLIMBS					
Paw(s): Flexed/ Rotated	L	2	0.24	0-1	(0-5.6)
	F	2	0.03	0-1	(0-0.6)
Paw: Short digits	L	1	0.12	0-1	(0-5.6)
	F	1	0.01	0-1	(0-0.7)
Limb(s): Rotated	L	2	0.24	0-1	(0-16.7)
	F	2	0.03	0-1	(0-1.9)
Limb(s): Absent	L	1	0.12	0-1	(0-5.3)
	F	1	0.01	0-1	(0-0.6)
TAIL					
Short	L	7	0.86	0-1	(0-20.0)
	F	10	0.14	0-4	(0-2.3)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SOFT TISSUE ALTERATIONS
NZW RABBITS

PERIOD	JANUARY 1996 - JANUARY 1998
# STUDIES	38
# LITTERS EXAMINED	674
# FETUSES EXAMINED	5739

	ALTERATION		N	%	RANGE/STUDY N	RANGE/STUDY %
BRAIN						
	Dilated lateral ventricles	L	2	0.30	0-1	(0-5.0)
	(Moderate) (Grade 2)	F	2	0.03	0-1	(0-0.6)
EYE(S)						
	Circumcorneal hemorrhage	L	20	2.97	0-2	(0-16.7)
		F	21	0.36	0-3	(0-1.9)
	Microphthalmia	L	1	0.15	0-1	(0-5.0)
		F	1	0.02	0-1	(0-0.6)
HEART						
	Large	L	1	0.15	0-1	(0-5.9)
		F	1	0.02	0-1	(0-0.6)
	Three ventricles	L	1	0.15	0-1	(0-5.9)
		F	1	0.02	0-1	(0-0.6)
VESSELS						
	Persistent truncus	L	1	0.15	0-1	(0-5.0)
	arteriosis	F	1	0.02	0-1	(0-0.6)
	Innominate, absent	L	1	0.15	0-1	(0-5.3)
		F	1	0.02	0-1	(0-0.7)
	Two pulmonary arteries	L	1	0.15	0-1	(0-5.9)
		F	1	0.02	0-1	(0-0.6)
LUNGS						
	One or more lobes, partial	L	69	10.24	0-5	(0-27.8)
	or complete agenesis	F	90	1.57	0-9	(0-5.0)
	Right apical and cardiac	L	1	0.15	0-1	(0-5.3)
	lobes fused	F	1	0.02	0-1	(0-0.6)
DIAPHRAGM						
	Hernia	L	1	0.15	0-1	(0-5.0)
		F	1	0.02	0-1	(0-0.6)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SOFT TISSUE ALTERATIONS
NZW RABBITS

ALTERATION				RANGE/STUDY	
		N	%	N	%
KIDNEY(S)					
Absent	L	1	0.15	0-1	(0-5.6)
	F	1	0.02	0-1	(0-0.6)
Displaced caudally	L	1	0.15	0-1	(0-4.3)
	F	1	0.02	0-1	(0-0.5)
SPLEEN					
Pale	L	1	0.15	0-1	(0-7.1)
	F	1	0.02	0-1	(0-0.9)
GONADS					
Right testis displaced caudally	L	1	0.15	0-1	(0-5.9)
	F	1	0.02	0-1	(0-0.6)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SKELETAL ALTERATIONS
NZW RABBITS

PERIOD	JANUARY 1996 - JANUARY 1998
# STUDIES	37
# LITTERS EXAMINED	668
# FETUSES EXAMINED	5684

ALTERATIONS				RANGE / STUDY		# OF STUDIES WITH
SKULL		N	%	N	%	ALTERATION
Summarization of all irregular ossification of skull	L	185	27.69	0-11	(0-66.7)	35
	F	237	4.17	0-17	(0-9.8)	
Anterior Fontanelle						
: Irregularly shaped	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.5)	
Posterior Fontanelle						
: Enlarged (Slight)	L	1	0.15	0-1	(0-5.3)	1
(Grade 1)	F	1	0.02	0-1	(0-0.6)	
Frontals						
: Irregular suture	L	18	2.69	0-3	(0-16.7)	13
	F	20	0.35	0-3	(0-1.8)	
: Interfrontals present	L	17	2.54	0-3	(0-15.0)	13
	F	17	0.30	0-3	(0-1.7)	
: Fused	L	5	0.75	0-2	(0-10.5)	4
	F	5	0.09	0-2	(0-1.1)	
: Two segments	L	1	0.15	0-1	(0-5.9)	1
	F	1	0.02	0-1	(0-0.7)	
: Suture large	L	1	0.15	0-1	(0-5.0)	1
	F	1	0.02	0-1	(0-0.6)	
: Small	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.6)	
Parietal(s)						
: Contain holes	L	3	0.45	0-1	(0-5.6)	3
	F	3	0.05	0-1	(0-0.6)	
: Fused and small	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.6)	
: Interparietals	L	1	0.15	0-1	(0-5.3)	1
irregularly shaped	F	1	0.02	0-1	(0-0.5)	
: Interparietals incom-	L	1	0.15	0-1	(0-16.7)	1
pletely ossified	F	1	0.02	0-1	(0-1.9)	

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SKELETAL ALTERATIONS
NZW RABBITS

ALTERATIONS		N	%	RANGE / STUDY N	RANGE / STUDY %	# OF STUDIES WITH ALTERATION
SKULL (CONT.)						
Nasals						
: Irregular suture	L	4	0.60	0-2	(0-10.0)	3
	F	4	0.07	0-2	(0-1.2)	
: Internasals	L	26	3.89	0-3	(0-15.8)	18
	F	29	0.51	0-4	(0-2.3)	
: Intranasals	L	16	2.40	0-2	(0-16.7)	13
	F	16	0.28	0-2	(0-1.9)	
: Displaced suture	L	116	17.36	0-8	(0-40.0)	34
	F	129	2.27	0-9	(0-5.2)	
: Fused	L	10	1.50	0-2	(0-10.0)	9
	F	11	0.19	0-2	(0-1.3)	
: Small	L	1	0.15	0-1	(0-5.0)	1
	F	1	0.02	0-1	(0-0.6)	
Nasal/Frontal sutures:	L	12	1.80	0-2	(0-11.1)	9
irregular and/or misaligned	F	13	0.23	0-3	(0-1.9)	
Premaxillae: fused	L	1	0.15	0-1	(0-5.9)	1
	F	1	0.02	0-1	(0-0.7)	
Premaxillae: not ossified	L	1	0.15	0-1	(0-5.0)	1
	F	1	0.02	0-1	(0-0.6)	
Maxillae: fused	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.6)	
Supraoccipitals: irregularly	L	1	0.15	0-1	(0-5.3)	1
shaped	F	1	0.02	0-1	(0-0.5)	
Eye socket: small	L	2	0.30	0-1	(0-5.0)	2
	F	2	0.04	0-1	(0-0.6)	
Skull: extra ossification	L	1	0.15	0-1	(0-5.6)	1
	F	1	0.02	0-1	(0-0.6)	
HYOID						
A1a(e), angulated	L	108	16.17	0-8	(0-40.0)	34
	F	137	2.41	0-18	(0-9.9)	
Small	L	1	0.15	0-1	(0-5.3)	1
	F	4	0.07	0-4	(0-2.4)	
Irregularly shaped	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.6)	

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SKELETAL ALTERATIONS
NZW RABBITS

ALTERATIONS		N	%	RANGE / STUDY N	RANGE / STUDY %	# OF STUDIES WITH ALTERATION
VERTEBRAE						
Cervical						
: Centrum, unilateral ossification	L	2	0.30	0-1	(0-5.6)	2
	F	2	0.04	0-1	(0-0.7)	
: Arches and/or Centra, fused	L	3	0.45	0-1	(0-5.6)	3
	F	3	0.05	0-1	(0-0.7)	
: Hemivertebra	L	3	0.45	0-1	(0-5.9)	3
	F	3	0.05	0-1	(0-0.6)	
: Centrum, asymmetric	L	1	0.15	0-1	(0-5.6)	1
	F	1	0.02	0-1	(0-0.7)	
: Centra, bifid	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.6)	
Thoracic						
: Hemivertebra	L	10	1.50	0-1	(0-5.9)	10
	F	11	0.19	0-2	(0-1.1)	
: Arches and/or Centra, fused	L	5	0.75	0-2	(0-10.5)	4
	F	6	0.10	0-2	(0-1.2)	
: Centrum, unilateral ossification	L	5	0.75	0-1	(0-5.3)	5
	F	5	0.09	0-1	(0-0.6)	
: Centra, one or more asymmetric	L	1	0.15	0-1	(0-5.6)	1
	F	1	0.02	0-1	(0-0.6)	
: Centrum, bifid	L	4	0.60	0-2	(0-10.5)	3
	F	4	0.07	0-2	(0-1.2)	
: Centra, not ossified	L	1	0.15	0-1	(0-4.5)	1
	F	1	0.02	0-1	(0-0.6)	
: Arch, absent	L	1	0.15	0-1	(0-5.6)	1
	F	1	0.02	0-1	(0-0.6)	
: Arch, small	L	3	0.45	0-1	(0-5.9)	3
	F	3	0.05	0-1	(0-0.6)	
Lumbar						
: Hemivertebra	L	2	0.30	0-1	(0-5.9)	2
	F	2	0.04	0-1	(0-0.8)	
: Centrum, unilateral ossification	L	1	0.15	0-1	(0-5.0)	1
	F	1	0.02	0-1	(0-0.6)	
: Arch, small	L	2	0.30	0-1	(0-5.0)	2
	F	2	0.04	0-1	(0-0.6)	
: Centrum, not ossified	L	1	0.15	0-1	(0-5.0)	1
	F	1	0.02	0-1	(0-0.6)	

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SKELETAL ALTERATIONS
NZW RABBITS

ALTERATIONS		N	%	RANGE / STUDY N	RANGE / STUDY %	# OF STUDIES WITH ALTERATION
VERTEBRAE (CONT.)						
Sacral						
: Arches open	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.5)	
Caudal						
: One or more	L	30	4.49	0-3	(0-16.7)	24
misaligned	F	31	0.54	0-3	(0-2.0)	
: Fused	L	8	1.20	0-1	(0-5.6)	8
	F	10	0.18	0-3	(0-1.6)	
: 11 present	L	1	0.15	0-1	(0-4.3)	1
	F	1	0.02	0-1	(0-0.5)	
: 12 present	L	1	0.15	0-1	(0-4.3)	1
	F	2	0.04	0-2	(0-1.0)	
: 13 to 14 present	L	3	0.45	0-1	(0-5.6)	3
	F	3	0.05	0-1	(0-0.6)	
: 15 present	L	1	0.15	0-1	(0-4.3)	1
	F	1	0.02	0-1	(0-0.5)	
: Irregularly shaped	L	1	0.15	0-1	(0-5.0)	1
	F	1	0.02	0-1	(0-0.6)	
VERTEBRAE/RIB						
Interrelated Vertebral / Rib	L	3	0.45	0-3	(0-15.8)	1
malformations	F	3	0.05	0-3	(0-1.6)	
RIBS						
Cervical Rib present	L	2	0.30	0-1	(0-5.6)	2
	F	2	0.04	0-1	(0-0.6)	
Two or more, fused	L	6	0.90	0-2	(0-10.5)	5
	F	6	0.10	0-2	(0-1.2)	
Bases proximate	L	10	1.50	0-2	(0-9.1)	9
	F	10	0.18	0-2	(0-1.1)	
One or more, split	L	8	1.20	0-1	(0-5.6)	8
	F	8	0.14	0-1	(0-0.6)	
One or more, thickened areas	L	27	4.04	0-3	(0-16.7)	19
	F	29	0.51	0-3	(0-2.2)	
Flat	L	2	0.30	0-1	(0-5.6)	2
	F	2	0.04	0-1	(0-0.6)	

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SKELETAL ALTERATIONS
NZW RABBITS

ALTERATIONS		N	%	RANGE / STUDY N	RANGE / STUDY %	# OF STUDIES WITH ALTERATION
RIBS (CONT.)						
Extra rib	L	1	0.15	0-1	(0-4.3)	1
	F	1	0.02	0-1	(0-0.5)	
Small	L	2	0.30	0-1	(0-5.9)	2
	F	2	0.04	0-1	(0-0.6)	
Broad	L	1	0.15	0-1	(0-5.9)	1
	F	1	0.02	0-1	(0-0.8)	
Bent	L	1	0.15	0-1	(0-4.5)	1
	F	1	0.02	0-1	(0-0.6)	
MANUBRIUM						
Duplicated	L	1	0.15	0-1	(0-5.9)	1
	F	1	0.02	0-1	(0-0.7)	
Fused	L	3	0.45	0-2	(0-10.5)	2
	F	3	0.05	0-2	(0-1.1)	
STERNEBRAE						
Two or more, fused	L	64	9.58	0-5	(0-27.8)	31
	F	78	1.37	0-7	(0-3.9)	
One or more, asymmetric	L	6	0.90	0-2	(0-10.5)	5
	F	6	0.10	0-2	(0-1.2)	
One or more, incompletely	L	11	1.65	0-2	(0-10.5)	9
or not ossified	F	11	0.19	0-2	(0-1.2)	
Duplicated	L	1	0.15	0-1	(0-5.9)	1
	F	1	0.02	0-1	(0-0.7)	
PELVIS						
Pubis(es): incompletely	L	4	0.60	0-1	(0-5.9)	4
or not ossified	F	5	0.09	0-2	(0-1.1)	
SCAPULAE						
Ala(e): irregularly	L	4	0.60	0-2	(0-10.5)	3
shaped	F	4	0.07	0-2	(0-1.2)	
Ala(e): wavy	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.6)	

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SKELETAL ALTERATIONS
NZW RABBITS

ALTERATIONS		N	%	RANGE / STUDY N	RANGE / STUDY %	# OF STUDIES WITH ALTERATION
SCAPULAE (CONT.)						
Misaligned	L	5	0.75	0-2	(0-10.5)	4
	F	5	0.09	0-2	(0-1.2)	
Bent	L	1	0.15	0-1	(0-5.0)	1
	F	1	0.02	0-1	(0-0.6)	
FORELIMB(S)						
1 Phalanx present	L	1	0.15	0-1	(0-5.6)	1
	F	1	0.02	0-1	(0-0.7)	
0 Phalanges present	L	1	0.15	0-1	(0-5.6)	1
	F	1	0.02	0-1	(0-0.7)	
Humerus, Radius, Ulna, Carpals, Metacarpals, Fore-digits and Forephalanges absent	L	1	0.15	0-1	(0-5.3)	1
	F	1	0.02	0-1	(0-0.6)	

L: LITTER INCIDENCE
F: FETAL INCIDENCE

APPENDIX F

STATEMENT OF THE STUDY DIRECTOR

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

PROTOCOL 418-012: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS
SPONSOR'S STUDY NUMBER: 6295.10

STATEMENT OF THE STUDY DIRECTOR

This final report accurately reflects the raw data obtained during the performance of the study. No significant deviations from the U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations; Final Rule[a], the Japanese Ministry of Health and Welfare (MHW) *Good Laboratory Practice Standard for Safety Studies on Drugs*[b] and the European Economic Community (EEC) *Council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice*[c] occurred that affected the quality or integrity of the study.

11-Jan-99

Raymond G. York, Ph.D., DABT Date
Associate Director of Research
and Study Director

---

a. U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.

b. Japanese Ministry of Health and Welfare (1988). *Good Laboratory Practice Standard for Safety Studies on Drugs*, MHW Ordinance Number 21, March 26, 1997.

c. European Economic Community (1989). *Council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice*. Official Journal of the European Communities: Legislation. 32(No. L 315; 28 October): 1-17.

APPENDIX G

QUALITY ASSURANCE UNIT FINAL REPORT STATEMENT

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

QUALITY ASSURANCE UNIT FINAL REPORT STATEMENT

Study Director: Raymond G. York, Ph.D., DABT

Executive Director of Research: Mildred S. Christian, Ph.D., Fellow, ATS

Protocol 418-012: Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits
Sponsor's Study Number: 6295.10

The draft protocol for this study was audited for adherence to U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations, Japanese Ministry of Health and Welfare (MHW); Good Laboratory Practice Standard for Safety Studies on Drugs, and European Economic Community (1989) council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice on 10 AUG 98.

Critical phases of this study were inspected five times; study information and raw data were audited twice (see tables 1 and 2 for dates and phases/data).

The draft final report and the raw data for this study [except for Appendix F, the Pilot Report, which was conducted in the spirit of Good Laboratory Practice (GLP)] were compared and audited for accuracy, for adherence to protocol requirements, and for adherence to U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations, Japanese Ministry of Health and Welfare (MHW); Good Laboratory Practice Standard for Safety Studies on Drugs, and European Economic Community (1989) council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice between 04 DEC 98 and 17 DEC 98, for revisions requested by the Sponsor 23 DEC 98, and for finalization on 11 JAN 99.

This study was conducted according to U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations, Japanese Ministry of Health and Welfare (MHW); Good Laboratory Practice Standard for Safety Studies on Drugs, and European Economic Community (1989) council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice.

Barbara J. Patterson 11 Jan 99

Barbara J. Patterson, B.A. Date
Director of Operations
and Compliance

Heather L. Rabuttino 11 Jan 99

Heather L. Rabuttino, M.S. Date
Quality Assurance Supervisor
and Principal Auditor

TABLE 1

CRITICAL PHASES INSPECTED

Test Article Administration - Gavage

Date of inspection: 25 AUG 98

Date results reported to the Study Director and Management: 12 SEP 98

Test Article Preparation

Date of inspection: 04 SEP 98

Date results reported to the Study Director and Management: 12 SEP 98

Blood Collection

Date of inspection: 10 SEP 98

Date results reported to the Study Director and Management: 10 SEP 98

Caesarean-Sectioning

Dates of inspection: 10 SEP 98, 18 SEP 98

Dates results reported to the Study Director and Management:
10 SEP 98, 24 SEP 98

TABLE 2

RAW DATA AUDIT(S)

The following study information and raw data were audited on 12 OCT 98:

Vehicle receipt, preparation and use.
Test article receipt, preparation and use.
Test article packing lists.

The results of this audit were reported to the Study Director and Management on 13 OCT 98.

The following study information and raw data were audited on 13 OCT 98:

Protocol.
Protocol amendments.
List of personnel and computer operator codes.
Error codes and codes for clinical sign observations.
Animal receipt, randomization, and acclimation.
Veterinary examination.
In-life transaction record.
Feed consumption.
Caesarean-sectioning.
Maternal gross observations.
Fetal gross observations.
Fetal fixative assignment.
Fetal visceral examination.
Fetal skeletal examination.
Necropsy.
Organ weights.
Tissue packing lists.
General comments.
Study maintenance records.
Tempscribes.
Feed and water analyses.
Edit requests.
Dosage volumes.
Deviations.
Data review page.
Blood collection.

The results of this audit were reported to the Study Director and Management on 14 OCT 98.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

An Epidemiologic Investigation of Clinical Chemistries, Hematology and Hormones in Relation to Serum Levels of Perfluorooctane Sulfonate in Male Fluorochemical Production Employees

Following reports of the finding of organic fluorine in sera samples, a fluorochemical medical surveillance program began at 3M's Decatur manufacturing facility in the late 1970's. The surveillance program has generally consisted of annual or biannual tests of clinical chemistries, pulmonary function, blood counts and a biomonitor of fluorochemical exposure. A total organic fluorine measurement was routinely done until 1993. This measures the amount of fluorine that was covalently bound to carbon in the serum sample. When test data were available, a company physician reviewed each employee's results. These physicians did not, and have not, found abnormalities in individuals that they felt were related to fluorochemical exposure. That is, medical conditions, medications and lifestyle factors adequately explained the laboratory abnormalities (which one expects to find in this type of program.)

Beginning in 1994, the 3M Decatur (Alabama) plant medical surveillance program incorporated a serum measurement of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). Total organic fluorine was not measured. A formal report was written of the aggregate analyses conducted of the medical surveillance clinical program data for the Decatur (Alabama) and Antwerp (Belgium) employees who voluntarily participated in 1994, 1995 and 1997. The findings from this aggregate

analysis suggested that, among these participating Antwerp and Decatur male fluorochemical production employees, significant hematological, clinical chemistry and hormonal abnormalities were not associated with serum PFOS levels up to 6 ppm. It was not possible to derive inferences from the few employees with serum PFOS levels > 6 ppm. Limitations of this study include its cross-sectional design, the voluntary participation rates, the few subjects exposed at the highest levels, and the lower levels of serum PFOS measured among these employees compared to those estimated to cause effects in laboratory animals. Results of the hepatic and lipid clinical chemistry tests were published in *the Journal of Occupational and Environmental Medicine* (1999;41:799-806). In the Spring of 2000, medical surveillance will again be offered to 3M fluorochemical production employees at the Antwerp and Decatur manufacturing sites.

April 22, 1998

An Epidemiologic Investigation of Clinical Chemistries, Hematology and Hormones in Relation to Serum Levels of Perfluorooctane Sulfonate in Male Fluorochemical Production Employees

Geary W. Olsen, D.V.M., Ph.D.

Jean M. Burris, R.N., M.P.H.

Jeffrey H. Mandel, M.D., M.P.H.

Larry R. Zobel, M.D., M.P.H.

Medical Department, 3M Company, 220-3W-05, St. Paul, MN 55144

ABSTRACT

3M manufactures products which contain chemical compounds, either as intentional components or residual impurities, that have as a parent molecule, perfluorooctane sulfonyl fluoride. These chemicals include: perfluorooctane sulfonate (PFOS), N-ethyl perfluorooctanesulfonamide, N-ethyl perfluorooctanesulfonamido ethanol, N-methyl perfluoroctanesulfonamido ethanol and chemicals derived from it, and the mixture of mono-, di- and tri [N-ethyl perfluorooctane sulfonamidoethyl] phosphates. There may be other precursors in the workplace. These molecules enter a number of product applications (e.g., surfactants, food packaging additives, polymers). These compounds may be expected to transform metabolically, to an undetermined degree, to PFOS as an end-stage metabolite. Potassium perfluorooctane sulfonate ($C_8F_{17}OSO_2K^+$) is, itself, a surfactant used as a wetting and foaming agent in industrial and commercial processes.

Subchronic studies in rats and primates suggest there may be a potential for cumulative toxicity with PFOS over time with the primary effect related to metabolic wasting. Although the mechanism of toxicity is not fully understood, toxicity may be due to an effect on peroxisome proliferation, fatty acid metabolism, membrane function, protein synthesis and/or mitochondrial bioenergetics.

Medical surveillance has been routinely performed on 3M fluorochemical production workers (in Decatur, Alabama and Antwerp, Belgium) with potential exposure to PFOS and/or to perfluorinated precursors that may metabolically degrade to PFOS. The purpose of this study was to provide an analysis of the hematology (hematocrit, hemoglobin, red blood cells, white blood cells and platelet count), clinical chemistries

(alkaline phosphatase, gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin, blood urea nitrogen, creatinine, glucose, cholesterol, low density lipoproteins, high density lipoproteins and triglycerides) and hormonal parameters (cortisol, dehydroepiandrosterone sulfate, estradiol, follicle stimulating hormone, 17-alpha hydroxyprogesterone, luteinizing hormone, prolactin, sex hormone binding globulin, free testosterone, bound testosterone, and thyroid stimulating hormone) in relation to serum PFOS as determined by high performance liquid chromatography mass spectrometry methods. These relationships were assessed in fluorochemical production employees from two time periods, 1995 (N = 178) and 1997 (N = 149).

Descriptive simple and stratified analyses, Pearson correlation coefficients, analysis of variance and multivariable regression were used to evaluate for possible associations between PFOS and each hematological and clinical chemistry test and hormonal assay. Age, body mass index, current alcohol consumption (drinks per day) and cigarette use (cigarettes smoked per day) were potential confounding factors that were considered in the analyses. Multivariable regression models were fitted with PFOS analyzed as a continuous variable using linear as well as non-linear transformations in order to maximize the possibility of finding associations between PFOS and the parameters of interest.

Four categorizations of serum PFOS levels were assessed in relation to the response variables: 0 - < 1 ppm; 1 - < 3 ppm; 3 - < 6 ppm; and $\geq$ 6 ppm. In 1995, mean serum PFOS levels by category were 0.49 ppm, 1.82 ppm, 4.12 ppm and 8.17 ppm, respectively. In 1997, mean serum PFOS levels by category were 0.52 ppm, 1.78 ppm,

3.87 ppm and 7.20 ppm, respectively. For both years, 95 percent of the employees' serum PFOS levels were below 6 ppm. Although the two plant populations differed by age, body mass index and alcohol consumption, no consistent associations, by both plant locations and year, were observed between the clinical chemistries, hematology and hormone parameters and the employees' serum PFOS levels.

The findings from this study suggest that, among these Antwerp and Decatur male fluorochemical production employees, significant hematological, clinical chemistry and hormonal abnormalities are not associated with serum PFOS levels up to 6 ppm. It is not possible to derive inferences from the few employees with serum PFOS levels $\geq$ 6 ppm. Limitations of this study include its cross-sectional design, the voluntary participation rates, the few subjects exposed at the highest levels, and the lower levels of serum PFOS measured among these employees compared to those that caused effects in laboratory animals.

INTRODUCTION

3M manufactures products which contain chemical compounds, either as intentional components or residual impurities, that have as a parent molecule, perfluorooctane sulfonyl fluoride. These chemicals include: perfluorooctane sulfonate (PFOS), N-ethyl perfluorooctanesulfonamide, N-ethyl perfluorooctanesulfonamido ethanol, N-methyl perfluoroctanesulfonamido ethanol and chemicals derived from it, and the mixture of mono-, di- and tri [N-ethyl perfluorooctane sulfonamidoethyl] phosphates. There may be other precursors in the workplace. These molecules enter a number of product applications (e.g., surfactants, food packaging additives, polymers). These compounds can be expected to be transformed metabolically, to an undetermined degree, to PFOS as an end-stage metabolite [Gibson et al., 1983]. Potassium perfluorooctane sulfonate ($C_8F_{17}OSO_2K^+$) is, itself, a surfactant used as a wetting and foaming agent in industrial and commercial processes.

Potassium perfluorooctane sulfonate is readily absorbed by ingestion [Johnson and Ober, 1979; O'Malley and Ebbens, 1980]. Ninety five percent of a single oral dose of [^{14}C] PFOS administered to male rats was absorbed within 24 hours [Johnson and Ober, 1979].

After a single, 24-hour occluded dermal exposure to PFOS at a dose of 5000 mg/kg, total serum organic fluorine concentrations were 10.3 and 0.9 ppm for male and female albino rabbits, respectively [O'Malley and Ebbens, 1980]. Twenty eight days after dosing, total serum organic fluorine concentrations had risen to 130.2 and 128.0 ppm for male and female albino rabbits, respectively. On the other hand, no quantifiable

organic fluorine could be detected 28 days after a single, 24 hour occluded dermal exposure to a 0.06% solution of PFOS in water at doses of 0, 0.003, 0.06, and 0.3 mg PFOS solution/kg, respectively, to 3 male and 3 female albino rabbits per dose group [Glaza, 1995].

Once in the body, PFOS concentrates primarily in the liver of rats [Johnson et al., 1979]. Eighty-nine days after a single intravenous dose (mean 4.2 mg/kg) of radiolabeled PFOS, mean tissue concentrations (μg PFOS equivalent/g tissue) were: liver, 20.56; plasma, 2.21; kidney, 1.09; lung, 1.06; spleen, 0.51; bone marrow, 0.46; red blood cells, 0.45; adrenals, 0.41; testes, 0.36; skin, 0.35; muscle, 0.29; subcutaneous fat, 0.20; eye, 0.16; abdominal fat, ≤ 0.08; and brain, < 0.05. Johnson et al. [1979] observed that 30.2 percent of the dose 89 days after administration had been excreted in the urine and 12.6 percent in the feces. Analyses of the urine, feces and tissues have suggested that PFOS is not metabolized [Johnson et al., 1984]. The plasma half-life was calculated to be 7.5 days after a single oral dose of radiolabeled PFOS (mean dose, 4.2 mg/kg) in solution to three male rats [Johnson and Ober, 1979].

There appears to be significant enterohepatic circulation of PFOS with both urinary and fecal excretion [Johnson et al., 1979; 1980; 1984]. In male rats, cholestyramine administered in the feed decreased the retention of radiolabeled PFOS in liver, plasma, and red blood cells, 3.8, 7.7 and 6.0 fold, respectively, and increased its elimination via feces 9.5 fold after the rats were given intravenous radiolabeled PFOS (mean dose, 3.4 mg/kg) [Johnson et al., 1980; 1984]. There was a lower clearance rate of ^{14}C in the urine compared to control animals because of the increased rate of fecal elimination. Cholestyramine is a bile acid sequestrant that acts by binding bile acids in

the intestinal tract. This reduces bile acid resorption and its return to the liver. Decreased flow of bile acids in the enterohepatic circulation results in increased conversion of hepatic cholesterol into bile acids. This results in a decline in hepatic cholesterol concentration and the stimulation of low density lipoprotein (LDL) receptor synthesis, which subsequently produces a decline in serum LDL cholesterol levels.

Upon acute exposure, PFOS was moderately toxic by oral administration [Gabriel, 1976; Dean et al., 1978; Rusch and Rinehart, 1979], but not dermal [O'Malley and Ebbens, 1980]. There have been two acute oral toxicity studies reported [Gabriel, 1976; Dean et al., 1978]. In the more recent study PFOS was suspended in a 20% acetone/80% corn oil mixture and administered orally by gavage levels to 5 male and 5 female rats per group at the following dosages: 100, 215, 464 and 1000 mg/kg [Dean et al., 1978]. Animals were observed for 14 days. The acute oral LD50 values (95% confidence limits in parentheses) were male rats, 233 (160 - 339) mg/kg; female rats 271 (200 -369) mg/kg; combined male and female rats; 251 (199 - 318) mg/kg. Clinical signs included diarrhea, hypoactivity, decreased limb tone, ataxia, corneal opacity, ptosis, piloerection, prostration and tremors. In the previous study, PFOS was administered in water and the LD50 in the rat was determined to be 1.25 - 2.50 g/kg [Gabriel, 1976]. Gabriel's results appear to be inconsistent with subsequent toxicity studies.

In an acute inhalation toxicity study [Rusch and Rinehart, 1979], a series of one-hour inhalation exposures in rats at exposure concentrations of PFOS at 24.09, 7.05, 6.49, 4.88, 2.86, 1.89 and 0.0 mg/L produced 100 percent mortality at the highest level and partial mortality (10 - 80%) at all other PFOS levels. Observations included dyspnea, tremors, convulsions, hypersensitivity, hypoactivity, excessive salivation and lacrimation

and general poor condition. The LC50 was determined to be 5.2 mg/L (95% CI = 4.4 - 6.4 mg/L).

PFOS has been shown to be a potent inducer of hepatic peroxisomes and fatty acid beta-oxidation in the rat [Ikeda et al., 1987] and mouse [Sohlenius et al., 1993]. After feeding male rats for two weeks with a powdered chow containing 0.02% PFOS, hepatic catalase, fatty acyl-CoA, carnitine acetyl transferase and carnitine palmitoyl transferase increased by 1.74, 4.90, 6.84 and 1.69 fold, respectively, compared to control animals [Ikeda et al., 1987]. PFOS also induced cytochrome P-450 activity. Male mice administered perfluorooctane sulfonic acid at a concentration of 0.05% weight/weight in the diet for 5 days resulted in weight loss and increased peroxisomal fatty acid beta-oxidation, peroxisomal catalase activity, Ω-hydroxylation of lauric acid, cytosolic epoxide hydrolase activity and cytosolic DT-diaphorase activity [Sohlenius et al., 1993].

Haughom and Spydevold [1992] fed 0.02% perfluorooctane sulfonic acid in the diet for 7 - 14 days to male Wistar rats which resulted in increased liver weight, liver triacylglycerol, liver free cholesterol and decreased liver cholesterol ester as well as decreased serum cholesterol and triacylglycerol levels. There was reduced cholesterol synthesis from acetate, pyruvate and hydroxymethyl glutarate but no reduction in synthesis from mevalonic acid in the hepatocytes from the treated rats. The activity of liver hydroxymethyl glutaric acid-Co-A reductase (HMG-CoA) and acyl-CoA cholesterol acyltransferase (ACAT) was reduced. Haughom and Spydevold [1992] suggested that the hypolipidemic effect of perfluorooctane sulfonic acid may be due to downregulation of HMG-CoA reductase and ACAT with enhanced fatty acid oxidation in the liver. This would subsequently reduce very low density lipoprotein (VLDL) production by the liver.

Recently, Nabbefeld et al. [1998] tested the hypothesis that PFOS and other fluorocompounds may act as peroxisome proliferators by displacing fatty acids from liver fatty acid binding protein (L-FABP). 10 μM PFOS caused a 66 percent reduction of the fluorescently labeled fatty acid analog 11-(5-dimethylaminonapthalenesulfphonyl)-undecanoic acid from L-FABP *in vitro*. Comparable results were observed for bovine serum albumin. These findings demonstrated that PFOS has a high affinity for fatty acid carrier proteins and can displace the endogenous ligand.

Results from three subchronic studies have been reported [Goldenthal et al., 1978a; 1978b; 1979]. PFOS was fed in the diet of Charles River CD rats at 0 (control), 30, 100, 300, 1,000 and 3,000 ppm for 90 days [Goldenthal et al., 1978a]. At the 300, 1,000 and 3,000 ppm dosage level, all rats died prior to scheduled termination. Toxicity signs included emaciation, convulsions, ocular and anogenital discharges, increased sensitivity to external stimuli and reduced motor activity. Histopathology showed compound-related lesions which included hepatic hypertrophy and necrosis, thymic and splenic follicular atrophy, bone marrow hypocellularity and atrophy of mesenteric lymph nodes, small intestinal villi and skeletal muscle. Among the 100 ppm dose group there was weight loss, elevated plasma creatinine phosphokinase, alkaline phosphatase, blood glucose and blood urea nitrogen, decreased hemoglobin, hematocrit, erythrocyte and leukocyte counts, hepatic enlargement and necrosis, and stomach discoloration and hemorrhage. Among the 30 ppm dose group there was weight loss, elevated plasma glutamate-pyruvate transaminase and plasma glutamate oxalacetate transaminase, and liver discoloration.

In a subchronic (90 day) study [Goldenthal et al., 1979], two male and two female rhesus monkeys per group received 0 (control), 10, 30, 100 and 300 mg/kg/day of PFOS by oral gavage. All animals (except controls) died within 20 days and timing was related to dosage. Monkeys treated with 300 mg/kg/day died between the second and fourth day. Monkeys treated with 100 mg/kg/day died between the 3rd and 5th day. Monkeys treated at 30 mg/kg/day died between the 7th and 10th day and those treated at 10 mg/kg/day died between the 11th and 20th day of the study. Signs of toxicity at each dosage level were comparable and included anorexia, diarrhea, decreased activity, emesis, weight loss, marked weakness, prostration, and general body tremors. There were no consistent histopathologic changes with exposure. Adrenal changes, including congestion, hemorrhage and lipid depletion of the adrenal cortex, were observed in all dose groups.

An additional subchronic rhesus monkey study was subsequently initiated at much lower dosages [Goldenthal, 1978b]. PFOS was administered by oral gavage to two male and two female monkeys at dosages of 0, 0.5,1.5 or 4.5 mg/kg/day for 90 days. Animals treated at the 4.5 mg/kg/day dosage level died or were sacrificed *in extremis* by the seventh week with signs of gastrointestinal toxicity comparable to those observed in the previous rhesus monkey study by Goldenthal et al. [1978a]. Also, in the 4.5 mg/kg/day dose group, mean serum cholesterol levels declined from 183 mg/100 ml to 99 within 30 days. SGOT increased from 36 to 95 u/l and alkaline phosphatase decreased from 1088 to 590 u/l. SGPT remained unchanged. Histopathology showed compound-related marked diffuse lipid depletion of the adrenals as well as diffuse atrophy of the pancreatic exocrine cells. Animals in the 0.5 mg/kg/day and 1.5 mg/kg/day dosage groups survived to the end of the study. Occasional diarrhea, anorexia and emesis were observed. There

was a decrease in serum alkaline phosphatase and inorganic phosphate in the 1.5 mg/kg/day group and a slight decrease in alkaline phosphatase in the 0.5 mg/kg/day group at the end of 90 days. Histopathology was unremarkable in both dosage groups.

A no observable adverse effect level (NOAEL) was not identified from any of the above three subchronic (90 day) studies. The results from these three subchronic studies suggest there may be a potential for cumulative toxicity over time with the primary toxic effect related to metabolic wasting. This may be due to an effect on peroxisome proliferation, fatty acid metabolism, membrane function, protein synthesis and/or mitochondrial bioenergetics.

To date, there are no data regarding the chronic toxicity and carcinogenicity of PFOS. PFOS was not observed to be mutagenic in several *Salmonella typhimurium* strains with or without metabolic activation [Jagannath and Brusic, 1978]. PFOS was negative in an *in vivo* mouse bone marrow micronucleus assay [Murli, 1996].

There have been two teratology studies conducted with PFOS. Oral administration, via corn oil, of PFOS at doses of 0, 1, 5 and 10 mg/kg/day to pregnant rats during days 6 - 15 of gestation resulted in fetuses with what was initially reported as teratogenic changes in the eye. [Gortner et al., 1980]. These fetal lens abnormalities were subsequently interpreted to be artifacts of the tissue sectioning process. Maternal body weights in the high dose group were significantly reduced but no significant treatment-related teratogenic or embryotoxic effects were reported. In the second teratology study, PFOS was administered in corn oil by oral gavage to groups of 25 pregnant rats on days 6 - 15 of gestation at doses of 0, 1, 5 and 10 mg/kg/day [Wetzel et al., 1983]. Maternal body weights and food consumption at 5 and 10 mg/kg/day were

significantly reduced compared to the control animals. Two female rats in the high dose group died before day 20. Clinical signs in surviving dams included hunching, thinness, alopecia, rough haircoat and anorexia. Treatment-related effects, primarily occurring in the high dose group, included increase resorptions and fetal death, decreased fetal body weight, delayed skeletal ossification, cleft palate, subcutaneous edema and cryptorchidism.

During the past 15 to 20 years there have been several endeavors designed to ascertain the health and exposure status of workers involved with fluorochemical production at the company's Decatur, Alabama and Antwerp, Belgium plants. Medical surveillance has been routinely conducted of fluorochemical production workers at both plants. Medical surveillance activities analyzed for total serum organic fluorine levels until the mid-1990's when serum PFOS determination, quantifiable by liquid chromatography mass spectrometry, became incorporated in the biennial medical surveillance examinations. However, we are aware of one occasion in 1979 where the serum of 5 Decatur employees was measured for PFOS by electron capture gas chromatograph and microwave plasma detection methods [Central Analytical Laboratory, 1979]. Total serum organic fluorine levels for these five employees were 10.1, 5.7, 9.4, 11.8 and 4.1 ppm. The percent of PFOS found was 60%, 70%, 80%, 55% and 65% of the total serum organic fluorine levels, respectively. In 1981, selected clinical chemistries and hematology values of Decatur employees in the chemical plant were compared to those results of employees in the adjacent 3M film plant [Roach, 1982; Schuman, 1982]. There were no significant correlation coefficients between total serum organic fluorine and gamma glutamyl transferase, serum glutamic oxaloacetic

transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, cholesterol, hemoglobin or red blood counts. However, this analysis was limited in scope (no dose response analysis), did not analyze specifically for PFOS, and did not account for several potential confounding factors. Another research initiative into the health status of Decatur employees was a retrospective cohort mortality study (1961 - 1991) conducted of former and current employees who had worked at least one year at the Decatur plant [Mandel and Johnson, 1995]. Vital status was determined for 99.7% of the 1,957 cohort members who had worked in the chemical and film plants. A total of 74 deaths were identified compared to 117.7 expected (U.S. rates). Among male employees who had worked only in the Decatur chemical plant, there were 32 deaths compared to 44.1 expected. There were no specific causes of death that had significantly elevated standardized mortality ratios. Because of its more recent construction in the 1970's, there has not been a retrospective cohort mortality study conducted of employees at the Antwerp plant.

The purpose of this report is to provide an aggregate analysis of the hematological, clinical chemistry and hormonal parameters, as measured in the medical surveillance examinations of Antwerp and Decatur employees in two separate time periods, in relation to the workers' serum PFOS levels. Although female employees also participated in these medical surveillance examinations, their actual numbers were too few to provide meaningful statistical analysis.

METHODS

PFOS Production

PFOS production began in Antwerp in 1976 and in Decatur in 1961. In general, perfluorinated chemicals are produced via an electrochemical process: a solution of organic substrate is electrolyzed in anhydrous hydrogen fluoride at a low voltage (Simons and Bryce, 1954). Basically, the products of this electrolysis cell reaction are highly fluorinated compounds with the end-product defined by the starting material. Products manufactured at these two plants include: Scotchgard™ brand fiber, leather and carpet protector; Light Water™ brand aqueous film forming foam (AFFF); Scotchban™ paper treatment; Kel-F™ brand plastic and Fluorel™ brand elastomers.

Subject Selection

General medical surveillance occurs biennially for employees at both of these plants. Participation is voluntary with approximately 100 Antwerp and 250 Decatur employees eligible for surveillance. A total of 88 Antwerp employees participated in the medical surveillance examinations in the Spring, 1995 and 90 Decatur employees participated in the Fall, 1994. In the Fall of 1997, a total of 149 employees (Antwerp = 65; Decatur = 84) participated in medical surveillance examinations. For purposes of brevity, these time periods will be referred to as 1995 and 1997. Altogether, 61 employees participated in both examination years (1995 and 1997). This lower number was due to a large turnover of employees at both plant locations during 1996-1997. For each time period the surveillance consisted of a medical questionnaire, measurement of height, weight and blood pressure, standard clinical chemistry and hematology tests, and determination of serum PFOS levels.

In 1995, several hormones were also analyzed for male employees who were judged *a priori* to have had likely PFOS exposure (i.e., those working in or in the immediate vicinity of the PFOS production area). Of the 88 Antwerp employees, 50 had hormone measurements. Of the 90 Decatur employees, 38 underwent hormone measurements.

PFOS Analysis

In 1995 the analysis for serum PFOS was conducted by 3M'S Environmental Technology Services in St. Paul, Minnesota. The method used tetrabutylammonium to ion-pair with PFOS in serum. The ion-pairs were then extracted with ethyl acetate. The abstraction product was then analyzed using high performance liquid chromatography-thermospray mass spectrometry [Johnson et al., 1996]. In 1997 the serum samples were analyzed by TurboIonSpray liquid chromatography/mass spectrometry using selected ion monitoring in the negative ion mode by Advanced Bioanalytical Services, Inc. [Anderson et al., 1997a; 1997b]. The lower limit of quantitation was 0.1 μg/mL for PFOS.

Laboratory Analyses

For both time periods, the United Laboratory Services (St. Paul, Minnesota) performed the standard hematological and clinical chemistry tests. These included the following hematological tests: hematocrit (percent), hemoglobin (gm/dl), red blood cells (RBC, 1000/mm^3), white blood cells (WBC, 1000/ mm^3) and platelet count (1000/ mm^3); and the following clinical chemistry tests: alkaline phosphatase (IU/L), gamma glutamyl transferase (GGT, IU/L), aspartate aminotransferase (AST, IU/L) formerly

known as serum glutamic oxaloacetic acid (SGOT), alanine aminotransferase (ALT, IU/L) formerly known as serum glutamic oxaloacetic transaminase (SGPT), total and direct bilirubin (mg/dl), blood urea nitrogen (BUN, mg/dl), serum creatinine (mg/dl), glucose (mg/dl), cholesterol (mg/dl), high density cholesterol (HDL, mg/dl) and triglycerides (mg/dl). Low density lipoprotein (mg/dl) was calculated as the following: LDL = [cholesterol - HDL - (tryglycerides/5)].

Eleven hormones were assayed in 1995: cortisol, dihydroepiandrosterone sulfate (DHEAS), estradiol, follicle stimulating hormone (FSH), 17 alpha hydroxyprogesterone (17-HP), free testosterone, total testosterone, luteinizing hormone (LH), prolactin, thyroid stimulating hormone (TSH) and sex hormone binding globulin (SHBG). All but SHBG (Endocrine Science Reference Laboratory, Tarzana, CA) were analyzed at the University of Minnesota's Endocrinology Laboratory.

Cortisol was assayed using a fluorescence polarization immunoassay (Abbott TDx). Radioimmunoassays (RIA) were used for DHEAS (Pantex), estradiol (modified Pantex), 17-HP (modified CIS) and total testosterone (Diagnostic Product Corp. Coat-A Count). Free testosterone was determined using equilibrium dialysis. LH, FSH and prolactin were assayed using a microparticle enzyme immunoassay (Abbott Imx). TSH was determined using a chemiluminescence immunometric assay (Nichols). SHBG was assessed via a radioimmunoassay after chromatographic sample purification (Endocrine Science Reference Laboratory). Bound testosterone was calculated as total testosterone less free testosterone.

Data Analysis

Descriptive simple and stratified analyses, Pearson correlation coefficients, ANOVA and ordinary multivariable regression were used to evaluate associations between PFOS and each hematological and clinical chemistry test and hormonal assay. Age, body mass index, current alcohol consumption (drinks per day) and cigarette use (cigarettes smoked per day) were potential confounding factors that were considered in the analyses. For stratified analyses, employees were divided into four PFOS categories: 0-1 ppm, 1 - < 3 ppm, 3 - < 6 ppm and $\geq$ 6 ppm in order to determine if an effect existed at the highest serum PFOS levels. Other categorical cutoff points were also used which provided similar results. For multivariable regression analyses, PFOS and the potential confounders of age, body mass index (BMI), alcohol use and cigarette use were examined as continuous explanatory variables in the models. Multivariable regression models were fitted with PFOS analyzed as a continuous variable using linear as well as non-linear transformations (quadratic, square, square root and inverse) in order to maximize the possibility of finding associations between PFOS and the dependent variable of interest. Linear and nonlinear relationships were examined by residual diagnostics using studentized and Cook's distance values. Natural log transformations of the dependent variables were performed, when necessary, to normalize variables and to enhance model fit. Traditional stepwise selection procedures were also employed (selection in and out of model was set at $p = 0.1$) as well as taking into account other covariants that may be on the biologic pathway of effect [Greenland, 1989]. We did not examine changes in measured PFOS between the two time periods because the estimated half-life of PFOS is at least two years. Study results were analyzed using the SAS System [1990].

Analyses are presented by plant, year and the three major groups of participants: all employees who participated in each year (1995: N = 178; 1997: N = 149); only those employees (N = 61) who participated in both years; and only those employees (N = 88) who participated in the hormone measurements in 1995.

RESULTS

The distribution of employees, by serum PFOS exposure categorization, is presented in Table 1. Whereas 20 percent of the Decatur employees had exposures at ≥ 3 ppm for both years, this proportion in Antwerp went from 25 percent in 1995 to 13 percent in 1997. For both years 95 percent of the measured serum PFOS levels were below 6 ppm. There were no PFOS measurements ≥ 6 ppm in Antwerp in 1997.

The overall mean values of PFOS, demographic, serum chemistry and hematological parameters for both locations, as well as each location separately, are presented in Tables 2 and 3, respectively. In particular, the Antwerp male employee population was significantly younger than Decatur, had lower body mass indices and higher self-reported daily consumption of alcohol. In addition, their clinical profiles were also different for several tests. The Antwerp employees had lower mean alkaline phosphatase, creatinine, glucose and triglyceride values and higher total bilirubin, HDL and hematocrit values.

Presented in Table 4 are the Pearson correlation coefficients between PFOS and the selected parameters of interest by both locations combined, each location separately, and by year of examination. In 1995, variables that were significantly ($p < .05$) correlated with PFOS for both locations combined included total bilirubin, white blood

cells and platelets. Although creatinine was not significantly correlated when both locations were examined, it was negatively correlated with PFOS in Antwerp but positively correlated in Decatur. In 1997, variables that were significantly correlated with PFOS for both locations combined were BMI, ALT, direct bilirubin, cholesterol, LDL and hematocrit. In addition, GGT and triglycerides were significantly positively correlated with PFOS among only Antwerp employees.

Provided in Table 5 are the mean, median, standard deviation and range of the covariates and the clinical chemistries and hematological parameters by four levels of PFOS categorization (0 - < 1, 1 - < 3, 3 < 6 and $\geq$ 6 ppm) for both years. Several observations are noteworthy. First, the mean for the $\geq$ 6 ppm PFOS category was one order of magnitude higher than the lowest PFOS category (0 - < 1 ppm) for both years. Also, the means of the four PFOS categories were significantly different from each other. Second, the youngest employees had the lowest serum levels of PFOS. Third, there was only one variable, total bilirubin, which had significant ($p < .05$) F tests for differences in means in both years of analysis. Besides total bilirubin, the only other variable in which the mean of the higher levels of PFOS exposure (3 - < 6 ppm or $\geq$ 6 ppm) was significantly different from the lowest category level of PFOS exposure (0 - < 1 ppm) was for WBC's in 1995. This was not observed in 1997. The lowest mean platelet count was observed at the highest PFOS exposure category in both years although the mean platelet counts by PFOS categories were not significantly different from each other.

Provided in the next two tables are the mean, median, standard deviation and range of the covariates and the clinical chemistries and hematological parameters by the four levels of PFOS categorization for each plant for 1995 (Table 6) and 1997 (Table 7).

In 1995 (Table 6) in Antwerp only, alcohol consumption was associated with higher PFOS levels. Mean serum creatinine levels declined in Antwerp but increased in Decatur employees. Antwerp employees in the 3 - < 6 ppm PFOS category smoked more cigarettes and had higher WBC levels. In 1997, Antwerp employees in the lowest PFOS exposure category were significantly younger than their counterparts. Antwerp employees in the higher PFOS category levels had higher mean alcohol consumption levels. Mean cholesterol, LDL and triglyceride levels trended upwards by PFOS exposure categories for Antwerp employees.

Linear and nonlinear relationships between PFOS and the dependent variables of interest, taking into account the potential confounding affects of age, BMI, alcohol and cigarettes, resulted in numerous analyses. For purposes of brevity, linear regression models are presented in Table 8 which show the effect that the parameter of interest, PFOS, has on the various dependent variables, adjusted for age, body mass index, alcohol and cigarette use. These covariates were analyzed as continuous variables. In the case of serum creatinine and total bilirubin, a quadratic ($PFOS + PFOS^2$) analysis provided the best statistical model of the data adjusted for the four potential confounders. The natural log transformation of total bilirubin, GGT and glucose provided the best fit for these response variables. PFOS was significantly associated ($p < .10$) in both years for only one clinical parameter: total bilirubin. PFOS was associated in one of the two years for the following variables: direct bilirubin, creatinine, cholesterol, LDL, HDL, hematocrit, hemoglobin and platelet count.

Those variables that were observed to be associated in at least one year in the regression models in Table 8 are separated by plant location and year in Table 9. After

separate analyses by employee population, only two variables, total bilirubin and HDL, remained significantly (negatively) associated with PFOS for at least one plant location for both time periods. Total bilirubin showed a significant negative association with PFOS (quadratic relation) for employees at the Decatur plant in both years. There were no significant associations among the Antwerp population between PFOS and total bilirubin. HDL was significantly negatively associated with PFOS in Antwerp in both 1995 and 1997 but was not significantly associated with PFOS in Decatur in either year. As for inconsistent associations observed in Table 8, direct bilirubin was not significantly associated with PFOS in either plant location (Table 9). The quadratic association for PFOS with creatinine was observed in Antwerp in 1995 and Decatur in 1997 but not in Antwerp in 1997 or Decatur in 1995. Cholesterol (and LDL) was observed to be positively associated with PFOS only in Decatur in 1997. Hematocrit and hemoglobin were associated with PFOS only in Decatur in 1997. Platelet counts were observed to be significantly negatively associated with PFOS only in Decatur in 1995.

Traditional stepwise regression modeling techniques were also used as well as testing models with variables that would be considered on the biological pathway of effect for any dependent variable. The associations (or lack thereof) from these analyses were similar to what has been presented in Tables 8 and 9. For purposes of brevity these analyses are not shown.

To further understand the association between total bilirubin and PFOS, scatter plots are presented for both time periods and by location in Appendix A. In addition, unconjugated bilirubin was also calculated (total bilirubin - direct) and these scatter plots are presented in Appendix B. Table 10 is a summary of these scatter plots from both

Appendices. The strongest associations appeared to be quadratic in nature primarily for the Decatur location and the percent of variability explained ranged between 3 (1995 data) and 7 percent (1997 data). The linear component of the quadratic was negative in direction. The upward trend appeared to occur around 6 ppm PFOS where the data are sparse. These simple linear and quadratic models were not influenced by any one employee according to residual diagnostics.

To further understand the possible association between HDL and PFOS, scatter plots are presented for both times and by location in Appendix C. Both locations combined resulted in significant negative linear and nonlinear (quadratic) associations in 1995 although the percent of variability explained in these models ranged between 3 and 5 percent. No significant associations were observed for each plant location in 1995. There were no significant negative associations between HDL and PFOS in 1997 for either the combined locations or each separate plant site.

Provided in Tables 11 through 14 are the analyses restricted to the 61 employees who participated in surveillance in both years. Table 11 provides the mean values for each parameter for the employees who participated in both exams compared to those who participated in only one of the two years. Overall, there were few differences. The mean age of the 61 employees was lower than that of the 1995 employees who didn't participate in 1997. Conversely, the mean age of the 61 employees was higher than that of the 1997 employees who didn't participate in 1995. Cholesterol and LDL were significantly higher in the 61 participants in 1997. Tables 12 and 13 present the mean values by plant location for 1995 and 1997, respectively. Of these 61 employees, 27 were from Antwerp and 34 from Decatur. Of noteworthy importance are the differences

between the 27 Antwerp employees and their fellow employees in 1997 (Table 13). The 27 Antwerp employees had significantly higher mean PFOS exposures, were significantly older, had greater BMI's and higher cholesterol values. Multivariable regression analyses for the 61 employees are presented in Table 14. The only significant association with PFOS appeared to be with serum creatinine (quadratic) in 1995.

Regardless of plant location, mean PFOS levels were higher for those employees who were selected for hormone measurements in 1995 (Table 15). This was expected as these employees were selected with the *a priori* belief that their serum measurements would be higher due to their workplace experience. For example, of the 42 employees in 1995 whose serum PFOS levels were ≥ 3 ppm, 76% had hormone measurements.

Presented in Table 16 are the mean values for PFOS, demographic, serum chemistries and hematology for those employees who had hormone measurements compared to those employees who did not in 1995. Those employees who had hormone measurements were younger, higher users of alcohol (Antwerp only) and cigarettes (both locations), and had lower serum creatinine (Antwerp only) and higher WBC levels (both locations). The latter observation is confounded by cigarette smoking as among non-smokers, those selected for hormone measurements had a mean WBC of 6.24 compared to 6.03 for non-selected employees ($p = .36$). Among smokers, those selected had a mean WBC of 8.69 compared to 8.06 for non-selected employees ($p = .23$). All other clinical parameters were comparable, by PFOS exposure categories, between subjects who had hormone measurements and those who did not in 1995 (Tables 17 and 18).

The Pearson correlation coefficients between PFOS and the hormones tested among the 88 employees were the following: cortisol (.07), DHEAS (-.13), estradiol

(.09), FSH (.06), 17-hydroxyprogesterone (-.04), LH (.03), prolactin (.06), SHBG (.11), free testosterone (-.06), bound testosterone (.06), TSH (.01). None were statistically significant.

Presented in Table 19 are the mean, median, standard deviation and range of the various hormones by the four PFOS categories: 0- <1 ppm, 1 - < 3 ppm, 3 - < 6 ppm and ≥ 6 ppm. Several observations are noteworthy. First, the mean age of the lowest PFOS exposure category was 10 years less than that of the highest exposure category. Therefore it was not unexpected to observe that the mean DHEAS, 17-HP, free testosterone and bound testosterone levels of this lowest exposure category were greater than the means of the higher PFOS exposure categorizations. Adjusting for the differences in age (as well as the other three potential confounders) in the regression models (Table 20) resulted in no significant associations between PFOS and the hormones analyzed, except for estradiol. With estradiol, a quadratic model provided the best fit of the data and both PFOS terms were significant. Upon residual diagnostics it was determined that this estradiol model was influenced by one specific employee (employee A). The influence of employee A is best seen in Figures 1 and 2 which are simple scatter plots of both the linear and quadratic fits of estradiol and PFOS, with and without employee A, respectively. Employee A had a 12.83 ppm serum level of PFOS which was the highest value recorded in 1995. His estradiol value was 92 pg/dl (see upper right hand corner of Figure 1). Employee's A estradiol value was also influenced by the fact that his body mass index was 33 kg/m^2. Exclusion of this employee resulted in a nonsignificant quadratic equation. The variability (R^2) of the data explained went from 7.6 percent to 2.1 percent upon exclusion of this employee. The slope of the linear equation changed from

positive to negative although it was nonsignificant in both Figures 1 and 2. Finally, it should be noted that the estradiol models in Table 20, with and without employee A, did predict the known positive assocation between estradiol and body mass index.

DISCUSSION

We conducted two cross-sectional analyses of surveillance data to examine the associations between serum PFOS levels and several hematological, clinical chemistry and hormonal parameters in male fluorochemical production employees. For both years, 95 percent of the measured serum PFOS levels were below 6 ppm. Because the Antwerp and Decatur employees were dissimilar by age, body mass indices and self-reported alcohol use, we conducted combined as well as separate analyses by plant location. These three demographic differences likely explain why the Antwerp employees had lower mean serum levels of alkaline phosphatase, HDL, triglycerides and blood glucose [Davern and Scharschmidt, 1993; Lewis, 1994; Friedman, 1998; Fu, 1998; Wolf, 1998].

In the present study, alkaline phosphatase, GGT, AST and ALT values were not significantly associated with the measured serum PFOS levels. This was an *a priori* question due to the fact that PFOS: 1) is a peroxisome proliferator in the rat [Ikeda et al., 1987; Sohlenius et al., 1993]; 2) resulted in slight to marked increases in plasma glutamic oxalacetic and pryuvic transaminase levels in a 90 day study of rats fed diets which contained PFOS at 100 ppm along with hypertrophy and liver necrosis observed at histopathology [Goldenthal, 1978a]; and 3) increased SGOT and decreased alkaline phosphatase levels in monkeys after administration, by oral gavage, for 30 days of doses of 4.5 mg/kg/day of PFOS [Goldenthal, 1978b]. On the other hand, SGPT values

remained constant and no histopathologic abnormalities were noted in the livers of these monkeys which died by the 7th week of the study. No significant liver enzymatic or histopathology changes occurred in monkeys in the 0.5 and 1.5 mg/kg/day dose groups.

We did observe a quadratic association with total bilirubin among only the Decatur employees. We do not suspect this is a biological association because the bilirubin levels were within the normal reference range. Also, the percent variability explained of total bilirubin by PFOS in the regression models was low. The Antwerp employees' total bilirubin levels were significantly higher than the Decatur employees' levels. We offer several possible explanations for this observation. First, we suspect there may be a greater prevalence of Gilbert's syndrome [Lidofsky and Scharschmidt, 1993; Friedman, 1998] among the Antwerp employees. In 1995, 15 (17%) Antwerp employees had total bilirubin values $\geq$ 1.2 mg/dl compared to 3 (3%) Decatur employees' levels. In 1997, there were 9 (15%) Antwerp and 2 (2%) Decatur employees with total bilirubin values $\geq$ 1.2 mg/dl. However, there was not a concomitant decline in bilirubin conjugation (i.e., direct bilirubin levels) as might be expected among individuals diagnosed with Gilbert's syndrome. Nevertheless, exclusion of these possible Gilbert's syndrome employees still resulted in higher mean total bilirubin values among the Antwerp employees in both years. Secondly, there were four Antwerp employees who self-reported hepatitis A histories and one employee self-reported a history of Hepatitis B. Fish and shellfish consumption is likely much greater in Antwerp than Decatur due to its vicinity near the North Atlantic. Third, bilirubin is a tetrapyrrole that is an end-product of heme degradation [Lidofsky and Scharschmidt, 1993]. Bilirubin levels may be increased due to disorders of bilirubin metabolism, liver

disease and obstruction of the bile ducts. Other hematological and clinical chemistry results did not suggest these conditions existed among the Decatur employees. Fourth, post-collection procedures may result in error. Total bilirubin determination may be falsely depressed if hemolysis is present because of increased absorbence in the blank [Kaplan and Pesce, 1984]. Bilirubin is also sensitive to and destroyed by light and heat. We are uncertain whether these factors could have contributed to the lower total bilirubin levels in the Decatur samples in both years. Finally, the linear component of the quadratic association observed among Decatur employees is negative in direction in relation with their measured PFOS levels. That is, total bilirubin levels declined with increasing PFOS levels. We would expect a positive association if PFOS impaired bilirubin conjugation. The trend upwards in the quadratic appears to occur at levels 6 ppm and higher where the data are sparse. We conclude that the association observed among only the Decatur employees is unlikely to be related to serum levels of PFOS.

We did observe a positive association between serum PFOS and serum cholesterol levels in the 1997 time period for Decatur employees. This result is unlikely to have a biological explanation as PFOS is a known peroxisome proliferator in the rat and was shown to have hypolipidemic properties in rhesus monkeys [Ikeda et al., 1987; Sohlenius et al., 1993; Goldenthal 1978b; 1979]. Rhesus monkeys fed PFOS at 4.5 mg/kg in their chow had serum cholesterol values reduced from 183 mg/L to 99 mg/L within 30 days. Rhesus monkeys fed 1.5 mg/kg in the chow had cholesterol levels reduced from 195 mg/kg to 111 mg/kg within 90 days [Goldenthal 1978b]. As for HDL, although the multivariable analyses were suggestive of a negative association between HDL and PFOS

in Antwerp (but not Decatur), the scatter plots presented in Appendix C do not support the notion of a biological association between PFOS and HDL.

It should be noted that total organic fluorine levels, primarily consisting of perfluorooctanoic acid (PFOA, $C_7F_{15}COO^-$), a seven carbon perfluorinated carboxylic acid, were reported to reduce the effect that alcohol has on HDL levels among higher exposed male PFOA production workers in Cottage Grove, Minnesota [Gilliland and Mandel, 1995]. However, this finding was not observed in subsequent analyses of these employees (Olsen et al., unpublished findings). This observation by Gilliland and Mandel was testable in the present study as both Antwerp and Decatur employees had measurable quantities of PFOA. We did not observe a significant negative modulation of the effect of alcohol consumption on HDL levels among Antwerp and Decatur employees with higher serum PFOA levels although their serum levels were approximately 3 to 5-fold less, on average, than that reported in Cottage Grove employees [Olsen et al., 1998]. The Antwerp and Decatur employees were exposed to PFOA, not in its actual production, but rather in its use as a surfactant in the production of fluoropolymers. In 1995 the mean serum PFOA level among the Antwerp and Decatur employees combined was 1.46 ppm (range 0 - 13.20 ppm) and in 1997 the mean serum level was 1.57 ppm (range 0.11 - 11.10). Stratified by plant location, the 1995 and 1997 mean serum PFOA levels were 1.19 and 1.78 ppm in the Antwerp employees and 1.72 and 1.40 ppm in the Decatur employees, respectively.

The multivariable regression models showed a negative association between PFOS and platelet counts at PFOS levels above 6 ppm in 1995 and this trend was also apparent, although to a lesser extent, in 1997. Nevertheless, platelet levels were well

within the normal reference range in both time periods. This association is not supported by a 90 day subchronic toxicity study which showed no decline in platelet counts for monkeys fed 0.5, 1.5 or 4.5 mg/kg for up to 90 days [Goldenthal, 1978b]. Mean platelet counts among the 1.5 mg/kg/day and 0.5 mg/kg/day dose groups were 226 and 231 (10^3/cmm) compared to 218 in the control group [Goldenthal, 1978b]. There were no platelet counts reported in the 90 day rat study although at the end of 3 months of study there were slight to moderate decreases in hemoglobin, hematocrit and erythrocyte counts observed for male and female rats in the 100 ppm dose group [Goldenthal, 1978a]. No consistent associations were observed between PFOS and hemoglobin, hematocrit or RBC values in the present epidemiologic investigation. In a prior subchronic rhesus monkey study that was aborted early due to all animals died by the 20th day, mean platelet counts were 203, 219, 136, 172 and 185 for the 300 mg/kg/day, 100 mg/kg/day, 30 mg/kg/day, 10 mg/kg/day and control groups, respectively [Goldenthal, 1979].

After controlling for age, a confounder for male testosterone hormone levels [Dali et al., 1981; Griffin and Wilson, 1994], we observed no significant associations with serum PFOS measurements. We did observe a quadratic association between estradiol and PFOS. Upon further examination, this finding was influenced by one particular employee who had the highest PFOS level but was confounded by the individual's large body mass index. Exclusion of this employee resulted in nonsignificant findings. Thus, any interpretation with estradiol is difficult because of the influence this one employee has on the statistical analyses.

It should be noted that perfluorooctanoic acid (PFOA), at approximately 50 - 100 ppm levels in serum, enhances the aromatase conversion of testosterone to estradiol in the

rat [Cook et al., 1992; Biegel et al., 1995]. However, PFOA production workers in Cottage Grove with serum levels up to 30 ppm appeared not to have altered serum estradiol levels [Olsen et al., 1998]. Again, like HDL, this was a testable hypothesis among the Antwerp and Decatur employees although their serum PFOA levels were lower than Cottage Grove employees. We did not observe any significant positive association between estradiol and serum PFOA levels in these Antwerp and Decatur employees.

Several methodological issues should be considered in evaluating the results from this study. First, the cross-sectional design does not allow for a direct analysis of the temporality of an association. Second, the voluntary participation rates in medical surveillance were not ideal as among eligible employees we had 88 and 65 percent participation in Antwerp for 1995 and 1997, respectively, but only 35 to 40 percent in Decatur for both years. Third, given the suspected long half-life of PFOS (at least two years), it may be conceivable that there may be some biological accommodation to the effects of PFOS which would minimize the possibility of finding an association. Fourth, it is known in laboratory animals that PFOS concentrates primarily in the liver. Serum measurements of PFOS may not adequately reflect body burden. Fifth, the two cross-sectional analyses cannot be viewed as independent populations as 61 employees were studied in both years. This was due, in part, to a large turnover of employees at both plants between examinations. Sixth, there could be measurement error in important confounding variables. Analysis of the data of the 61 subjects who participated in both years showed that there was excellent correlation for the confounding factors of BMI ($r = .92$, $p = .0001$), self-reported aspects of alcohol consumption ($r = .88$, $p = .0001$) and

cigarette smoking ($r = .79$, $p = .0001$). As expected, these 61 employees' serum PFOS levels for the two years were highly correlated ($r = .92$, $p = .0001$). Seventh, the quality of medical surveillance data, prior to its use for studying an *a priori* hypothesis, can often be evaluated by whether known positive associations are observed. In this regard, we observed various expected associations including cigarette smoking and elevated white blood cell counts and large body mass indices associated with elevated liver transaminase levels [Olsen et al., 1991; Burns et al., 1997]. Finally, the pulsatile nature of some of the hormones studied (e.g., FSH, LH, testosterone) has resulted in prior recommendations that mean hormone measurements should be the result of pooled blood from multiple samples taken at short intervals [Goldzieher et al., 1976]. In our study multiple samples were not feasible because of the low probability of employees voluntarily giving three serum samples over a 45 -60 minute period of time.

In summary, we conducted two cross-sectional analyses and did not observe consistent associations by plant location or time for several hematological parameters, serum chemistries and reproductive hormones with measured serum PFOS levels in male fluorochemical production employees. Ninety-five percent of the employees had serum PFOS levels below 6 ppm. Our findings suggest that, among these Antwerp and Decatur male fluorochemical production employees, significant hematological, clinical chemistry and hormonal abnormalities were not associated with serum PFOS concentrations less than 6 ppm. Any inferences derived from the few employees with serum PFOS levels ≥ 6 ppm would be tenuous, at best. Limitations of this study include its cross-sectional design, the voluntary participation rates, the few subjects exposed at the highest levels,

and the lower levels of serum PFOS measured among these employees compared to those that caused effects in two species of laboratory animals.

Acknowledgements

The authors gratefully acknowledge the assistance of Michele Burlew, Martha McGough, Jane Quarfoth and the helpful comments of Drs. John Butenhoff, Marvin Case and Andrew Seacat.

References

Anderson DJ, Mulvana DE (1997a). Analytical report for the determination of perfluorooctanoate and perfluorooctanesulfonate in human serum by LC/MS. Ithaca:Advanced Bioanalytical Services, Inc., August 25, 1997.

Anderson DJ, Mulvana DE (1997b). Analytical report for the determination of perfluorooctanoate and perfluorooctanesulfonate in human serum by LC/MS. Ithaca:Advanced Bioanalytical Services, Inc., September 22, 1997.

Biegel LB, Liu RCM, Hurtt ME, Cook JC (1995). Effects of ammonium perfluorooctanoate on Leydig cell function: in vitro, in vivo, and ex vivo studies. Toxicol Appl Pharmacol 134:18-25.

Burns CJ, Boswell JM, Olsen GW (1997). Liver enzyme activity and body mass index. J Occup Env Med 38:1248-1252.

Central Analytical Laboratory (1979). Characterization of Decatur RF Values. 3M Project Number 91721100, Report No. 7230, October 4, 1979.

Cook JC, Murray SM, Frame SR, Hurtt ME. Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. Toxicol Appl Pharmacol 113:209-217.

Davern TJ, Scharschmidt BF (1993). (In) Feldman M, Sleisenger MH, Scharschmidt BF (eds), Gastrointestinal and Liver Disease, 6th Edition. Philadelphia:W.B. Saunders Co., pages 1112 - 1122.

Dali WS, Kuller LH, LaPorte RE, Gutai JP, Falvo-Gerard L, Caggiula A (1981). The epidemiology of plasma testosterone levels in middle-aged men. Am J Epidemiol 114:804-816.

Friedman LS (1998). Liver, biliary tract and pancreas. (In) Tierney LM, McPhee SJ, Papadakis WA (eds), Medical Diagnosis and Treatment, 37th Edition. Stamford (CT):Appleton and Lange pages 628-630.

Fu, PC (1991). Lipids, lipoproteins, and apolipoproteins. (In) Howanitz JH, Howanitz PJ (eds), Laboratory medicine: test selection and interpretation. New York:Churchill Livingstone Inc., pages 173-190.

Gabriel KL (1976). Acute oral toxicity in rats of T-1389. Biosearch, Inc., Philadelphia, PA.

Gibson SJ, Johnson JD, Ober RE (1983). Absorption and biotransformation of N-ethyl FOSE and tissue distribution and elimination of carbon-14 after administration of N-ethyl FOSE-^{14}C in feed. St. Paul:Riker Laboratories, Inc.

Gilliland FD, Mandel JS (1996). Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins, and cholesterol: a study of occupationally exposed men. A J Ind Med 29:560-568.

Glaza SM (1995). Single-dose dermal absorption/toxicity study of T-6049 in rabbits. HWI 6329-130, Hazelton Laboratories, Madison, WI.

Goldenthal EI, Jessup DC, Geil RG, Jefferson ND, Arceo RJ (1978a). Ninety day subacute rat toxicity study. Study No. 137-085, International Research Development Corp., Mattawan, MI.

Goldenthal EI, Jessup DC, Geil RG, Jefferson ND (1978b). Ninety-day subacute rhesus monkey toxicity study. Study No 137-085, International Research Development Corp., Mattawan, MI.

Goldenthal EI, Jessup DC, Geil RG, Jefferson ND (1979). 90-day subacute rhesus monkey toxicity study. Study No. 137-087, International Research Development Corp., Mattawan, MI.

Goldzieher JW, Dozier TS, Smith KD, Steinberger E. Improving the diagnositic reliability of rapidly fluctuating plasma hormone levels by optimized multiple-sampling techniques. J Clin Endocrinol Metab 43:824-830.

Gortner EG, Lamprecht EG, Case MT (1980). Oral teratology study of FC-95 in rats. Expt No. 060TR0008, Riker Laboratories, Inc., St. Paul, MN.

Greenland, S (1989). Modeling and variable selection in epidemiologic analysis. Am J Public Health 79:340-349.

Griffin JE, Wilson JD (1994). Disorders of the testes. (In) Isselbacker KJ, Braunwald E, Wilson JD, Martini JB, Fauci AS, Karper DL (eds):) Harrison's Principles of Internal Medicine. New York:McGraw-Hill, 1994:2006-2017.

Haughom B, Spydevold O (1992). The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluoroctane sulphonic acid (PFOSA) and clofibric acid. Biochemica et Biophysica Acta 1128:65-72.

Ikeda T, Fukuda K, Mori I, Enomoto M, Komai T, Suga T (1987). Induction of cytochrome P-450 and peroxisome proliferation in rat liver by perfluorinated octanesulfonic acid. In: Peroxisomes in Biology and Medicine, Fahimi HD, Sies H (eds), Springer Verlag, New York, pages 304-308.

Jagannath DR, Brusic D (1978). Mutagenicity evaluation of T-2014CoC in the Ames Salmonella/microsome plate test. Litton Bionetics, Inc., Kensington, MD.

Johnson JD, Gibson SJ, Ober RE (1979). Extent and route of excretion and tissue distribution of total carbon-14 in rats after a single i.v. dose of FC-95-^{14}C. Project No. 8900310200, Riker Laboratories, Inc., St. Paul, MN.

Johnson JD, Ober RE (1979). Absorption of FC-95-^{14}C in rats after a single oral dose. Project No. 8900310200, Riker Laboratories, Inc., St. Paul, MN.

Johnson JD, Gibson SJ, Ober RE (1980). Enhanced elimination of FC-95-^{14}C and FC-143-^{14}C in rats with cholestyramine treatment. Project No. 8900310200, Riker Laboratories, Inc., St. Paul, MN.

Johnson JD, Gibson SJ, Ober RE (1984). Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C] perfluorooctanoate or potassium [^{14}C] perfluorooctanesulfonate. Fund Appl Toxicol 4:972-976.

Johnson JD, Wolter JT, Colaizy GE, Rethwill PA, Nelson RM (1996). Quantification of perflurooctanoate and perfluorooctanesulfonate in human serum using ion-pair extraction and high performance liquid chromatography - thermospray mass spectrometry with automated sample preparation. J Chromatography Biomed Appl (submitted for publication).

Kaplan and Pesce (1984). Clincal Chemistry. New York:C.V. Mosby Co., page 1238.

Lewis JA (1994). Illustrated Guide to Diagnostic Tests. Springhouse (PA): Springhouse Corp., pages 100 - 107, 194-207.

Lidofsky S, Scharschmidt BF (1993). (In) Feldman M, Sleisenger MH, Scharschmidt BF (eds), Gastrointestinal and Liver Disease, 6th Edition. Philadelphia:W.B. Saunders Co., pages 220-224.

Mandel JS, Johnson RA (1995). Mortality study of employees at 3M plant in Decatur, Alabama. Division of Environmental and Occupational Health, University of Minnesota, March 13, 1995.

Murli H (1996). Mutagenicity test on T-6295 in an *in vivo* mouse micronucleus assay. CHV study No. 17403-0-455, Corning Hazelton Inc., (CHV), Vienna, VA.

Nabbefeld J, Butenhoff, J, Bass N, Seacat N (1998). Displacement of a fluorescently labeled fatty acid analogue from fatty acid carrier proteins by wyeth-14,643, ammonium perfluorooctanoate, potassium perfluorooctane sulfonate and other known peroxisome proliferators. Toxicologist (in press).

Olsen GW, Kusch GD, Stafford BA, Gudmundsen SL, Currier MF (1991). The positive known association design: a quality assurance method for occupational health surveillance data. J Occup Med 33:998-1000.

Olsen GW, Gilliland FD, Burlew MM, Burris JM, Mandel JS, Mandel JH (1998). An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. J Occup Env Med (in press).

O'Malley KD, Ebbens KL (1980). Twenty-eight day percutaneous absorption study with FC-95 in albino rabbits. Expt No. 0979AB0632, Riker Laboratories, Inc., St. Paul, MN.

Roach DE (1982). Fluorochemical Control Study. 3M unpublished report. May 25, 1982.

Rusch GM, Rinehart WE (1979). An acute inhalation toxicity study of T-2306CoC in the rat. Bio/dynamics, Inc.

SAS Institute, Inc. (1990). SAS Users Guide: Statistics. Version 6, SAS Institute, Inc., Cary, NC.

Schuman LM (1982). Letter to Dr. Frank Ubel (3M Medical Director) concerning Fluorochemical Control Study. April 29, 1982.

Sohlenius AK, Eriksson AM, Hogstrom C, Kimland M, DePierre JW (1993). Perfluorooctane sulfonic acid is a potent inducer of peroxisomal fatty acid beta-oxidation and other activities known to be affected by peroxisome proliferators in mouse liver. Pharmacol Toxicol 72:90-93.

Wetzel LT, Burdock GA, Durloo RS, Colpean BR (1983). Rat teratology study. Project No. 154-160, Hazelton Laboratories America, Inc., Vienna, VA.

Wolf PL (1991). Liver function. (In) Howanitz JH, Howanitz PJ (eds), Laboratory medicine: test selection and interpretation. New York:Churchill Livingstone Inc., pages 173-190.

Table 1

Distribution of Employees by Year, Location and PFOS Exposure Level (ppm)

	1995 Data								1997 Data							
	All Employees		Antwerp		Decatur				All Employees		Antwerp		Decatur			
PFOS	N	(%)	N	(%)	N	(%)	N		(%)	N	(%)	N	(%)			
0 - < 1 ppm	45	25	34	39	11	12			60	40	31	48	29	35		
1 - < 3 ppm	91	51	32	36	59	66			63	43	25	38	38	45		
3 - < 6 ppm	35	20	19	22	16	18			21	14	9	14	12	14		
>= 6 ppm	7	4	3	3	4	4			5	3	0	0	5	6		
	178	(100)	88	(100)	90	(100)			149	(100)	65	(100)	84	(100)		

Table 2

Mean, Median, Standard Deviation and Range of Study Parameters, Both Locations, 1995 and 1997

	1995 Data				1997 Data			
Variable	Mean	Median	Std Dev	Range	Mean	Median	Std. Dev.	Range
PFOS	2.19	1.70	1.87	0 - 12.83	1.75	1.30	1.60	0.10 - 9.93
Age	40.6	40.0	8.76	21 - 60	39.3	40.0	9.7	21 - 63
Alcohol	0.7	0.1	1.1	0 - 6.0	0.5	0.1	1.0	0 - 7.1
BMI	26.6	25.8	5.1	17.9 - 60.7	27.1	26.0	5.2	18.1 - 48.5
Cigarettes	6.3	0.0	10.7	0 - 40	6.1	0.0	10.3	0 - 40
Alk Phos	86.2	85.0	24.6	31 - 191	79.8	77.0	20.8	29 - 163
GGT	44.4	36.0	32.1	2 - 293	31.6	.24.0	25.9	10 - 179
AST	27.6	26.0	10.9	13 - 96	26.2	25.0	7.0	13 - 56
ALT	45.9	42.0	18.6	18 - 183	32.6	30.0	14.4	10 - 89
T. Bilirubin	0.72	0.70	0.37	0.20 - 2.90	0.67	0.60	0.32	0.30 - 2.30
D. Bilirubin	0.21	0.20	0.05	0.10 - 0.40	0.13	0.10	0.06	0.10 - 0.40

Table 2
(Continued)

Variable	Mean	Median	Std Dev	Range	Mean	Median	Std. Dev.	Range
BUN	15.9	15.0	3.8	8 - 26	14.4	14.0	3.0	6 - 26
Creatinine	1.0	1.0	0.2	0.6 - 1.6	0.9	0.9	0.1	0.6 - 1.4
Glucose	86.7	85.0	18.8	60 - 260	90.7	86.0	29.9	58 - 303
Cholesterol	215.9	214.0	42.5	100 - 340	211.2	213.0	40.7	110 - 365
LDL	136.6	134.0	39.1	28 - 261	135.3	135.0	35.0	50 - 290
HDL	48.5	48.0	12.7	23 - 94	45.5	45.0	10.6	19 - 74
Triglycerides	151.0	126.0	99.9	34 - 651	156.5	122.0	130.0	38 - 1209
Hematocrit	46.3	47.0	2.7	38.3 - 52.0	45.8	46.0	2.6	39 - 52.9
Hemoglobin	15.3	15.3	0.9	13 - 17.4	15.4	15.5	0.9	12.5 - 17.3
RBC	4.9	4.9	0.3	4.0 - 5.74	5.1	4.8	0.3	4.1 - 5.9
MCH	31.1	31.0	1.5	26.0 - 36.9	30.5	31.0	1.5	26.2 - 34.1
MCHC	33.1	33.0	0.7	31.3 - 34.7	33.6	34.0	0.5	31.8 - 34.9
MCV	93.7	94.0	4.9	79.7 - 114.6	90.7	91.0	.42	80.0 - 101.0
WBC	6.9	6.6	1.9	3.6 - 15.5	6.5	6.2	1.7	3.8 - 13.2
Platelets	226.4	223.0	45.4	122 - 367	225.7	230.0	50.4	106 - 406

Table 3

Mean Values of PFOS, Demographic, Serum Chemistry and Hematologic Parameters for Antwerp and Decatur, 1995 and 1997 Examinations

	1995 Data		1997 Data	
Variable	Antwerp	Decatur	Antwerp	Decatur
PFOS (ppm)	1.93	2.44	1.48	1.96
Age	37***	45	33***	44
BMI	23.9***	29.2	23.5***	30.0
Cigarettes	4.7*	7.9	5.5	6.6
Alcohol	1.3***	0.2	1.1***	0.1
Alk Phosphatase	75***	97	70***	87
GGT	41	48	26*	36
AST	26*	29	27	26
ALT	44	47	31	34
Total bilirubin	0.86***	0.58	0.80***	0.58
Direct bilirubin	0.22	0.21	0.15***	0.12
BUN	17.0***	14.8	14.9	14.1
Creatinine	0.9***	1.1	0.9**	1.0
Glucose	81***	92	81***	98
Cholesterol	214	218	206	215
LDL	138	136	134	137
HDL	54***	43	50***	42
Triglycerides	115***	187	111***	192

Table 3
(continued)

Variable	1995 Data		1997 Data	
	Antwerp	Decatur	Antwerp	Decatur
Hematocrit	47***	46	46*	45
Hemoglobin	15.4	15.2	15.4	15.3
RBC	4.9	5.0	5.1	5.1
MCH	31.4***	30.7	30.5	30.4
MCHC	32.9***	33.4	33.3***	33.7
MVC	95.7***	91.8	91.6*	90.0
WBC	6.4***	7.5	6.5	6.4
Platelets	224	229	237*	217

* $p < .05$; ** $p < .01$; *** $p < .001$

Table 4

Correlation Coefficients Between PFOS and Selected Variables by Location and Year of Examination

	1995 Data			1997 Data		
Variable	Both Locations	Antwerp	Decatur	Both Locations	Antwerp	Decatur
Age	.12	.10	.04	.20*	.41***	-.01
Alcohol	.09	.25*	.06	.10	.40***	-.07
BMI	.01	-.14	-.05	.18*	.22	.09
Cigarettes	.14	.19	.08	-.03	.08	-.08
BUN	-.05	-.04	.01	.01	.13	-.01
Creatinine	.05	-.37***	.28**	.08	-.12	.12
Glucose	-.02	-.17	-.02	.10	.04	.06
Alkaline Phosphatase	.06	.07	-.06	.07	.09	-.02
GGT	.01	.02	-.04	.07	.26*	-.06
AST	.06	.06	.0002	.003	.03	.001

Table 4
(continued)

Variable	1995 Data			1997 Data		
	Both Locations	Antwerp	Decatur	Both Locations	Antwerp	Decatur
ALT	-.01	-.03	-.03	.16*	.11	.16
Total Bilirubin	-.15*	-.13	-.07	-.13	-.12	-.07
Direct Bilirubin	-.03	-.13	.09	-.18*	-.13	-.17
Cholesterol	-.02	.02	-.08	.25**	.34**	.19
LDL	.01	.02	.02	.22**	.30*	.17
HDL	-.17*	-.14	-.12	-.07	-.05	.0005
Triglycerides	.04	.11	-.10	.10	.39***	-.01
Hematocrit	.002	.10	-.0003	-.16*	-.05	-.20
Hemoglobin	-.01	.05	-.02	-.15	-.05	-.20
Red Blood Cells	-.03	-.11	.01	-.11	-.15	-.10
MCH	.04	.21*	-.04	-.04	.17	-.10

Table 4
(continued)

Variable	1995 Data			1997 Data		
	Both Locations	Antwerp	Decatur	Both Locations	Antwerp	Decatur
MCHC	-.03	-.11	-.09	.01	-.03	-.06
MCV	.05	.25*	-.01	-.03	.20	-.09
White Blood Cells	.18*	.27**	.04	.01	.18	-.09
Platelets	-.15*	-.06	-.25*	-.11	-.06	-.10

* $p < .05$; ** $p < .01$; *** $p < .001$

Table 5

Mean, Median, Standard Deviation (SD) of Mean and Range of PFOS, Demographic, Serum Chemistries and Hematological for Antwerp and Decatur Employees Combined, 1995 (N=178) and 1997 (N=147)

PFOS* (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
				PFOS (ppm)				
0 - < 1ppm	0.49[1]	0.5	0.27	000 - 0.90	0.52[1]	0.52	0.27	0.10 - 0.97
1 - < 3ppm	1.82[1]	1.77	0.58	1.00 - 2.91	1.78[1]	1.64	0.56	1.02 - 2.89
3 - < 6ppm	4.12[1]	3.97	0.81	3.00 - 5.80	3.87[1]	3.59	0.70	3.09 - 5.30
≥ 6ppm	8.17[1]	7.73	2.52	6.06 - 12.83	7.20[1]	6.68	1.59	6.05 - 9.3
	F value = 321.9, p < .0001				F value = 367.6, p < .0001			
				Age (yrs)				
0 - < 1 ppm	37	36	8	21 - 58	36	34	11	21 - 62
1 - < 3 ppm	42[2]	41	9	25 - 60	42[2]	41	9	24 - 63
3 - < 6 ppm	40	40	7	26 - 55	41	42	5	32 - 54
≥ 6 ppm	45	43	7	37 - 56	42	45	9	29 - 52
	F value = 3.7, p = .02				F value = 5.1, p = .002			

Table 5 (continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				Alcohol (drinks/day)				
0 - < 1ppm	0.8	0.6	0.9	0.0 - 3.6	0.5	0.1	0.8	0 - 4.3
1 - < 3 ppm	0.5	0.1	0.7	0.0 - 3.6	0.5	0.1	0.8	0 - 5.0
3 - < 6 ppm	1.2	0.3	1.9	0.0 - 6.0	1.0	0.1	1.8	0 - 7.1
≥ 6ppm	0.7	0.0	1.1	0.0 - 2.9	0.2	0.1	0.2	0.1 - 0.8
	F value = 4.0, p = .009				F value = 1.8, p = .15			
				BMI (kg/m^2)				
0 - < 1 ppm	25.5	24.8	4.2	17.9 - 38.7	26.0	24.9	4.9	20.1 - 41.7
1 - < 3 ppm	27.7	26.3	5.8	19.6 - 60.7	27.7	26.4	5.7	18.1 - 48.5
3 - < 6 ppm	24.9[3]	25.0	3.8	17.9 - 32.5	27.3	27.9	4.4	19.1 - 36.0
≥ 6 ppm	27.7	29.4	4.2	20.6 - 33.0	30.8	29.7	4.0	26.1 - 36.2
	F value = 3.7, p = .02				F value = 2.1, p = .10			
				Cigarettes (per day)				
0 - < 1 ppm	2.6	0.0	6.4	0.0 - 25.0	4.7	0.0	9.4	0 - 40
1 - < 3 ppm	6.8	0.0	11.3	0.0 - 40.0	8.2	0.0	11.3	0 - 40
3 - < 6 ppm	10.6	8.0	12.4	0.0 - 40.0	4.1	0.0	8.3	0 - 30
≥ 6 ppm	0.4	0.0	1.1	0.0 - 3.0	6.0	0.0	13.4	0 - 30
	F value = 4.8, p = .003				F value = 1.5, p = .23			

Table 5 (continued)

PFOS (ppm)	1995 Data Mean	1995 Data Median	1995 Data SD	1995 Data Range	1997 Data Mean	1997 Data Median	1997 Data SD	1997 Data Range
BUN								
0 - < 1ppm	16.5	15	3.5	11 - 26	14.5	14.0	2.8	9.0 - 21.0
1 - <3 ppm	15.4	15.0	4.0	8.0 - 26.0	14.2	14.0	3.2	6.0 - 26.0
3 - < 6 ppm	16.4	16.0	3.7	10.0 - 23.0	15.0	15.0	2.9	9.0 - 20.0
≥ 6 ppm	15.1	14.0	4.2	10.0 - 21.0	13.8	12.0	4.1	9.0 - 19.0
	F value = 1.1, p = .36				F value = 0.5, p = 0.67			
Creatinine								
0 - < 1ppm	1.0	1.0	0.2	0.7 - 1.6	0.9	0.9	0.1	0.6 - 1.2
1 - <3 ppm	1.0	1.0	0.2	0.7 - 1.6	0.9	0.9	0.1	0.7 - 1.3
3 - < 6 ppm	0.9	0.9	0.2	0.7 - 1.2	0.9	0.9	0.1	0.7 - 1.1
≥ 6 ppm	1.1	1.2	0.3	0.6 - 1.6	1.0	0.9	0.2	0.8 - 1.4
	F value = 2.3, p = .08				F value = 0.4, p = 0.78			
Glucose								
0 - < 1 ppm	85	85	15	66 - 170	87	85	17	58 - 174
1 - <3 ppm	86	86	22	60 - 260	93	84	39	65 - 303
3 - < 6 ppm	84	83	12	66 - 114	95	89	25	74 - 192
≥ 6 ppm	84	83	14	71 - 105	89	88	7	80 - 97
	F value = 0.9, p = .44				F value = 0.6, p = .59			

Table 5 (continued)

PFOS (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
				Alkaline Phosphatase				
0 - < 1 ppm	80	78	22	31 - 158	77	73	17	49 - 132
1 - <3 ppm	89	89	27	49 - 191	83	79	23	41 - 163
3 - < 6 ppm	86	85	21	32 - 124	76	74	22	29 - 120
≥ 6 ppm	88	85	24	63 - 136	88	84	18	65 - 114
	F value = 1.3, p = .28				F value = 1.17, p = .32			
				GGT				
0 - < 1 ppm	43	31	28	16 - 155	28	22	20	10 - 142
1 - <3 ppm	47	36	39	2 - 293	36	25	33	10 - 179
3 - < 6 ppm	40	39	15	21 - 80	28	27	14	13 - 71
≥ 6 ppm	43	33	18	28 - 79	33	37	12	17 - 48
	F value = 0.5, p = .71				F value = 1.1, p = .34			
				AST				
0 - < 1 ppm	27	25	13	15 - 96	27	25	7	13 - 53
1 - <3 ppm	29	26	12	14 - 90	26	25	7	15 - 56
3 - < 6 ppm	25	24	5	13 - 37	25	23	7	14 - 43
≥ 6 ppm	33	33	6	26 - 43	29	28	3	26 - 34
	F value - 1.8, p = .14				F value = 0.5, p = .67			

Table 5 (continued)

PFOS (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
				ALT				
0 - < 1 ppm	48	43	20	27 - 118	31	30	11	13 - 60
1 - <3 ppm	46	42	21	18 - 183	33	29	16	10 - 89
3 - < 6 ppm	42	41	7	30 - 59	34	31	18	14 - 82
≥ 6 ppm	51	49	17	29 - 82	41	45	10	25 - 49
	F value = 1.0, p = .38				F value = 0.9, p = .46			
				Total Bilirubin				
0 - < 1 ppm	0.88	0.70	0.50	0.40 - 2.90	0.77	0.60	0.40	0.30 - 2.30
1 - <3 ppm	0.66[2]	0.60	0.30	0.20 - 1.50	0.61[2]	0.60	0.21	0.30 - 1.30
3 - < 6 ppm	0.64[2]	0.60	0.28	0.20 - 1.40	0.63	0.50	0.31	0.40 - 1.30
≥ 6 ppm	0.76	0.70	0.23	0.50 - 1.20	0.58	0.50	0.24	0.40 - 1.00
	F value = 4.4, p = .005				F value = 2,9, p = .04			
				Direct Bilirubin				
0 - < 1 ppm	0.22	0.20	0.05	0.02 - 0.40	0.15	0.10	0.07	0.10 - 0.40
1 - <3 ppm	0.21	0.20	0.06	0.10 - 0.40	0.12[2]	0.10	0.04	0.10 - 0.20
3 - < 6 ppm	0.21	0.20	0.04	0.20 - 0.30	0.12	0.10	0.04	0.10 - 0.20
≥ 6 ppm	0.20	0.20	0.02	0.10 0.30	0.10	0.10	0.00	0.10 - 0.10
	F value = 0.6, p = .58				F value = 3.5, p = .02			

Table 5 (continued)

PFOS (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
				Cholesterol				
0 - <1 ppm	219	215	47	100 - 340	198	197	40	110 - 280
1 - <3 ppm	216	213	43	118 - 315	216	219	42	116 - 365
3 - <6 ppm	214	214	35	128 - 278	229[2]	224	29	192 - 321
≥ 6 ppm	213	221	36	160 - 251	229	238	26	186 - 250
	F value = 0.1, p =.96				F value = 4.3, p =.006			
				LDL				
0 - <1 ppm	140	137	43	29 - 261	124	128	34	50 - 205
1 - < 3 ppm	134	134	40	44 - 234	141	134	38	61 - 290
3 - <6 ppm	137	135	34	65 - 190	148[2]	142	24	111 - 196
≥ 6 ppm	142	136	32	95 - 178	145	156	26	103 - 164
	F value = 0.2 , p =.87				F value = 3.7, p =.01			
				HDL				
0 - <1 ppm	53	53	13	31 - 94	46	48	11	19 - 74
1- <3 ppm	48	47	13	26 -94	44	45	10	28 - 69
3 - <6 ppm	45	46	11	23 - 74	48	47	10	32 - 69
≥ 6 ppm	45	46	9	34 - 61	40	38	4	37 - 45
	F value = 2.9, p =.04				F value = 1.1, p =.34			
				Triglycerides				
0 - <1 ppm	129	96	98	41 - 622	148	107	162	38 - 1209
1- <3 ppm	161	133	107	41 - 651	156	122	108	44 - 534
3 - <6 ppm	158	142	88	34 - 413	166	158	92	45 - 394
≥ 6 ppm	132	151	45	64 - 187	220	191	83	149 - 352
	F value = 1.1, p =.35				F value = 0.5, p =.67			

Table 5 (continued)

PFOS (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
				Hematocrit				
0 - <1 ppm	47	47	2	43 - 51	46	46	2	40 - 52
1 - <3 ppm	46	46	3	38 - 52	45	46	3	39 - 53
3 - <6 ppm	47	47	2	41 - 52	46	44	3	39 - 50
≥ 6 ppm	47	48	2	44 - 49	45	44	2	42 - 47
	F value = 2.4, p =.07				F value = 2.1, p = .11			
				Hemoglobin				
0 - <1 ppm	15.5	15.5	0.7	14.0 - 16.7	15.5	15.5	0.8	13.5 - 17.0
1 - <3 ppm	15.2	15.2	1.0	13.0 - 17.4	15.4	15.5	0.9	13.3 - 17.3
3 - <6 ppm	15.5	15.5	0.8	13.6 - 17.4	15.0	14.7	1.0	12.5 - 16.7
≥ 6 ppm	15.5	15.4	0.7	14.7 - 16.2	15.1	15.0	0.7	14.1 - 16.2
	F value = 2.2, p =.10				F value = 1.8, p =.15			
				RBC				
0 - <1 ppm	5.0	5.0	0.3	4.3 - 5.7	5.1	5.2	0.3	4.3 - 5.9
1 - <3 ppm	4.9	4.9	0.3	4.3 - 5.7	5.0	5.1	0.3	4.1 - 5.5
3 - <6 ppm	5.0	5.0	0.2	4.6 - 5.5	5.0	5.0	0.3	4.4 - 5.7
≥ 6 ppm	5.0	4.9	0.5	4.0 - 5.7	5.0	4.9	0.3	4.7 - 5.5
	F value = 0.4, p = .75				F value = 1.4, p = .25			

Table 5 (continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				MCH				
0 - <1 ppm	31.3	31.2	1.6	27.7 - 34.3	30.4	30.3	1.5	27.6 - 34.1
1 - <3 ppm	30.9	30.9	1.4	26.4 - 34.3	30.6	30.5	1.2	26.7 - 33.8
3 - <6 ppm	31.2	31.3	1.5	26.0 - 33.6	30.2	31.2	1.9	26.2 - 32.9
≥ 6 ppm	31.2	30.5	2.8	28.2 - 36.9	30.5	30.5	2.1	27.5 - 33.4
	F value = 0.9 , p =.45				F value = 0.6, p = .65			
				MCHC				
0 - <1 ppm	33.1	33.2	0.7	31.9 - 34.7	33.6	33.6	0.5	32.2 - 34.9
1 - <3 ppm	33.2	33.1	0.6	31.7 - 34.5	33.6	33.6	0.5	31.8 - 34.6
3 - <6 ppm	33.1	32.6	0.7	31.3 - 34.3	33.5	33.5	0.7	32.0 - 34.4
≥ 6 ppm	33.1	33.3	0.6	32.2 - 34.0	33.9	33.9	0.5	33.4 - 34.6
	F value = 0.2, p = .90				F value = 0.7, p = .56			
				MCV				
0 - <1 ppm	94	95	5.2	85-106	90	90	4.4	83 - 101
1 - <3 ppm	93	94	4.3	80-104	91	91	3.6	81 - 99
3 - <6 ppm	94	94	4.8	81-104	90	91	5.2	80 - 97
≥ 6 ppm	94	92	9.7	85-115	90	91	5.7	81 - 96
	F value = 1.1, p = .35				F value = 0.6, p = .59			

Table 5 (continued)

PFOS (ppm)	1995 Data Mean	1995 Data Median	1995 Data SD	1995 Data Range	1997 Data Mean	1997 Data Median	1997 Data SD	1997 Data Range
				WBC				
0 - <1 ppm	6.1	6.0	1.3	4.1 - 9.4	6.1	5.8	1.4	3.8 - 10.3
1 - <3 ppm	7.0[2]	6.8	2.0	3.6 - 15.5	6.9	6.6	1.9	3.8 - 13.2
3 - <6 ppm	7.6[2]	6.9	2.2	4.1 - 13.3	6.2	6.1	1.5	4.0 - 10.0
≥ 6 ppm	7.0	6.9	0.6	6.4 - 7.8	6.2	7.1	1.5	4.2 - 7.4
	F value = 4.3, p =.006				F value = 2.2, p =.09			
				Platelets				
0 - <1 ppm	226	224	40	159 - 309	227	220	53	106 - 406
1 - <3 ppm	229	224	47	122 - 367	229	223	49	124 - 359
3 - <6 ppm	228	226	45	132 - 344	220	221	45	147 - 316
≥ 6 ppm	185	182	50	132 - 277	199	191	58	146 - 295
	F value = 2.1, p =.10				F value = 0.6, p = 0.60			

1. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of the other PFOS categories.
2. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of 0 - < 1 ppm PFOS category.
3. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of 1 - < 3 ppm category.

* Sample Size	1995 Data	1997 Data
0 - <1 ppm	45	60
1 - <3 ppm	91	63
3 - <6 ppm	35	21
≥ 6 ppm	7	5

Table 6

Mean, Median (Med), Standard Deviation (SD) of Mean and Range of PFOS, Demographic, Serum Chemistries and Hematological Values by Plant Location, 1995

PFOS* (ppm)	1995 Data							
	Antwerp				Decatur			
	Mean	Med	SD	Range	Mean	Med	SD	Range
				PFOS				
0 < 1	0.46[1]	0.45	0.29	0.00 - 0.90	0.60[1]	0.63	0.19	0.25 - 0.88
1 < 3	1.69[1]	1.50	0.57	1.00 - 2.90	1.89[1]	1.89	0.58	1.00 - 2.91
3 < 6	3.96[1]	3.70	0.79	3.00 - 5.60	4.31[1]	4.23	0.82	3.11 - 5.80
≥ 6	8.17[1]	8.50	1.92	6.10 - 9.90	8.17[1]	6.90	3.20	6.06 - 12.83
	F value = 241.0, p = .0001				F value = 114.5, p = .0001			
				Age				
0 < 1	36	36	7	21 - 52	40	39	11	29 - 58
1 < 3	36	34	9	25 - 60	46	46	7	30 - 58
3 < 6	37	37	7	28 - 51	44	44	7	26 - 55
≥ 6	40	37	6	37 - 47	48	47	7	42 - 56
	F value = 0.5, p = .71				F value = 2.0, p = .12			
				Alcohol				
0 < 1	1.0	0.7	1.0	0.0 - 3.6	0.1	0.0	0.3	0.0 - 0.9
1 < 3	1.0	0.8	0.9	0.0 - 3.6	0.2	0.0	0.4	0.0 - 2.0
3 < 6	2.0	1.3	2.2	0.0 - 6.0	0.4	0.0	0.9	0.0 - 3.4
≥ 6	1.7	1.4	1.1	0.7 - 2.9	0.0	0.0	0.0	0.0 - 0.1
	F value = 2.9, p = .04				F value = 1.1, p = .37			
				BMI				
0 < 1	24.1	24.3	2.4	17.9 - 28.1	30.1	28.0	5.3	22.8 - 38.7
1 < 3	24.3	23.8	2.4	19.6 - 31.6	29.6	28.3	6.2	22.3 - 60.7
3 < 6	23.1	23.0	3.3	17.9 - 31.4	27.0	27.5	3.4	19.1 - 32.5
≥ 6	23.7	24.7	2.8	20.6 - 25.8	30.7	30.2	1.6	29.4 - 33.0
	F value = 0.9, p = .47				F value = 1.1, p = .35			

Table 6
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				Cigarettes				
0 < 1	3.0	0.0	7.0	0 - 25	1.4	0.0	3.8	0 - 13
1 < 3	4.1	0.0	7.0	0 - 23	8.3	0.0	12.9	0 - 40
3 < 6	9.1[3]	7.5	9.0	0 - 25	12.5	0.0	15.7	0 - 40
≥ 6	1.0	0.0	1.7	0 - 3	0	0.0	0	0 - 0
	F = 3.1, p = .03				F value = 2.3, p = .09			
				Alkaline Phosphatase				
0 < 1	75	75	16	31 - 104	96	99	29	47 - 158
1 < 3	73	66	19	49 - 108	98	95	27	49 - 191
3 < 6	79	78	20	32 -12195	95	96	18	58 - 124.
≥ 6	74	74	11	63 - 85	98	91	27	73 - 136
	F value = 0.5, p = .69				F value = 0.1, p = .98			
				GGT				
0 < 1	39	29	22	16 - 111	55	41	41	21 - 155
1 < 3	48	30	58	12 - 293	46	40	24	2 - 118
3 < 6	34	34	11	21 - 55	47	48	16	21 - 80
≥ 6	31	32	3	28 - 33	52	49	20	30 - 79
	F value = 0.7, p = .55				F value = 0.5, p = .71			
				AST				
0 < 1	26	23	13	15 - 96	32	31	9	19 - 52
1 < 3	27	26	14	14 - 90	29	27	10	17 - 85
3 < 6	23	22	5	13 - 35	27	26	5	21 - 37
≥ 6	33	35	6	26 - 37	34	32	7	28 - 43
	F value = 0.9, p = .45				F value = 0.9, p = .44			

Table 6
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				ALT				
0 < 1	46	42	18	27 - 108	55	47	24	37 - 118
1 < 3	45	40	18	26 - 122	47	42	22	18 - 183
3 < 6	40	39	6	30 - 52	43	42	9	30 - 59
≥ 6	50	38	28	29 - 82	52	53	8	41 - 59
	F value = 0.7, p = .59				F value = 0.8, p = .49			
				Total Bilirubin				
0 < 1	0.96	0.80	0.55	0.40 - 2.90	0.65	0.60	0.16	0.40 - 0.90
1 < 3	0.83	0.80	0.26	0.40 - 1.30	0.57	0.50	0.28	0.20 - 1.50
3 < 6	0.75	0.70	0.30	0.30 - 1.40	0.51	0.50	0.18	0.20 - 1.00
≥ 6	0.93	0.90	0.25	0.70 - 1.20	0.63	0.65	0.10	0.50 - 0.70
	F value = 1.2, p = .31				F value = 0.7, p = .54			
				Direct Bilirubin				
0 < 1	0.23	0.20	0.06	0.20 - 0.40	0.20	0.20	0.00	0.20 - 0.20
1 < 3	0.22	0.20	0.04	0.20 - 0.30	0.20	0.20	0.06	0.10 - 0.40
3 < 6	0.21	0.20	0.03	0.20 - 0.30	0.20	0.20	0.04	0.20 - 0.30
≥ 6	0.20	0.20	0.00	0.20 - 0.20	0.20	0.20	0.08	0.10 - 0.30
	F value = 0.7, p = .55				F value = 0.4, p = .74			
				BUN				
0 < 1	17.0	17.0	3.6	12.0 - 26.0	14.8	15.0	2.7	11.0 - 21.0
1 < 3	16.7	16.0	3.8	11.0 - 26.0	14.7	14.0	4.0	8.0 - 24.0
3 < 6	17.3	17.0	3.8	10.0 - 23.0	15.2	15.0	3.1	10.0 - 23.0
≥ 6	17.3	21.0	6.4	10.0 - 21.0	13.5	13.5	0.06	13.0 - 14.0
	F value = 0.1, p = .95				F value = 0.2, p = .87			
				Creatinine				
0 < 1	1.0	0.9	0.2	0.8 - 1.6	1.1	1.1	0.2	0.7 - 1.3
1 < 3	0.9	0.9	0.1	0.7 - 1.1	1.1	1.1	0.2	0.9 - 1.6
3 < 6	0.9[3]	0.9	0.1	0.7 - 1.0	1.1	1.1	0.1	0.8 - 1.2
≥ 6	0.8	0.8	0.2	0.6 - 0.9	1.3	1.3	0.2	1.2 - 1.6
	F value = 4.7, p = .004				F value = 3.8, p = .01			

Table 6
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				Glucose				
0 < 1	83	83	8	66 - 103	90	86	28	66 -170
1 < 3	82	81	13	60 - 126	93	89	25	67 - 260
3 < 6	80	82	9	66 - 101	89	89	14	66 -114
≥ 6	72	72	2	71 - 74	93	92	12	83 - 105
	F value = 1.3, p = .29				F value = 0.1, p = .94			
				Cholesterol				
0 < 1	220	219	50	100 - 340	215	208	39	154 - 276
1 < 3	206	211	49	118 - 315	221	218	39	132 - 300
3 < 6	217	215	30	178 - 266	209	213	42	128 - 278
≥ 6	223	221	16	208 - 240	206	206	47	160 - 251
	F value = 0.6, p = .61				F value = 0.5, p = .69			
				LDL				
0 < 1	140	138	45	29 - 261	139	130	38	79 - 192
1 < 3	131	124	46	44 - 220	136	136	36	62 - 234
3 < 6	143	139	27	99 - 189	131	128	41	65 - 190
≥ 6	144	136	20	130 - 168	139	142	42	95 - 178
	F value = 0.4, p = .76				F value = 0.1, p = .94			
				HDL				
0 < 1	56	57	13	31 - 94	43	41	9	31 - 59
1 < 3	53	51	13	33 - 79	45	44	12	26 - 94
3 < 6	50	49	11	31 - 74	39	39	9	23 - 51
≥ 6	53	49	7	48 - 61	39	39	5	34 - 46
	F value = 1.1, p = .35				F value = 1.4, p = .25			
				Triglycerides				
0 < 1	117	93	98	41 - 622	167	151	94	62 - 307
1 < 3	105	75	65	41 - 368	191	146	114	61 - 651
3 < 6	126	112	64	34 - 278	199	198	99	78 - 413
≥ 6	129	116	52	85 - 187	135	153	48	64 - 168
	F value = 0.3, p = .81				F value = 0.5, p = .67			

Table 6
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				Hematocrit				
0 < 1	47	47	2	43 -51	46	45	2	43 - 50
1 < 3	46	47	2	42 - 51	45	45	3	38 - 52
3 < 6	48	47	2	44 - 51	46	46	3	41 - 52
≥ 6	47	48	1	46 - 48	46	46	2	44 - 49
	F value = 1.5, p = 23.				F value = 0.3, p = .83			
				Hemoglobin				
0 < 1	15.5	15.5	0.8	14.0 - 16.7	15.5	15.4	0.7	14.5 - 16.4
1 < 3	15.2	15.3	0.7	13.8 - 16.6	15.1	15.1	1.1	13.0 - 17.4
3 < 6	15.6	15.6	0.5	14.6 - 16.7	15.3	15.3	1.0	13.6 - 17.4
≥ 6	15.4	15.4	0.6	14.8 - 16.0	15.5	15.6	0.8	14.7 - 16.2
	F value = 1.4, p = .26				F value = 0.7, p = .58			
				RBC				
0 < 1	4.9	5.0	0.3	4.3 - 5.7	5.1	5.1	0.2	4.8 - 5.5
1 < 3	4.9	4.9	0.2	4.3 - 5.4	4.9	4.9	0.3	4.3 -5.7
3 < 6	5.0	5.0	0.2	4.7 - 5.3	5.0	4.9	0.3	4.6 - 5.5
≥ 6	4.7	4.9	0.6	4.0 - 5.2	5.2	5.1	0.4	4.8 - 5.7
	F value = 0.9, p = .46				F value = 1.1, p = .35			
				MCH				
0 < 1	31.5	31.3	1.5	27.7 - 34.3	30.7	30.7	1.6	28.7 - 33.5
1 < 3	31.2	31.0	1.2	29.5 - 34.3	30.7	30.9	1.5	26.4 - 33.1
3 < 6	31.5	31.9	1.2	29.1 - 33.3	30.9	31.2	1.7	26.0 - 33.6
≥ 6	33.0	32.5	3.7	29.6 - 36.9	29.9	30.4	1.2	28.2 - 30.7
	F value = 1.4, p = .25				F value = 0.4, p = .74			
				MCHC				
0 < 1	32.9	33.0	0.6	31.9 - 34.5	33.8	33.9	0.6	32.9 - 34.7
1 < 3	32.9	32.8	0.6	31.7 - 33.8	33.3	33.3	0.5	31.9 - 34.5
3 < 6	32.8	32.7	0.7	31.3 - 34.2	33.4	33.4	0.6	32.1 - 34.3
≥ 6	32.6	32.4	0.5	32.2 - 33.1	33.5	33.4	0.3	33.3 - 34.0
	F value = 0.4, p = .78				F value = 2.2, p = .10			

Table 6
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				MCV				
0 < 1	96	96	5	85 -106	91	90	4	85 - 99
1 < 3	95	95	3	90 - 104	92	93	4	80 - 100
3 < 6	96	97	4	88 - 104	92	92	5	81 - 101
≥ 6	101	98	12	91 - 115	89	90	3	85 - 92
	F value = 1.8, p = .16				F value = 0.8, p = .50			
				WBC				
0 < 1	5.9	5.7	1.3	4.1 - 9.4	6.8	6.9	1.1	5.0 - 9.0
1 < 3	6.1	6.1	1.3	3.8 - 8.8	7.5	7.0	2.2	3.6 - 15.5
3 < 6	7.5[2]	6.9	2.5	4.1 - 13.3	7.7	7.4	1.9	4.9 - 11.5
≥ 6	6.5	6.4	0.2	6.4 - 6.7	7.4	7.4	0.4	6.9 - 7.8
	F value = 4.5, p = .006				F value = 0.5, p = .72			
				Platelets				
0 < 1	224	222	42	159 - 309	233	246	35	162 - 271
1 < 3	222	220	41	153 - 318	233	225	50	122 - 367
3 < 6	237[4]	234	52	132 - 344	218	214	34	172 - 287
≥ 6	162	151	36	132 - 202	202	194	56	143 - 277
	F value = 2.6, p = .06				F value = 1.0, p = .40			

*Sample sizes:

FC95 Level	Antwerp	Decatur
0 < 1 ppm	34	11
1 < 3 ppm	32	59
3 < 6 ppm	19	16
≥ 6 ppm	3	4
	88	90

1. Significantly different ($p < .05$, Bonferroni (Dunn) t-test) than the remaining three PFOS exposure categories.
2. Significantly different ($p < .05$, Bonferroni (Dunn) t-test) than the 0 - < 1 ppm and the 1 - < 3 ppm categories.
3. Significantly different ($p < .05$, Bonferroni (Dunn) t-test) than the 0 - < 1 ppm category.
4. Significantly different ($p < .05$, Bonferroni (Dunn) t-test) than the 3 - < 6 ppm category.

Table 7

Mean, Median (Med), Standard Deviation (SD) of Mean and Range of PFOS, Demographic, Serum Chemistries and Hematological Values by Plant Location, 1997

PFOS* (ppm)	1997 Data							
	Antwerp				Decatur			
	Mean	Med	SD	Range	Mean	Med	SD	Range
				PFOS				
0 - < 1	0.46[1]	0.37	0.27	0.10 - 0.94	0.60[1]	0.59	0.26	0.10 - 0.97
1 - < 3	1.89[1]	1.79	0.61	1.02 - 2.89	1.71[1]	1.53	0.52	1.04 - 2.85
3 - < 6	3.87[1]	3.66	0.59	3.22 - 4.83	3.87[1]	3.51	0.81	3.09 - 5.30
≥ 6	-	-	-	-	7.20[1]	6.68	1.59	6.05 - 9.93
	F value = 195.3, p = .0001				F value = 218.1, p = .0001			
				Age				
0 - < 1	29	28	6	21 - 50	43	44	9	23 - 62
1 - < 3	37[3]	37	9	24 - 63	45	45	8	31 - 62
3 - < 6	37[3]	37	3	32 - 40	45	44	4	36 - 54
≥ 6	-	-	-	-	42	45	9	29 - 52
	F value = 10.4, p = .0001				F value = 0.5, p = .69			
				Alcohol				
0 - < 1	0.8	0.5	1.0	0.0 - 4.3	0.2	0.1	0.4	0.1 - 2.0
1 - < 3	1.0	0.7	1.1	0.0 - 5.0	0.1	0.1	0.1	0.1 - 0.3
3 - < 6	2.2[3]	1.4	2.3	0.0 - 7.1	0.1	0.1	0.1	0.1 - 0.3
≥ 6	-	-	-	-	0.2	0.1	0.3	0.1 - 0.8
	F value = 4.3, p = .02				F value = 0.8, p = .49			
				BMI				
0 - < 1	22.9	21.9	2.1	20.2 - 28.3	29.3	28.9	4.9	22.4 - 41.7
1 - < 3	24.2	23.9	2.8	18.1 - 30.4	30.0	29.0	6.0	20.2 - 48.5
3 - < 6	23.6	24.9	3.1	19.2 - 28.3	30.0	29.3	3.0	25.4 - 36.0
≥ 6	-	-	-	-	30.8	29.7	4.0	26.1 - 36.2
	F value = 2.0, p = .15				F value = 0.2, p = .91			

Table 7
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				Cigarettes				
0 - < 1	4.2	0.0	6.7	0 - 20	5.3	0.0	11.6	0 - 40
1 - < 3	7.3	2.0	8.1	0 - 20	8.7	0.0	13.0	0 - 40
3 - < 6	5	0.0	7.8	0 - 20	3.3	0.0	9.0	0 - 30
≥ 6	-	-	-	-	6.0	0.0	13.4	0 - 30
	F = 1.3, p = .28				F value = 0.8, p = .51			
				Alkaline Phosphatase				
0 - < 1	69	68	14	49 - 110	86	84	16	55 - 132
1 - < 3	74	74	16	41 - 113	88	83	26	41 - 163
3 - < 6	64	59	24	29 - 120	85	83	15	61 - 109
≥ 6	-	-	-	-	88	84	18	65 - 114
	F value = 1.5, p = .22				F value = 0.1, p = .96			
				GGT				
0 - < 1	21	17	10	10 - 50	36	32	25	13 - 142
1 - < 3	34	24	34	10 - 144	37	27	33	13 - 179
3 - < 6	25	22	10	14 - 43	31	28	15	13 - 71
≥ 6	-	-	-	-	33	37	12	17 - 48
	F value = 2.5, p = .09				F value = 0.2, p = .91			
				AST				
0 - < 1	27	26	7	17 - 53	26	25	7	13 - 48
1 - < 3	27	25	7	15 - 48	26	25	7	18 - 56
3 - < 6	25	24	4	19 - 30	25	23	9	14 -43
≥ 6	-	-	-	-	29	28	3	26 - 34
	F value = 0.2, p = .80				F value = 0.4, p = .77			

Table 7
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				ALT				
0 - < 1	30	25	12	13 - 60	33	31	11	17 - 57
1 - < 3	33	31	16	13 - 87	33	28	16	10 - 89
3 - < 6	28	23	10	14 - 46	38	33	21	17 - 82
≥ 6	-	-	-	-	41	45	10	25 - 49
	F value = 0.6, p = .58				F value = 0.9, p = .45			
				Total Bilirubin				
0 - < 1	0.90	0.80	0.46	0.40 - 2.30	0.63	0.60	0.30	0.30 - 1.40
1 - < 3	0.68	0.70	0.23	0.30 - 1.30	0.56	0.50	0.18	0.30 - 1.00
3 - < 6	0.79	0.70	0.40	0.30 - 1.30	0.51	0.50	0.16	0.30 - 0.90
≥ 6	-	-	-	-	0.58	0.50	0.24	0.40 - 1.00
	F value = 2.3, p = .11				F value = 1.0, p = .41			
				Direct Bilirubin				
0 - < 1	0.16	0.20	0.08	0.10 - 0.40	0.13	0.10	0.06	0.10 - 0.30
1 - < 3	0.13	0.10	0.05	0.10 - 0.20	0.11	0.10	0.03	0.10 - 0.20
3 - < 6	0.14	0.10	0.05	0.10 - 0.20	0.11	0.10	0.03	0.10 - 0.20
≥ 6	-	-	-	-	0.10	0.10	0.00	0.10 - 0.10
	F value = 2.2, p = .12				F value= 1.3, p = .28			
				BUN				
0 - < 1	14.3	14.0	2.1	11.0 - 19.0	14.7	14.0	3.3	9.0 - 21.0
1 - < 3	15.2	15.0	2.6	10.0 - 20.0	13.5	13.5	3.5	6.0 - 26.0
3 - < 6	16.0	16.0	2.7	12.0 - 20.0	14.3	14.0	3.0	9.0 - 19.0
≥ 6	-	-	-	-	13.8	12.0	4.1	9.0 - 19.0
	F value = 2.1, p = .13				F value = 0.7, p = .56			
				Creatinine				
0 - < 1	0.9	0.9	0.1	0.7 - 1.2	1.0	1.0	0.1	0.6 - 1.2
1 - < 3	0.9	0.9	0.1	0.7 - 1.3	1.0	1.0	0.1	0.7 - 1.3
3 - < 6	0.9	0.9	0.1	0.8 - 1.1	1.0	1.0	0.1	0.7 - 1.1
≥ 6	-	-	-	-	1.0	0.9	0.2	0.8 - 1.4
	F value = 0.3, p = .76				F value = 0.2, p = .89			

Table 7
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				Glucose				
0 - < 1	81	82	10	63 - 114	93	91	21	58 - 174
1 - < 3	79	78	9	65 - 102	102	89	47	75 - 303
3 - < 6	84	85	8	74 - 96	103	93	30	79 - 192
≥ 6	-	-	-	-	89	88	7	80 - 97
	F value = 0.8, p = .47				F value = 0.5, p = .67			
				Cholesterol				
0 - < 1	193	190	41	110 - 277	204	208	38	145 - 277
1 - < 3	213	205	48	116 - 365	218	226	39	152 - 290
3 - < 6	228	223	38	192 - 321	230	230	23	197 - 280
≥ 6	-	-	-	-	229	238	26	186 - 250
	F value = 2.9, p = .07				F value = 2.0, p = .13			
				LDL				
0 - < 1	122	114	35	57 - 205	127	133	33	50 - 178
1 - < 3	143	134	42	67 - 290	139	135	35	61 - 196
3 - < 6	147	141	24	111 - 195	149	144	25	113 - 196
≥ 6	-	-	-	-	145	156	26	103 - 164
	F value = 3.0, p = .06				F value = 1.6, p = .19			
				HDL				
0 - < 1	51	50	12	19 - 74	42	41	9	26 - 59
1 - < 3	48	46	10	34 - 68	42	41	10	28 - 69
3 - < 6	51	50	10	39 - 69	45	45	10	32 - 62
≥ 6	-	-	-	-	40	38	4	37 - 45
	F value = 0.8, p = .47				F value = 0.5, p = .67			
				Triglycerides				
0 - < 1	99	92	41	38 - 175	200	128	219	46 - 1209
1 - < 3	112	95	58	44 - 290	185	147	124	63 - 534
3 - < 6	150	122	88	65 - 362	179	183	98	45 - 394
≥ 6	-	-	-	-	220	191	83	149 - 352
	F value = 2.9, p = .06				F value = 0.1, p = .95			

Table 7
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				Hematocrit				
0 - < 1	47	47	3	42 - 52	46	45	2	40 - 50
1 - < 3	46	47	3	40 - 53	46	46	3	39 - 51
3 - < 6	46	45	3	42 - 50	44	44	2	39 - 48
≥ 6	-	-	-	-	45	44	2	42 - 47
	F value = 0.7, p = .51				F value = 1.6, p = .18			
				Hemoglobin				
0 - < 1	15.6	15.5	0.8	14.2 - 17.0	15.4	15.4	0.7	13.5 - 16.9
1 - < 3	15.4	15.6	1.0	13.3 - 17.3	15.4	15.5	0.9	13.5 - 17.3
3 - < 6	15.2	14.7	1.0	14.0 - 16.7	14.8	14.7	1.0	12.5 - 16.5
≥ 6	-	-	-	-	15.1	15.0	0.8	14.1 - 16.2
	F value = 0.7, p = .48				F value = 1.7, p = .18			
				RBC				
0 - < 1	5.1	5.2	0.4	4.6 - 5.9	5.1	5.1	0.3	4.3 - 5.7
1 - < 3	5.0	5.1	0.3	4.1 - 5.5	5.1	5.1	0.3	4.2 - 5.5
3 - < 6	4.9	4.8	0.4	4.4 - 5.7	5.0	5.0	0.3	4.5 - 5.4
≥ 6	-	-	-	-	5.0	4.9	0.3	4.7 - 5.5
	F value = 1.7, p = .19				F value = 0.4, p = .78			
				MCH				
0 - < 1	30.4	30.4	1.3	27.9 - 33.5	30.3	30.2	1.8	27.6 - 34.1
1 - < 3	30.6	30.5	0.9	29.2 - 32.8	30.6	30.6	1.4	26.7 - 33.8
3 - < 6	30.9	31.4	1.4	28.8 - 32.7	29.7	30.5	2.2	26.2 - 32.9
≥ 6	-	-	-	-	30.5	30.5	2.1	27.5 - 33.4
	F value = 0.9, p = .40				F value = 1.0, p = .41			
				MCHC				
0 - < 1	33.4	33.4	0.5	32.2 - 34.0	33.8	33.7	0.5	32.8 - 34.9
1 - < 3	33.3	33.2	0.5	31.8 - 34.1	33.8	33.8	0.5	32.7 - 34.6
3 - < 6	33.4	33.5	0.5	32.4 - 34.3	33.6	33.9	0.8	32.0 - 34.4
≥ 6	-	-	-	-	33.9	33.9	0.5	33.4 - 34.6
	F value = 0.6, p = .54				F value = 0.6, p = .62			

Table 7
(continued)

PFOS (ppm)	Antwerp Mean	Antwerp Med	Antwerp SD	Antwerp Range	Decatur Mean	Decatur Med	Decatur SD	Decatur Range
				MCV				
0 - < 1	91	91	4	84 - 101	90	89	5	83 - 101
1 - < 3	92	92	3	87 - 99	91	90	4	81 - 99
3 - < 6	93	95	5	86 - 97	88	89	5	80 - 96
≥ 6	-	-	-	-	90	91	6	81 - 96
	F value = 1.3, p = .29				F value = 0.9, p = .45			
				WBC				
0 - < 1	6.0	5.7	1.3	3.8 - 8.8	6.3	5.9	1.6	4.0 - 10.3
1 - < 3	7.1	6.8	2.2	4.4 - 13.2	6.7	6.6	1.6	3.8 - 10.1
3 - < 6	6.4	6.0	1.8	4.2 - 10.0	6.1	6.1	1.3	4.0 - 8.9
≥ 6	-	-	-	-	6.2	7.1	1.5	4.2 - 7.4
	F value = 2.6, p = .08				F value = 0.8, p = .51			
				Platelets				
0 - < 1	237	232	55	126 - 406	215	207	50	106 - 363
1 - < 3	243	232	48	151 - 359	219	210	48	124 - 323
3 - < 6	215	225	41	147 - 263	224	219	50	159 - 316
≥ 6	-	-	-	-	199	191	58	146 - 295
	F value = 1.1, p = .35				F value = 0.3, p = .80			

*Sample sizes:

FC95 Level	Antwerp	Decatur
0 - < 1 ppm	31	29
1 - < 3 ppm	25	38
3 - < 6 ppm	9	12
≥ 6 ppm	0	5
	65	84

1. Significantly different (p < .05, Bonferroni (Dunn) t-test) than the remaining three PFOS exposure categories.
2. Significantly different (p < .05, Bonferroni (Dunn) t-test) than the 0 - < 1 ppm and the 1 - < 3 ppm categories.
3. Significantly different (p < .05, Bonferroni (Dunn) t-test) than the 0 - < 1 ppm category.

Table 8

Multivariable Regression of Serum Chemistries and Hematological Parameters - Examination of the Effect of PFOS Adjusting for Age, Alcohol, BMI and Cigarettes, Antwerp and Decatur Data Combined, 1995 and 1997 Examinations

Alkaline Phosphatase

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	52.99	11.14	.0001	40.87	9.60	.0001
PFOS	0.32	0.89	.72	0.19	1.03	.86
Age	0.08	0.20	.68	0.45	0.18	.01
Alcohol	-5.43	1.53	.0005	-2.09	1.61	.20
BMI	1.04	0.34	.002	0.69	0.34	.04
Cigarettes	0.85	0.15	.0001	0.49	0.15	.002
	$R^2 = .26$	Adj $R^2 = .24$		$R^2 = .19$	Adj $R^2 = .16$	

ln GGT

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	2.3816	0.2711	.0001	2.0471	0.2592	.0001
PFOS	0.0032	0.0217	.88	0.0178	0.0280	.53
Age	0.0040	0.0048	.41	0.0036	0.0049	.47
Alcohol	0.0605	0.0373	.11	0.0747	0.0438	.09
BMI	0.0372	0.0082	.0001	0.0365	0.0091	.0001
Cigarettes	0.0076	0.0038	.05	0.0030	0.0042	.48
	$R^2 = .14$	Adj $R^2 = .12$		$R^2 = .15$	Adj $R^2 = .12$	

AST

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	12.62	5.52	.02	23.25	3.50	.0001
PFOS	0.42	0.44	.35	-0.11	0.38	.77
Age	0.0005	0.10	.99	-0.07	0.07	.29
Alcohol	0.70	0.76	.36	0.40	0.59	.50
BMI	0.54	0.17	.002	0.23	0.12	.07
Cigarettes	-0.11	0.08	.14	-0.07	0.06	.20
	$R^2 = .08$	Adj $R^2 = .05$		$R^2 = .04$	Adj $R^2 = .00$	

Table 8 (continued)

ALT

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	28.54	9.46	.003	11.60	6.78	.09
PFOS	0.09	0.76	.91	0.97	0.73	.19
Age	-0.18	0.17	.28	-0.19	0.13	.14
Alcohol	-0.16	1.30	.90	0.31	1.14	.79
BMI	0.96	0.29	.001	1.00	0.24	.0001
Cigarettes	-0.14	0.13	.28	-0.09	0.11	.40
	$R^2 = .08$	Adj $R^2 = .05$		$R^2 = .13$	Adj $R^2 = .10$	

ln Total Bilirubin

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.2742	0.1958	.16	-0.1395	0.1945	.47
PFOS	-0.0984	0.0368	.008	-0.1620	0.0515	.002
$PFOS^2$	0.0086	0.0039	.03	0.0188	0.0070	.009
Age	0.0052	0.0035	.14	0.0015	0.0037	.70
Alcohol	0.0738	0.0268	.007	0.1220	0.0327	.0003
BMI	-0.0273	0.0059	.0001	-0.0097	0.0068	.16
Cigarettes	-0.0158	0.0028	.0001	-0.0054	0.0031	.09
	$R^2 = .32$	Adj $R^2 = .30$		$R^2 = .18$	Adj $R^2 = .14$	

Direct Bilirubin

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.2196	0.0259	.0001	0.1929	0.0264	.0001
PFOS	-0.0009	0.0021	.68	-0.0061	0.0029	.03
Age	0.0006	0.0005	.17	-0.0002	0.0005	.69
Alcohol	0.0074	0.0036	.04	0.0106	0.0045	.02
BMI	-0.0012	0.0008	.13	-0.0017	0.0009	.07
Cigarettes	-0.0007	0.0004	.06	-0.0009	0.0004	.05
	$R^2 = .06$	Adj $R^2 = .03$		$R^2 = .14$	Adj $R^2 = .11$	

Table 8 (continued)

BUN

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	18.64	1.94	.0001	13.22	1.47	.0001
PFOS	-0.02	0.15	.91	-0.08	0.16	.60
Age	-0.03	0.03	.39	0.08	0.03	.005
Alcohol	0.37	0.27	.17	0.50	0.25	.05
BMI	-0.05	0.06	.42	-0.07	0.05	.20
Cigarettes	-0.08	.03	.004	-0.05	0.02	.05
	$R^2 = .08$	Adj $R^2 = .05$		$R^2 = .09$	Adj $R^2 = .06$	

Creatinine

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.5744	0.0779	.0001	0.9478	0.0602	.0001
PFOS	-0.0223	0.0146	.13	-0.0302	0.0159	.06
$PFOS^2$	0.0033	0.0016	.04	0.0055	0.0022	.01
Age	0.0059	0.0013	.0001	0.0027	0.0012	0.02
Alcohol	-0.0260	0.0106	.02	-0.0242	0.0101	.02
BMI	0.0086	0.0023	.0003	-0.0022	0.0021	.30
Cigarettes	-0.0005	0.0011	.65	-0.0019	0.0010	.05
	$R^2 = .29$	Adj $R^2 = .27$		$R^2 = .16$	Adj $R^2 = .13$	

ln Glucose

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	3.8943	0.0732	.0001	3.8597	0.0919	.0001
PFOS	-0.0039	0.0058	.51	0.0014	0.0098	.89
Age	0.0045	0.0013	.0006	0.0025	0.0017	.15
Alcohol	-0.0026	0.0101	.79	-0.0068	0.0154	.66
BMI	0.0143	0.0022	.0001	0.0195	0.0032	.0001
Cigarettes	-0.0003	0.0010	.78	-0.0013	0.0015	.39
	$R^2 = .31$	Adj $R^2 = .29$		$R^2 = .30$	Adj $R^2 = .27$	

Table 8 (continued)

Cholesterol

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	190.1	22.1	.0001	155.5	19.0	.0001
PFOS	-0.99	1.77	.58	4.66	2.04	.02
Age	0.74	0.39	.06	1.39	0.36	.0002
Alcohol	-0.40	3.04	.90	7.50	3.18	.02
BMI	-0.04	0.67	.95	-0.40	0.67	.55
Cigarettes	-0.18	0.31	.56	-0.08	0.30	.80
	$R^2 = .02$	Adj $R^2 = .00$		$R^2 = .17$	Adj $R^2 = .14$	

LDL

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	135.26	20.27	.0001	114.69	17.06	.0001
PFOS	0.50	1.61	.76	4.01	1.82	.03
Age	0.58	0.36	.11	1.04	0.32	.002
Alcohol	-3.33	2.78	.23	4.29	2.84	.13
BMI	-6.67	0.61	.28	-1.09	0.60	.07
Cigarettes	-0.52	0.28	.07	-0.02	0.28	.95
	$R^2 = .05$	Adj $R^2 = .02$		$R^2 = .13$	Adj $R^2 = .10$	

HDL

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	70.82	5.73	.0001	66.71	4.52	.0001
PFOS	-1.17	0.46	.01	-0.22	0.49	.65
Age	-0.11	0.10	.27	0.12	0.09	.17
Alcohol	3.53	0.79	.0001	2.01	0.76	.009
BMI	-0.63	0.17	.0004	-0.95	0.16	.0001
Cigarettes	-0.17	0.08	0.03	-0.16	0.07	.03
	$R^2 = .28$	Adj $R^2 = .26$		$R^2 = .29$	Adj $R^2 = .26$	

Table 8 (continued)

Triglycerides

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	-103.29	45.97	.03	-187.99	58.18	.002
PFOS	-1.78	3.68	.63	-0.33	6.28	.96
Age	1.54	0.82	.06	1.37	1.11	.22
Alcohol	-0.43	6.32	.95	14.68	9.82	.14
BMI	6.71	1.39	.0001	10.06	2.05	.0001
Cigarettes	2.70	0.64	.0001	1.87	0.94	.05
	$R^2 = .24$	Adj $R^2 = .22$		$R^2 = .23$	Adj $R^2 = .20$	

Hematocrit

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	48.01	1.36	.0001	46.73	1.28	.0001
PFOS	-0.06	0.11	.57	-0.25	0.14	.07
Age	0.0005	0.02	.98	-0.03	0.02	.20
Alcohol	0.10	0.19	.58	0.15	0.22	0.50
BMI	-0.08	0.04	.06	0.01	0.05	0.75
Cigarettes	0.05	0.02	.007	0.04	0.02	.04
	$R^2 = .08$	Adj $R^2 = .05$		$R^2 = .08$	Adj $R^2 = .04$	

Hemoglobin

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	15.78	0.45	.0001	15.43	0.43	.0001
PFOS	-0.02	0.04	.53	-0.08	0.05	.07
Age	0.004	0.008	.57	-0.006	0.01	.46
Alcohol	-0.02	0.06	.77	0.01	0.07	.85
BMI	-0.03	0.01	.06	0.008	0.02	.59
Cigarettes	0.02	0.006	.02	0.02	0.007	.03
	$R^2 = .06$	Adj $R^2 = .03$		$R^2 = .06$	Adj $R^2 = .03$	

Table 8 (continued)

RBC

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	4.8766	0.1559	.0001	5.1416	0.1626	.0001
PFOS	-0.0026	0.0123	.83	-0.0208	0.1756	.24
Age	-0.0022	0.0028	.42	-0.0068	0.0031	.03
Alcohol	-0.0323	0.0213	.13	-0.0111	0.0274	.69
BMI	0.0069	0.0047	.14	0.0089	0.0057	.12
Cigarettes	-0.0006	0.0022	.77	-0.0025	0.0026	.35
	$R^2 = .04$	Adj $R^2 = .01$		$R^2 = .06$	Adj $R^2 = .02$	

MCH

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	32.292	0.725	.0001	30.062	0.706	.0001
PFOS	-0.015	0.058	.79	-0.047	0.076	.54
Age	0.23	0.013	.08	0.028	0.013	.04
Alcohol	0.189	0.099	.06	0.095	0.119	.43
BMI	-0.093	0.022	.0001	-0.035	0.025	.16
Cigarettes	0.0347	0.010	.001	0.046	0.011	.0001
	$R^2 = .20$	Adj $R^2 = .18$		$R^2 = .13$	Adj $R^2 = .10$	

MCHC

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	32.8525	0.3341	.0001	33.0109	0.2675	.0001
PFOS	-0.0015	0.0266	.96	-0.0092	0.0289	.75
Age	0.0097	0.0059	.11	0.0101	0.0051	.05
Alcohol	-0.1121	0.0458	.02	-0.0589	0.0451	.19
BMI	-0.0006	0.0100	.95	0.0072	0.0094	.45
Cigarettes	-0.0041	0.0047	.39	0.0016	0.0043	.72
	$R^2 = .07$	Adj $R^2 = .05$		$R^2 = .07$	Adj $R^2 = .04$	

Table 8 (continued)

MCV

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	98.29	2.29	.0001	90.91	1.98	.0001
PFOS	-0.03	0.18	.88	-0.09	0.21	.67
Age	0.04	0.04	.33	0.06	0.04	.15
Alcohol	0.88	0.31	.01	0.49	0.33	.14
BMI	-0.28	0.07	.0001	-0.12	0.07	.08
Cigarettes	0.12	0.03	.0005	0.13	0.03	.0001
	$R^2 = .23$	Adj $R^2 = .21$		$R^2 = .15$	Adj $R^2 = .12$	

Platelets

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	272.49	23.37	.0001	260.92	25.27	.0001
PFOS	-3.37	1.86	.07	-2.91	2.73	.29
Age	-0.88	0.41	.04	-0.25	0.48	.61
Alcohol	-4.80	3.20	.14	2.37	4.26	.58
BMI	-0.04	0.70	.95	-0.73	0.89	.41
Cigarettes	0.21	0.33	0.52	-0.30	0.41	.46
	$R^2 = .06$	Adj $R^2 = .03$		$R^2 = .03$	Adj $R^2 = .00$	

WBC

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	3.9538	0.8120	.0001	5.1296	0.6927	.0001
PFOS	0.0893	0.0646	.17	-0.0048	0.0748	.95
Age	0.0214	0.0144	.14	0.0139	0.0132	.30
Alcohol	-0.0860	0.1111	.44	0.2471	0.1169	.04
BMI	0.0492	0.0244	.05	0.0045	0.0244	.86
Cigarettes	0.1009	0.0115	.0001	0.0878	0.0111	.0001
	$R^2 = .36$	Adj $R^2 = .34$		$R^2 = .33$	Adj $R^2 = .31$	

Table 9

Multivariable Regression of Total Bilirubin, Direct Bilirubin, Creatinine Cholesterol, LDL, HDL, Heamtocrit, Hemoglobin and Platelets - Examination of the Effect of PFOS Adjusting for Age, Alcohol, BMI and Cigarettes, by Location, 1995 and 1997

ln Total Bilirubin - Both Locations

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.2742	0.1958	.16	-0.1395	0.1945	.47
PFOS	-0.0984	0.0368	.008	-0.1620	0.0515	.002
$PFOS^2$	0.0086	0.0039	.03	0.0188	0.0070	.009
Age	0.0052	0.0035	.14	0.0015	0.0037	.70
Alcohol	0.0738	0.0268	.007	0.1220	0.0327	.0003
BMI	-0.0273	0.0059	.0001	-0.0097	0.0068	.16
Cigarettes	-0.0158	0.0028	.0001	-0.0054	0.0031	.09
	$R^2 = .32$	Adj $R^2 = .30$		$R^2 = .18$	Adj $R^2 = .14$	

ln Total Bilirubin - Antwerp Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	-0.0776	0.0475	.85	-0.3130	0.5586	0.58
PFOS	-0.0473	0.0575	.41	-0.2157	0.1779	.23
$PFOS^2$	0.0035	0.0072	.63	0.0336	0.0385	.39
Age	0.0062	0.0055	.27	-0.0036	0.0081	.66
Alcohol	0.0398	0.0316	.21	0.0826	0.0441	.07
BMI	-0.0115	0.0161	.48	0.0114	0.0222	.61
Cigarettes	-0.0176	0.0056	.002	-0.0102	0.0074	.17
	$R^2 = .15$	Adj $R^2 = .08$		$R^2 = .14$	Adj $R^2 = .05$	

ln Total Bilirubin - Decatur Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	-0.4462	0.3154	.16	-0.9486	0.2932	.002
PFOS	-0.0862	0.0509	.09	-0.1160	0.0578	.05
$PFOS^2$	0.0081	0.0048	.10	0.0144	0.0072	.05
Age	0.0128	0.0048	.01	0.0134	0.0046	.005
Alcohol	-0.0045	0.0828	.59	0.1708	0.1607	.29
BMI	-0.0179	0.0068	.01	-0.0045	0.0073	.54
Cigarettes	-0.0117	0.0031	.0004	-0.0031	0.0032	.33
	$R^2 = .29$	Adj $R^2 = .24$		$R^2 = .17$	Adj $R^2 = .10$	

Table 9
(continued)

HDL - Both Locations

	1995 Data (N = 178)			1997 Data (N = 149)		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	70.82	5.73	.0001	66.71	4.52	.0001
PFOS	-1.17	0.46	.01	-0.22	0.49	.65
Age	-0.11	0.10	.27	0.12	0.09	.17
Alcohol	3.53	0.79	.0001	2.01	0.76	.009
BMI	-0.63	0.17	.0004	-0.95	0.16	.0001
Cigarettes	-0.17	0.08	0.03	-0.16	0.07	.03
	$R^2 = .28$	Adj $R^2 = .26$		$R^2 = .28$	Adj $R^2 = .26$	

HDL - Antwerp Only

	1995 Data (N = 86)			1997 Data (N = 63)		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	73.44	12.69	.0001	57.30	12.12	.0001
PFOS	-1.55	0.73	.04	-2.26	1.17	.06
Age	0.01	0.17	.93	0.43	0.17	.01
Alcohol	3.31	0.98	.001	2.78	0.99	.007
BMI	-0.87	0.50	.09	-0.82	0.48	.10
Cigarettes	-0.19	0.17	.28	-0.45	0.16	.007
	$R^2 = .16$	Adj $R^2 = .11$		$R^2 = .28$	Adj $R^2 = .21$	

HDL - Decatur Only

	1995 Data (N = 85)			1997 Data (N = 83)		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	66.35	9.42	.0001	69.17	7.01	.0001
PFOS	-0.75	0.60	.22	0.17	0.50	.73
Age	-0.14	0.14	.32	0.01	0.11	.96
Alcohol	4.71	2.48	.06	-3.07	3.87	.43
BMI	-0.49	0.21	.02	-0.90	0.18	.0001
Cigarettes	-0.16	0.09	.09	-0.04	0.08	.61
	$R^2 = .14$	Adj $R^2 = .09$		$R^2 = .27$	Adj $R^2 = .22$	

Table 9
(continued)

Platelets - Both Locations

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	272.49	23.37	.0001	260.92	25.27	.0001
PFOS	-3.37	1.86	.07	-2.91	2.73	.29
Age	-0.88	0.41	.04	-0.25	0.48	.61
Alcohol	-4.80	3.20	.14	2.37	4.26	.58
BMI	-0.04	0.70	.95	-0.73	0.89	.41
Cigarettes	0.21	0.33	.52	-0.30	0.41	.46
	$R^2 = .06$	Adj $R^2 = .03$		$R^2 = .03$	Adj $R^2 = .00$	

Platelets, Antwerp Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	294.81	48.19	.0001	258.21	66.58	.0003
PFOS	-2.57	2.77	.36	-1.99	6.44	.76
Age	-0.35	0.65	.60	0.41	0.91	.65
Alcohol	-3.65	3.74	.33	-0.87	5.42	.87
BMI	-2.23	1.91	.25	-1.16	2.65	.66
Cigarettes	0.82	0.65	.21	-0.70	0.90	.44
	$R^2 = .06$	Adj $R^2 = .00$		$R^2 = .02$	Adj $R^2 = .00$	

Platelets, Decatur Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	353.90	39.04	.0001	225.66	42.96	.0001
PFOS	-6.08	2.50	.02	-2.75	3.09	.38
Age	-1.99	0.59	.001	-0.16	0.69	.82
Alcohol	5.36	10.14	.60	8.45	23.70	.72
BMI	-0.64	0.84	.45	0.14	1.10	.90
Cigarettes	-0.35	0.40	.38	-0.19	0.47	.69
	$R^2 = .19$	Adj $R^2 = .14$		$R^2 = .01$	Adj $R^2 = .00$	

Table 9
(continued)

Creatinine - Both Locations

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.5744	0.0779	.0001	0.9478	0.0602	.0001
PFOS	-0.0223	0.0146	.13	-0.0302	0.0159	.06
$PFOS^2$	0.0033	0.0016	.04	0.0055	0.0022	.01
Age	0.0059	0.0013	.0001	0.0027	0.0012	0.02
Alcohol	-0.0260	0.0106	.02	-0.0242	0.0101	.02
BMI	0.0086	0.0023	.0003	-0.0022	0.0021	.30
Cigarettes	-0.0005	0.0011	.65	-0.0019	0.0010	.05
	$R^2 = .29$	Adj $R^2 = .27$		$R^2 = .16$	Adj $R^2 = .13$	

Creatinine - Antwerp Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.7319	0.1382	.0001	0.8600	0.1430	.0001
PFOS	-0.0324	0.0195	.10	-0.0453	0.0455	.32
$PFOS^2$	0.0008	0.0024	.74	0.0074	0.0099	.46
Age	0.0044	0.0019	.02	0.0033	0.0021	.11
Alcohol	-0.0055	0.0107	.61	-0.0142	0.0113	.21
BMI	0.0037	0.0055	.50	-0.0012	0.0057	.83
Cigarettes	0.0017	0.0019	.36	0.0041	0.0019	.04
	$R^2 = .20$	Adj $R^2 = .14$		$R^2 = .14$	Adj $R^2 = .05$	

Creatinine - Decatur Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.8641	0.1300	.0001	1.1363	0.1021	.0001
PFOS	-0.0093	0.0206	.65	-0.0430	0.0201	.04
$PFOS^2$	0.0036	0.0019	.07	0.0067	0.0025	.009
Age	0.0032	0.0020	.11	0.0006	0.0016	.72
Alcohol	0.0082	0.0336	.81	-0.1133	0.0559	.05
BMI	0.0032	0.0028	.26	-0.0038	0.0026	.14
Cigarettes	-0.0030	0.0013	.02	-0.0034	0.0011	.003
	$R^2 = .23$	Adj $R^2 = .18$		$R^2 = .27$	Adj $R^2 = .22$	

Table 9
(continued)

Cholesterol - Both Locations

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	190.1	22.1	.0001	155.5	19.0	.0001
PFOS	-0.99	1.77	.58	4.66	2.04	.02
Age	0.74	0.39	.06	1.39	0.36	.0002
Alcohol	-0.40	3.04	.90	7.50	3.18	.02
BMI	-0.04	0.67	.95	-0.40	0.67	.55
Cigarettes	-0.18	0.31	.56	-0.08	0.30	.80
	$R^2 = .02$	Adj $R^2 = .00$		$R^2 = .17$	Adj $R^2 = .14$	

Cholesterol - Antwerp Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	62.17	43.72	.16	93.11	49.20	.06
PFOS	-1.22	2.51	.63	2.37	4.74	.62
Age	2.60	0.59	.0001	2.54	0.67	.0004
Alcohol	1.13	3.39	.74	7.49	3.98	.06
BMI	2.28	1.73	.19	0.78	1.95	.69
Cigarettes	0.81	0.59	.17	-0.23	0.66	.72
	$R^2 = .25$	Adj $R^2 = .20$		$R^2 = .33$	Adj $R^2 = .27$	

Cholesterol - Decatur Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	304.27	33.14	.0001	207.01	31.55	.0001
PFOS	-1.03	2.19	.64	4.26	2.27	.06
Age	-1.05	0.51	.04	0.48	0.51	.35
Alcohol	-21.16	8.88	.02	19.54	17.41	.27
BMI	-1.03	.73	.17	-.78	0.81	.34
Cigarettes	-0.40	0.33	.23	-0.11	.35	.75
	$R^2 = .14$	Adj $R^2 = .09$		$R^2 = .07$	Adj $R^2 = .01$	

Table 9
(continued)

LDL

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	135.26	20.27	.0001	114.69	17.06	.0001
PFOS	0.50	1.61	.76	4.01	1.82	.03
Age	0.58	0.36	.11	1.04	0.32	.002
Alcohol	-3.33	2.78	.23	4.29	2.84	.13
BMI	-6.67	0.61	.28	-1.09	0.60	.07
Cigarettes	-0.52	0.28	.07	-0.02	0.28	.95
	$R^2 = .05$	Adj $R^2 = .02$		$R^2 = .13$	Adj $R^2 = .10$	

LDL - Antwerp Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	12.14	41.44	.77	40.88	42.42	.34
PFOS	0.24	2.38	.92	1.50	4.08	.71
Age	2.15	0.56	.0003	2.22	0.58	.0003
Alcohol	-3.04	3.21	.35	4.12	3.43	.23
BMI	2.04	1.64	.22	0.48	1.68	.78
Cigarettes	0.35	0.56	.53	0.26	0.57	.65
	$R^2 = .20$	Adj $R^2 = .15$		$R^2 = .29$	Adj $R^2 = .23$	

LDL - Decatur Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	218.24	30.58	.0001	160.50	28.17	.0001
PFOS	1.06	1.96	.59	3.55	2.03	.08
Age	-0.79	0.46	.09	0.20	0.45	.65
Alcohol	-22.95	8.04	.006	-12.68	28.72	.66
BMI	-1.38	0.67	.04	-1.27	0.72	.08
Cigarettes	-0.65	0.30	.03	-0.13	0.31	.67
	$R^2 = .20$	Adj $R^2 = .15$		$R^2 = .07$	Adj $R^2 = .01$	

Table 9
(continued)

Hematocrit - Both Locations

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	48.01	1.36	.0001	46.73	1.28	.0001
PFOS	-0.06	0.11	,57	-0.25	0.14	.07
Age	0.0005	0.02	.98	-0.03	0.02	.20
Alcohol	0.10	0.19	.58	0.15	0.22	0.50
BMI	-0.08	0.04	.06	0.01	0.05	0.75
Cigarettes	0.05	0.02	.007	0.04	0.02	.04
	$R^2 = .08$	Adj $R^2 = .05$		$R^2 = .08$	Adj $R^2 = .04$	

Hematocrit - Antwerp Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	44.00	2.30	.0001	45.63	3.63	.0001
PFOS	0.06	0.13	.63	-0.29	0.35	.42
Age	0.06	0.03	.06	0.04	0.50	.37
Alcohol	-0.12	0.18	.51	0.06	0.30	.84
BMI	0.02	0.09	.80	-0.02	0.14	.89
Cigarettes	0.05	0.03	.14	0.01	0.05	.79
	$R^2 = .08$	Adj $R^2 = .03$		$R^2 = .01$	Adj $R^2 = .00$	

Hematocrit - Decatur Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	45.56	2.62	.0001	45.69	1.95	.0001
PFOS	-0.05	0.17	.79	-0.28	0.14	.05
Age	0.01	0.04	.89	-0.05	0.03	.09
Alcohol	-0.56	0.68	.41	-1.52	1.08	.16
BMI	-0.02	0.06	.71	0.08	0.05	.11
Cigarettes	0.08	0.03	.004	0.06	0.02	.006
	$R^2 = .10$	Adj $R^2 = .04$		$R^2 = .19$	Adj $R^2 = .13$	

Table 9
(continued)

Hemoglobin - Both Locations

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	15.78	0.45	.0001	15.43	0.43	.0001
PFOS	-0.02	0.04	.53	-0.08	0.05	.07
Age	0.004	0.008	.57	-0.006	0.01	.46
Alcohol	-0.02	0.06	.77	0.01	0.07	.85
BMI	-0.03	0.01	.06	0.008	0.02	.59
Cigarettes	0.02	0.006	.02	0.02	0.007	.03
	$R^2 = .06$	Adj $R^2 = .03$		$R^2 = .06$	Adj $R^2 = .03$	

Hemoglobin - Antwerp Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	14.72	0.74	.0001	15.32	1.15	.0001
PFOS	0.008	0.04	.86	-0.10	0.11	.40
Age	0.02	0.01	.02	0.02	0.02	.33
Alcohol	-0.04	0.06	.51	0.02	0.09	.81
BMI	-0.007	0.03	.81	-0.01	0.05	.81
Cigarettes	0.005	0.01	.61	-0.006	0.02	.70
	$R^2 = .08$	Adj $R^2 = .02$		$R^2 = .03$	Adj $R^2 = .00$	

Hemoglobin - Decatur Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	15.87	0.90	.0001	15.58	0.71	.0001
PFOS	-0.03	0.06	.63	-0.10	0.05	.07
Age	-0.004	0.01	.79	-0.02	0.01	.12
Alcohol	-0.22	0.23	.35	-0.47	0.39	.23
BMI	-0.02	0.02	.32	0.02	0.02	.25
Cigarettes	0.02	0.009	.01	0.02	0.008	.004
	$R^2 = .10$	Adj $R^2 = .04$		$R^2 = .17$	Adj $R^2 = .12$	

Table 9
(continued)

Direct Bilirubin - Both Locations

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.2196	0.0259	.0001	0.1929	0.0264	.0001
PFOS	-0.0009	0.0021	.68	-0.0061	0.0029	.03
Age	0.0006	0.0005	.17	-0.0002	0.0005	.69
Alcohol	0.0074	0.0036	.04	0.0106	0.0045	.02
BMI	-0.0012	0.0008	.13	-0.0017	0.0009	.07
Cigarettes	-0.0007	0.0004	.06	-0.0009	0.0004	.05
	$R^2 = .06$	Adj $R^2 = .03$		$R^2 = .14$	Adj $R^2 = .11$	

Direct Bilirubin - Antwerp Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.1794	0.0461	.0002	0.1963	0.0813	.02
PFOS	-0.0034	0.0026	.21	-0.0077	0.0079	.33
Age	0.00004	0.0006	.95	-0.0017	0.0011	.13
Alcohol	0.0102	0.0036	.006	0.0112	0.0066	.10
BMI	0.0017	0.0018	.36	0.0006	0.0032	.84
Cigarettes	-0.0016	0.0006	0.009	-0.0017	0.0011	.12
	$R^2 = .17$	Adj $R^2 = .12$		$R^2 = .14$	Adj $R^2 = .06$	

Direct Bilirubin - Decatur Only

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.1972	0.0478	.0001	0.1205	0.0347	.0008
PFOS	0.0025	0.0032	.44	-0.0038	0.0025	.13
Age	0.0012	0.0007	0.11	0.0014	0.0006	.02
Alcohol	-0.0114	0.0128	.37	-0.0042	0.0191	.83
BMI	-0.0016	0.0011	.13	-0.0018	0.0009	.04
Cigarettes	-0.0001	0.0005	.79	-0.0004	0.0004	.27
	$R^2 = .07$	Adj $R^2 = .02$		$R^2 = .15$	Adj $R^2 = .10$	

Table 10

Summaries of Simple Linear and Quadratic Regression Models for Total and Unconjugated Bilirubin, 1995 and 1997

	1995 Data				1997 Data			
	Total Bilirubin		Unconjugated		Total Bilirubin		Unconjugated	
	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
Both Locations								
R^2	.002	.052	.024	.059	.016	.046	.012	.046
Intercept	0.78247	0.86532	0.56837	0.65239	0.8175	0.78963	0.57745	0.64247
PFOS(p value)	-0.02927 (.047)	-0.00918 (.03)	-0.0285 (.04)	-0.09941 (.002)	-0.02583 (.12)	-0.10456 (.01)	-0.01940 (.18)	-0.09138 (.01)
$PFOS^2$ (p value)		0.00846 (.02)		0.00858 (.01)		0.01200 (.03)		0.01097 (.03)
Antwerp								
R^2	.018	.028	.017	.028	.014	.052	.012	.050
Intercept	0.92001	0.96548	0.69447	0.73919	0.85170	0.96350	0.69161	0.78708
PFOS (p value)	-0.02927 (.21)	-0.07872 (.18)	-0.02604 (.23)	-0.07468 (.17)	-0.03647 (.35)	-0.22847 (.08)	-0.02872 (.39)	-0.19268 (.09)
$PFOS^2$ (p value)		0.00697 (.31)		0.00686 (.32)		0.04620 (.20)		0.03945 (.13)
Decatur								
R^2	.004	.010	.008	.029	0.009	.071	.002	.077
Intercept	0.59712	0.66974	0.39803	0.48279	0.59609	0.68287	0.47268	0.55440
PFOS (p value)	-0.014363 (.53)	-0.05867 (.09)	-0.01166 (.39)	-0.06966 (.03)	-0.00894 (.53)	-0.09408 (.02)	-0.00489 (.69)	-0.08508 (.01)
$PFOS^2$ (p value)		0.00518 (.12)		0.00605 (.05)		0.001143 (.02)		0.01076 (.01)

Table 11

Mean Values of PFOS, Demographic, Serum Chemistry and Hematologic Parameters for Antwerp and Decatur Combined Locations, 1995 and 1997 Examinations for Employees Who Participated and Did Not Participate in Both Years

	1995 Data		1997 Data	
Variable	Both Years (N = 61)	Only 1995 (N = 117)	Both Years (N = 61)	Only 1997 (N = 88)
PFOS (ppm)	2.40	2.08	2.34***	1.34
Age	39*	42	41*	38
BMI	27.1	26.3	27.3	26.9
Cigarettes	5.4	6.7	5.2	6.8
Alcohol	0.8	0.6	0.4*	0.4
Alk Phosphatase	87	86	79	80
GGT	44	44	37*	27
AST	29	27	26	26
ALT	46	46	33	32
Total bilirubin	0.71	0.72	0.65	0.69
Direct bilirubin	0.21	0.22	0.13	0.13
BUN	16.2	15.7	14.6	14.3
Creatinine	1.0	1.0	1.0	1.0
Glucose	86	87	93	89
Cholesterol	212	213	225***	201
LDL	142	134	145***	128
HDL	50	47	46	45

Table 11
(continued)

Variable	1995 Data		1997 Data	
	Antwerp	Decatur	Antwerp	Decatur
Triglycerides	139	157	167	149
Hematocrit	45	47	46	46
Hemoglobin	15.0	15.5	15.3	15.4
RBC	4.9	5.0	5.0*	5.1
MCH	30.8	31.2	30.8*	30.2
MCHC	33.2	33.1	33.6	33.6
MVC	93	94	92**	90
WBC	6.6	7.0	6.5	6.4
Platelets	226	227	228	224

* $p < .05$; ** $p < .01$; *** $p < .001$

Table 12

Mean Values of PFOS, Demographic, Serum Chemistry and Hematologic Parameters for Antwerp and Decatur, 1995 Examinations for Employees Who Participated and Did not Participate in Both 1995 and 1997

	Antwerp		Decatur	
Variable	Both Years (N = 27)	Only 1995 (N = 61)	Both Years (N = 34)	Only 1995 (N = 56)
PFOS (ppm)	2.30	1.76	2.48	2.42
Age	34	37	42*	46
BMI	24.0	23.9	29.6	29.0
Cigarettes	5.4	4.3	5.4	9.4
Alcohol	1.7*	1.0	0.1	0.2
Alk Phosphatase	75	75	96	98
GGT	46	39	43	50
AST	28	25	30	29
ALT	43	45	48	47
Total bilirubin	0.91	0.84	0.54	0.59
Direct bilirubin	0.23	0.22	0.19	0.21
BUN	17.7	16.7	15.0	15.0
Creatinine	0.9	0.9	1.1	1.1
Glucose	78*	83	92	92
Cholesterol	209	216	232**	209
LDL	133	140	150**	128
HDL	56	53	46	42

Table 12
(continued)

Variable	1995 Data		1997 Data	
	Antwerp	Decatur	Antwerp	Decatur
Triglycerides	100	122	170	197
Hematocrit	46	47	44**	46
Hemoglobin	15.3	15.5	14.8**	15.4
RBC	4.9	4.9	4.9*	5.0
MCH	31.2	31.5	30.5	30.8
MCHC	33.0	32.8	33.4	33.4
MVC	95	96	91	92
WBC	6.4	6.3	6.8*	7.8
Platelets	222	225	23.0	228

* $p < .05$; ** $p < .01$; *** $p < .001$

Table 13

Mean Values of PFOS, Demographic, Serum Chemistry and Hematologic Parameters for Antwerp and Decatur, 1997 Examinations for Employees Who Participated and Did not Participate in Both Years

	Antwerp		Decatur	
Variable	Both Years (N = 27)	Only 1997 (N = 38)	Both Years (N = 34)	Only 1997 (N = 50)
PFOS (ppm)	2.33***	0.88	2.35	1.69
Age	37***	30	45	44
BMI	24.4*	22.8	29.5	29.9
Cigarettes	5.9	5.2	4.7	7.9
Alcohol	1.0	0.7	0.1	0.2
Alk Phosphatase	72	69	85	88
GGT	33*	21	41	32
AST	27	26	26	26
ALT	32	30	34	34
Total bilirubin	0.77	0.81	0.56	0.59
Direct bilirubin	0.14	0.16	0.12	0.11
BUN	15.2	14.6	14.1	14.0
Creatinine	0.9	0.9	1.0	1.0
Glucose	80	81	103	95
Cholesterol	224***	192	226*	208
LDL	148	123	143	132
HDL	51	49	43	42

Table 13
(continued)

Variable	1995 Data		1997 Data	
	Antwerp	Decatur	Antwerp	Decatur
Triglycerides	125	101	201	185
Hematocrit	46	46	45	46
Hemoglobin	15.5	15.4	15.2	15.4
RBC	5.0	5.1	4.9**	5.1
MCH	30.8	30.4	30.9	30.0
MCHC	33.4	33.3	33.7	33.8
MVC	92	91	91*	89
WBC	6.8	6.3	6.4	6.5
Platelets	241	234	218	217

* $p < .05$; ** $p < .01$; *** $p < .001$

Table 14

Multivariable Regression of Serum Chemistries and Hematological Parameters - Examination of the Effect of PFOS Adjusting for Age, Alcohol, BMI and Cigarettes, Antwerp and Decatur Employees (N = 61) Who Participated in Both the 1995 and 1997 Examinations

Alkaline Phospatase

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	55.23	22.22	.02	45.74	22.07	.04
PFOS	-1.71	1.77	.34	0.46	1.71	.79
Age	0.42	0.43	.33	0.33	0.37	.37
Alcohol	-5.34	2.47	.04	-2.93	2.36	.22
BMI	0.70	0.67	.30	0.62	0.61	.31
Cigarettes	0.82	0.34	.02	0.76	0.32	.02
	$R^2 = .24$	Adj $R^2 = .17$		$R^2 = .15$	Adj $R^2 = .07$	

ln GGT

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	2.5359	0.5561	.0001	2.5660	0.5965	.0001
PFOS	-0.0028	0.0443	.95	-0.0357	0.0461	.44
Age	-0.0078	0.0107	.47	-0.0095	0.0010	.35
Alcohol	0.0795	0.0619	.20	0.0673	0.0638	.30
BMI	0.0469	0.0167	.007	0.0463	0.0165	.007
Cigarettes	0.0023	0.0084	.79	-0.0025	0.0087	.77
	$R^2 = .14$	Adj $R^2 = .06$		$R^2 = .14$	Adj $R^2 = .07$	

SGOT

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	15.27	11.19	.18	21.68	6.80	.002
PFOS	0.40	0.89	.65	-0.22	0.53	.67
Age	0.17	0.22	.42	0.001	0.11	.99
Alcohol	1.01	1.25	.42	0.47	.73	52
BMI	0.24	0.34	.47	0.17	0.19	.38
Cigarettes	-0.26	0.17	.13	0.07	0.10	.49
	$R^2 = .08$	Adj $R^2 = .00$		$R^2 = .03$	Adj $R^2 = .00$	

Table 14
(continued)

SGPT

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	26.32	17.83	.15	15.34	13.86	.27
PFOS	-0.03	1.42	.98	0.27	1.07	.80
Age	-0.20	0.34	.57	-0.34	0.23	.14
Alcohol	0.13	1.98	.95	0.22	1.48	.88
BMI	1.05	0.54	.06	1.15	0.38	.004
Cigarettes	-0.25	0.27	.35	-0.04	0.20	.83
	$R^2 = .10$	Adj $R^2 = .02$		$R^2 = .18$	Adj $R^2 = .11$	

ln Total Bilirubin

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.7086	0.3971	.08	-0.3739	0.3996	.35
PFOS	-0.0874	0.0806	.28	0.0202	0.0308	.51
$PFOS^2$	0.0081	0.0068	.24	-0.0014	0.0026	.58
Age	-0.0030	0.0073	.69	0.0072	0.0068	.29
Alcohol	0.0344	0.0436	.43	0.1045	0.0426	.02
BMI	-0.0319	0.0115	.01	-0.0182	0.0112	.11
Cigarettes	-0.0130	0.0060	.03	-0.0105	0.0058	.08
	$R^2 = .23$	Adj $R^2 = .14$		$R^2 = .19$	Adj $R^2 = .10$	

Direct Bilirubin

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.2579	0.0469	.0001	0.1711	0.0462	.0005
PFOS	-0.0027	0.0037	.47	-0.0026	0.0036	.47
Age	-0.0019	0.0009	.04	0.0004	0.0008	.60
Alcohol	0.0049	0.0052	.35	0.0089	0.0049	.08
BMI	0.0010	0.0014	.49	-0.0021	0.0013	.10
Cigarettes	-0.0005	0.0007	.50	-0.0008	0.0007	.22
	$R^2 = .13$	Adj $R^2 = .05$		$R^2 = .14$	Adj $R^2 = .07$	

Table 14
(continued)

BUN

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	18.33	3.62	.0001	14.06	3.09	.0001
PFOS	-0.09	0.29	.75	0.29	0.24	.22
Age	-0.01	0.07	.87	0.07	0.05	.16
Alcohol	0.59	0.40	.15	0.18	0.33	.59
BMI	-0.04	0.11	.71	-0.12	0.09	.18
Cigarettes	-0.15	0.05	.007	-0.03	0.04	.55
	$R^2 = .16$	Adj $R^2 = .09$		$R^2 = .08$	Adj $R^2 = .00$	

Creatinine

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	0.5185	0.1308	.0002	0.8785	0.1333	.0001
PFOS	-0.0515	0.0264	.06	0.0168	0.0103	.11
$PFOS^2$	0.0068	0.0022	.004	-0.0005	0.0009	.57
Age	0.0073	0.0024	.004	0.0036	0.0023	.12
Alcohol	-0.0100	0.0143	.49	-0.0266	0.0142	.07
BMI	0.0099	0.0038	.01	-0.0030	0.0037	.43
Cigarettes	-0.0003	0.0019	.89	-0.0028	0.0020	.15
	$R^2 = .49$	Adj $R^2 = .43$		$R^2 = .18$	Adj $R^2 = .09$	

ln Glucose

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	3.7479	0.1311	.0001	3.4111	0.2013	.0001
PFOS	-0.0017	0.0103	.87	0.0036	0.0156	.82
Age	0.0008	0.0025	.74	0.0063	0.0034	.07
Alcohol	-0.0050	0.0145	.73	0.0005	0.0215	.98
BMI	0.0236	0.0039	.0001	0.0306	0.0056	.0001
Cigarettes	0.0023	0.0020	.26	-0.0051	0.0029	.09
	$R^2 = .46$	Adj $R^2 = .41$		$R^2 = .48$	Adj $R^2 = .43$	

Table 14
(continued)

Cholesterol

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	184.37	34.59	.0001	179.50	38.87	.0001
PFOS	-1.15	2.76	.68	3.75	3.01	.22
Age	1.37	0.67	.04	0.73	0.65	.27
Alcohol	-2.72	3.85	.48	6.57	4.16	.12
BMI	-0.24	1.04	.81	0.09	1.07	.94
Cigarettes	-0.82	0.52	.12	-0.07	0.57	.90
	$R^2 = .13$	Adj $R^2 = .05$		$R^2 = .09$	Adj $R^2 = .01$	

LDL

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	115.89	30.35	.0004	150.88	34.90	.0001
PFOS	0.36	2.38	.88	2.68	2.70	.33
Age	1.26	0.58	.88	0.44	0.58	.45
Alcohol	-3.74	3.35	.27	3.81	3.73	.31
BMI	-0.61	0.91	.51	-1.20	0.96	.22
Cigarettes	-0.72	0.45	.12	-0.10	0.51	.85
	$R^2 = .15$	Adj $R^2 = .07$		$R^2 = .07$	Adj $R^2 = .00$	

HDL

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	75.25	11.01	.0001	68.22	8.91	.0001
PFOS	-0.68	0.86	.44	-0.33	0.69	.63
Age	-0.0005	0.21	.99	0.11	0.15	.47
Alcohol	2.31	1.21	.06	2.10	0.95	.03
BMI	-0.86	0.33	.01	-0.97	0.25	.0002
Cigarettes	-0.30	0.17	.08	-0.12	0.13	.37
	$R^2 = .25$	Adj $R^2 = .18$		$R^2 = .34$	Adj $R^2 = .28$	

Table 14
(continued)

Triglycerides

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	-89.52	66.79	.19	-198.05	82.02	.02
PFOS	-3.78	5.32	.48	7.03	6.35	.27
Age	0.72	1.29	.58	0.87	1.37	.53
Alcohol	-1.93	7.43	.80	3.28	8.77	.71
BMI	7.50	2.00	.0004	11.26	2.26	.0001
Cigarettes	1.40	1.01	.17	0.73	1.19	.54
	$R^2 = .26$	Adj $R^2 = .19$		$R^2 = .38$	Adj $R^2 = .32$	

Hematocrit

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	50.87	2.15	.0001	46.61	2.56	.0001
PFOS	-0.03	0.17	.87	-0.20	0.20	.33
Age	-0.05	0.04	.19	-0.04	0.04	.38
Alcohol	0.09	0.24	.70	0.31	0.27	.26
BMI	-0.13	0.06	.04	0.02	0.07	.77
Cigarettes	0.05	0.04	.21	0.06	0.04	.14
	$R^2 = .20$	Adj $R^2 = .13$		$R^2 = .10$	Adj $R^2 = .02$	

Hemoglobin

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	17.09	0.72	.0001	15.55	0.88	.0001
PFOS	-0.02	0.06	.70	-0.07	0.07	.33
Age	-0.01	0.01	.36	-0.01	0.01	.44
Alcohol	-0.002	0.08	.98	0.05	0.09	.61
BMI	-0.06	0.02	.01	0.01	0.02	.70
Cigarettes	0.003	0.01	.79	0.02	0.01	.12
	$R^2 = .18$	Adj $R^2 = .10$		$R^2 = .07$	Adj $R^2 = .00$	

Table 14
(continued)

RBC

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	5.21	0.22	.0001	5.02	0.30	.0001
PFOS	0.002	0.018	.89	-0.003	0.024	.88
Age	-0.003	0.004	.55	-0.003	0.005	.53
Alcohol	-0.006	0.025	.81	0.025	0.033	.44
BMI	-0.007	0.007	.28	0.004	0.008	.64
Cigarettes	-0.005	0.004	.18	-0.005	0.004	.28
	$R^2 = .06$	Adj $R^2 = .03$		$R^2 = .04$	Adj $R^2 = .04$	

MCH

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	32.97	1.25	.0001	31.13	1.43	.0001
PFOS	-0.05	0.10	.60	-0.11	0.11	.31
Age	-0.01	0.02	.67	-0.004	0.024	.85
Alcohol	0.04	0.14	.79	-0.06	0.15	.71
BMI	-0.07	0.04	.07	-0.007	0.039	.86
Cigarettes	0.04	0.02	.06	0.07	0.02	.001
	$R^2 = .18$	Adj $R^2 = .10$		$R^2 = .19$	Adj $R^2 = .12$	

MCHC

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	33.55	0.53	.0001	33.40	0.50	.0001
PFOS	-0.03	0.04	.55	-0.005	0.039	.89
Age	0.01	0.01	.20	0.005	0.008	.55
Alcohol	-0.08	0.06	.18	-0.10	0.05	.07
BMI	-0.02	0.02	.17	0.001	0.014	.92
Cigarettes	-0.02	0.01	.01	0.003	0.007	.69
	$R^2 = .18$	Adj $R^2 = .10$		$R^2 = .09$	Adj $R^2 = .01$	

Table 14
(continued)

MCV

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	98.33	3.59	.0001	92.85	3.82	.0001
PFOS	-0.09	0.29	.76	-0.28	0.30	.34
Age	-0.07	0.07	.34	-0.02	0.06	.76
Alcohol	0.30	0.40	.45	0.19	0.41	.65
BMI	-0.15	0.11	.18	-0.03	0.11	.78
Cigarettes	0.19	0.06	.003	0.20	0.06	.0008
	$R^2 = .27$	Adj $R^2 = .20$		$R^2 = .22$	Adj $R^2 = .15$	

Platelets

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	295.55	37.09	.0001	392.12	48.22	.0001
PFOS	-3.96	2.96	.19	-3.84	3.73	.31
Age	-0.84	0.71	.24	-2.31	0.81	.006
Alcohol	-10.54	4.13	.01	-6.59	5.16	.21
BMI	-0.54	1.12	.63	-1.85	1.33	.17
Cigarettes	-0.78	0.63	.22	-0.82	0.70	.25
	$R^2 = .19$	Adj $R^2 = .11$		$R^2 = .21$	Adj $R^2 = .14$	

WBC

	1995 Data			1997 Data		
	Parameter	SE	p value	Parameter	SE	p value
Intercept	3.10	1.19	.01	4.46	1.36	.002
PFOS	0.03	0.09	.77	-0.04	0.11	.70
Age	0.002	0.023	.93	-0.007	0.02	.75
Alcohol	0.07	0.13	.62	0.21	0.15	.15
BMI	0.10	0.04	.007	0.06	0.04	.09
Cigarettes	0.12	0.02	.0001	0.11	0.02	.0001
	$R^2 = .43$	Adj $R^2 = .38$		$R^2 = .40$	Adj $R^2 = .34$	

Table 15

Employee Distribution as to Plant Location and Whether Hormones Were Measured, 1995

PFOS (ppm)	Total	Both Locations: Hormones Measured Yes	Both Locations: Hormones Measured No	By Location: Antwerp Hormones Measured Yes	By Location: Antwerp Hormones Measured No	By Location: Decatur Hormones Measured Yes	By Location: Decatur Hormones Measured No
0 - < 1	45	10 (22%)	35 (78%)	9 (27%)	25 (73%)	1 (9%)	10 (91%)
1 - < 3	91	46 (51%)	45 (49%)	21 (66%)	11 (34%)	25 (43%)	34 (57%)
3 - < 6	35	27 (77%)	8 (23%)	18 (95%)	1 (5%)	9 (56%)	7 (44%)
>= 6	7	5 (71%)	2 (29%)	2 (67%)	1 (33%)	3 (75%)	1 (25%)
	178	88	90	50	38	38	52

Table 16

Mean Values for PFOS, Demographic, Serum Chemistries and Hematology, by Plant Location and Whether Hormones Were Measured, 1995

	Both Locations Hormones Measured		Antwerp Hormones Measured		Decatur Hormones Measured	
Variable	Yes N = 88	No N = 90	Yes N = 50	No N = 38	Yes N = 38	No N = 52
PFOS	2.87***	1.52	2.69***	0.92	3.10**	1.96
Age	38.4***	42.7	35.0*	38.4	42.9	45.9
Alcohol	1.0**	0.4	1.6**	0.8	0.2	0.2
BMI	26.5	26.7	23.6	24.4	30.3	28.5
Cigarettes	8.3*	4.3	6.7**	1.9	10.3	6.0
BUN	16.3	15.5	17.1	16.8	15.1	14.5
Creatinine	0.97*	1.02	0.87***	0.94	1.10	1.08
Glucose	86	88	80	84	94	90
AlkalinePhosphatase	86	86	77	73	99	96

Table 16
(continued)

Variable	Both Locations Hormones Measured Yes N = 88	Both Locations Hormones Measured No N = 90	Antwerp Hormones Measured Yes N = 50	Antwerp Hormones Measured No N = 38	Decatur Hormones Measured Yes N = 38	Decatur Hormones Measured No N = 52
GGT	47	42	45	37	50	46
AST	29	27	27	24	31	29
ALT	47	45	44	45	51	45
Total Bilirubin	0.7	0.8	0.8	0.9	0.5	0.6
Direct Bilirubin	0.2	0.2	0.2	0.2	0.2	0.2
Cholesterol	217	215	210	220	227	211
LDL	136	137	133	144	141	132
HDL	49	48	52	55	43	43
Triglycerides	158	144	124	104	205	174
Hematocrit	47	46	47	47	46	45
Hemoglobin	15.4	15.3	15.4	15.4	15.3	15.1

Table 16
(continued)

Variable	Both Locations Hormones Measured Yes N = 88	Both Locations Hormones Measured No N = 90	Antwerp Hormones Measured Yes N = 50	Antwerp Hormones Measured No N = 38	Decatur Hormones Measured Yes N = 38	Decatur Hormones Measured No N = 52
RBC	5.0	4.9	4.9	4.9	5.0	4.9
MCH	31.1	31.0	31.5	31.3	30.5	30.8
MCHC	33.0*	33.3	32.9	32.8	33.2*	33.5
MCV	94	93	96	95	92	92
WBC	7.3**	6.5	6.8***	5.7	8.0*	7.0
Platelets	230	223	233**	212	226	231

* $p < .05$; ** $p < .01$; *** $p < .001$

Table 17

Mean, Median (Med), Standard Deviation (SD) of Mean and Range of PFOS, Demographic, Serum Chemistries and Hematological Values for N = 88 Employees, Antwerp and Decatur Combined, who Had Hormone Measurements, 1995

PFOS (ppm)	Mean	Med	SD	Range
		PFOS		
0 - < 1	0.68[1]	0.75	0.21	0.37-0.90
1 - < 3	1.96[1]	1.96	0.62	1.00-2.90
3 - < 6	4.15[1]	3.97	0.85	3.00-5.80
≥ 6	8.67[1]	8.50	2.85	6.06-12.83
	F value = 121.3, p = .0001			
		Age		
0 - < 1	32.7	32.5	6.3	21.0-43.0
1 - < 3	38.7	39.0	8.4	25.0-58.0
3 - < 6	39.0	39.0	7.6	26.0-54.0
≥ 6	43.0	42.0	7.8	37.0-56.0
	F value = 2.4, p = .08			
		Alcohol		
0 - < 1	1.4	1.1	1.2	0.0-3.6
1 - < 3	0.6	0.2	0.8	0.0-3.6
3 - < 6	1.4	0.5	2.0	0.0-6.0
≥ 6	0.9	0.0	1.3	0.0-2.9
	F value = 2.1, p = .11			
		BMI		
0 - < 1	24.5	24.5	2.4	20.4-28.0
1 - < 3	27.9	26.2	7.2	19.6-60.7
3 - < 6	24.7	24.2	4.2	17.9-32.5
≥ 6	27.5	29.4	4.9	20.6-33.0
	F value = 2.1, p = .10			

Table 17
(continued)

PFOS (ppm)	Mean	Med	SD	Range
		Cigarettes		
0 - < 1	6.0	0.0	10.5	0.0-25.0
1 - < 3	7.7	0.0	11.2	0.0-35.0
3 - < 6	11.6	12.0	11.8	0.0-40.0
≥ 6	0.6	0.0	1.3	0.0-3.0
	F = 1.8, p = .16			
		BUN		
0 - < 1	17.0	16.0	3.1	14.0-22.0
1 - < 3	15.8	15.0	3.8	8.0-26.0
3 - < 6	17.0	17.0	3.8	10.0-23.0
≥ 6	14.4	14.0	4.0	10.0-21.0
	F value = 1.1, p = .35			
		Creatinine		
0 - < 1	1.0	1.0	0.1	0.8-1.1
1 - < 3	1.0	0.9	0.2	0.7-1.6
3 - < 6	0.9	0.9	0.1	0.7-1.2
≥ 6	1.1	1.2	0.4	0.6-1.6
	F value = 2.7, p = .05			
		Glucose		
0 - < 1	82	82	7	70-93
1 - < 3	89	84	29	62-260
3 - < 6	81	82	12	66-112
≥ 6	87	83	16	71-105
	F value = 0.8, p = .52			
		Alkaline Phosphatase		
0 - < 1	82	82	14	66-103
1 - < 3	88	89	25	49-146
3 - < 6	85	82	21	32-124
≥ 6	88	74	29	63-136
	F value = 0.3, p = .84			

Table 17
(continued)

PFOS (ppm)	Mean	Med	SD	Range
		GGT		
0 - < 1	38	28	27	23-111
1 - < 3	53	41	49	2-293
3 - < 6	39	39	15	21-80
≥ 6	48	49	19	32-79
	F value = 0.9, p = .44			
		AST		
0 - < 1	31	25	23	17-96
1 - < 3	30	27	14	14-90
3 - < 6	25	24	6	13-37
≥ 6	31	30	4	26-37
	F value = 1.2, p = .31			
		ALT		
0 - < 1	52	48	21	36-108
1 - < 3	48	43	24	25-183
3 - < 6	42	41	7	30-59
≥ 6	54	57	20	29-82
	F value =1.1, p = .36			
		Total Bilirubin		
0 - < 1	0.86	0.70	0.45	0.40-2.00
1 - < 3	0.67	0.65	0.28	0.20-1.30
3 - < 6	0.65	0.60	0.31	0.20-1.40
≥ 6	0.68	0.70	0.15	0.50-0.90
	F value = 1.2, p = .30			
		Direct Bilirubin		
0 - < 1	0.22	0.20	0.04	0.20-0.30
1 - < 3	0.20	0.20	0.05	0.10-0.30
3 - < 6	0.22	0.20	0.04	0.20-0.30
≥ 6	0.22	0.20	0.04	0.20-0.30
	F value = 0.6, p = .63			

Table 17
(continued)

PFOS (ppm)	Mean	Med	SD	Range
		Cholesterol		
0 - < 1	213	207	33	180-290
1 - < 3	220	227	42	144-315
3 - < 6	217	214	29	171-270
≥ 6	200	208	34	160-240
	F value = 0.5, p = .69			
		LDL		
0 - < 1	129	128	22	106-177
1 - < 3	136	146	39	65-228
3 - < 6	139	135	29	84-190
≥ 6	129	130	29	95-172
	F value = 0.3, p = .85			
		HDL		
0 - < 1	52	57	12	31-63
1 - < 3	50	46	15	28-94
3 - < 6	46	48	12	23-74
≥ 6	45	46	10	34-61
	F value = 0.6, p = .64			
		Triglycerides		
0 - < 1	157	121	167	41-622
1 - < 3	163	129	118	41-651
3 - < 6	156	138	97	34-413
≥ 6	128	151	52	64-187
	F value = 0.1, p = .94			
		Hematocrit		
0 - < 1	47	48	2	44-49
1 - < 3	46	46	3	39-52
3 - < 6	47	47	2	43-52
≥ 6	47	48	1	45-49
	F value = 2.3, p = .08			

Table 17
(continued)

PFOS (ppm)	Mean	Med	SD	Range
		Hemoglobin		
0 - < 1	15.6	15.8	0.5	14.6-16.1
1 - < 3	15.2	15.3	0.9	13.0-17.1
3 - < 6	15.6	15.6	0.7	13.8-17.4
≥ 6	15.5	15.4	0.7	14.8-16.2
	F value = 1.8, p = .16			
		RBC		
0 - < 1	4.9	5.0	0.3	4.3-5.2
1 - < 3	4.9	4.9	0.3	4.3-5.7
3 - < 6	5.0	5.0	0.2	4.6-5.4
≥ 6	5.0	5.2	0.7	4.0-5.7
	F value = 0.4, p = .74			
		MCH		
0 - < 1	32.0	31.7	1.1	30.8-33.9
1 - < 3	30.8	30.8	1.4	27.3-33.2
3 - < 6	31.2	31.8	1.5	26.0-33.3
≥ 6	31.1	30.2	3.4	28.2-36.9
	F value = 1.9, p = .14			
		MCHC		
0 - < 1	32.9	33.1	0.8	31.9-34.5
1 - < 3	33.0	33.1	0.7	31.7-34.5
3 - < 6	32.9	32.8	0.7	31.3-34.2
≥ 6	33.1	33.3	0.8	32.2-34.0
	F value = 0.2, p = .93			
		MCV		
0 - < 1	97	95	5	92-106
1 - < 3	93	93	4	83-101
3 - < 6	95	95	5	81-104
≥ 6	94	91	12	85-115
	F value = 1.9, p = .13			

Table 17
(continued)

PFOS (ppm)	Mean	Med	SD	Range
		WBC		
0 - < 1	6.7	6.5	1.8	4.4-9.4
1 - < 3	7.3	6.9	2.2	3.6-15.5
3 - < 6	7.7	7.5	2.3	4.1-13.3
≥ 6	7.1	6.9	0.6	6.4-7.8
	F value = 0.7, p = .59			
		Platelets		
0 - < 1	243	230	43	189-309
1 - < 3	234	227	44	153-365
3 - < 6	229	230	50	132-344
≥ 6	177	182	29	143-205
	F value = 2.7, p = .05			

1. Significantly different ($p < .05$) than the remaining three PFOS exposure categories.
2. Significantly different ($p < .05$) than the 0 - < 1 ppm PFOS category.
3. Significantly different ($p < .05$) than the 1 - < 3 ppm PFOS category.
4. Significantly different ($p < .05$) than the 3 - < 6 ppm PFOS category.
5. Significantly different ($p < .05$) than the ≥ 6ppm PFOS category.

Sample sizes:

PFOS Level	Both Locations	Antwerp	Decatur
0 - < 1 ppm	10	9	1
1 - < 3 ppm	46	21	25
3 - < 6 ppm	27	18	9
≥ 6 ppm	5	2	3
	88	50	38

Table 18

Multivariable Regression of Serum Chemistries and Hematological Parameters in Relation to PFOS Adjusting for Age, Alcohol, BMI and Cigarettes, Antwerp and Decatur Data Combined, For Those Employees Who Had Hormone Measurements in 1995

Alkaline Phosphatase

Variable	Parameter	SE	p value
Intercept	64.57	15.23	.001
PFOS	0.002	1.11	.99
Age	0.13	0.30	.66
Alcohol	-4.17	1.75	.02
BMI	0.60	0.39	.13
Cigarettes	0.60	0.20	.004
	$R^2 = .22$	Adj $R^2 = .17$	

ln GGT

Variable	Parameter	SE	p value
Intercept	1.9570	0.4203	.0001
PFOS	0.0077	0.0301	.80
Age	0.0157	0.0084	.07
Alcohol	0.1043	0.0484	.03
BMI	0.0341	0.0107	.002
Cigarettes	0.0096	0.0056	.09
	$R^2 = .20$	Adj $R^2 = .15$	

AST

Variable	Parameter	SE	p value
Intercept	16.13	9.90	.11
PFOS	-0.13	.72	.86
Age	0.006	0.20	.98
Alcohol	1.02	1.14	.37
BMI	0.51	0.25	.04
Cigarettes	-0.20	0.13	.13
	$R^2 = .08$	Adj $R^2 = .02$	

Table 18
(continued)

ALT

Variable	Parameter	SE	p value
Intercept	25.19	14.22	.08
PFOS	-0.12	1.04	.90
Age	-0.04	0.28	.89
Alcohol	0.20	1.64	.90
BMI	0.95	0.36	.01
Cigarettes	-0.20	0.19	.29
	$R^2 = .10$	Adj $R^2 = .04$	

ln Total Bilirubin

Variable	Parameter	SE	p value
Intercept	0.6133	0.2726	.03
PFOS	-0.0885	0.0518	.09
$PFOS^2$	0.0073	0.0048	.13
Age	-0.0025	0.0054	.64
Alcohol	0.0235	0.0309	.45
BMI	-0.0270	0.0068	.0002
Cigarettes	-0.0162	0.0036	.0001
	$R^2 = .39$	Adj $R^2 = .34$	

Direct Bilirubin

Variable	Parameter	SE	p value
Intercept	0.225	0.0335	.0001
PFOS	0.001	0.002	.64
Age	0.0007	0.0007	.31
Alcohol	0.004	0.004	.26
BMI	-0.002	0.0009	0.05
Cigarettes	-0.0003	0.0004	.44
	$R^2 = .09$	Adj $R^2 = .03$	

Table 18
(continued)

BUN

Variable	Parameter	SE	p value
Intercept	18.73	2.72	.0001
PFOS	-0.10	0.20	.60
Age	-0.05	0.05	.35
Alcohol	0.37	0.31	.24
BMI	-0.003	0.07	.97
Cigarettes	-0.05	0.04	.14
	$R^2 = .08$	Adj $R^2 = .02$	

Creatinine

Variable	Parameter	SE	p value
Intercept	0.5031	0.1178	.0001
PFOS	-0.0488	0.0223	.03
$PFOS^2$	0.0051	0.0020	.01
Age	0.0086	0.0023	.0004
Alcohol	-0.0119	0.0133	.38
BMI	0.0090	0.0029	.003
Cigarettes	-0.0017	0.0016	.28
	$R^2 = .37$	Adj $R^2 = .32$	

ln Glucose

Variable	Parameter	SE	p value
Intercept	3.8241	0.1090	.0001
PFOS	-0.0029	0.0079	.71
Age	0.0051	0.0022	.02
Alcohol	-0.0052	0.0125	.68
BMI	0.0166	0.0028	.0001
Cigarettes	-0.0018	0.0014	.22
	$R^2 = .42$	Adj $R^2 = .38$	

Table 18
(continued)

Cholesterol

Variable	Parameter	SE	p value
Intercept	198.04	26.88	.0001
PFOS	-2.83	1.97	.15
Age	1.26	0.54	.02
Alcohol	-1.32	3.09	.67
BMI	-0.73	0.68	.29
Cigarettes	-0.07	0.35	.85
	$R^2 = .09$	Adj $R^2 = .03$	

LDL

Variable	Parameter	SE	p value
Intercept	152.37	23.90	.0001
PFOS	-0.45	1.74	.80
Age	0.70	0.48	.14
Alcohol	-5.58	2.75	.05
BMI	-1.19	0.61	.05
Cigarettes	-0.59	0.32	.07
	$R^2 = .14$	Adj $R^2 = .08$	

HDL

Variable	Parameter	SE	p value
Intercept	77.68	8.51	.0001
PFOS	-0.66	0.62	.29
Age	-0.31	0.17	.07
Alcohol	2.68	0.98	.008
BMI	-0.66	0.22	.003
Cigarettes	-0.07	0.11	.56
	$R^2 = .32$	Adj $R^2 = .28$	

Table 18
(continued)

Triglycerides

Variable	Parameter	SE	p value
Intercept	-178.52	71.97	.02
PFOS	-7.89	5.26	.14
Age	4.22	1.44	.005
Alcohol	9.90	8.28	.24
BMI	6.07	1.83	.001
Cigarettes	3.17	0.95	.001
	$R^2 = .32$	Adj $R^2 = .28$	

Hematocrit

Variable	Parameter	SE	p value
Intercept	48.03	1.66	.0001
PFOS	-0.05	0.12	.70
Age	0.02	0.03	.48
Alcohol	-0.003	0.19	.99
BMI	-0.10	0.04	.02
Cigarettes	0.04	0.02	.05
	$R^2 = .12$	Adj $R^2 = .06$	

Hemoglobin

Variable	Parameter	SE	p value
Intercept	15.76	0.58	.0001
PFOS	-0.02	0.04	.67
Age	-0.04	0.07	.28
Alcohol	0.01	0.01	.58
BMI	-0.03	0.01	.03
Cigarettes	0.009	0.008	.25
	$R^2 = .09$	Adj $R^2 = .03$	

Table 18
(continued)

MCH

Variable	Parameter	SE	p value
Intercept	33.52	1.05	.0001
PFOS	0.04	0.08	.59
Age	-0.006	0.02	.79
Alcohol	0.11	0.12	.35
BMI	-0.10	0.03	.0003
Cigarettes	0.03	0.01	.03
	$R^2 = .24$		Adj $R^2 = .20$

MCHC

Variable	Parameter	SE	p value
Intercept	32.87	0.50	.0001
PFOS	-0.001	.037	.98
Age	0.008	0.01	.42
Alcohol	-0.08	0.06	.19
BMI	-0.0006	0.01	.96
Cigarettes	-0.01	0.007	0.08
	$R^2 = .07$	Adj $R^2 = .02$	

MCV

Variable	Parameter	SE	p value
Intercept	101.97	3.36	.0001
PFOS	0.14	0.25	.56
Age	-0.04	0.07	.53
Alcohol	0.56	0.39	.15
BMI	-0.31	0.09	.0005
Cigarettes	0.12	0.04	0.007
	$R^2 = .28$	Adj $R^2 = .23$	

Table 18
(continued)

RBC

Variable	Parameter	SE	p value
Intercept	4.673	0.214	.0001
PFOS	-0.009	0.016	.58
Age	0.005	0.004	.28
Alcohol	-0.026	0.025	.29
BMI	0.006	0.005	.24
Cigarettes	-0.002	0.003	.48
	$R^2 = .08$	Adj $R^2 = .02$	

Platelets

Variable	Parameter	SE	p value
Intercept	320.83	32.21	.0001
PFOS	-6.73	2.36	.006
Age	-0.94	0.64	.15
Alcohol	-6.95	3.70	.06
BMI	-1.26	0.82	.13
Cigarettes	0.43	0.43	.32
	$R^2 = .18$	Adj $R^2 = .13$	

WBC

Variable	Parameter	SE	p value
Intercept	3.24	1.32	.02
PFOS	-0.03	0.10	.74
Age	0.07	0.03	.01
Alcohol	0.03	0.15	.82
BMI	0.03	0.03	.42
Cigarettes	0.09	0.02	.0001
	$R^2 = .35$	Adj $R^2 = .31$	

Table 19

Mean, Median (Med), Standard Deviation (SD) of Mean and Range of PFOS, Hormonal Measurements for N = 88 Employees, Antwerp and Decatur Combined, 1995

PFOS (ppm)	Mean	Med	SD	Range
		Cortisol		
0 - < 1	19.3	17.5	7.0-29.0	
1 - < 3	17.7	18.0	7.2	1.0-42.0
3 - < 6	21.4	23.0	7.2	7.0-31.0
≥ 6	17.0	19.0	6.2	9.0-23.0
	F = 1.7, p = .18			
		DHEA-S		
0 - < 1	388[3,5]	358	168	88-605
1 - < 3	234[2,4]	210	95	69-460
3 - < 6	316[3]	318	106	90-530
≥ 6	194[2]	190	19	176-215
	F = 8.3, p = .0001			
		Estradiol		
0 - < 1	67.1	67.0	12.8	50.0-87.0
1 - < 3	60.3	59.0	15.2	35.0-101.0
3 - < 6	60.5	61.0	10.1	42.0-81.0
≥ 6	64.8	65.0	18.7	47.0-92.0
	F = 0.8, p = .49			
		FSH		
0 - < 1	3.8	3.5	1.5	2.0-6.0
1 - < 3	5.6	4.0	4.2	1.0-26.0
3 - < 6	5.6	4.0	3.9	2.0-18.0
≥ 6	6.6	6.0	3.4	3.0-12.0
	F value = 0.8, p = 0.48			
		17-Hydroxyprogesterone		
0 - < 1	170	164	41	121-245
1 - < 3	131	123	52	53-294
3 - < 6	150	153	52	65-245
≥ 6	119	98	4590-197	
	F value = 2.4, p = .08			

Table 19
(continued)

PFOS (ppm)	Mean	Med	SD	Range
		LH		
0 - < 1	3.8	4.0	0.9	2.0-5.0
1 - < 3	4.6	4.0	3.0	1.0-21.0
3 - < 6	4.6	5.0	1.9	2.0-9.0
≥ 6	4.8	5.0	1.3	3.0-6.0
	F value = 0.3, p = .81			
		Prolactin		
0 - < 1	13.5	13.0	7.0	6.0-29.0
1 - < 3	11.8	11.0	5.0	3.0-30.0
3 - < 6	13.4	10.0	7.9	5.0-39.0
≥ 6	13.6	10.0	6.3	9.0-24.0
	F value = 0.5, p = .67			
		SHBG		
0 - < 1	0.9	0.9	0.3	0.5-1.3
1 - < 3	1.0	0.9	0.4	0.4-1.9
3 - < 6	1.0	0.9	0.3	0.4-1.7
≥ 6	1.2	1.3	0.6	0.6-2.1
	F = 1.1, p = .35			
		Free testosterone		
0 - < 1	20.5[3]	20.2	5.2	10.2-28.2
1 - < 3	16.2[2]	16.1	3.3	8.9-27.1
3 - < 6	17.7	18.2	3.2	12.2-25.2
≥ 6	17.5	17.7	2.0	15.3-20.5
	F value = 4.5, p = .006			
		Bound testosterone		
0 - < 1	739[3]	757	175	5281094
1 - < 3	580[2,4]	589	110	278-762
3 - < 6	676[3]	659	171	410-1039
≥ 6	711	752	160	462-883
	F value = 5.2, p = .003			

Table 19
(continued)

PFOS (ppm)	Mean	Med	SD	Range
		TSH		
0 - < 1	1.9	1.4	1.3	0.6-4.5
1 - < 3	1.0	1.5	1.4	0.5-8.1
3 - < 6	1.5	1.4	0.8	0.5-3.4
≥ 6	2.0	1.6	1.2	0.7-3.8

F value = 0.6, p = .62

Sample sizes:

PFOS Level	Both Locations	Antwerp	Decatur
0 - < 1 ppm	10	9	1
1 - < 3 ppm	46	21	25
3 - < 6 ppm	27	18	9
≥ 6 ppm	5	2	3
	88	50	38

Table 20

Multivariable Regression Analysis of Hormones in Relation to PFOS
Adjusting for Age, Alcohol, BMI and Cigarettes,
Antwerp and Decatur Data Combined, 1995

Cortisol

Variable	Parameter	SE	p value
Intercept	27.13	4.84	.0001
PFOS	0.27	0.35	.45
Age	-0.10	0.10	.31
Alcohol	1.56	0.56	.006
BMI	-0.25	0.12	.04
Cigarettes	0.01	0.06	.87
	$R^2 = .22$	Adj $R^2 = .17$	

DHEAS

Variable	Parameter	SE	p value
Intercept	499.17	76.98	.0001
PFOS	-2.95	5.63	.60
Age	-5.30	1.54	.0009
Alcohol	17.88	8.84	.05
BMI	-1.82	1.95	.35
Cigarettes	2.25	1.01	.03
	$R^2 = .28$	Adj $R^2 = .24$	

Estradiol

Variable	Parameter	SE	p value
Intercept	53.56	9.76	.0001
PFOS	-3.82	1.85	.04
$PFOS^2$	0.44	0.17	.01
Age	-0.13	0.19	.51
Alcohol	2.42	1.10	.03
BMI	0.57	0.24	.02
Cigarettes	0.07	0.13	.58
	$R^2 = .18$	Adj $R^2 = .12$	

Table 20
(continued)

Estradiol (without employee C)

Variable	Parameter	SE	p value
Intercept	53.43	9.84	.0001
PFOS	-3.56	2.39	.14
$PFOS^2$	0.40	0.28	.15
Age	-0.13	0.19	.51
Alcohol	2.43	1.11	.03
BMI	0.56	0.25	.03
Cigarettes	0.69	0.13	.60
	$R^2 = .13$	Adj $R^2 = .06$	

FSH

Variable	Parameter	SE	p value
Intercept	3.64	2.79	.20
PFOS	0.02	0.20	.91
Age	0.14	0.06	.02
Alcohol	0.04	0.32	.89
BMI	-0.13	0.07	.07
Cigarettes	0.005	0.04	.90
	$R^2 = .10$	Adj $R^2 = .05$	

17-Hydroxyprogesterone

Variable	Parameter	SE	p value
Intercept	306.10	32.69	.0001
PFOS	-0.04	2.36	.99
Age	-2.13	0.67	.002
Alcohol	-0.76	3.68	.84
BMI	-3.47	0.82	.0001
Cigarettes	0.79	0.42	.07
	$R^2 = .33$	Adj $R^2 = .28$	

Table 20
(continued)

LH

Variable	Parameter	SE	p value
Intercept	5.65	1.86	.003
PFOS	0.05	0.13	.69
Age	-0.02	0.04	.69
Alcohol	-0.24	0.21	.26
BMI	-0.03	0.05	.56
Cigarettes	0.03	0.02	.21
	$R^2 = .04$	Adj $R^2 = .02$	

Prolactin

Variable	Parameter	SE	p value
Intercept	16.67	3.99	.0001
PFOS	0.34	0.29	.25
Age	-0.15	0.08	.07
Alcohol	1.75	0.46	.0003
BMI	0.004	0.10	.97
Cigarettes	-0.14	0.05	.01
	$R^2 = .31$	Adj $R^2 = .27$	

SHBG

Variable	Parameter	SE	p value
Intercept	0.03	0.24	.89
PFOS	0.005	0.02	.77
Age	0.02	0.005	.0001
Alcohol	-0.005	0.03	.86
BMI	0.005	0.006	.40
Cigarettes	0.004	0.003	.19
	$R^2 = .28$	Adj $R^2 = .23$	

Table 20
(continued)

Free Testosterone

Variable	Parameter	SE	p value
Intercept	27.37	2.57	.0001
PFOS	0.02	0.19	.90
Age	-0.14	0.05	.01
Alcohol	-0.11	0.30	.69
BMI	-0.19	0.06	.005
Cigarettes	0.01	0.03	.68
	$R^2 = .19$	Adj $R^2 = .14$	

Bound Testosterone

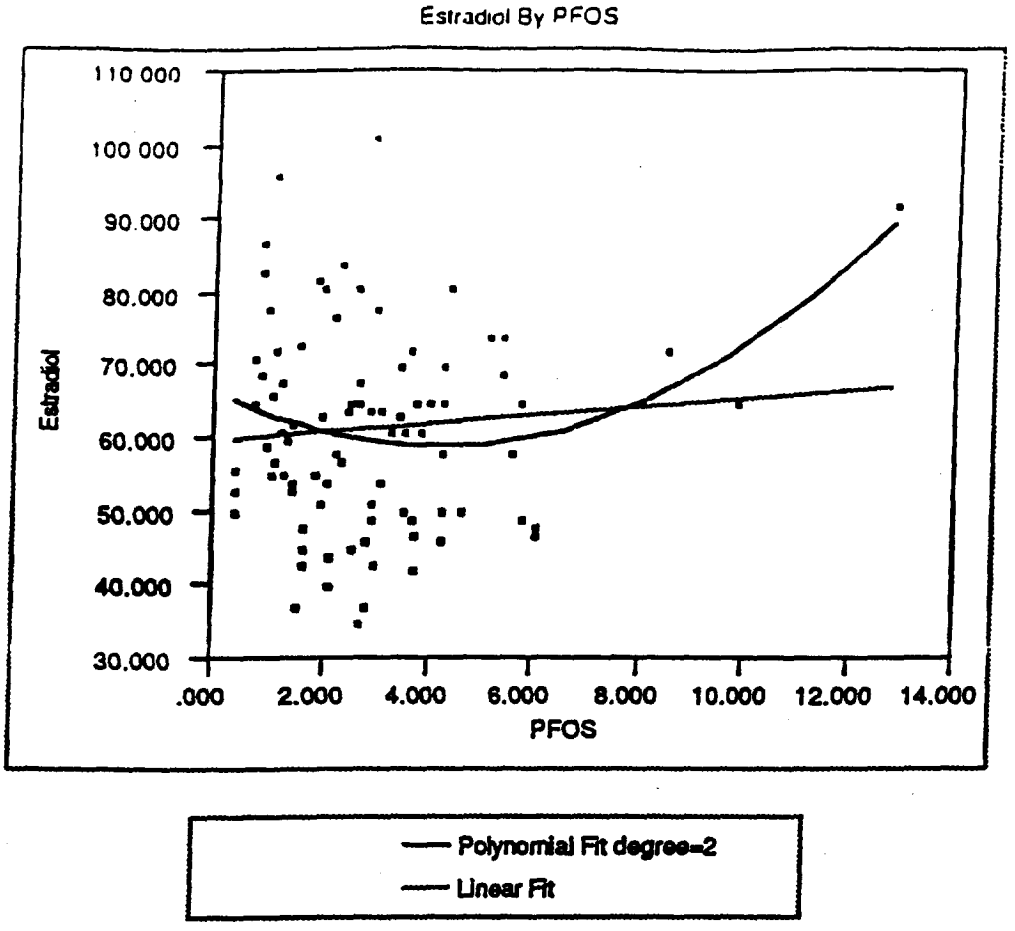
Variable	Parameter	SE	p value
Intercept	993.88	100.93	.0001
PFOS	6.91	7.28	.35
Age	-2.43	2.06	.24
Alcohol	7.41	11.37	.52
BMI	-11.43	2.52	.0001
Cigarettes	1.22	1.31	.35
	$R^2 = .28$	Adj $R^2 = .23$	

TSH

Variable	Parameter	SE	p value
Intercept	-0.873	0.826	.29
PFOS	0.004	0.060	.95
Age	0.003	0.016	.85
Alcohol	0.078	0.095	.41
BMI	0.096	0.021	.0001
Cigarettes	-0.011	0.011	.32
	$R^2 = .23$	Adj $R^2 = .18$	

FIGURE 1

Scatter Plot of Estradiol and PFOS, Both Locations Combined, 1995



Polynomial Fit degree=2

Estradiol = 66.4703 – 3.60448 PFOS + 0.42197 PFOS^2

Summary of Fit

RSquare	0.07616
RSquare Adj	0.054423
Root Mean Square Error	13.33314
Mean of Response	61.36364
Observations (or Sum Wgts)	88

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1245.701	622.850	3.5036
Error	85	15110.663	177.773	Prob>F
C Total	87	16356.364		0.0345

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	66.470276	3.632698	18.30	<.0001
PFOS	-3.604477	1.7997	-2.00	0.0484
PFOS^2	0.4219692	0.167705	2.52	0.0137

Estradiol = 59.7222 + 0.57198 PFOS

Summary of Fit

RSquare	0.00735
RSquare Adj	-0.00419
Root Mean Square Error	13.74017
Mean of Response	61.36364
Observations (or Sum Wgts)	88

Analysis of Variance

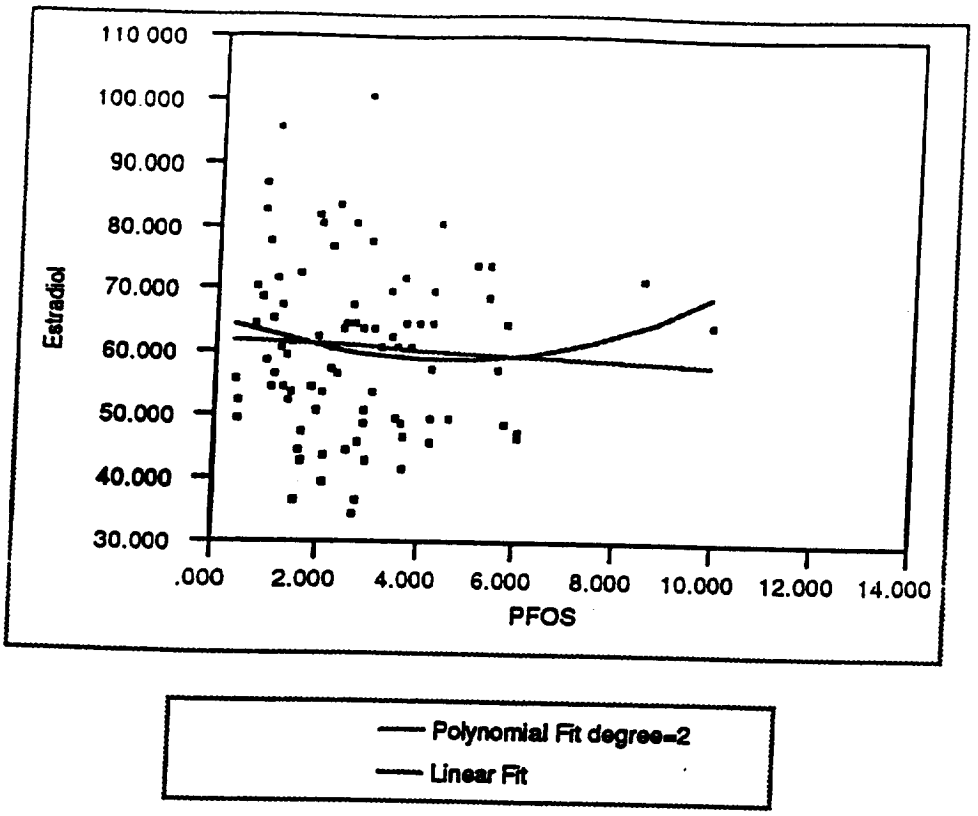
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	120.226	120.226	0.6368
Error	86	16236.138	188.792	Prob>F
C Total	87	16356.364		0.4271

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	59.722236	2.525093	23.65	<.0001
PFOS	0.5719843	0.716766	0.80	0.4271

FIGURE 2

Scatter Plot of Estradiol and PFOS, Both Locations Cominbed, 1995
Without Employee A



Polynomial Fit degree=2

Estradiol = 65.8629 – 3.10569 PFOS + 0.34755 PFOS^2

Summary of Fit

RSquare	0.020552
RSquare Adj	-0.00277
Root Mean Square Error	13.40325
Mean of Response	61.01149
Observations (or Sum Wgts)	87

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	316.639	158.320	0.8813
Error	84	15090.349	179.647	Prob>F
C Total	86	15406.989		0.4180

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	65.862871	4.074112	16.17	<.0001
PFOS	-3.105693	2.339491	-1.33	0.1879
PFOS^2	0.3475472	0.278213	1.25	0.2151

Linear Fit

Estradiol = 62.0271 - 0.36861 PFOS

Summary of Fit

RSquare	0.002356
RSquare Adj	-0.00938
Root Mean Square Error	13.44737
Mean of Response	61.01149
Observations (or Sum Wgts)	87

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	36.295	36.295	0.2007
Error	85	15370.694	180.832	Prob>F
C Total	86	15406.989		0.6553

Parameter Estimates

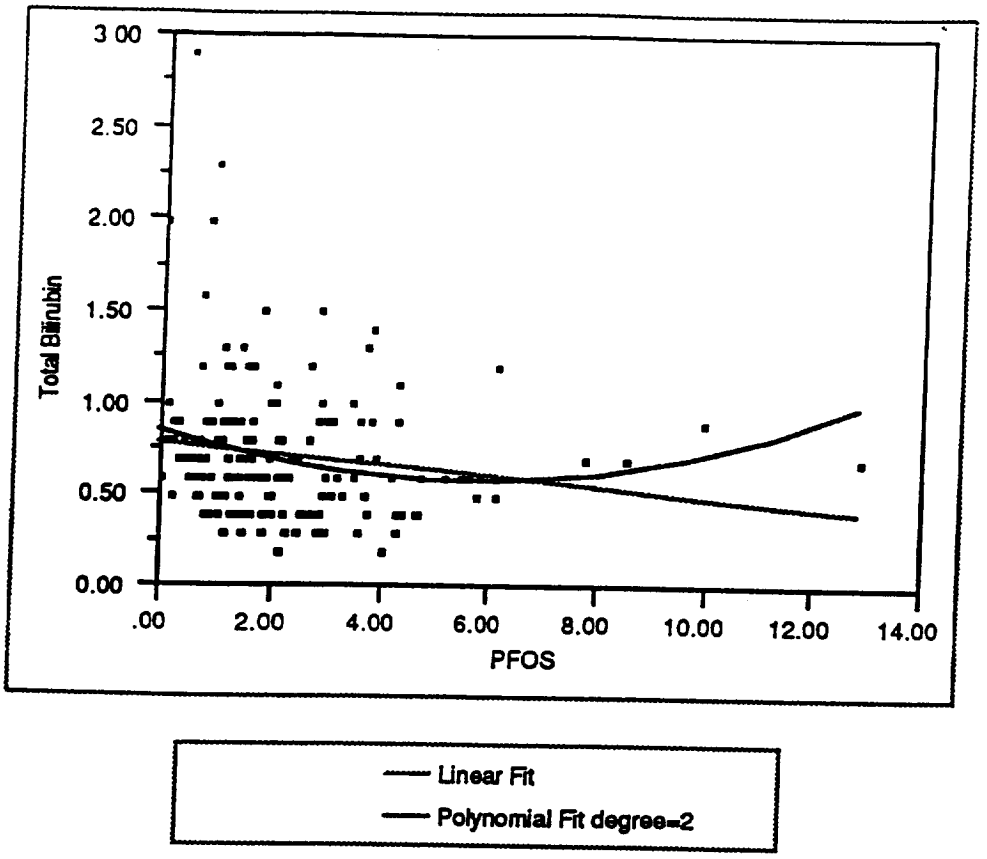
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	62.027069	2.686488	23.09	<.0001
PFOS	-0.368607	0.822768	-0.45	0.6553

APPENDIX A

Total Bilirubin and PFOS Scatter Plots

Scatter Plot of Total Bilirubin and PFOS, Both Locations Combined, 1995

Total Bilirubin By PFOS



Linear Fit

Total Bilirubin = 0.78247 – 0.02927 PFOS

Summary of Fit

RSquare	0.022279
RSquare Adj	0.016692
Root Mean Square Error	0.363488
Mean of Response	0.718644
Observations (or Sum Wgts)	177

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.526856	0.526856	3.9876
Error	175	23.121618	0.132124	Prob>F
C Total	176	23.648475		0.0474

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.7824732	0.04205	18.61	<.0001
PFOS	-0.029269	0.014657	-2.00	0.0474

Polynomial Fit degree=2

Total Bilirubin = 0.86532 – 0.09918 PFOS + 0.00846 PFOS^2

Summary of Fit

RSquare	0.051674
RSquare Adj	0.040773
Root Mean Square Error	0.359009
Mean of Response	0.718644
Observations (or Sum Wgts)	177

Analysis of Variance

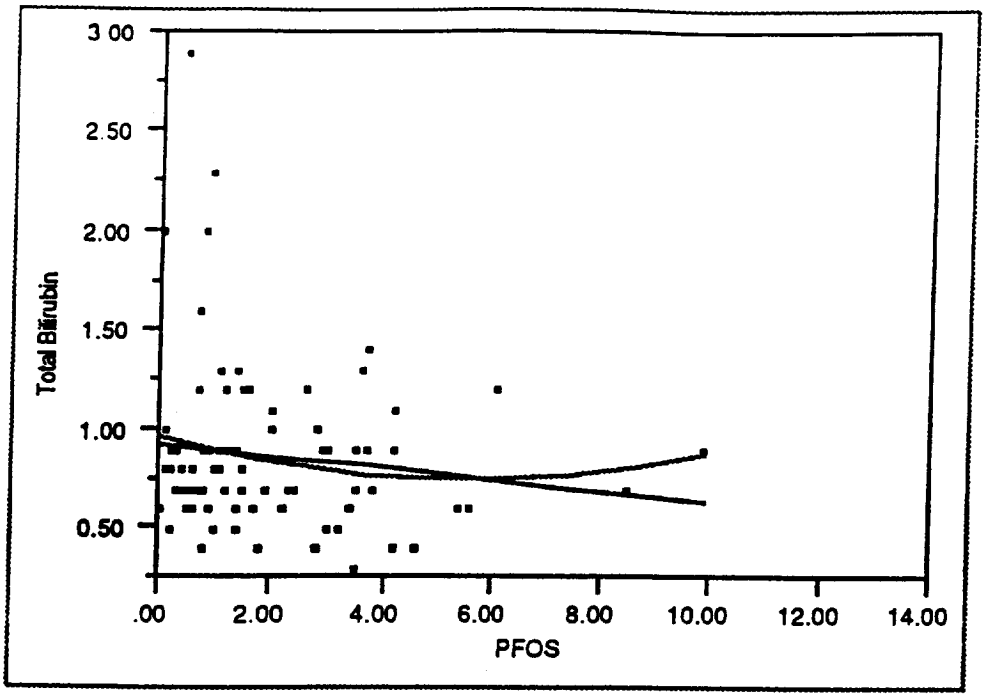
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1.222006	0.611003	4.7406
Error	174	22.426468	0.128888	Prob>F
C Total	176	23.648475		0.0099

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.865318	0.054748	15.81	<.0001
PFOS	-0.099182	0.033404	-2.97	0.0034
PFOS^2	0.008459	0.003642	2.32	0.0214

Scatter Plot of Total Bilirubin and PFOS, Antwerp, 1995

Total Bilirubin By PFOS



Linear Fit

Polynomial Fit degree=2

Linear Fit

Total Bilirubin = 0.92001 – 0.02927 PFOS

Summary of Fit

RSquare	0.017786
RSquare Adj	0.006365
Root Mean Square Error	0.407279
Mean of Response	0.863636
Observations (or Sum Wgts)	88

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.258313	0.258313	1.5573
Error	86	14.265323	0.165876	Prob>F
C Total	87	14.523636		0.2155

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.9200069	0.062654	14.68	<.0001
PFOS	-0.029266	0.023452	-1.25	0.2155

Polynomial Fit degree=2

Total Bilirubin = 0.96548 – 0.07872 PFOS + 0.00697 PFOS^2

Summary of Fit

RSquare	0.027505
RSquare Adj	0.004623
Root Mean Square Error	0.407635
Mean of Response	0.863636
Observations (or Sum Wgts)	88

Analysis of Variance

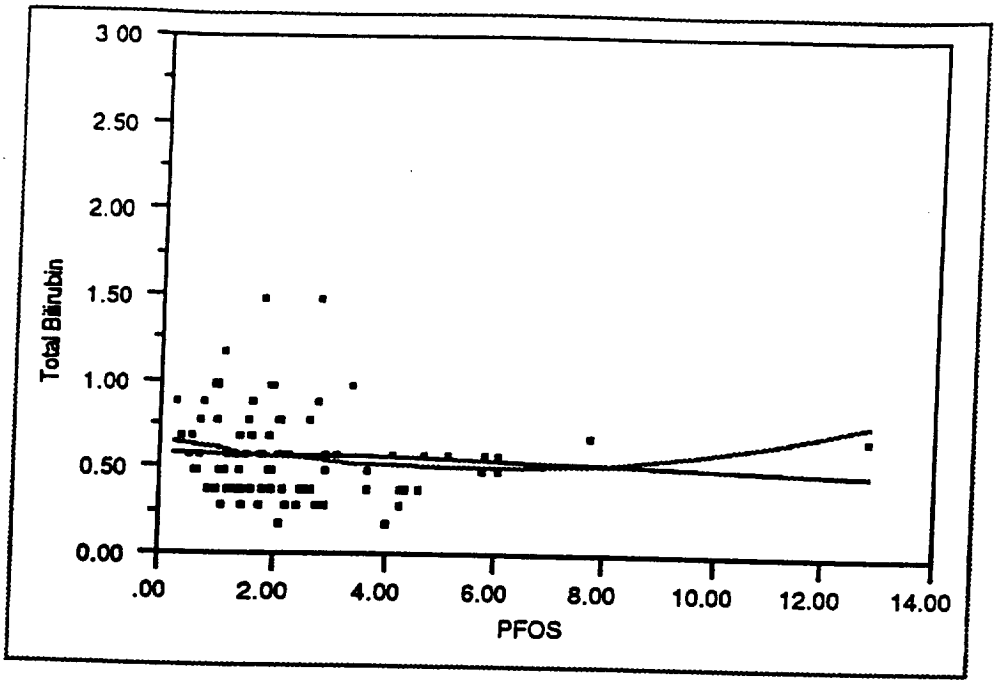
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.399470	0.199735	1.2020
Error	85	14.124166	0.166167	Prob>F
C Total	87	14.523636		0.3056

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.9654789	0.07979	12.10	<.0001
PFOS	-0.078719	0.058565	-1.34	0.1825
PFOS^2	0.0069749	0.007568	0.92	0.3593

Scatter Plot of Total Bilirubin and PFOS, Decatur, 1995

Total Bilirubin By PFOS



— Linear Fit

— Polynomial Fit degree=2

Linear Fit

Total Bilirubin = 0.59712 – 0.00898 PFOS

Summary of Fit

RSquare	0.004472
RSquare Adj	-0.00697
Root Mean Square Error	0.249626
Mean of Response	0.575281
Observations (or Sum Wgts)	89

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.0243540	0.024354	0.3908
Error	87	5.4212639	0.062313	Prob>F
C Total	88	5.4456180		0.5335

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.5971232	0.043827	13.62	<.0001
PFOS	-0.008979	0.014363	-0.63	0.5335

Polynomial Fit degree=2

Total Bilirubin = 0.66974 – 0.05867 PFOS + 0.00518 PFOS^2

Summary of Fit

RSquare	0.032545

RSquare Adj	0.010046
Root Mean Square Error	0.247508
Mean of Response	0.575281
Observations (or Sum Wgts)	89

Analysis of Variance

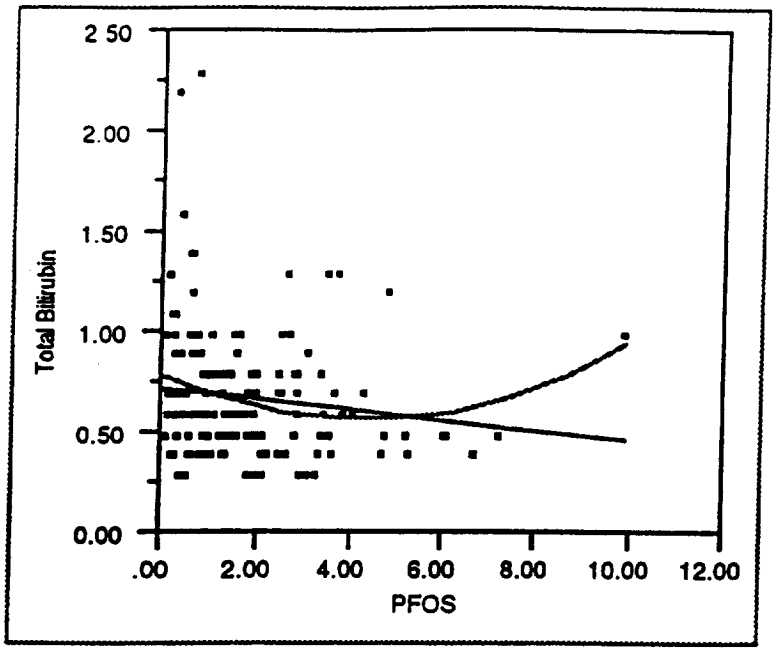
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.1772262	0.088613	1.4465
Error	86	5.2683918	0.061260	Prob>F
C Total	88	5.4456180		0.2411

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.6697418	0.063258	10.59	<.0001
PFOS	-0.058672	0.034531	-1.70	0.0929
PFOS^2	0.0051833	0.003281	1.58	0.1178

Scatter Plot of Total Bilirubin and PFOS, Both Locations Combined, 1997

Total Bilirubin By PFOS



—— Linear Fit

------ Polynomial Fit degree=2

Linear Fit

Total Bilirubin = 0.7185 – 0.02583 PFOS

Summary of Fit

RSquare	0.016221
RSquare Adj	0.009483
Root Mean Square Error	0.322046
Mean of Response	0.672973
Observations (or Sum Wgts)	148

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.249674	0.249674	2.4073
Error	146	15.142218	0.103714	Prob>F
C Total	147	15.391892		0.1229

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.7184974	0.039518	18.18	<.0001
PFOS	-0.025825	0.016645	-1.55	0.1229

Polynomial Fit degree=2

Total Bilirubin = 0.78963 – 0.10456 PFOS + 0.012 PFOS^2

Summary of Fit

RSquare	0.045789
RSquare Adj	0.032628

Root Mean Square Error	0.318261
Mean of Response	0.672973
Observations (or Sum Wgts)	148

Analysis of Variance

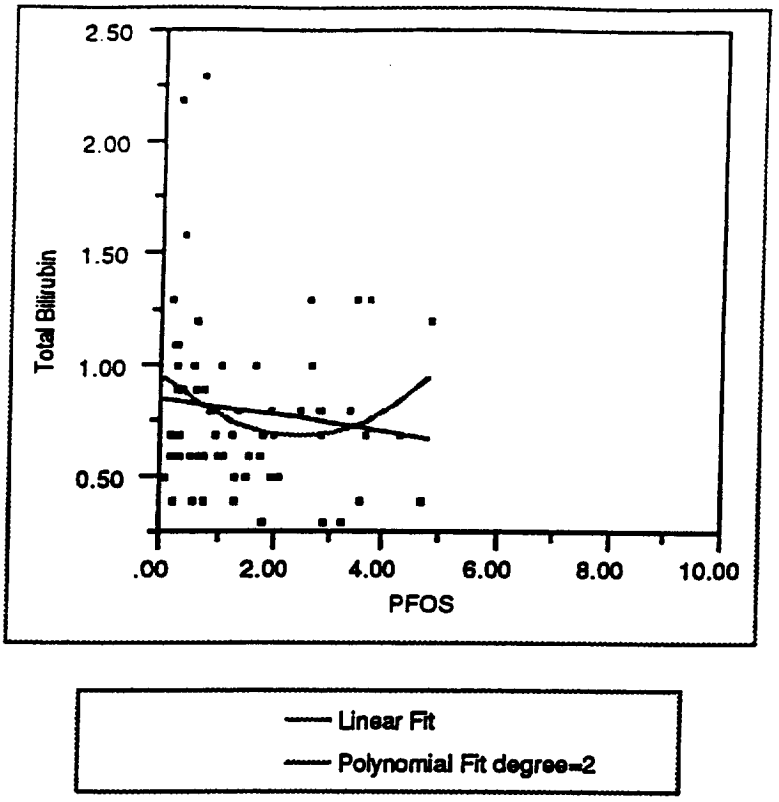
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.704787	0.352393	3.4790
Error	145	14.687105	0.101290	Prob>F
C Total	147	15.391892		0.0334

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.7896264	0.05149	15.34	<.0001
PFOS	-0.104564	0.040625	-2.57	0.0111
PFOS^2	0.0120049	0.005663	2.12	0.0357

Scatter Plot of Total Bilirubin and PFOS, Antwerp, 1997

Total Bilirubin By PFOS



Linear Fit

Total Bilirubin = 0.8517 – 0.03647 PFOS

Summary of Fit

RSquare	0.014291
RSquare Adj	-0.00161
Root Mean Square Error	0.385334
Mean of Response	0.796875
Observations (or Sum Wgts)	64

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.1334670	0.133467	0.8989
Error	62	9.2059080	0.148482	Prob>F
C Total	63	9.3393750		0.3468

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.8516999	0.075259	11.32	<.0001
PFOS	-0.036474	0.038471	-0.95	0.3468

Polynomial Fit degree=2

Total Bilirubin = 0.9635 – 0.22847 PFOS + 0.0462 PFOS^2

Summary of Fit

RSquare	0.051848
RSquare Adj	0.020761

Root Mean Square Error	0.318261
Mean of Response	0.672973
Observations (or Sum Wgts)	148

Analysis of Variance

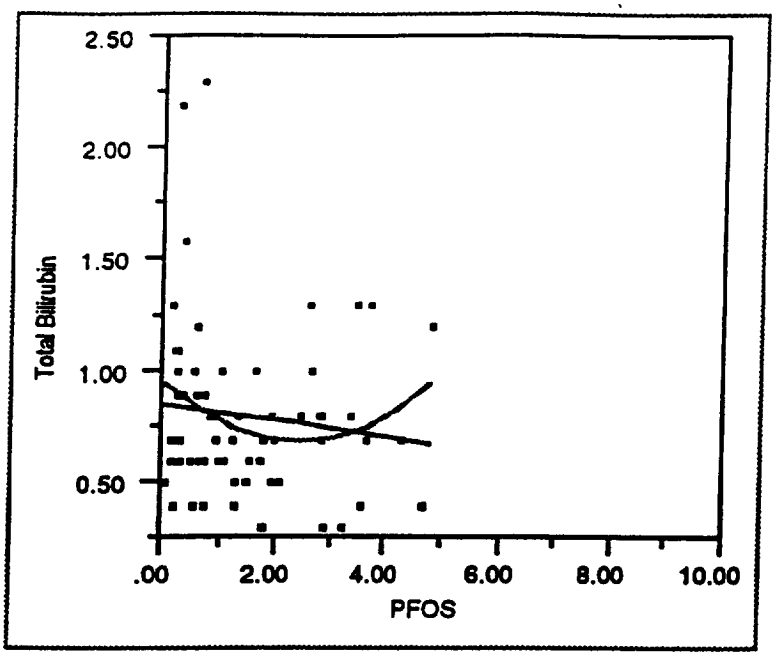
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.704787	0.352393	3.4790
Error	145	14.687105	0.101290	Prob>F
C Total	147	15.391892		0.0334

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.7896264	0.05149	15.34	<.0001
PFOS	-0.104564	0.040625	-2.57	0.0111
PFOS^2	0.0120049	0.005663	2.12	0.0357

Scatter Plot of Total Bilirubin and PFOS, Antwerp, 1997

Total Bilirubin By PFOS



Linear Fit

Polynomial Fit degree=2

Linear Fit

Total Bilirubin = 0.8517 – 0.03647 PFOS

Summary of Fit

RSquare	0.014291
RSquare Adj	-0.00161
Root Mean Square Error	0.385334
Mean of Response	0.796875
Observations (or Sum Wgts)	64

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.1334670	0.133467	0.8989
Error	62	9.2059080	0.148482	Prob>F
C Total	63	9.3393750		0.3468

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.8516999	0.075259	11.32	<.0001
PFOS	-0.036474	0.038471	-0.95	0.3468

Polynomial Fit degree=2

Total Bilirubin = 0.9635 – 0.22847 PFOS + 0.0462 PFOS^2

Summary of Fit

RSquare	0.051848
RSquare Adj	0.020761

Root Mean Square Error	0.381007
Mean of Response	0.796875
Observations (or Sum Wgts)	64

Analysis of Variance

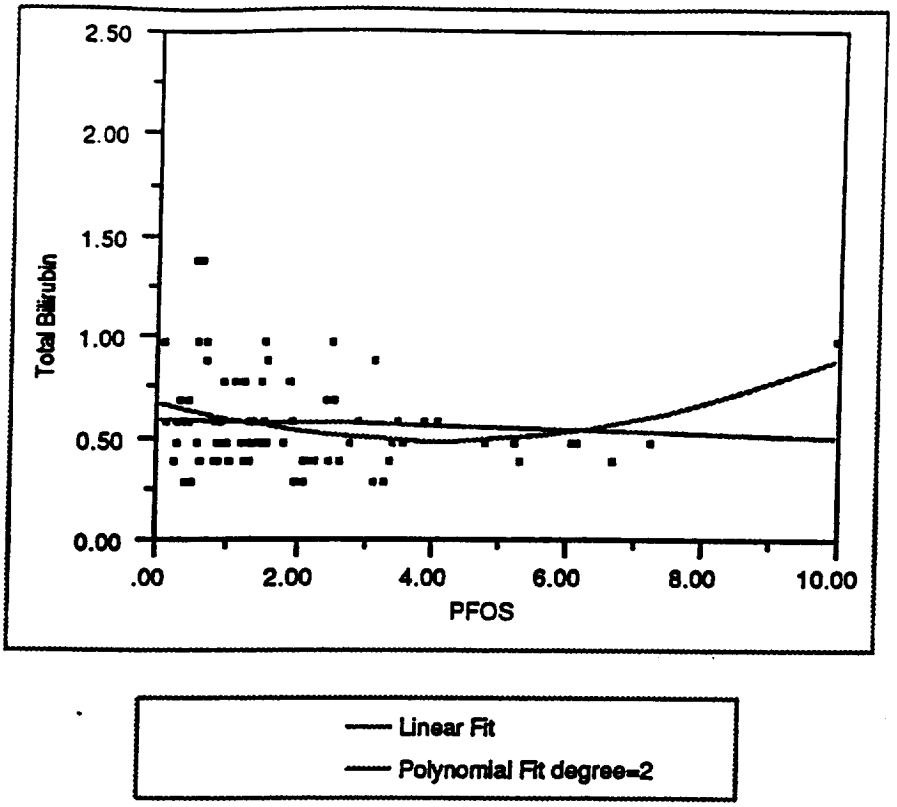
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.4842255	0.242113	1.6678
Error	61	8.8551495	0.145166	Prob>F
C Total	63	9.3393750		0.1971

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.963501	0.103492	9.31	<.0001
PFOS	-0.228466	0.129238	-1.77	0.0821
PFOS^2	0.0461951	0.029718	1.55	0.1253

Scatter Plot of Total Bilirubin and PFOS, Decatur, 1997

Total Bilirubin By PFOS



Linear Fit

Total Bilirubin = 0.59609 – 0.00894 PFOS

Summary of Fit

RSquare	0.004924
RSquare Adj	-0.00721
Root Mean Square Error	0.229
Mean of Response	0.578571
Observations (or Sum Wgts)	84

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.0212775	0.021278	0.4057
Error	82	4.3001510	0.052441	Prob>F
C Total	83	4.3214286		0.5259

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.5960926	0.037161	16.04	<.0001
PFOS	-0.008937	0.01403	-0.64	0.5259

Polynomial Fit degree=2

Total Bilirubin = 0.68287 – 0.09408 PFOS + 0.01143 PFOS^2

Summary of Fit

RSquare	0.071405
RSquare Adj	0.048477

Root Mean Square Error	0.222579
Mean of Response	0.578571
Observations (or Sum Wgts)	84

Analysis of Variance

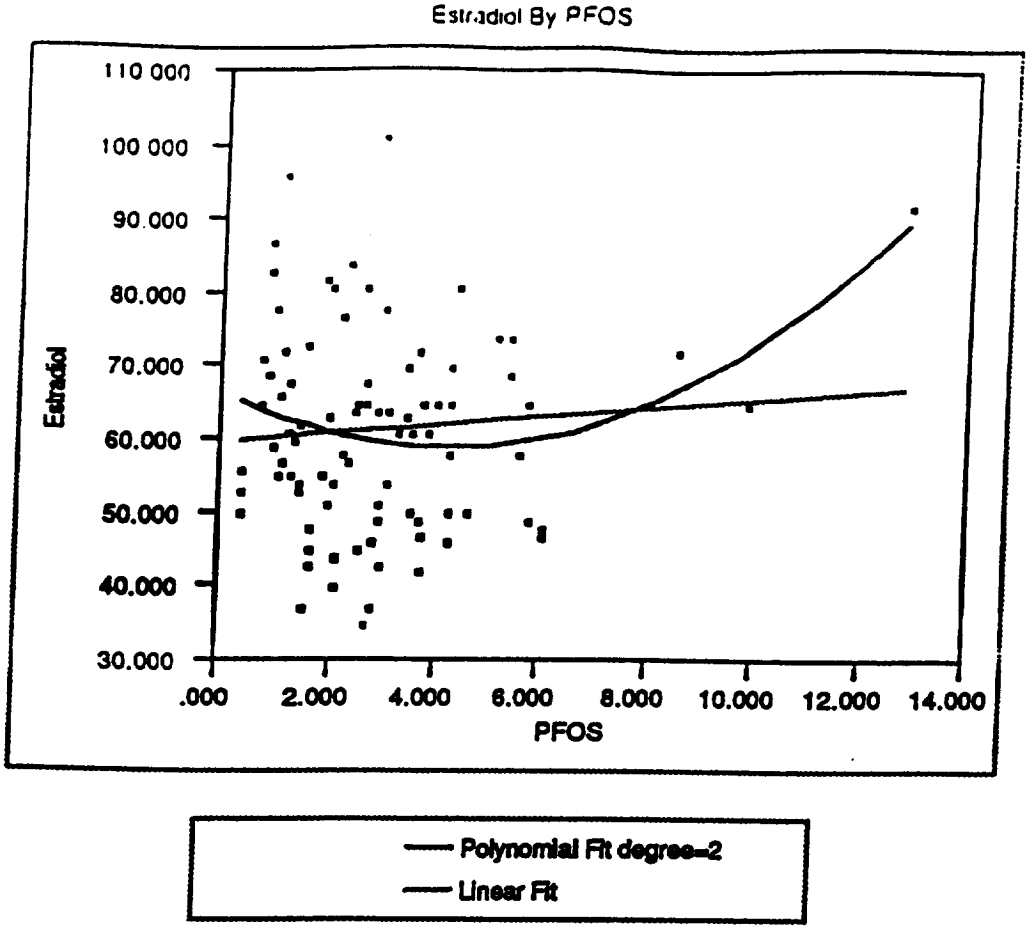
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.3085734	0.154287	3.1143
Error	81	4.0128552	0.049541	Prob>F
C Total	83	4.3214286		0.0498

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.6828676	0.05102	13.38	<.0001
PFOS	-0.094082	0.037896	-2.48	0.0151
PFOS^2	0.011426	0.004745	2.41	0.0183

FIGURE 1

Scatter Plot of Estradiol and PFOS, Both Locations Combined, 1995



Polynomial Fit degree=2

Estradiol = 66.4703 – 3.60448 PFOS + 0.42197 PFOS^2

Summary of Fit

RSquare	0.07616
RSquare Adj	0.054423
Root Mean Square Error	13.33314
Mean of Response	61.36364
Observations (or Sum Wgts)	88

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1245.701	622.850	3.5036
Error	85	15110.663	177.773	Prob>F
C Total	87	16356.364		0.0345

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	66.470276	3.632698	18.30	<.0001
PFOS	-3.604477	1.7997	-2.00	0.0484
PFOS^2	0.4219692	0.167705	2.52	0.0137

Estradiol = 59.7222 + 0.57198 PFOS

Summary of Fit

RSquare	0.00735
RSquare Adj	-0.00419
Root Mean Square Error	13.74017
Mean of Response	61.36364
Observations (or Sum Wgts)	88

Analysis of Variance

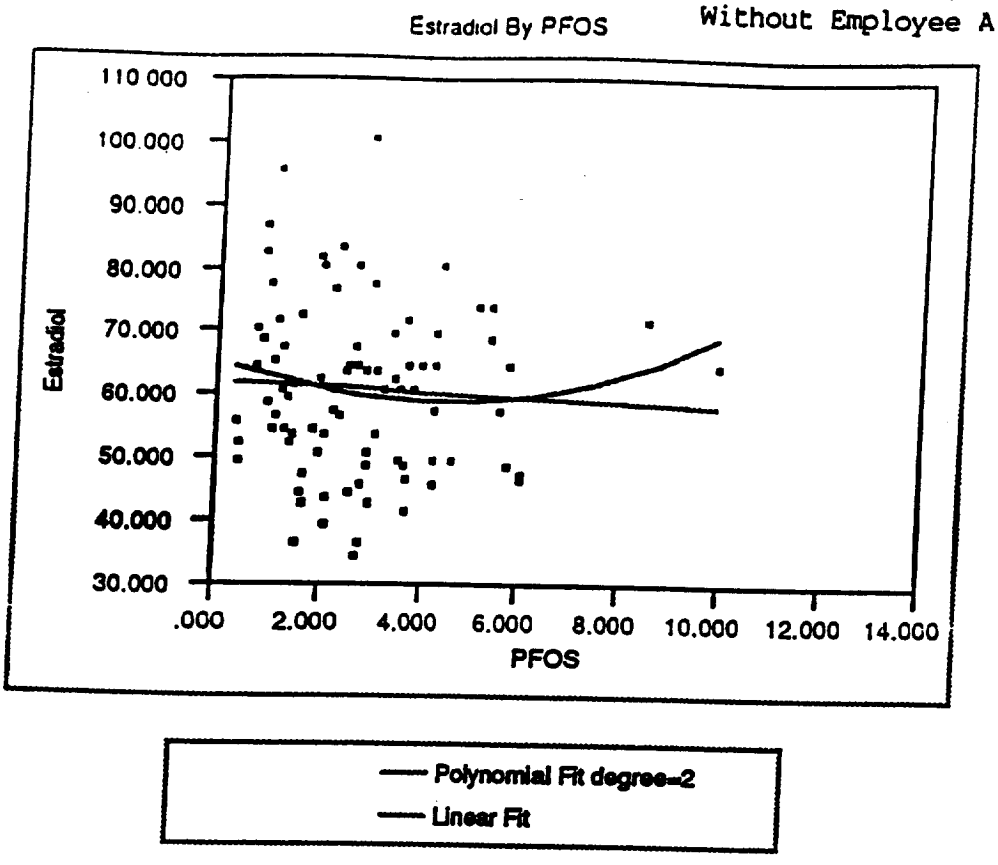
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	120.226	120.226	0.6368
Error	86	16236.138	188.792	Prob>F
C Total	87	16356.364		0.4271

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	59.722236	2.525093	23.65	<.0001
PFOS	0.5719843	0.716766	0.80	0.4271

FIGURE 2

Scatter Plot of Estradiol and PFOS, Both Locations Cominbed, 1995
Without Employee A



Polynomial Fit degree=2

Estradiol = 65.8629 – 3.10569 PFOS + 0.34755 PFOS^2

Summary of Fit

RSquare	0.020552
RSquare Adj	-0.00277
Root Mean Square Error	13.40325
Mean of Response	61.01149
Observations (or Sum Wgts)	87

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	316.639	158.320	0.8813
Error	84	15090.349	179.647	Prob>F
C Total	86	15406.989		0.4180

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	65.862871	4.074112	16.17	<.0001
PFOS	-3.105693	2.339491	-1.33	0.1879
PFOS^2	0.3475472	0.278213	1.25	0.2151

Linear Fit

Estradiol = 62.0271 - 0.36861 PFOS

Summary of Fit

RSquare	0.002356
RSquare Adj	-0.00938
Root Mean Square Error	13.44737
Mean of Response	61.01149
Observations (or Sum Wgts)	87

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	36.295	36.295	0.2007
Error	85	15370.694	180.832	Prob>F
C Total	86	15406.989		0.6553

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	62.027069	2.686488	23.09	<.0001
PFOS	-0.368607	0.822768	-0.45	0.6553

APPENDIX B

Unconjugated Bilirubin and PFOS Scatter Plots

DRAFT

RSquare Adj	-0 02446
Root Mean Square Error	9 349875
Mean of Response	42 33333
Observations (or Sum Wgts)	84

Analysis of Variance

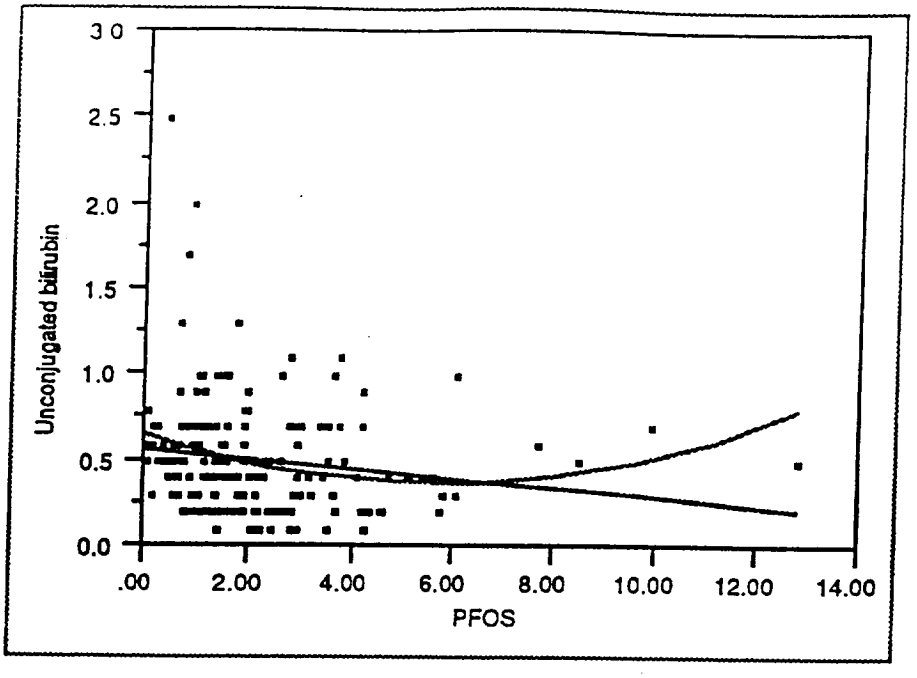
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1.6333	0.8166	0.0093
Error	81	7081.0334	87.4202	Prob>F
C Total	83	7082.6667		0.9907

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	42.534847	2.143189	19.85	<.0001
PFOS	-0.20021	1.591892	-0.13	0.9002
PFOS^2	0.0272273	0.199313	0.14	0.8917

Scatter Plot of Unconjugated Bilirubin and PFOS, Both Locations Combined, 1995

Unconjugated bilirubin By PFOS



—— Linear Fit

—— Polynomial Fit degree=2

Linear Fit

Unconjugated bilirubin = 0.56837 – 0.0285 PFOS

Summary of Fit

RSquare	0.024418
RSquare Adj	0.018843
Root Mean Square Error	0.337753
Mean of Response	0.506215
Observations (or Sum Wgts)	177

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.499660	0.499660	4.3800
Error	175	19.963503	0.114077	Prob>F
C Total	176	20.463164		0.0378

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.5683746	0.039072	14.55	<.0001
PFOS	-0.028503	0.013619	-2.09	0.0378

Polynomial Fit degree=2

Unconjugated bilirubin = 0.65239 – 0.09941 PFOS + 0.00858 PFOS^2

Summary of Fit

RSquare	0.059356
RSquare Adj	0.048544

Root Mean Square Error	0.332602
Mean of Response	0.506215
Observations (or Sum Wgts)	177

Analysis of Variance

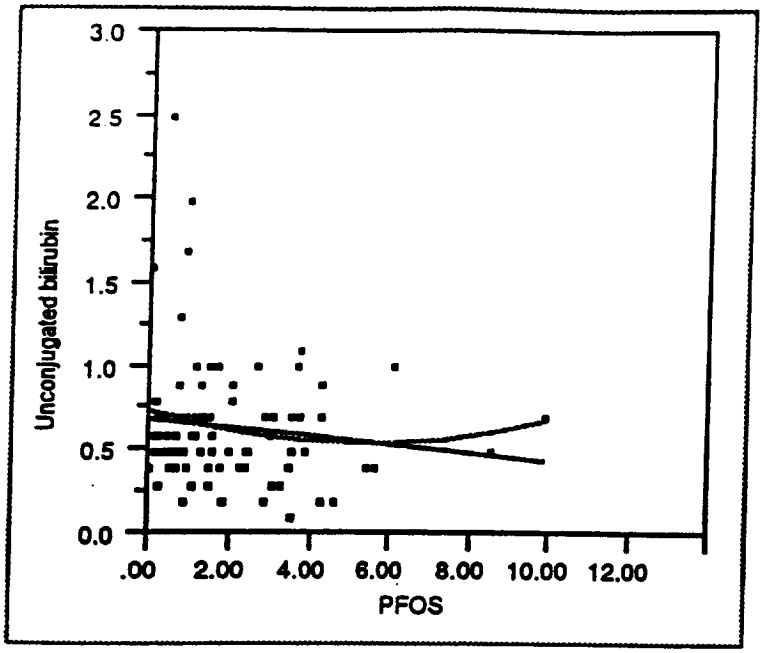
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1.214616	0.607308	5.4898
Error	174	19.248548	0.110624	Prob>F
C Total	176	20.463164		0.0049

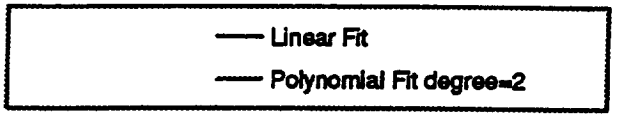
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.6523913	0.050721	12.86	<.0001
PFOS	-0.099406	0.030947	-3.21	0.0016
PFOS^2	0.0085787	0.003374	2.54	0.0119

Scatter Plot of Unconjugated Bilirubin and PFOS, Antwerp, 1995

Unconjugated bilirubin By PFOS





Linear Fit

Unconjugated bilirubin = 0.69447 – 0.02604 PFOS

Summary of Fit

RSquare	0.016651
RSquare Adj	0.005217
Root Mean Square Error	0.374674
Mean of Response	0.644318
Observations (or Sum Wgts)	88

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.204426	0.204426	1.4562
Error	86	12.072733	0.140381	Prob>F
C Total	87	12.277159		0.2308

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.6944654	0.057638	12.05	<.0001
PFOS	-0.026035	0.021575	-1.21	0.2308

Polynomial Fit degree=2

Unconjugated bilirubin = 0.73919 – 0.07468 PFOS + 0.00686 PFOS^2

Summary of Fit

RSquare	0.027775
RSquare Adj	0.004899

Root Mean Square Error	0.374734
Mean of Response	0.644318
Observations (or Sum Wgts)	88

Analysis of Variance

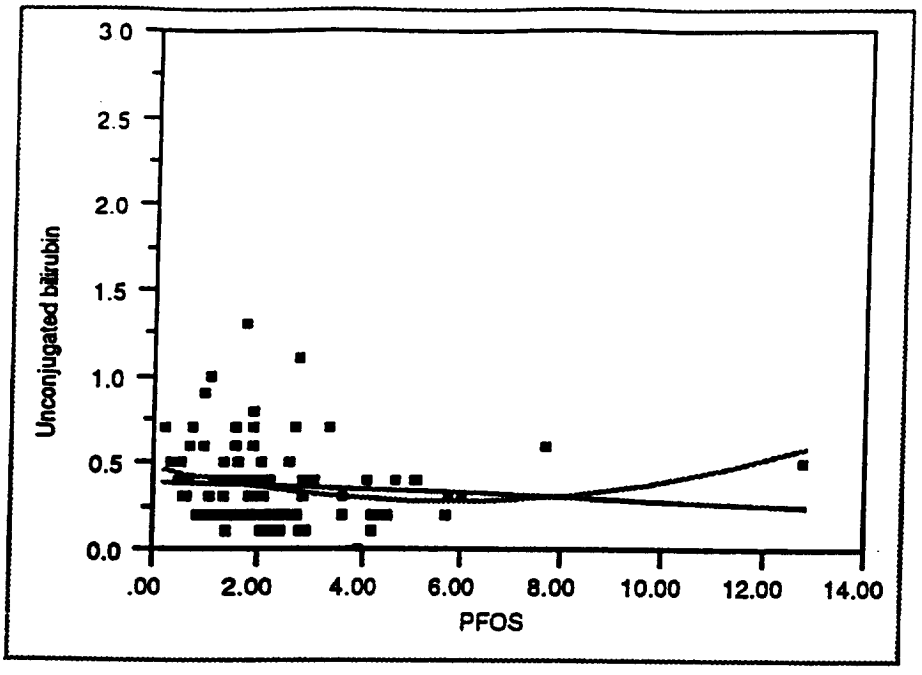
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.340994	0.170497	1.2141
Error	85	11.936165	0.140425	Prob>F
C Total	87	12.277159		0.3021

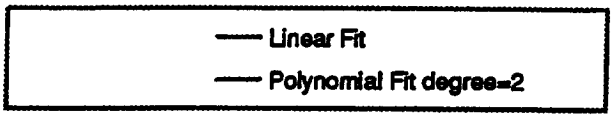
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.7391923	0.07335	10.08	<.0001
PFOS	-0.074678	0.053838	-1.39	0.1690
PFOS^2	0.0068606	0.006957	0.99	0.3268

Scatter Plot of Unconjugated Bilirubin and PFOS, Decatur, 1995

Unconjugated bilirubin By PFOS





Linear Fit

Unconjugated bilirubin = 0.39803 – 0.01166 PFOS

Summary of Fit

RSquare	0.008475
RSquare Adj	-0.00292
Root Mean Square Error	0.235059
Mean of Response	0.369663
Observations (or Sum Wgts)	89

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.0410886	0.041089	0.7436
Error	87	4.8070013	0.055253	Prob>F
C Total	88	4.8480899		0.3909

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.3980339	0.04127	9.64	<.0001
PFOS	-0.011663	0.013525	-0.86	0.3909

Polynomial Fit degree=2

Unconjugated bilirubin = 0.48279 – 0.06966 PFOS + 0.00605 PFOS^2

Summary of Fit

RSquare	0.051431

RSquare Adj	0.029371
Root Mean Square Error	0.231244
Mean of Response	0.369663
Observations (or Sum Wgts)	89

Analysis of Variance

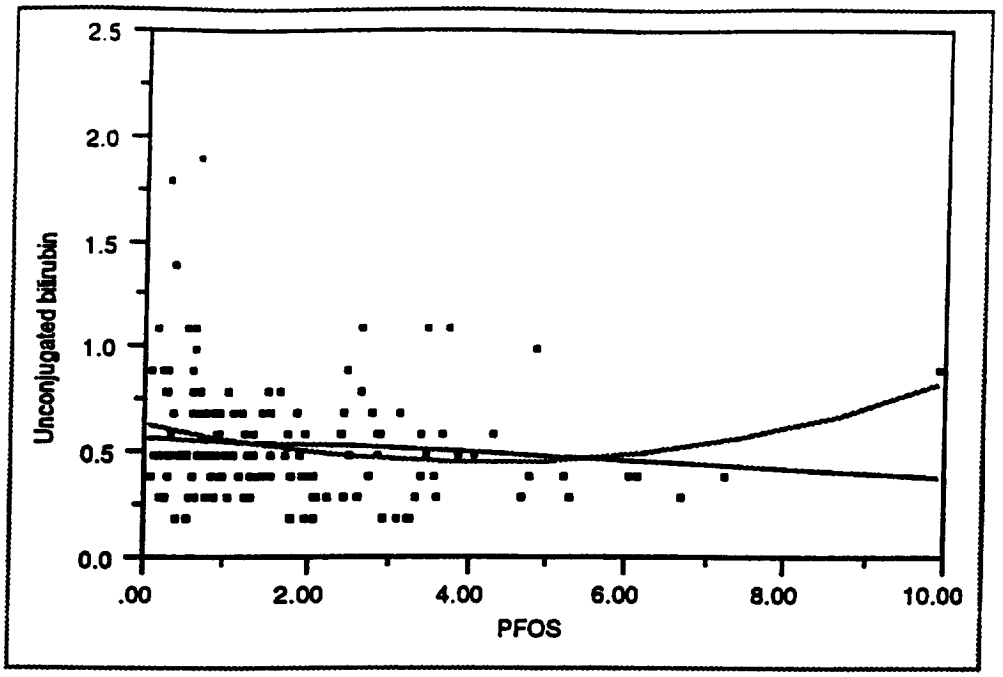
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.2493418	0.124671	2.3314
Error	86	4.5987481	0.053474	Prob>F
C Total	88	4.8480899		0.1033

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.4827917	0.059102	8.17	<.0001
PFOS	-0.069663	0.032262	-2.16	0.0336
PFOS^2	0.0060498	0.003066	1.97	0.0517

Scatter Plot of Unconjugated Bilirubin and PFOS, Both Locations Combined, 1997

Unconjugated bilirubin By PFOS



Linear Fit

Polynomial Fit degree=2

Linear Fit

Unconjugated bilirubin = 0.57745 – 0.0194 PFOS

Summary of Fit

RSquare	0.012338
RSquare Adj	0.005573
Root Mean Square Error	0.277986
Mean of Response	0.543243
Observations (or Sum Wgts)	148

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.140939	0.140939	1.8238
Error	146	11.282304	0.077276	Prob>F
C Total	147	11.423243		0.1789

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.577447	0.034111	16.93	<.0001
PFOS	-0.019403	0.014368	-1.35	0.1789

Polynomial Fit degree=2

Unconjugated bilirubin = 0.64247 – 0.09138 PFOS + 0.01097 PFOS^2

Summary of Fit

RSquare	0.045634

RSquare Adj	0.03247
Root Mean Square Error	0.274201
Mean of Response	0.543243
Observations (or Sum Wgts)	148

Analysis of Variance

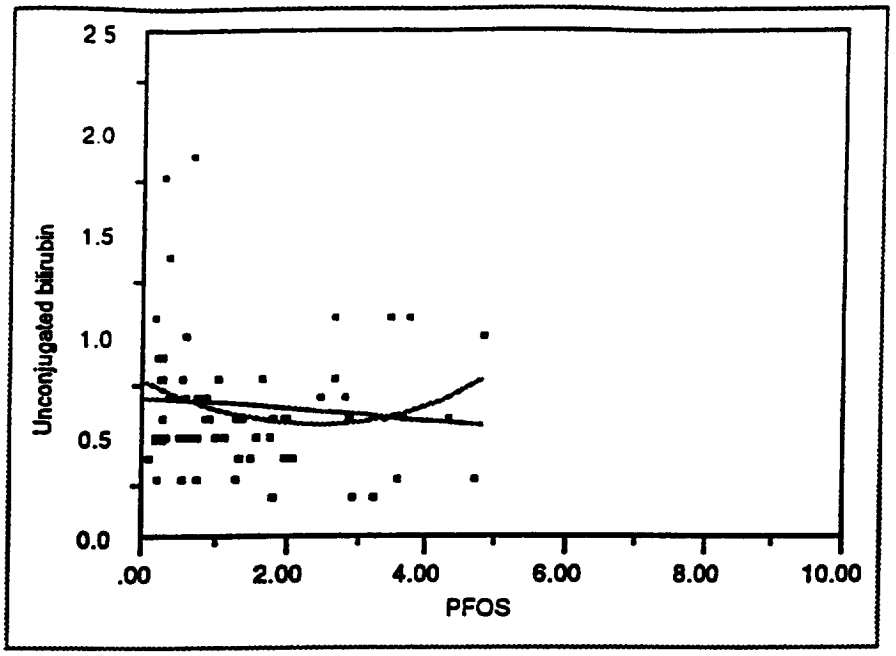
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.521284	0.260642	3.4666
Error	145	10.901959	0.075186	Prob>F
C Total	147	11.423243		0.0338

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.6424713	0.044361	14.48	<.0001
PFOS	-0.091384	0.035001	-2.61	0.0100
PFOS^2	0.0109746	0.004879	2.25	0.0260

Scatter Plot of Unconjugated Bilirubin and PFOS, Antwerp, 1997

Unconjugated bilirubin By PFOS



—— Linear Fit

—— Polynomial Fit degree=2

Linear Fit

Unconjugated bilirubin = 0.69161 – 0.02872 PFOS

Summary of Fit

RSquare	0.01217
RSquare Adj	-0.00376
Root Mean Square Error	0.329151
Mean of Response	0.648438
Observations (or Sum Wgts)	64

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.0827520	0.082752	0.7638
Error	62	6.7170917	0.108340	Prob>F
C Total	63	6.7998437		0.3855

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.6916073	0.064286	10.76	<.0001
PFOS	-0.02872	0.032862	-0.87	0.3855

Polynomial Fit degree=2

Unconjugated bilirubin = 0.78708 – 0.19268 PFOS + 0.03945 PFOS^2

Summary of Fit

RSquare	0.049788
RSquare Adj	0.018634

Root Mean Square Error	0.325458
Mean of Response	0.648438
Observations (or Sum Wgts)	64

Analysis of Variance

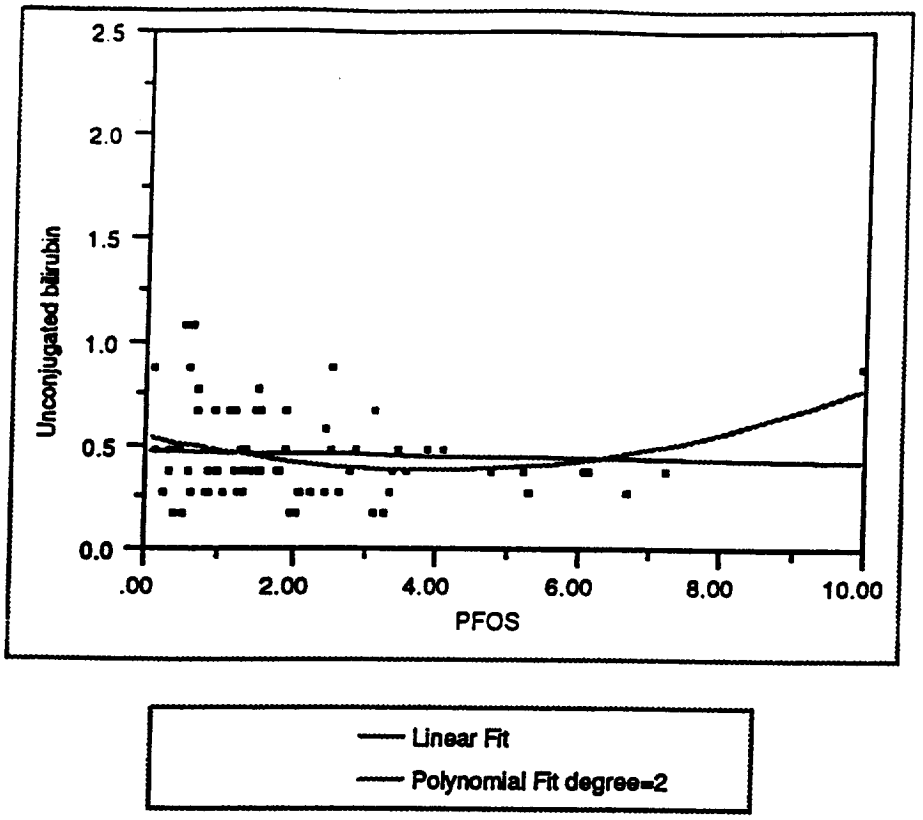
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.3385540	0.169277	1.5981
Error	61	6.4612897	0.105923	Prob>F
C Total	63	6.7998437		0.2106

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.7870832	0.088403	8.90	<.0001
PFOS	-0.192677	0.110395	-1.75	0.0860
PFOS^2	0.0394497	0.025386	1.55	0.1254

Scatter Plot of Unconjugated Bilirubin and PFOS, Decatur, 1997

Unconjugated bilirubin By PFOS



Linear Fit

Unconjugated bilirubin = 0.47268 – 0.00489 PFOS

Summary of Fit

RSquare	0.001886
RSquare Adj	-0.01029
Root Mean Square Error	0.202702
Mean of Response	0.463095
Observations (or Sum Wgts)	84

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.0063673	0.006367	0.1550
Error	82	3.3692280	0.041088	Prob>F
C Total	83	3.3755952		0.6949

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.47268	0.032893	14.37	<.0001
PFOS	-0.004889	0.012419	-0.39	0.6949

Polynomial Fit degree=2

Unconjugated bilirubin = 0.5544 – 0.08508 PFOS + 0.01076 PFOS^2

Summary of Fit

RSquare	0.077372

RSquare Adj	0.054591
Root Mean Square Error	0.196086
Mean of Response	0.463095
Observations (or Sum Wgts)	84

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.2611750	0.130588	3.3963
Error	81	3.1144202	0.038450	Prob>F
C Total	83	3.3755952		0.0383

Parameter Estimates

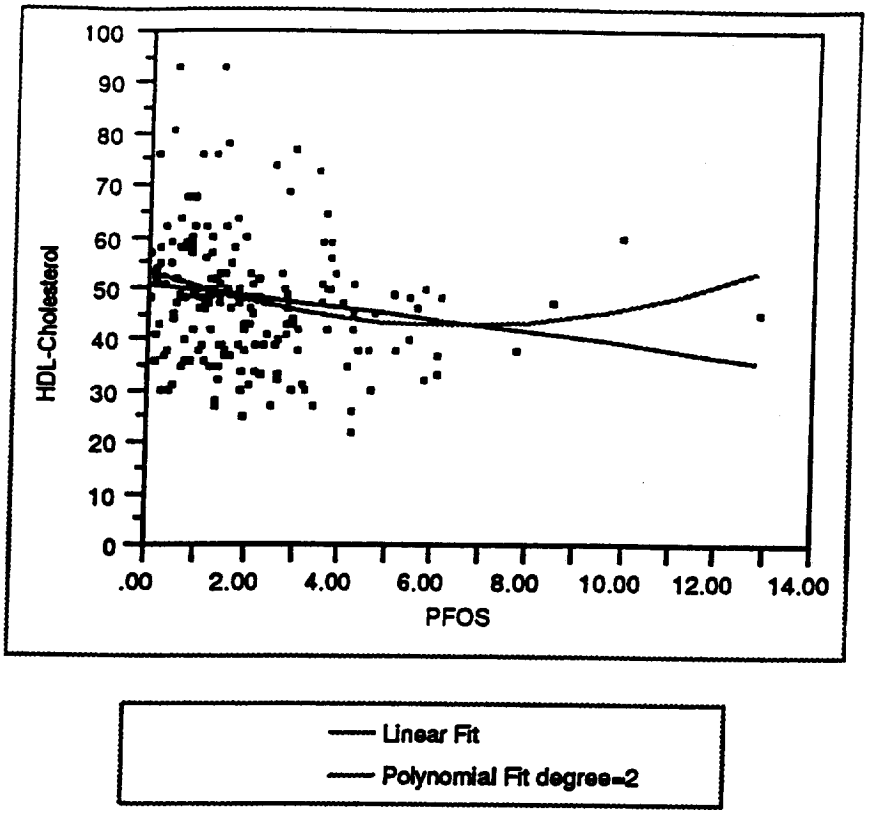
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.5544015	0.044947	12.33	<.0001
PFOS	-0.085075	0.033385	-2.55	0.0127
PFOS^2	0.0107606	0.00418	2.57	0.0119

APPENDIX C

HDL and PFOS Scatter Plots

Scatter Plot of HDL Cholesterol and PFOS, Both Locations Combined, 1995

HDL-Cholesterol By PFOS



Linear Fit

HDL-Cholesterol = 51.0197 – 1.17509 PFOS

Summary of Fit

RSquare	0.030337
RSquare Adj	0.0247
Root Mean Square Error	12.55611
Mean of Response	48.45977
Observations (or Sum Wgts)	174

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	848.392	848.392	5.3813
Error	172	27116.827	157.656	Prob>F
C Total	173	27965.218		0.0215

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	51.01971	1.457347	35.01	<.0001
PFOS	-1.17509	0.506557	-2.32	0.0215

Polynomial Fit degree=2

HDL-Cholesterol = 53.5286 – 3.30537 PFOS + 0.25758 PFOS^2

Summary of Fit

RSquare	0.053266
RSquare Adj	0.042193

Root Mean Square Error	12.443
Mean of Response	48.45977
Observations (or Sum Wgts)	174

Analysis of Variance

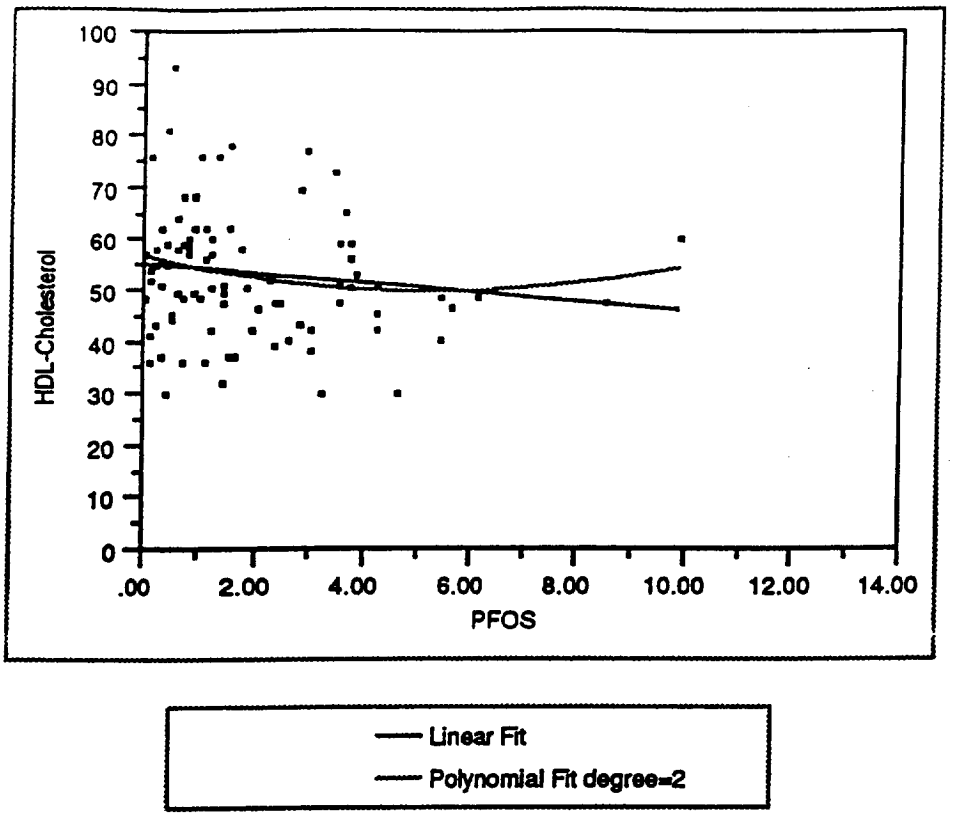
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1489.605	744.802	4.8105
Error	171	26475.614	154.828	Prob>F
C Total	173	27965.218		0.0093

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	53.5286	1.898856	28.19	<.0001
PFOS	-3.305373	1.160937	-2.85	0.0050
PFOS^2	0.257576	0.12657	2.04	0.0434

Scatter Plot of HDL and PFOS, Antwerp, 1995

HDL-Cholesterol By PFOS



Linear Fit

HDL-Cholesterol = 55.2981 – 0.93355 PFOS

Summary of Fit

RSquare	0.019736
RSquare Adj	0.008337
Root Mean Square Error	12.32088
Mean of Response	53.5
Observations (or Sum Wgts)	88

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	262.842	262.842	1.7315
Error	86	13055.158	151.804	Prob>F
C Total	87	13318.000		0.1917

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	55.298149	1.895379	29.18	<.0001
PFOS	-0.933553	0.709469	-1.32	0.1917

Polynomial Fit degree=2

HDL-Cholesterol = 56.91 – 2.68656 PFOS + 0.24725 PFOS^2

Summary of Fit

RSquare	0.033054

RSquare Adj	0.010302
Root Mean Square Error	12.30867
Mean of Response	53.5
Observations (or Sum Wgts)	88

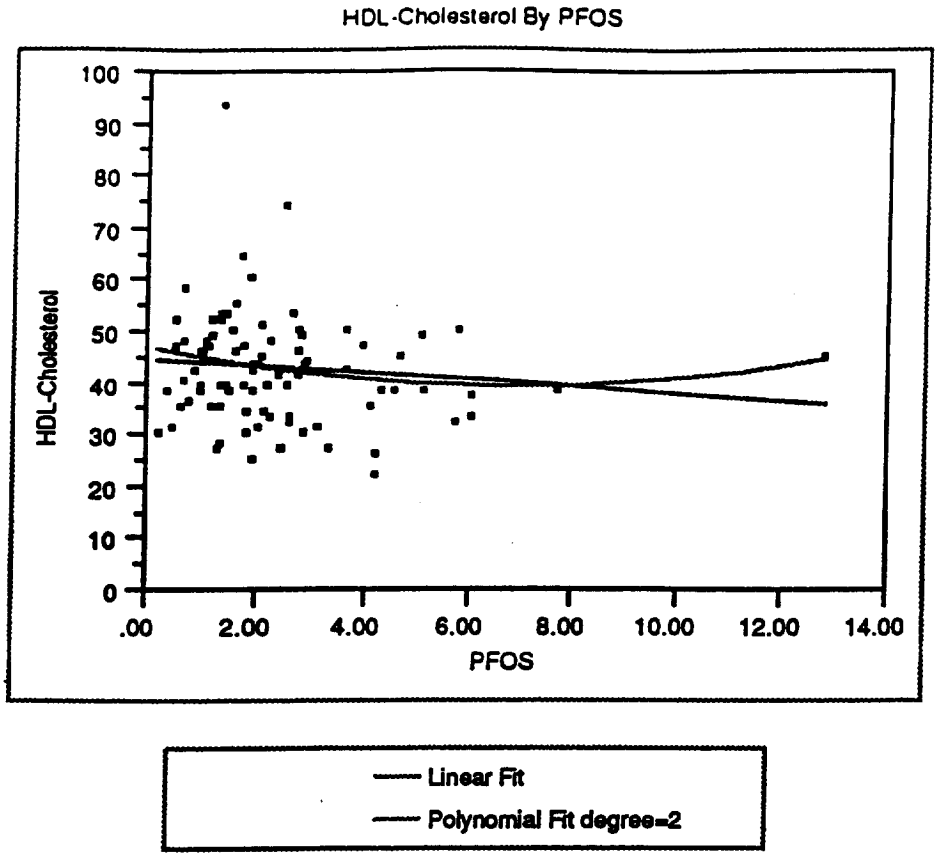
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	440.212	220.106	1.4528
Error	85	12877.788	151.503	Prob>F
C Total	87	13318.000		0.2397

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	56.910034	2.409276	23.62	<.0001
PFOS	-2.686556	1.768392	-1.52	0.1324
PFOS^2	0.2472466	0.228508	1.08	0.2823

Scatter Plot of HDL and PFOS, Decatur, 1995



Linear Fit

HDL-Cholesterol = 45.0317 – 0.7097 PFOS

Summary of Fit

RSquare	0.014998
RSquare Adj	0.003272
Root Mean Square Error	10.89577
Mean of Response	43.30233
Observations (or Sum Wgts)	86

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	151.843	151.843	1.2790
Error	84	9972.296	118.718	Prob>F
C Total	85	10124.140		0.2613

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	45.031672	1.928382	23.35	<.0001
PFOS	-0.709695	0.627527	-1.13	0.2613

Polynomial Fit degree=2

HDL-Cholesterol = 47.1802 – 2.18908 PFOS + 0.15422 PFOS^2

Summary of Fit

RSquare	0.028286

RSquare Adj	0.004872
Root Mean Square Error	10.88702
Mean of Response	43.30233
Observations (or Sum Wgts)	86

Analysis of Variance

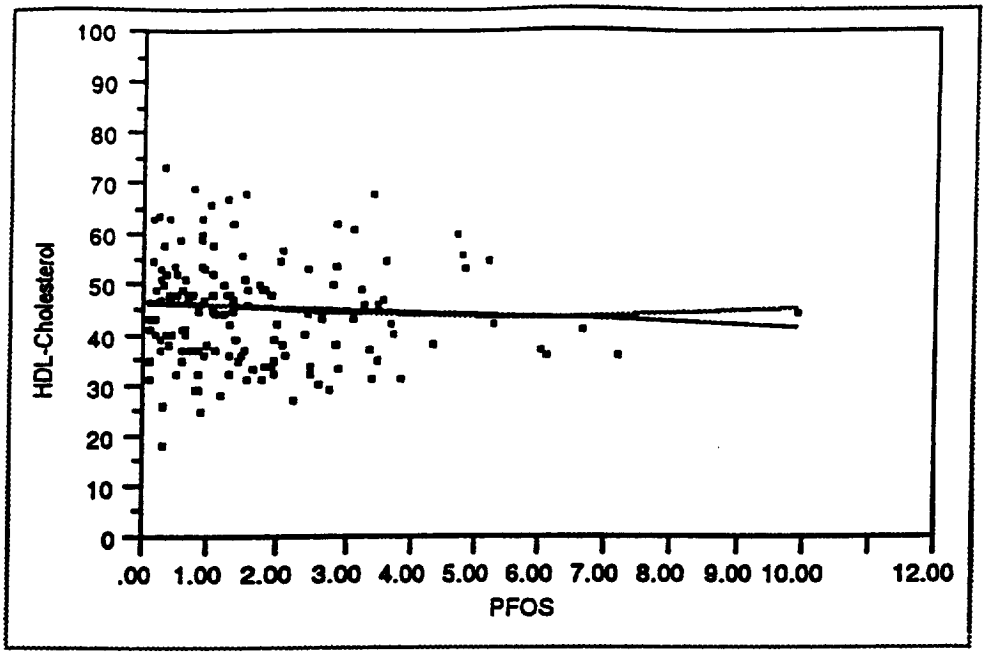
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	286.376	143.188	1.2081
Error	83	9837.764	118.527	Prob>F
C Total	85	10124.140		0.3040

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	47.180201	2.789208	16.92	<.0001
PFOS	-2.189082	1.523604	-1.44	0.1545
PFOS^2	0.1542222	0.144758	1.07	0.2898

Scatter Plot of HDL and PFOS, Both Locations Combined, 1997

HDL-Cholesterol By PFOS



Linear Fit

Polynomial Fit degree=2

Linear Fit

HDL-Cholesterol = 46.3275 – 0.45133 PFOS

Summary of Fit

RSquare	0.004651
RSquare Adj	-0.00212
Root Mean Square Error	10.57408
Mean of Response	45.53691
Observations (or Sum Wgts)	149

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	76.804	76.804	0.6869
Error	147	16436.243	111.811	Prob>F
C Total	148	16513.047		0.4086

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	46.327525	1.288559	35.95	<.0001
PFOS	-0.451333	0.544563	-0.83	0.4086

Polynomial Fit degree=2

HDL-Cholesterol = 46.8473 – 1.03203 PFOS + 0.08884 PFOS^2

Summary of Fit

RSquare	0.006173
RSquare Adj	-0.00744

Root Mean Square Error	10.60212
Mean of Response	45.53691
Observations (or Sum Wgts)	149

Analysis of Variance

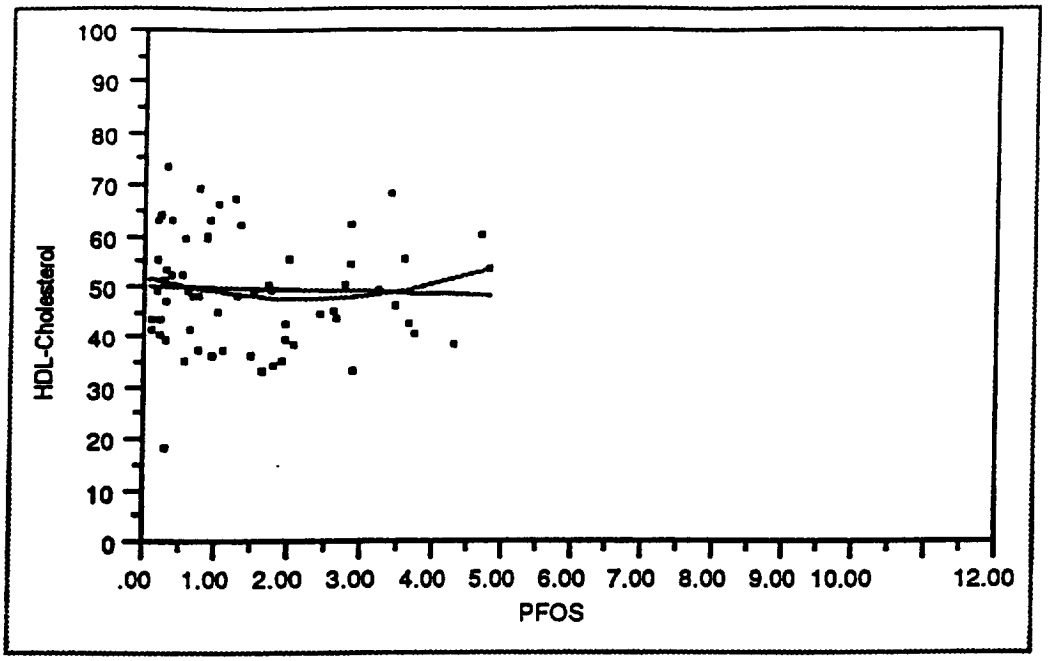
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	101.936	50.968	0.4534
Error	146	16411.111	112.405	Prob>F
C Total	148	16513.047		0.6363

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	46.847332	1.696367	27.62	<.0001
PFOS	-1.032032	1.343985	-0.77	0.4438
PFOS^2	0.0888404	0.187882	0.47	0.6370

Scatter Plot of HDL and PFOS, Antwerp, 1997

HDL-Cholesterol By PFOS



Linear Fit

Polynomial Fit degree=2

Linear Fit

HDL-Cholesterol = 50.2864 – 0.41131 PFOS

Summary of Fit

RSquare	0.00232
RSquare Adj	-0.01352
Root Mean Square Error	10.86492
Mean of Response	49.67692
Observations (or Sum Wgts)	65

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	17.2928	17.293	0.1465
Error	63	7436.9226	118.046	Prob>F
C Total	64	7454.2154		0.7032

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	50.286404	2.086111	24.11	<.0001
PFOS	-0.41131	1.074642	-0.38	0.7032

Polynomial Fit degree=2

HDL-Cholesterol = 52.4254 – 4.16781 PFOS + 0.90954 PFOS^2

Summary of Fit

RSquare	0.020968
RSquare Adj	-0.01061

Root Mean Square Error	10.84934
Mean of Response	49.67692
Observations (or Sum Wgts)	65

Analysis of Variance

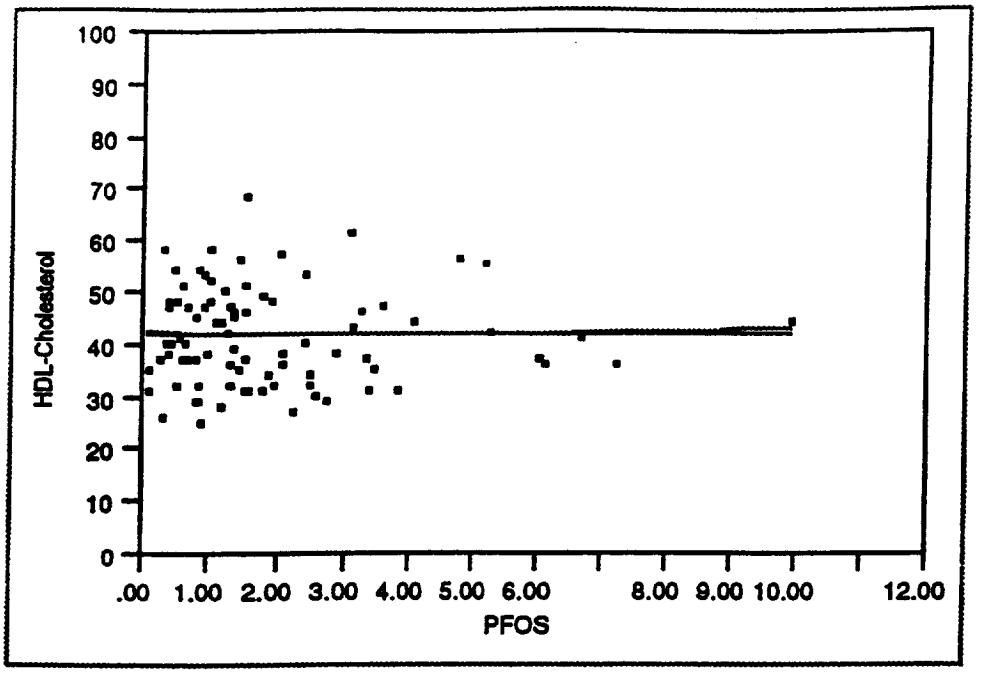
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	156.3026	78.151	0.6639
Error	62	7297.9128	117.708	Prob>F
C Total	64	7454.2154		0.5184

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	52.425357	2.865909	18.29	<.0001
PFOS	-4.167812	3.619456	-1.15	0.2539
PFOS^2	0.9095417	0.836957	1.09	0.2814

Scatter Plot of HDL and PFOS, Decatur, 1995

HDL-Cholesterol By PFOS



— Linear Fit

— Polynomial Fit degree=2

Linear Fit

HDL-Cholesterol = 42.3281 + 0.00268 PFOS

Summary of Fit

RSquare	2.712e-7
RSquare Adj	-0.01219
Root Mean Square Error	9.293759
Mean of Response	42.33333
Observations (or Sum Wgts)	84

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.0019	0.0019	0.0000
Error	82	7082.6647	86.3740	Prob>F
C Total	83	7082.6667		0.9962

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	42.328069	1.508131	28.07	<.0001
PFOS	0.002685	0.569385	0.00	0.9962

Polynomial Fit degree=2

HDL-Cholesterol = 42.5348 – 0.20021 PFOS + 0.02723 PFOS^2

Summary of Fit

RSquare	0.000231

Serum Perfluorooctane Sulfonate and Hepatic and Lipid Clinical Chemistry Tests in Fluorochemical Production Employees

Geary W. Olsen, DVM, PhD
Jean M. Burris, RN, MPH
Jeffrey H. Mandel, MD, MPH
Larry R. Zobel, MD, MPH

The 3M Company manufactures fluorochemicals, which have as a precursor perfluorooctane sulfonyl fluoride ($C_8F_{17}SO_2F$). These compounds may be expected to transform metabolically, to an undetermined degree, to perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$) as an end-stage metabolite. Subchronic studies in rats and primates indicate a potential for cumulative toxicity with PFOS with the primary effect related to metabolic wasting with hypolipidemia as a consistent finding. Biennial medical surveillance has been offered to the company's fluorochemical production workers located in Decatur, Alabama, and Antwerp, Belgium. In 1995, the mean serum PFOS level, as measured by high-performance liquid chromatography mass spectrometry, for 178 male employees was 2.19 parts per million (ppm; range, 0.00 to 12.83 ppm), and in 1997, for 149 male employees, it was 1.75 ppm (0.10 to 9.93 ppm). Our analyses suggest that among these production employees, there were no substantial changes in serum hepatic enzymes, cholesterol, or lipoproteins associated with PFOS levels less than 6 ppm. It was not possible to derive inferences from the few employees who had serum PFOS levels ≥ 6 ppm. These results may be due to the lower levels of serum PFOS measured among these production employees, compared to those suspected to cause effects in laboratory animals.

The 3M Company manufactures products that contain fluorochemicals, either as intentional components or residual impurities, that have as a precursor molecule perfluorooctane sulfonyl fluoride ($C_8F_{17}SO_2F$). Workplace exposure may occur by inhalation, ingestion, and dermal routes. These fluorochemicals may transform metabolically, to an undetermined degree, to perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3^-$) as an end-stage metabolite.[1] Potassium perfluorooctane sulfonate ($C_8F_{17}SO_3^-K^+$) is itself a surfactant used as a wetting and foaming agent in industrial and commercial processes.

PFOS is known to concentrate primarily in the liver and, to a 10-fold lesser extent, in the plasma of rats.[2] There appears to be significant enterohepatic circulation of PFOS with both urinary and fecal excretion.[2,3] Cholestyramine decreased the retention of radiolabeled PFOS in the liver and plasma and increased its elimination via feces.[3] Subchronic studies in rats and primates suggest there is cumulative toxicity with PFOS.[4–7] Lowered serum total cholesterol levels appear to be an early consistent finding, with cumulative toxicity resulting in metabolic wasting and ultimately death. Although the mechanism of toxicity in laboratory animals is not fully understood, it may be due to an effect on fatty acid transport and metabolism, membrane function, peroxisome proliferation, and/or mitochondrial bioenergetics.[8–11]

From the Medical Department, 3M Company, St. Paul, Minn.
Address correspondence to: Geary Olsen, DVM, PhD, Medical Department, 3M Company, 220-3W-05, St. Paul, MN 55144.
Copyright © by American College of Occupational and Environmental Medicine

Voluntary biennial medical surveillance of fluorochemical production employees has been routinely performed since the late 1970s at 3M's Decatur, Alabama, and Antwerp, Belgium, locations. Total serum organic fluorine levels were analyzed until the mid-1990s when serum PFOS determination, quantifiable by high-performance liquid chromatography mass spectrometry, became available. The purpose of this study was to provide a cross-sectional analysis of the medical surveillance data in relation to the employees' serum PFOS levels.

Methods

Fluorochemical Production

Fluorochemical production began in Decatur in 1961 and Antwerp in 1976. In general, perfluorinated chemicals are produced via an electrochemical process: a solution of organic substrate is electrolyzed in anhydrous hydrogen fluoride at a low voltage.[12] The products of this electrolysis cell reaction are highly fluorinated compounds, with the end-product defined by the starting material. Potential for workplace exposure to those fluorochemicals that metabolize to PFOS can occur in the electrochemical cell, the reactor, mixing, drumming, and packaging areas, as well as in the plant's quality assurance and research and development laboratories.

Subject Selection

Voluntary fluorochemical medical surveillance examinations are offered biennially to approximately 200 Antwerp and 300 Decatur production employees. In 1995, a total of 178 male employees (Antwerp: $n = 88$; Decatur: $n = 90$) participated in the medical surveillance examinations. In 1997, 149 male employees (Antwerp: $n = 65$; Decatur: $n = 84$) participated. (There were too few female employees to include in the data analysis.) Sixty-one employees participated in both years. This reduction was due to a large turnover of employees at both plant locations during 1996 and 1997. The surveillance consisted of a medical questionnaire; measurement of height, weight, and blood pressure; standard clinical chemistry and hematology tests; and determination of serum PFOS levels.

PFOS Analysis

In 1995, the analysis for serum PFOS was conducted by 3M's Environmental Laboratory in St. Paul, Minnesota. The method used tetrabutylammonium to ion-pair with PFOS in serum.[13] The ion-pairs were then extracted with ethyl acetate. The abstraction product was then analyzed using high-performance liquid chromatography–thermospray mass spectrometry. In 1997, the serum samples were analyzed by liquid chromatography/mass spectrometry, using selected ion monitoring in the negative-ion mode.[14,15]

Laboratory Analyses

For both time periods and plant locations, United Laboratory Services (St. Paul, MN) performed the standard hematological and serum chemistry tests. These included the following: alkaline phosphatase (IU/L), gamma glutamyl transferase (IU/L), aspartate aminotransferase (IU/L), alanine aminotransferase (IU/L), total and direct bilirubin (mg/dL), cholesterol (mg/dL), low-density protein (mg/dL), high-density cholesterol (HDL; mg/dL) and triglycerides (mg/dL). Clinical chemistry, hematology, and serum PFOS determinations were performed on overnight fasted blood samples.

Data Analysis

Descriptive, simple, and stratified analyses, analyses of variance, and ordinary multivariable regression were used to evaluate associations between PFOS and each hematological and clinical chemistry test. Age, body mass index (BMI; kg/m^2), current alcohol consumption (drinks per day), and cigarette use (cigarettes smoked per day) were potential confounding factors that were considered in the analyses. For stratified analyses, employees were divided into four PFOS categories (0 to <1 ppm, 1 to <3 ppm, 3 to <6 ppm, and ≥6 ppm) to determine whether an effect could be detected at the highest serum PFOS levels. Other categorical levels were used that provided similar results. For multivariable regression analyses, serum PFOS and the potential confounders of age, BMI, alcohol use, and cigarette use were examined as continuous and categorical explanatory variables in the models. Multivariable regression models were fitted, with PFOS analyzed as a continuous variable, using linear as well as non-linear transformations in order to maximize the possibility of finding associations between PFOS and the dependent variable of interest. Natural log transformations of the dependent variables were performed, when necessary, to normalize variables and to enhance model fit. Traditional stepwise selection procedures were also utilized (selection in and out of model was set at $P = 0.1$), as well as taking into account other covariates that may be on the biologic pathway of effect.[16] We did not examine changes in measured PFOS between the two time periods because the estimated half-life of PFOS in the serum is believed to range between 1000 and 1500 days (J. H. Mandel, MD, unpublished data based on 4 years of serum measurements of three retirees, 1998). Study results were analyzed using the SAS System.[17]

Results

The distribution of employees, by serum PFOS exposure categorization, is presented in Table 1. Whereas 20% of the Decatur employees had exposures at ≥3 ppm for both years, this proportion in Antwerp declined from 25% in 1995 to 13% in 1997. For both years, 95% of the employees' serum PFOS levels were below 6 ppm. There were no PFOS measurements ≥6 ppm in Antwerp in 1997.

TABLE 1
Distribution of Employees, by Year, Location, and Perfluorooctane Sulfonate (PFOS) Exposure Level (in parts per million [ppm])

	1995 Data						1997 Data					
	All Employees		Antwerp		Decatur		All Employees		Antwerp		Decatur	
PFOS	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
0 to <1 ppm	45	25	34	39	11	12	60	40	31	48	29	35
1 to <3 ppm	91	51	32	36	59	66	63	43	25	38	38	45
3 to <6 ppm	35	20	19	22	16	18	21	14	9	14	12	14
≥6 ppm	7	4	3	3	4	4	5	3	0	0	5	6
Total	178	100	88	100	90	100	149	100	65	100	84	100

TABLE 2
Mean Values of PFOS, Demographic, Serum Chemistry, and Hematologic Parameters for Antwerp and Decatur, 1995 and 1997 Examinations[a]

	1995 Data		1997 Data	
Variable	Antwerp	Decatur	Antwerp	Decatur
PFOS, ppm	1.93	2.44	1.48	1.96
Age, years	37***	45	33***	44
BMI, kg/m^2	23.9***	29.2	23.5***	30.0
Cigarettes, no. per day	4.7*	7.9	5.5	6.6
Alcohol, drinks per day	1.3***	0.2	1.1***	0.1
Alk Phos, IU/L	75***	97	70***	87
GGT, IU/L	41	48	26*	36
AST, IU/L	26*	29	27	26
ALT, IU/L	44	47	31	34
T. bilirubin, mg/dL	0.86***	0.58	0.80***	0.58
D. bilirubin, mg/dL	0.22	0.21	0.15***	0.12
Cholesterol, mg/dL	214	218	206	215
LDL, mg/dL	138	136	134	137
HDL, mg/dL	54***	43	50***	42
Triglycerides, mg/dL	115***	187	111***	192

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[a] BMI, body mass index; Alk Phos, alkaline phosphatase; GGT, gamma glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T., total; D., direct; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

The mean values for PFOS, demographic, and liver and lipid test results, by location, are presented in Table 2. The Antwerp male employee population was significantly younger than that at Decatur, had lower BMIs, and had higher self-reported daily consumption of alcohol. In addition, their clinical profiles were different for several tests. For both time periods, the Antwerp employees had lower mean alkaline phosphatase and triglyceride values and higher total bilirubin and HDL values.

Table 3 lists the mean, median, standard deviation, and range of the covariates and hepatic enzymes, cholesterol, and lipoproteins by four levels of PFOS categorization (0 to <1, 1 to <3, 3 to <6, and ≥6 ppm) for the combined populations for each surveillance year. (Hematology and other clinical chemistry data were unremarkable and are not shown.) Several observations are noteworthy. First, the mean for the ≥6 ppm PFOS category was one order of magnitude higher than the lowest PFOS category (0 to <1 ppm) for both years. Also, the means of the four PFOS categories were significantly different from each other. Second, there was only one variable, total bilirubin, that had significant ($P < 0.05$) F tests for differences in means in both years of analysis. Stratification by plant location and surveillance year did not result in significant findings for total bilirubin or direct bilirubin (Table 4). Third, mean serum cholesterol levels remained constant (1995) or increased (1997) with higher PFOS category levels, although the HDL mean values trended lower among those employees with the highest PFOS categorizations. Stratification by plant location and surveillance year did not result in significantly different mean cholesterol or HDL values by PFOS categories (Table 5). Finally, it should be noted that employees in the highest PFOS category were older and had higher BMIs than did employees in the lowest PFOS category. Furthermore, in 1997, the employees with ≥6 ppm serum PFOS levels were only from Decatur (Table 1); thus the analyses may be confounded because Decatur employees were generally older and heavier than Antwerp employees. For example, in 1997 the mean HDL level for the ≥6 ppm group was 40 mg/dL (Table 3). However, this mean value was solely from Decatur employees.

Linear and nonlinear relationships between PFOS and the dependent variables of interest, taking into account the potential confounding affects of age, BMI, alcohol, and cigarettes, resulted in many analyses. Total bilirubin showed a significant

TABLE 3
Mean, Median, Standard Deviation (SD) of Mean and Range of PFOS, by Demographic and Serum Chemistries for Antwerp and Decatur Employees Combined, for 1995 (n = 178) and 1997 (n = 147)

	1995 Data				1997 Data			
PFOS* (ppm)	Mean	Median	SD	Range	Mean	Median	SD	Range
PFOS, ppm								
0 to <1	0.49[1]	0.50	0.27	0.00 to 0.90	0.52[1]	0.52	0.27	0.10 to 0.97
1 to <3	1.82[1]	1.77	0.58	1.00 to 2.91	1.78[1]	1.64	0.56	1.02 to 2.89
3 to <6	4.12[1]	3.97	0.81	3.00 to 5.80	3.87[1]	3.59	0.70	3.09 to 5.30
≥6	8.17[1]	7.73	2.52	6.06 to 12.83	7.20[1]	6.68	1.59	6.05 to 9.3
	F value = 321.9, P < 0.0001				F value = 367.6, P < 0.0001			
Age, years								
0 to <1	37	36	8	21 to 58	36	34	11	21 to 62
1 to <3	42[2]	41	9	25 to 60	42[2]	41	9	24 to 63
3 to <6	40	40	7	26 to 55	41	42	5	32 to 54
≥6	45	43	7	37 to 56	42	45	9	29 to 52
	F value = 3.7, P = 0.02				F value = 5.1, P = 0.002			
Alcohol, drinks per day								
0 to <1	0.8	0.6	0.9	0 to 3.6	0.5	0.1	0.8	0 to 4.3
1 to <3	0.5	0.1	0.7	0 to 3.6	0.5	0.1	0.8	0 to 5.0
3 to <6	1.2	0.3	1.9	0 to 6.0	1.0	0.1	1.8	0 to 7.1
≥6	0.7	0.0	1.1	0 to 2.9	0.2	0.1	0.2	0.1 to 0.8
	F value = 4.0, P = 0.009				F value = 1.8, P = 0.15			
BMI, kg/m^2								
0 to <1	25.5	24.8	4.2	17.9 to 38.7	26.0	24.9	4.9	20.1 to 41.7
1 to <3	27.7	26.3	5.8	19.6 to 60.7	27.7	26.4	5.7	18.1 to 48.5
3 to <6	24.9[3]	25.0	3.8	17.9 to 32.5	27.3	27.9	4.4	19.1 to 36.0
≥6	27.7	29.4	4.2	20.6 to 33.0	30.8	29.7	4.0	26.1 to 36.2
	F value = 3.7, P = 0.02				F value = 2.1, P = 0.10			
Cigarettes, no. per day								
0 to <1	2.6	0.0	6.4	0.0 to 25.0	4.7	0.0	9.4	0 to 40
1 to <3	6.8	0.0	11.3	0.0 to 40.0	8.2	0.0	11.3	0 to 40
3 to <6	10.6	8.0	12.4	0.0 to 40.0	4.1	0.0	8.3	0 to 30
≥6	0.4	0.0	1.1	0.0 to 3.0	6.0	0.0	13.4	0 to 30
	F value = 4.8, P = 0.003				F value = 1.5, P = 0.23			
Alkaline phosphatase, IU/L								
0 to <1	80	78	22	31 to 158	77	73	17	49 to 132
1 to <3	89	89	27	49 to 191	83	79	23	41 to 163
3 to <6	86	85	21	32 to 124	76	74	22	29 to 120
≥6	88	85	24	63 to 136	88	84	18	65 to 114
	F value = 1.3, P = 0.28				F value = 1.2, P = 0.32			
GGT, U/L								
0 to <1	43	31	28	16 to 155	28	22	20	10 to 142
1 to <3	47	36	39	2 to 293	36	25	33	10 to 179
3 to <6	40	39	15	21 to 80	28	27	14	13 to 71
≥6	43	33	18	28 to 79	33	37	12	17 to 48
	F value = 0.5, P = 0.71				F value = 1.1, P = 0.34			
AST, U/L								
0 to <1	27	25	13	15 to 96	27	25	7	13 to 53
1 to <3	29	26	12	14 to 90	26	25	7	15 to 56
3 to <6	25	24	5	13 to 37	25	23	7	14 to 43
≥6	33	33	6	26 to 43	29	28	3	26 to 34
	F value = 1.8, P = 0.14				F value = 0.5, P = 0.67			
ALT, IU/L								
0 to <1	48	43	20	27 to 118	31	30	11	13 to 60
1 to <3	46	42	21	18 to 183	33	29	16	10 to 89
3 to <6	42	41	7	30 to 59	34	31	18	14 to 82
≥6	51	49	17	29 to 82	41	45	10	25 to 49
	F value = 1.0, P = 0.38				F value = 0.9, P = 0.46			
T. bilirubin, mg/dL								
0 to <1	0.88	0.70	0.50	0.40 to 2.90	0.77	0.60	0.40	0.30 to 2.30
1 to <3	0.66[2]	0.60	0.30	0.20 to 1.50	0.61[2]	0.60	0.21	0.30 to 1.30
3 to <6	0.64[2]	0.60	0.28	0.20 to 1.40	0.63	0.50	0.31	0.40 to 1.30
≥6	0.76	0.70	0.23	0.50 to 1.20	0.58	0.50	0.24	0.40 to 1.00
	F value = 4.4, P = 0.005				F value = 2.9, P = 0.04			

TABLE 3
Continued.

PFOS* (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
D. bilirubin, mg/dL								
0 to <1	0.22	0.20	0.05	0.02 to 0.40	0.15	0.10	0.07	0.10 to 0.40
1 to <3	0.21	0.20	0.06	0.10 to 0.40	0.12[2]	0.10	0.04	0.10 to 0.20
3 to <6	0.21	0.20	0.04	0.20 to 0.30	0.12	0.10	0.04	0.10 to 0.20
≥6	0.20	0.20	0.02	0.10 to 0.30	0.10	0.10	0.00	0.10 to 0.10
	F value = 0.6, P = 0.58				F value = 3.5, P = 0.02			
Cholesterol, mg/dL								
0 to <1	219	215	47	100 to 340	198	197	40	110 to 280
1 to <3	216	213	43	118 to 315	216	219	42	116 to 365
3 to <6	214	214	35	128 to 278	229[2]	224	29	192 to 321
≥6	213	221	36	160 to 251	229	238	26	186 to 250
	F value = 0.1, P = 0.96				F value = 4.3, P = 0.006			
LDL, mg/dL								
0 to <1	140	137	43	29 to 261	124	128	34	50 to 205
1 to <3	134	134	40	44 to 234	141	134	38	61 to 290
3 to <6	137	135	34	65 to 190	148[2]	142	24	111 to 196
≥6	142	136	32	95 to 178	145	156	26	103 to 164
	F value = 0.2, P = 0.87				F value = 3.7, P = 0.01			
HDL, mg/dL								
0 to <1	53	53	13	31 to 94	46	48	11	19 to 74
1 to <3	48	47	13	26 to 94	44	45	10	28 to 69
3 to <6	45	46	11	23 to 74	48	47	10	32 to 69
≥6	45	46	9	34 to 61	40	38	4	37 to 45
	F value = 2.9, P = 0.04				F value = 1.1, P = 0.34			
Triglycerides, mg/dL								
0 to <1	129	96	98	41 to 622	148	107	162	38 to 1209
1 to <3	161	133	107	41 to 651	156	122	108	44 to 534
3 to <6	158	142	88	34 to 413	166	158	92	45 to 394
≥6	132	151	45	64 to 187	220	191	83	149 to 352
	F value = 1.1, P = 0.35				F value = 0.5, P = 0.67			

* See Table 1 for sample size by year.
[1] Mean is significantly different ($P < 0.05$, Bonferroni (Dunn) test) from the mean of the other PFOS categories.
[2] Mean is significantly different ($P < 0.05$, Bonferroni (Dunn) test) from the mean of 0 to <1 ppm PFOS category.
[3] Mean is significantly different ($P < 0.05$, Bonferroni (Dunn) test) from the mean of 1 to <3 ppm PFOS category.

quadratic association with PFOS for Decatur employees. That is, total bilirubin levels initially declined but subsequently increased with increasing PFOS levels. This association was not observed in the Antwerp employee population, which had higher total bilirubin levels. In 1997, serum cholesterol levels were positively associated (linearly) with serum PFOS, after adjustment for potential confounding factors among Decatur employees (data not shown). This association was not observed with serum PFOS and cholesterol in 1995 among Decatur employees or in either time period with the Antwerp employees. Stratification of the data by plant (Table 5) and multivariable analyses (data not shown) showed no consistent associations between PFOS and HDL. For both plants combined, HDL levels were significantly negatively associated (linearly) with PFOS in 1995, after adjustment for possible confounding factors, but this was not observed in 1997.

Discussion

We conducted two cross-sectional analyses of surveillance data to examine the associations between serum PFOS levels and several hematological and clinical chemistry tests in male fluorochemical production employees. For both years, 95% of the measured serum PFOS levels were below 6 ppm. Because the Antwerp and Decatur employees were dissimilar by age, BMIs, and self-reported alcohol use, we conducted combined as well as separate analyses by plant location. These three demographic factors explain, at least partially, why the Antwerp employees had lower mean serum triglycerides and higher HDL levels than the Decatur employees.[18–22]

Clinical hepatic enzyme tests were not associated with the employees' serum PFOS levels. This was an important question to address because PFOS has been reported (1) to be a peroxisome proliferator in rats[8,10]; (2) to result in increased plasma liver transaminase tests in a

TABLE 4
Mean, Median (Med), Standard Deviation (SD) of Mean and Range of PFOS, and Total and Direct Bilirubin Levels, by Year and Plant Location

	Antwerp				Decatur			
PFOS* (ppm)	Mean	Med	SD	Range	Mean	Med	SD	Range
1995 Data								
T. bilirubin, mg/dL								
0 to <1	0.96	0.80	0.55	0.40 to 2.90	0.65	0.60	0.16	0.40 to 0.90
1 to <3	0.83	0.80	0.26	0.40 to 1.30	0.57	0.50	0.28	0.20 to 1.50
3 to <6	0.75	0.70	0.30	0.30 to 1.40	0.51	0.50	0.18	0.20 to 1.00
≥6	0.93	0.90	0.25	0.70 to 1.20	0.63	0.65	0.10	0.50 to 0.70
	F value = 1.2, P = 0.31				F value = 0.7, P = 0.54			
D. bilirubin, mg/dL								
0 to <1	0.23	0.20	0.06	0.20 to 0.40	0.20	0.20	0.00	0.20 to 0.20
1 to <3	0.22	0.20	0.04	0.20 to 0.30	0.20	0.20	0.06	0.10 to 0.40
3 to <6	0.21	0.20	0.03	0.20 to 0.30	0.20	0.20	0.04	0.20 to 0.30
≥6	0.20	0.20	0.00	0.20 to 0.20	0.20	0.20	0.08	0.10 to 0.30
	F value = 0.7, P = 0.55				F value = 0.4, P = 0.74			
1997 Data								
T. bilirubin, mg/dL								
0 to <1	0.90	0.80	0.46	0.40 to 2.30	0.63	0.60	0.30	0.30 to 1.40
1 to <3	0.68	0.70	0.23	0.30 to 1.30	0.56	0.50	0.18	0.30 to 1.00
3 to <6	0.79	0.70	0.40	0.30 to 1.30	0.51	0.50	0.16	0.30 to 0.90
≥6	—	—	—	—	0.58	0.50	0.24	0.40 to 1.00
	F value = 2.3, P = 0.11				F value = 1.0, P = 0.41			
D. bilirubin, mg/dL								
0 to <1	0.16	0.20	0.08	0.10 to 0.40	0.13	0.10	0.06	0.10 to 0.30
1 to <3	0.13	0.10	0.05	0.10 to 0.20	0.11	0.10	0.03	0.10 to 0.20
3 to <6	0.14	0.10	0.05	0.10 to 0.20	0.11	0.10	0.03	0.10 to 0.20
≥6	—	—	—	—	0.10	0.10	0.00	0.10 to 0.10
	F value = 2.2, P = 0.12				F value = 1.3, P = 0.28			

* See Table 1 for sample sizes by year.

subchronic rat study[4]; and (3) to increase serum aspartate aminotransferase and lower alkaline phosphatase levels in a subchronic rhesus monkey study.[5]

There appears to be significant enterohepatic circulation of PFOS with both urinary and fecal excretion in the rat.[2,3] Although we observed a quadratic relation between PFOS and total bilirubin in our multivariable analyses with the Decatur employee population, interpretation of these data is difficult, given the narrow range of PFOS serum levels associated with the total bilirubin values. Interestingly, the Antwerp employees' total bilirubin levels were significantly higher than the Decatur employees' levels. We suspect there may be a greater prevalence of Gilbert's syndrome[20,23] among the Antwerp employees. In 1995, 15 (17%) Antwerp employees had total bilirubin values ≥1.2 mg/dL, compared with three (3%) Decatur employees. Comparable percentages also existed in 1997. We are uncertain whether post-collection factors, including hemolysis, light, and heat, could have contributed to the differences in total bilirubin levels between the Antwerp and Decatur employees.[18] Unfortunately, total bilirubin was not analyzed in two subchronic rhesus monkey studies, which resulted in death of all animals in the 4.5 mg/kg/day PFOS dose group or higher.[5,6] Ongoing toxicology studies in rats and primates should provide additional perspectives regarding any biological effect between PFOS and bilirubin levels.

Our data do not suggest a reduction in total serum cholesterol with PFOS at the serum levels measured among these production employees. PFOS is a peroxisome proliferator in the rat, and hypolipidemia has been consistently observed in subchronic rat and primate toxicology studies.[5–8,10] Rhesus monkeys that had been administered PFOS at 4.5 mg/kg/day had mean serum cholesterol values that decreased from 183 mg/dL to 99 mg/dL within 30 days.[5] Rhesus monkeys administered 1.5 mg/kg/day had mean cholesterol levels that decreased from 195 mg/dL to 111 mg/dL within 90 days. A no-observable-effect level was seen for the 0.5 mg/kg dose group at 90 days. Serum PFOS measurements were not determined in these rhesus monkeys. However, data from a recent cynomolgus monkey dose-range-finding study suggested that hypolipidemia may be initially associated with serum PFOS levels in the range of 100 to 200 ppm.[7] Haughom and Spydevold suggested that the hypolipidemic effect of PFOS may be due to reduced liver activity of hydroxymethyl glutaric acid-CoA reductase and acyl-CoA cholesterol acyltransferase, with enhanced fatty acid oxidation in the liver.[9] Nabbefeld et al observed that PFOS has a high affinity for the fatty acid carrier proteins albumin and L-fatty acid–binding

TABLE 5
Mean, Median (Med), Standard Deviation (SD) of Mean and Range of PFOS, and Total and HDL Cholesterol, by Year and Plant Location

	Antwerp				Decatur			
PFOS* (ppm)	Mean	Med	SD	Range	Mean	Med	SD	Range
1995 Data								
Cholesterol, mg/dL								
0 to <1	220	219	50	100 to 340	215	208	39	154 to 276
1 to <3	206	211	49	118 to 315	221	218	39	132 to 300
3 to <6	217	215	30	178 to 266	209	213	42	128 to 278
≥6	223	221	16	208 to 240	206	206	47	160 to 251
	F value = 0.6, P = 0.61				F value = 0.5, P = 0.69			
HDL, mg/dL								
0 to <1	56	57	13	31 to 94	43	41	9	31 to 59
1 to <3	53	51	13	33 to 79	45	44	12	26 to 94
3 to <6	50	49	11	31 to 74	39	39	9	23 to 51
≥6	53	49	7	48 to 61	39	39	5	34 to 46
	F value = 1.1, P = 0.35				F value = 1.4, P = 0.25			
1997 Data								
Cholesterol, mg/dL								
0 to <1	193	190	41	110 to 277	204	208	38	145 to 277
1 to <3	213	205	48	116 to 365	218	226	39	152 to 290
3 to <6	228	223	38	192 to 321	230	230	23	197 to 280
≥6	—	—	—	—	229	238	26	186 to 250
	F value = 2.9, P = 0.07				F value = 2.0, P = 0.13			
HDL, mg/dL								
0 to <1	51	50	12	19 to 74	42	41	9	26 to 59
1 to <3	48	46	10	34 to 68	42	41	10	28 to 69
3 to <6	51	50	10	39 to 69	45	45	10	32 to 62
≥6	—	—	—	—	40	38	4	37 to 45
	F value = 0.8, P = 0.47				F value = 0.5, P = 0.67			

* See Table 1 for sample sizes by year.

protein in vivo. This could potentially alter fatty acid transport, biochemistry, and metabolism, which conceivably could then lead to a decrease in cholesterol esterification and metabolic wasting.[11]

Several methodological issues should be considered in evaluating the results from our study. First, the cross-sectional design does not allow for a direct analysis of the temporality of an association. Second, the voluntary participation rates in the fluorochemical medical surveillance were not ideal among the eligible fluorochemical production employees. Third, the serum levels of PFOS that were measured may be below the no-effect level in laboratory animals. Fourth, PFOS concentrates primarily in the liver of laboratory animals. Serum measurements of PFOS may not adequately reflect body burden. Fifth, the two cross-sectional analyses cannot be viewed as independent populations. Sixty-one employees were studied in both years. This was due, in part, to a large turnover of employees at both plants between examinations. Sixth, there could be measurement error in important confounding variables. Analysis of the data of the 61 subjects who participated in both years showed that there was excellent correlation for the confounding factors of BMI (r = .92, P = 0.0001), self-reported aspects of alcohol consumption (r = .88, P = 0.0001), and cigarette smoking (r = .79, P = 0.0001). As expected, these 61 employees' serum PFOS levels for the 2 years were highly correlated (r = .92, P = 0.0001). Their results did not differ from those of the entire study population. Finally, the quality of medical surveillance data, prior to its use for studying an a priori hypothesis, can often be evaluated by whether known positive associations are observed. In this regard, we did observe various expected associations, such as cigarette smoking and elevated white blood cell counts and large BMIs, associated with elevated liver transaminase levels.[24,25]

In summary, our findings suggest that among these Antwerp and Decatur male fluorochemical production employees, there were no substantial changes in serum hepatic enzymes, cholesterol, or lipoproteins associated with PFOS levels less than 6 ppm. It is not possible to derive inferences from the few employees with serum levels ≥6 ppm. Limitations of this study include its cross-sectional design, the low voluntary participation rates among the employees, and the lower levels of serum PFOS measured among these employees, compared with those suspected to cause effects in laboratory animals.

Acknowledgment

The authors gratefully acknowledge the assistance of Michele Burlew.

References

1. Gibson SJ, Johnson JD, Ober RE. *Absorption and Biotransformation of N-Ethyl FOSE and Tissue Distribution and Elimination of Carbon-14 After Administration of N-Ethyl FOSE-^{14}C In Feed.* St. Paul, MN: Riker Laboratories Inc; 1983.
2. Johnson JD, Gibson SJ, Ober RE. *Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats After a Single i.v. Dose of FC-95-^{14}C. Project No. 8900310200.* St. Paul, MN: Riker Laboratories Inc; 1979.
3. Johnson JD, Gibson SJ, Ober RE. Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C] perfluorooctanoate or potassium [^{14}C] perfluorooctanesulfonate. *Fund Appl Toxicol.* 1984;4:972–976.
4. Goldenthal EI, Jessup DC, Geil RG, Jefferson ND, Arceo RJ. *Ninety-Day Subacute Rat Toxicity Study. Study No. 137-085.* Mattawan, MI: International Research Development Corp; November 1978.
5. Goldenthal EI, Jessup DC, Geil RG, Jefferson ND. *Ninety-Day Subacute Rhesus Monkey Toxicity Study. Study No 137-085.* Mattawan, MI: International Research Development Corp; December 1978.
6. Goldenthal EI, Jessup DC, Geil RG, Jefferson ND. *Ninety-Day Subacute Rhesus Monkey Toxicity Study. Study No. 137-087.* Mattawan, MI: International Research Development Corp; January 1979.
7. Thomford PJ. *Four-Week Capsule Toxicity Study With Perfluorooctane Sulfonic Acid Potassium Salt (PFOS) in Cynomologus Monkeys. Covance Study 6329–222.* Madison, WI: Covance Co; August 1998.
8. Ikeda T, Fukuda K, Mori I, Enomoto M, Komai T, Suga T. Induction of cytochrome P-450 and peroxisome proliferation in rat liver by perfluorinated octanesulfonic acid. In: Fahimi HD, Sies H, eds. *Peroxisomes in Biology and Medicine.* New York: Springer Verlag; 1987:304–308.
9. Haughom B, Spydevold O. The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluoroctane sulphonic acid (PFOSA) and clofibric acid. *Biochem Biophys Acta.* 1992;1128:65–72.
10. Sohlenius AK, Eriksson AM, Hogstrom C, Kimland M, DePierre JW. Perfluorooctane sulfonic acid is a potent inducer of peroxisomal fatty acid beta-oxidation and other activities known to be affected by peroxisome proliferators in mouse liver. *Pharmacol Toxicol.* 1993;72:90–93.
11. Nabbefeld J, Butenhoff, J, Bass N, Seacat A. Displacement of a fluorescently labeled fatty acid analogue from fatty acid carrier proteins by Wyeth-14,643, ammonium perfluorooctanoate, potassium perfluorooctane sulfonate and other known peroxisome proliferators (abstract). *Toxicologist.* 1998;42:395.
12. Bryce H. Industrial and utilitarian aspects of fluorine chemistry. In: Simons J, ed. *Fluorine Chemistry.* New York: Academic Press; 1964:297–492.
13. Johnson JD, Wolter JT, Colaizy GE, Rethwill PA, Nelson RM. *Quantification of Perflurooctanoate and Perfluorooctanesulfonate in Human Serum Using Ion-Pair Extraction and High Performance Liquid Chromatography-Thermospray Mass Spectrometry With Automated Sample Preparation.* St. Paul, MN: 3M Environmental Laboratory; 1996.
14. Anderson DJ, Mulvana DE. *Analytical Report for the Determination of Perfluorooctanoate and Perfluorooctanesulfonate in Human Serum by LC/MS.* Ithaca, NY: Advanced Bioanalytical Services Inc; August 1997.
15. Anderson DJ, Mulvana DE. *Analytical Report for the Determination of Perfluorooctanoate and Perfluorooctanesulfonate in Human Serum by LC/MS.* Ithaca, NY: Advanced Bioanalytical Services Inc; September 1997.
16. Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public Health.* 1989;79:340–349.
17. SAS Institute, Inc. *SAS Users Guide: Statistics. Version 6.* Cary, NC: SAS Institute, Inc; 1990.
18. Davern TJ, Scharschmidt BF. Biochemical liver tests. In: Feldman M, Sleisenger MH, Scharschmidt BF, eds. *Gastrointestinal and Liver Disease,* 6th ed. Philadelphia, PA: W.B. Saunders Co.; 1993: 1112–1122.
19. Lewis JA. *Illustrated Guide to Diagnostic Tests.* Springhouse, PA: Springhouse Corp; 1994:100–107, 194–207.
20. Friedman LS. Liver, biliary tract and pancreas. In: Tierney LM, McPhee SJ, Papadakis WA, eds. *Medical Diagnosis and Treatment,* 37th ed. Stamford, CT: Appleton and Lange; 1998:628–630.
21. Fu PC. Lipids, lipoproteins, and apolipoproteins. In: Howanitz JH, Howanitz PJ, eds. *Laboratory Medicine: Test Selection and Interpretation.* New York: Churchill Livingstone Inc; 1991:173–198.
22. Wolf PL. Liver function. In: Howanitz JH, Howanitz PJ, eds. *Laboratory Medicine: Test Selection and Interpretation.* New York: Churchill Livingstone Inc; 1991:67–84.
23. Lidofsky S, Scharschmidt BF. Jaundice. In: Feldman M, Sleisenger MH, Scharschmidt BF, eds. *Gastrointestinal and Liver Disease,* 6th ed. Philadelphia: W.B. Saunders Co; 1998:220–224.
24. Olsen GW, Kusch GD, Stafford BA, Gudmundsen SL, Currier MF. The positive known association design: a quality assurance method for occupational health surveillance data. *J Occup Med.* 1991;33: 998–1000.
25. Burns CJ, Boswell JM, Olsen GW. Liver enzyme activity and body mass index. *J Occup Environ Med.* 1997;38:1248–1252.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Mortality Study of Employees at 3M Plant in Decatur, Alabama

A retrospective cohort mortality study of employees, who worked at least one year (beginning in March, 1961) at the 3M chemical and film plants in Decatur Alabama, was conducted by University of Minnesota epidemiologists to determine whether the mortality experience of these employees was significantly different from that which would be expected. The cohorts consisted of 1,050 and 1,116 men who were ever employed in the chemical and film plants, respectively. Of these male employees, 485 and 547 were only employed in the chemical and film plants, respectively. A total of 318 female employees were identified (both plants combined). Vital status was searched through December 31, 1991 and determined for 99.7% of the cohort. Of the 74 deceased employees, death certificates were obtained for 72 (97.3%). Standardized Morality Ratios (SMRs) were calculated using U.S., Alabama and a regional Alabama counties for comparisons. There was no significantly elevated SMRs. Investigators recommended the study be updated in 1998 to cover an additional five years of mortality experience.

3M, in conjunction with epidemiologists from the University of Minnesota Division of Occupational and Environmental Health are in the midst of completing this updated retrospective cohort mortality study. Several methodologic improvements have occurred since the original study. Since the completion of the original mortality study which abstracted only the beginning and stop dates of employment from work history

data, 3M has now computerized the entire work history records of employees at the Decatur location. The computerization of this detailed job and department information, in conjunction with information about serum fluorochemical levels acquired from medical surveillance exams and the random sample (described above), will allow for the construction of a potential perfluorooctanesulfonate (PFOS) exposure matrix. This matrix will be used to more thoroughly assess the mortality experience of Decatur employees as it relates to the workers' occupational fluorochemical levels. Estimated date of the final report for this updated retrospective cohort mortality study is November 1, 2000.

FINAL REPORT

Mortality Study of Employees at 3M Plant in Decatur, Alabama

Submitted by:

Jack S. Mandel, Ph.D., M.P.H.
Rebecca A. Johnson, Ph.D., M.P.H.

Division of Environmental and Occupational Health
School of Public Health
University of Minnesota
Box 807 UMHC
420 Delaware St. S.E.
Minneapolis, MN 55455

March 13, 1995

Table of Contents

Page

List of Tables 3

Introduction 6

Methods 6

Definition of Cohort 6

Enumeration of Cohort and Ascertainment of Pertinent Employment Information 6

Follow-up of Cohort and Determination of Vital Status 8

Obtaining and Coding of Death Certificates 12

Selection of Comparison Populations 13

Study Database 13

Analysis 14

Results 16

Discussion 40

Recommendation 41

References 41

Appendices 42

List of Tables

Page

Table 1: Description of Ineligible Employees in Study Database (n = 1,212) 8

Table 2: Description of Final Employment Status and Vital Status for Men (n = 1,639) 10

Table 3: Description of Final Employment Status and Vital Status for Women (n = 318) 10

Table 4: Description of Employees Lost to Follow-up (n = 6) 11

Table 5: Description of State of Residence at Time of Death for Deceased Employees for Whom a Death Certificate Was Obtained (n = 72) 12

Table 6: Distribution of Deaths by Year of Death (n = 74) 12

Table 7: Characteristics of Men (n = 1,639) 16

Table 8: Characteristics of Women (n = 318) 16

Table 9: Distribution of Men by Age and Year of Entry into Follow-up (n = 1,639) 17

Table 10: Distribution of Women by Age and Year of Entry into Follow-up (n = 318) 18

Table 11: Distribution of Person-Years for Men by Age and Calendar Period (n = 1,639) 19

Table 12: Distribution of Person-Years for Women by Age and Calendar Period (n = 318) 20

Table 13: Distribution of Absolute Number at Risk by Calendar Period for Men (n = 1,639) 21

Table 14: Distribution of Absolute Number at Risk by Calendar Period for Women (n = 318) 21

Table 15: Selected Cause-Specific SMRs for Men by Comparison Population (n = 1,639) 23

Table 16: Cause-Specific SMRs for Men Using the U.S. as Comparison Population (n = 1,639) 25

Table 17: Cause-Specific SMRs for Women Using the U.S. as Comparison Population (n = 318) 28

Table 18: Cause-Specific SMRs for Men Ever Employed in Chemical Department(s) Using the U.S. as Comparison Population (n = 1,050) 29

Table 19: Cause-Specific SMRs for Men Only Employed in Chemical Department(s) Using the U.S. as Comparison Population (n = 485) 32

Table 20: Cause-Specific SMRs for Men Ever Employed in Film Department(s) Using the U.S. as Comparison Population (n = 1,116) 35

Table 21: Cause-Specific SMRs for Men Only Employed in Film Department(s) Using the U.S. as Comparison Population (n = 547) 38

Table 22: Cause-Specific SMRs for Men Using Alabama as Comparison Population (n = 1,639) 42

Table 23: Cause-Specific SMRs for Women Using Alabama as Comparison Population (n = 318) 45

Table 24: Cause-Specific SMRs for Men Using Alabama Counties as Comparison Population (n = 1,639) 46

Table 25: Cause-Specific SMRs for Women Using Alabama Counties as Comparison Population (n = 318) 48

Mortality Study of Employees
at 3M Plant
in Decatur, Alabama

Introduction

A retrospective cohort mortality study of employees who worked at least one year at the 3M Plant in Decatur, Alabama was conducted to determine whether the mortality experience of these employees was significantly different from that which would be expected in a comparable population. The major elements of the study were to: (1) define and completely enumerate the cohort; (2) ascertain pertinent employment information; (3) determine vital status as of December 31, 1991; (4) obtain death certificates for deceased cohort members and code the underlying causes of death; (5) select appropriate comparison populations; and (6) compare the number of deaths observed to the number of deaths expected, based on the mortality rates of the comparison populations.

Methods

Definition of Cohort

The cohort comprised all employees who had worked at least one year at the 3M Plant in Decatur, Alabama and at least one day after March 1, 1961. The cohort was followed from March 1, 1961 through December 31, 1991. March 1, 1961 was chosen as the begin date because 3M reported that the plant in Decatur began operations in March of 1961 and review of employment records revealed that very few employees started work prior to March 1, 1961. December 31, 1991 was chosen as the end date for the current follow-up study because National Death Index reporting was complete through that date.

Enumeration of Cohort and Ascertainment of Pertinent Employment Information

The 3M Plant at Decatur provided a computer file and computer-generated pages for 1,263 individuals actively employed anytime since 1986. The computer file only contained information about the most recent job or employment status, whereas the computer-generated page contained the entire job history. In addition, 3M provided photocopies of employment records for employees who were: (1) terminated during 1961 - 1971, (2) terminated since 1972, and (3) hired prior to 1986 and currently working.

To identify overlap among the record sources, a label was created for each employee in the computer file. If both a computer record and a photocopied employment record existed for an employee, the label was affixed to, and the computer-generated page was attached to, the photocopied employment record. Subsequently, new records were added to the study database for those employees who had photocopied employment records, but were not in the computer file. A total of 1,906 unique employees were added to the study database.

For all employees in the study database, the relevant employment information was abstracted from the photocopied employment records and/or computer generated pages. In those instances where there were minor discrepancies in employment dates between the photocopied employment record and the computer-generated page, the dates on the photocopied employment record were assumed to be correct.

Lists were sent to 3M for those employees for whom employment information was missing, insufficient, and/or inconsistent. 3M also was asked to provide missing demographic information (e.g., sex, date of birth, etc.) or information needed to determine the vital status of the employee or, if the employee was deceased, to obtain a copy of the death certificate. Where possible, 3M searched the Decatur Plant personnel records, corporate human resources computer databases, and microfilmed and microfiched corporate payroll records to provide additional information and resolve inconsistencies. However, despite 3M's efforts, a small number of employees were still missing a start date and/or end date. The date of first employment and the date of last employment are required for determining whether the employee worked for at least one year during the time period from March 1, 1961 through December 31, 1991. For such employees, either the employee was contacted directly and supplied the date(s) or a knowledgeable employee at the 3M Plant in Decatur estimated the date(s). The study databases and employment records were updated using the information provided by the various sources. Any information which remained missing was coded as unknown.

There were numerous quality control procedures to ensure the validity of information in the study database. Information acquired from the computer-generated pages or photocopied employment records, provided by 3M or its employees, or obtained during vital status follow-up was keyed and verified. All sources of information were reviewed to ensure that all eligible employees had been entered in the study database. The study database was examined for duplicate records. When duplicate records were identified, the best information from both records was combined into the first record and the second record

was marked as a duplicate. A random sample of records was selected and key employment information was reabstracted to determine abstracting error rates and to identify any systematic errors which may have occurred. The data were checked in order to identify missing or inconsistent information and 3M was asked to follow-up on this information. A comprehensive error checking program was written to examine whether: (1) the individual values for key variables were acceptable, and (2) the values for key variables were consistent with one another. Errors identified by this program were resolved and the study database was updated.

The study database included 3,181 records. Of these, 12 were duplicate records and 1,212 were ineligible employees. A description of ineligible employees is shown below in Table 1.

Table 1: Description of Ineligible Employees in Study Database (n = 1,212)

Reason for Ineligibility	N
Worked Less Than One Year	1,210
Did Not Work at 3M Plant in Decatur	1
Not 3M Employee	1
TOTAL	1,212

Thus, a total of 1,957 eligible employees (1,639 males and 318 females) were included in the final cohort.

Follow-up of Cohort and Determination of Vital Status

For those employees for whom vital status was not known (including those reported as deceased, but for whom a death certificate had not been obtained), follow-up included computerized death searches via the: (1) National Death Index (NDI), (2) Equifax Death Search, and (3) TRW FACS+ Summary. In addition, some employees who terminated employment prior to 1979 were actively traced through driver's license records, relatives, neighbors, and/or coworkers.

A computer file of employees for whom vital status was not known was submitted to NDI for matching against information for the years 1979 - 1991.

Equifax has information about deaths reported to the Social Security Administration (SSA) and from sources such as the military, corporate pension plans, insurance companies, etc. A computer file of employees for whom vital status was not known was submitted to Equifax.

TRW FACS+ Summary contains information about deaths and is maintained by the Credit Reporting Division of TRW. The majority of death information contained in TRW also is provided by the SSA. TRW was used to search for deaths among employees for whom vital status was not known. Currently, TRW typically provides the name, date of birth, and date of death for individuals who have been reported deceased. Throughout most of the study, however, TRW only provided information that there had been a reported death associated with the social security number, without providing additional identifying information or death information.

Because all of the computerized death searches were dependent upon having the correct social security number for an employee, efforts were made to ensure the accuracy of social security numbers. In addition to comparing social security numbers from the various sources of information (i.e., the computer file, employment records, etc.), TRW Social Search was used to verify the accuracy of social security numbers for employees. Some social security numbers were not found in TRW. For these employees, the social security numbers were assumed to be correct because there are a number of valid reasons why a person may not be included in TRW (e.g., a person may ask to be removed from the TRW file or a person may not have any credit activity). Additional tracing was done to resolve discrepancies in social security numbers.

Active tracing of employees was performed for all employees who terminated prior to 1979 (when NDI was implemented) and for whom vital status was not previously known. Driver's license records frequently were used to determine vital status. In addition, interactive software was used to access Metronet, a national consumer database, and addresses and/or telephone numbers of relatives, neighbors, and/or former employees were obtained. Other tracing sources included other 3M employees and law enforcement agencies. Once a tracing lead was identified, telephone tracers followed up to determine, directly or indirectly, the vital status of the employee.

The results of the vital status follow-up, along with the employment status as of the study end date, are shown in Tables 2 and 3 for males and females, respectively.

Table 2: Description of Final Employment Status and Vital Status for Men (n = 1,639)

Final Employment Status and Vital Status	N	%
Currently Employed	810	49.4
Retired		
Alive	59	3.6
Dead	10	0.6
Unknown	0	0.0
Terminated		
Alive	694	42.3
Dead	29	1.8
Unknown	6	0.4
Died While Employed	31	1.9
TOTAL	1,639	100.0*

* percentages may not add to 100 due to rounding

Table 3: Description of Final Employment Status and Vital Status for Women (n = 318)

Final Employment Status and Vital Status	N	%
Currently Employed	141	44.3
Retired		
Alive	9	2.8
Dead	0	0.0
Unknown	0	0.0
Terminated		
Alive	164	51.6
Dead	1	0.3
Unknown	0	0.0
Died While Employed	3	0.9
TOTAL	318	100.0*

* percentages may not add to 100 due to rounding

Six (0.3%) employees were lost to follow-up. For these employees, the last known date that the employee was alive is the date of termination of employment. A description of the employees who were lost to follow-up is shown in Table 4.

Table 4: Description of Employees Lost to Follow-up (n = 6)

Study ID	Sex	Month/Year of Birth	Month/Year of Employment	Job Title(s)
20067	male	08/49	08/73 - 10/75	general helper, process helper, material handler, resin handler
20958	male	12/37	02/65 - 02/71	general helper, process helper, process operator, chemical operator, foreman
21028	male	06/41	01/66 - 07/68	general helper, process helper, process operator, chemical operator
21057	male	01/46	04/66 - 07/67	general helper, process helper, process operator
21184	male	11/46	08/66 - 03/69	general helper, process operator
21639	male	08/45	01/69 - 09/77	general helper, process helper, packer, material handler helper, material handler, process operator, warehouseman, order filler, chemical operator

Obtaining and Coding of Death Certificates

Death certificates were requested from state vital statistics offices. If a death certificate could not be obtained or the death certificate obtained was not for the correct person, additional tracing was implemented. Overall, 72 (97.3%) death certificates were obtained for the 74 deceased employees (70 men and 4 women). A description of the state of residence at time of death for the 72 deceased employees for whom a death certificate was obtained is provided in Table 5. Ninety percent died in Alabama.

Table 5: Description of State of Residence at Time of Death for Deceased Employees for Whom a Death Certificate Was Obtained (n = 72)

State	N
Alabama	65
Florida	1
Georgia	1
Michigan	1
Minnesota	1
Ohio	1
Tennessee	1
Texas	1
TOTAL	72

The distribution of deaths by calendar year is shown in Table 6.

Table 6: Distribution of Deaths by Year of Death (n = 74)

Year of Death	N
1967 - 1969	3
1970 - 1974	9
1975 - 1979	9
1980 - 1984	12
1985 - 89	33
1990 - 1991	8
TOTAL	74

Death certificates were coded by a professional nosologist according to the Eighth Revision of the International Classification of Diseases (ICD 8). The underlying cause of death and any mention of cancer were coded.

Selection of Comparison Populations

Three comparison populations were selected: (1) the U.S., (2) the state of Alabama, and (3) Alabama counties where more than one-half of the county was within 100 miles of Decatur, excluding those counties in which there was a city with greater than 100,000 persons. Counties included in the regional comparison were: (1) Blount, (2) Calhoun, (3) Cherokee, (4) Colbert, (5) Cullman, (6) De Kalb, (7) Etowah, (8) Fayette, (9) Franklin, (10) Jackson, (11) Lamar, (12) Lauderdale, (13) Lawrence, (14) Limestone, (15) Marion, (16) Marshall, (17) Morgan, (18) St. Clair, (19) Shelby, (20) Talladega, (21) Tuscaloosa, (22) Walker, and (23) Winston. Based upon 1990 census figures, the combined population for these counties was 1,387,615 persons. Counties excluded due to a population center of 100,000 persons or more were: (1) Madison (large city is Huntsville), and (2) Jefferson (large city is Birmingham).

Race-, sex-, age-, and time-specific mortality rates, adjusted to ICD 8, were obtained for the three comparison populations from the Mortality and Population Data System (MPDS) at the University of Pittsburgh. No mortality rates are available from the MPDS prior to 1950 and only cancer mortality rates are available prior to 1962. The most current mortality rates available are for 1989.

Study Database

The final study database was a combination of data from the following sources: the computer file, computer-generated pages, photocopied employment records, 3M, employees, tracing/vital status follow-up, and death certificates. The following key information was available in the study database:

- study ID;
- first name, middle name, and last name;
- street address, city, state, and zip code;
- telephone number;
- social security number;
- sex;
- date of birth;

- date of first employment at 3M Plant in Decatur;
- date of last employment at 3M Plant in Decatur;
- number of lapsed months between date of first employment and date of last employment when not actually working at 3M Plant in Decatur;
- date first employed in chemical department(s);
- date last employed in chemical department(s);
- number of months worked in chemical department(s) (accounting for any lapses when not actually working in chemical department(s));
- flags indicating work in specific department(s) (i.e., worked in chemical department(s), worked in film department(s), etc.);
- final employment status (i.e., currently working, retired, otherwise terminated from employment, died while employed);
- vital status (i.e., alive, presumed alive, deceased, or unknown);
- date of death or date last known to be alive (if different from last date of employment or date of termination);
- underlying cause of death coded to ICD 8; and
- contributing causes of death or other significant conditions (if cancer) coded to ICD 8.

The necessary data files for analysis were prepared using the key information contained in the study database. As part of this process, default values were automatically substituted for any missing components of employment dates (i.e., 06 for a missing month and 15 for a missing day). If this automatic substitution for missing dates created a date that was chronologically out of sequence, a date was manually estimated and this estimated date was entered in the study database.

Analysis

The method of analysis was based upon a comparison of the observed to expected numbers of deaths specific for the cause of death, race, sex, age, and time. The estimate of the expected number of deaths was calculated by applying cause-, race-, sex-, age-, and time-specific rates for the comparison population to the person-years at risk. Follow-up began March 1, 1961. Cohort members did not contribute person-years until they had met the minimum length of work criterion (i.e., one year or more). Person-years of follow-up were contributed until death, loss to follow-up, or the end of the study (December 31, 1991). No information on race was available; thus, employees were assumed to be white. Deceased study members for whom a death certificate could not be obtained only were included in the "all causes of death" and "unknown cause of death" categories.

Standardized mortality ratios (SMRs) were computed using the Occupational Cohort Mortality Analysis Program (OCMAP).[1] Mortality rates for whites were used to calculate the expected numbers for all men and all women. For 1961, when mortality rates for noncancer causes were unavailable, the expected numbers of deaths were not calculated (i.e., the expected numbers of deaths were set to zero). There were no observed deaths prior to 1967. Mortality rates for 1989 were used to estimate mortality rates for subsequent years. SMRs were calculated using both OCMAP and the United States Death Rates (USDR) program developed by Monson.[2] Results were virtually identical.

The overall pattern of mortality was examined for men and women. For men, the pattern of mortality by department(s) worked also was examined. For this analysis, the following subgroups of employees were defined: (1) ever employed in the chemical department(s); (2) only employed in the chemical department(s); (3) ever employed in the film department(s); and (4) only employed in the film department(s). Department codes at the 3M Decatur Plant were defined by four-digit codes. The first two digits were '59' for film departments and were '76' for chemical departments. For 11 employees, at least one department was unknown; and, for 3 of these, all departments were unknown. Thus, these 3 employees could not be included in the subgroup analysis by department(s) worked.

Results

Characteristics of male and female cohort members are given in Tables 7 and 8, respectively. Men contributed 33,108 person-years of follow-up and women contributed 4,807 person-years. There were 70 (95%) deaths among men and 4 (5%) among women. On average, men entered the cohort at an earlier point in time (1971 versus 1977) and at a slightly younger age than women (25 versus 26).

Table 7: Characteristics of Men (n = 1,639)

Variable	Value
Number of Employees	1,639
Number of Person-Years	33,108
Number of Deaths	70
Average Age Started Work	25
Average Year of Entry	1971
Average Age at Death	47
Average Year of Death	1984

Table 8: Characteristics of Women (n = 318)

Variable	Value
Number of Employees	318
Number of Person-Years	4,807
Number of Deaths	4
Average Age Started Work	26
Average Year of Entry	1977
Average Age at Death	28
Average Year of Death	1980

In Table 9, the distribution of men by age and year of entry into follow-up is shown. Table 10 shows the same distribution for women. Most men entered the cohort during the late 1960s and early 1970s and between the ages of 20 and 30, whereas most women entered the cohort at similar ages, but between 1970 and 1980.

Table 9: Distribution of Men by Age and Year of Entry into Follow-up (n = 1,639)

	Year of Entry into Follow-up							
Age	1960 - 1964	1965 - 1969	1970 - 1974	1975 - 1979	1980 - 1984	1985 - 1989	1990 - 1991	TOTAL
<20	18	34	86	31	4	2	0	175
20 - 24	114	238	301	96	28	18	3	798
25 - 29	64	139	65	37	21	14	5	345
30 - 34	21	58	33	19	8	10	4	153
35 - 39	22	25	9	15	6	5	1	83
40 - 44	19	18	4	10	8	2	0	61
45 - 49	3	3	4	5	0	2	0	17
50 - 54	0	1	0	0	2	3	0	6
55 - 59	0	1	0	0	0	0	0	1
60+	0	0	0	0	0	0	0	0
TOTAL	261	517	502	213	77	56	13	1,639

Table 10: Distribution of Women by Age and Year of Entry into Follow-up (n = 318)

	Year of Entry into Follow-up							
Age	1960 - 1964	1965 - 1969	1970 - 1974	1975 - 1979	1980 - 1984	1985 - 1989	1990 - 1991	TOTAL
<20	2	12	18	16	4	0	0	52
20 - 24	4	22	23	50	4	9	1	113
25 - 29	3	4	4	47	4	5	3	70
30 - 34	2	3	2	15	1	4	2	29
35 - 39	1	2	3	16	3	3	2	30
40 - 44	0	0	0	8	3	2	0	13
45 - 49	0	0	0	5	1	1	0	7
50 - 54	0	0	0	3	0	0	0	3
55 - 59	0	0	0	1	0	0	0	1
60+	0	0	0	0	0	0	0	0
TOTAL	12	43	50	161	20	24	8	318

Tables 11 and 12 provide the distribution of person-years by age and calendar period for men and women, respectively.

Table 11: Distribution of Person-Years for Men by Age and Calendar Period (n = 1,639)

	Calendar Period							
Age	1960 - 1964	1965 - 1969	1970 - 1974	1975 - 1979	1980 - 1984	1985 - 1989	1990 - 1991	TOTAL*
<20	9	19	43	23	2	1	0	96
20 - 24	182	583	985	930	244	64	21	3,008
25 - 29	124	861	1,530	1,633	1,171	341	66	5,724
30 - 34	60	463	1,254	1,672	1,728	1,228	208	6,613
35 - 39	50	188	609	1,339	1,716	1,760	636	6,297
40 - 44	35	129	272	645	1,390	1,719	738	4,927
45 - 49	21	91	180	295	665	1,387	630	3,270
50 - 54	0	28	103	184	308	650	456	1,728
55 - 59	0	5	27	101	185	307	204	827
60 - 64	0	0	5	27	101	174	96	402
65 - 69	0	0	0	5	24	86	47	161
70 - 74	0	0	0	0	5	18	25	47
75 - 79	0	0	0	0	0	5	1	5
80 - 84	0	0	0	0	0	0	2	2
85+	0	0	0	0	0	0	0	0
TOTAL*	479	2,365	5,006	6,853	7,536	7,739	3,129	33,108

* totals may not be equal to sum of individual rows or columns due to rounding

Table 12: Distribution of Person-Years for Women by Age and Calendar Period (n = 318)

	Calendar Period							
Age	1960 - 1964	1965 - 1969	1970 - 1974	1975 - 1979	1980 - 1984	1985 - 1989	1990 - 1991	TOTAL*
<20	1	9	20	21	12	0	0	63
20 - 24	9	65	138	182	139	38	5	576
25 - 29	7	39	115	253	337	171	34	955
30 - 34	2	19	55	171	376	346	106	1,074
35 - 39	2	14	26	81	235	383	160	900
40 - 44	2	5	17	48	136	243	143	594
45 - 49	0	2	5	24	70	141	91	332
50 - 54	0	0	2	8	49	70	43	172
55 - 59	0	0	0	3	18	49	19	89
60 - 64	0	0	0	0	5	18	18	41
65 - 69	0	0	0	0	0	5	7	12
70 - 74	0	0	0	0	0	0	0	0
75 - 79	0	0	0	0	0	0	0	0
80 - 84	0	0	0	0	0	0	0	0
85+	0	0	0	0	0	0	0	0
TOTAL*	22	153	377	790	1,377	1,463	624	4,807

* totals may not be equal to sum of individual rows or columns due to rounding

The distribution of the absolute number of persons at risk by calendar period is provided in Table 13 for men and in Table 14 for women.

Table 13: Distribution of Absolute Number at Risk by Calendar Period for Men (n = 1,639)

Calendar Period	Number at Risk
1960 - 1964	261
1965 - 1969	778
1970 - 1974	1,274
1975 - 1979	1,477
1980 - 1984	1,545
1985 - 1989	1,591
1990 - 1991	1,571
TOTAL	1,639

Table 14: Distribution of Absolute Number at Risk by Calendar Period for Women (n = 318)

Calendar Period	Number at Risk
1960 - 1964	12
1965 - 1969	55
1970 - 1974	105
1975 - 1979	266
1980 - 1984	284
1985 - 1989	306
1990 - 1991	314
TOTAL	318

In the tables which follow, the observed number of deaths, the expected number of deaths, the observed to expected ratio (i.e., the SMR), and the 95% confidence interval (95% CI) are shown for specific causes of death. The observed number of deaths for a specific cause is the number of deaths from that cause that occurred during the study time period. The expected number of deaths for a specific cause is the number of deaths that would have occurred if the employees experienced the same cause-specific mortality rates as observed in the comparison population. The expected number of deaths was calculated by applying the race-, sex-, age-, and time period-specific rates for the comparison population to the

number of person-years of follow-up contributed by the cohort members. The observed number of deaths for a specific cause was divided by the expected number of deaths for that cause and multiplied by 100 to obtain the cause-specific SMR. An SMR may be interpreted as follows: (1) an SMR of 100 means that the observed and expected number of deaths were equal, (2) an SMR less than 100 means that fewer deaths occurred than expected, and (3) an SMR greater than 100 means more deaths occurred than expected. The 95% CI for the SMR provides an indication of the variability and statistical significance of the estimate of the SMR. The 95% CI means that there is a 95% chance that the interval includes the true value of the SMR. If the 95% CI does not include 100, the SMR is considered statistically significant.

In Table 15, SMRs for men are presented for selected cause of death categories for each of the three white comparison populations.

Table 15: Selected Cause-Specific SMRs for Men by Comparison Population (n = 1,639)

		SMR (95% Confidence Interval)		
		Comparison Population		
Cause of Death	Observed Deaths	U.S.	Alabama	Alabama Counties
All Causes of Death	70	62.9	54.8	52.0
		(49.0, 79.5)	(42.7, 69.3)	(40.5, 65.7)
All Malignant Neoplasms	15	68.4	60.9	59.9
		(38.3, 112.8)	(34.1, 100.4)	(33.5, 98.8)
Cerebrovascular Disease	1	33.5	27.8	26.5
		(0.8, 186.8)	(0.7, 155.2)	(0.7, 147.5)
All Heart Disease	17	59.1	53.2	49.1
		(34.4, 94.7)	(31.0, 85.1)	(28.6, 78.6)
Respiratory Disease	0	---	---	---
		(0.0, 91.2)	(0.0, 80.2)	(0.0, 73.9)
External Causes	25	74.5	59.0	55.0
		(48.2, 110.0)	(38.2, 87.1)	(35.6, 81.3)

SMRs based upon the Alabama and Alabama counties comparison populations were similar to, but lower than, those based upon the U.S. comparison population. Since the U.S death rates are more stable than the death rates for the state and regional comparison populations and because the U.S. commonly is used as a comparison population in occupational cohort mortality studies, SMRs based upon the U.S. comparison population will be presented in the results which follow. SMRs for men and women based upon the Alabama and Alabama counties comparison populations are provided in Tables 22 through 25 in the appendices.

There were 70 deaths among the men in this cohort. Using the U.S. as the comparison population, SMRs for men are shown in Table 16. The SMRs for all causes of death, heart disease, and respiratory disease (i.e., nonmalignant respiratory disease) were significantly less than 100. The SMR for all causes of death was 62.9 (95% CI = 49.0, 79.5) and the SMR for heart disease was 59.1 (95% CI = 34.4, 94.7). No respiratory disease deaths occurred, although 4 deaths were expected.

Other deficits occurred for all cancer and external causes of death. The SMR for all cancers was 68.4 (95% CI = 38.3, 112.8). Most of the specific cancer SMRs were less than 100, except for cancer of the bladder and other urinary organs and cancer of other lymphatic and hematopoietic tissue. For these two causes, the SMRs were based on only one or two deaths and were not statistically significant. For external causes of death, the SMR was 74.5 (95% CI = 48.2, 110.0). Less than the expected number of deaths occurred for motor vehicle accidents, suicides, and homicides and other external causes of death.

Table 16: Cause-Specific SMRs for Men Using the U.S. as Comparison Population (n = 1,639)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	70	111.3	62.9	(49.0, 79.5)
All Malignant Neoplasms	15	21.9	68.4	(38.3, 112.8)
Cancer of Buccal Cavity and Pharynx	0	0.6	---	(0.0, 628.2)
Cancer of Digestive Organs and Peritoneum	1	4.7	21.5	(0.5, 120.0)
Cancer of Esophagus	0	0.5	---	(0.0, 722.6)
Cancer of Stomach	0	0.7	---	(0.0, 531.8)
Cancer of Large Intestine	1	1.7	60.2	(1.5, 335.3)
Cancer of Rectum	0	0.4	---	(0.0, 1,003.1)
Cancer of Liver and Biliary Passages	0	0.4	---	(0.0, 919.1)
Cancer of Pancreas	0	1.0	---	(0.0, 379.1)
Cancer of All Other Digestive Organs	0	0.2	---	(0.0, 2,442.9)
Cancer of Respiratory System	7	7.6	91.8	(36.9, 189.2)
Cancer of Larynx	0	0.2	---	(0.0, 1,529.7)
Cancer of Bronchus, Trachea, and Lung	7	7.3	96.4	(38.7, 198.6)
Cancer of Other Respiratory Organs	0	0.1	---	(0.0, 3,109.0)
Cancer of Prostate	0	0.5	---	(0.0, 694.6)
Cancer of Testes and Other Male Genital Organs	0	0.3	---	(0.0, 1,123.9)
Cancer of Kidney	0	0.6	---	(0.0, 592.5)
Cancer of Bladder and Other Urinary Organs	1	0.3	341.3	(8.5, 1,901.6)

Table 16: (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Cancer of Brain and Other Central Nervous System	1	1.2	84.3	(2.1, 470.0)
Cancer of Thyroid and Other Endocrine Glands	0	0.1	—	(0.0, 3,749.2)
Cancer of Bone	0	0.1	—	(0.0, 3,035.4)
Cancer of All Lymphatic and Hematopoietic Tissue	3	3.0	101.1	(20.9, 295.5)
Lymphoma	0	0.4	—	(0.0, 997.3)
Hodgkin's Disease	0	0.5	—	(0.0, 804.7)
Leukemia and Aleukemia	1	1.2	86.4	(2.2, 481.4)
Cancer of Other Lymphatic and Hematopoietic Tissue	2	1.0	203.8	(24.7, 736.2)
Malignant Melanoma of Skin	1	0.9	116.8	(2.9, 650.6)
All Other Malignant Neoplasms	1	1.9	52.2	(1.3, 290.7)
Diabetes Mellitus	0	1.5	—	(0.0, 240.2)
Cerebrovascular Disease	1	3.0	33.5	(0.8, 186.8)
All Heart Disease	17	28.8	59.1	(34.4, 94.7)
Hypertension	0	0.1	—	(0.0, 2,652.5)
Respiratory Disease	0	4.0	—	(0.0, 91.2)
Ulcer of Stomach and Duodenum	0	0.3	—	(0.0, 1,407.9)
Cirrhosis of Liver	3	4.1	73.7	(15.2, 215.3)
Nephritis and Nephrosis	0	0.4	—	(0.0, 999.6)
External Causes	25	33.5	74.5	(48.2, 110.0)
Accidents	19	20.6	92.4	(55.6, 144.2)
Motor Vehicle Accidents	9	11.8	76.1	(34.8, 144.5)
All Other Accidents	10	8.8	113.6	(54.5, 208.9)
Suicides	4	7.9	50.8	(13.9, 130.1)

Table 16: (Continued)				
Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Homicides and Other External Causes	2	4.9	40.7	(4.9, 147.0)
Residual Causes*	7	12.8	54.5	(21.9, 112.3)
Unknown Causes†	2			

* all other causes of death combined

† no death certificate obtained; included only in all causes of death category

In Table 17, SMRs are provided for women, using the U.S. as the comparison population. No cause of death had an SMR significantly different from 100. There were 4 deaths among female employees and the all cause SMR was 62.6 (95% CI = 17.1, 160.4). Of the 4 deaths which occurred, 3 deaths were from external causes (SMR = 213.4; 95% CI = 44.0, 623.6). By examining the death certificates for the specific causes of death and circumstances surrounding the deaths, it was determined that the excess mortality among females due to external causes was not work-related. One of the external causes of death was due to arsenic poisoning and the other two external causes of death were due to motor vehicle accidents.

Table 17: Cause-Specific SMRs for Women Using the U.S. as Comparison Population (n = 318)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	4	6.4	62.6	(17.1, 160.4)
All Malignant Neoplasms	0	2.3	---	(0.0, 162.4)
Cerebrovascular Disease	1	0.3	376.0	(9.4, 2,095.3)
All Heart Disease	0	0.9	---	(0.0, 413.1)
Respiratory Disease	0	0.3	---	(0.0, 1,344.7)
External Causes	3	1.4	213.4	(44.0, 623.6)
Accidents	2	0.8	247.3	(29.9, 893.4)
Motor Vehicle Accidents	2	0.6	364.7	(44.1, 1,317.6)
All Other Accidents	0	0.3	---	(0.0, 1,415.3)
Suicides	0	0.4	---	(0.0, 992.2)
Homicides and Other External Causes	1	0.2	449.6	(11.2, 2,505.4)

Tables 18 through 21 present SMRs and 95% CIs for men by the department(s) worked subgroups.

Findings were similar for men ever employed in chemical department(s) (Table 18). A total of 57 deaths were observed for these men and the all cause SMR was significantly decreased (SMR = 70.0; 95% CI = 53.0, 90.6). The SMR for heart disease also was significantly less than 100 (SMR = 48.8; 95% CI = 24.4, 87.4). SMRs and 95% CIs for all cancer and external causes of death were 76.9 (95% CI = 40.9, 131.5) and 90.2 (95% CI = 55.1, 139.3), respectively.

Table 18: Cause-Specific SMRs for Men Ever Employed in Chemical Department(s) Using the U.S. as Comparison Population (n = 1,050)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	57	81.5	70.0	(53.0, 90.6)
All Malignant Neoplasms	13	16.9	76.9	(40.9, 131.5)
Cancer of Buccal Cavity and Pharynx	0	0.5	—	(0.0, 800.6)
Cancer of Digestive Organs and Peritoneum	1	3.6	27.5	(0.7, 153.2)
Cancer of Esophagus	0	0.4	—	(0.0, 913.3)
Cancer of Stomach	0	0.5	—	(0.0, 686.1)
Cancer of Large Intestine	1	1.3	76.9	(1.9, 428.5)
Cancer of Rectum	0	0.3	—	(0.0, 1,271.9)
Cancer of Liver and Biliary Passages	0	0.3	—	(0.0, 1,188.2)
Cancer of Pancreas	0	0.8	—	(0.0, 480.6)
Cancer of All Other Digestive Organs	0	0.1	—	(0.0, 3,203.2)
Cancer of Respiratory System	7	6.1	115.1	(46.3, 237.1)
Cancer of Larynx	0	0.2	—	(0.0, 1,891.9)
Cancer of Bronchus, Trachea, and Lung	7	5.8	120.7	(48.5, 248.7)
Cancer of Other Respiratory Organs	0	0.1	—	(0.0, 4,214.1)
Cancer of Prostate	0	0.5	—	(0.0, 805.9)
Cancer of Testes and Other Male Genital Organs	0	0.2	—	(0.0, 1,678.5)
Cancer of Kidney	0	0.5	—	(0.0, 768.2)
Cancer of Bladder and Other Urinary Organs	1	0.2	415.5	(10.4, 2,315.3)

Table 18: (Continued)				
Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Cancer of Brain and Other Central Nervous System	1	0.9	117.2	(2.9, 653.0)
Cancer of Thyroid and Other Endocrine Glands	0	0.1	—	(0.0, 5,113.2)
Cancer of Bone	0	0.1	—	(0.0, 4,368.3)
Cancer of All Lymphatic and Hematopoietic Tissue	2	2.2	92.9	(11.2, 335.5)
Lymphoma	0	0.3	—	(0.0, 1,341.4)
Hodgkin's Disease	0	0.3	—	(0.0, 1,163.3)
Leukemia and Aleukemia	1	0.8	120.0	(3.0, 668.8)
Cancer of Other Lymphatic and Hematopoietic Tissue	1	0.7	137.2	(3.4, 764.5)
Malignant Melanoma of Skin	0	0.6	—	(0.0, 614.7)
All Other Malignant Neoplasms	1	1.5	68.9	(1.7, 383.7)
Diabetes Mellitus	0	1.1	—	(0.0, 323.9)
Cerebrovascular Disease	1	2.3	43.4	(1.1, 241.7)
All Heart Disease	11	22.5	48.8	(24.4, 87.4)
Hypertension	0	0.1	—	(0.0, 3,389.9)
Respiratory Disease	0	3.2	—	(0.0, 116.3)
Ulcer of Stomach and Duodenum	0	0.2	—	(0.0, 1,796.3)
Cirrhosis of Liver	3	3.0	100.2	(20.7, 292.7)
Nephritis and Nephrosis	0	0.3	—	(0.0, 1,308.2)
External Causes	20	22.2	90.2	(55.1, 139.3)
Accidents	16	13.6	117.3	(67.0, 190.5)
Motor Vehicle Accidents	8	7.8	102.8	(44.4, 202.5)
All Other Accidents	8	5.9	135.7	(58.6, 267.4)
Suicides	3	5.2	57.7	(11.9, 168.6)

Table 18: (Continued)				
Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Homicides and Other External Causes	1	3.2	31.4	(0.8, 175.1)
Residual Causes*	7	9.0	77.6	(31.2, 159.9)
Unknown Causes†	2			

* all other causes of death combined

† no death certificate obtained; included only in all causes of death category

Thirty-two deaths occurred among men only employed in chemical department(s) (Table 19). The SMR for all causes of death was 72.5 and was not statistically significant (95% CI = 49.6, 102.4). For heart disease, the SMR was 53.4 (95% CI = 21.5, 110.1). The all cancer SMR was 93.6 (95% CI = 42.8, 177.8). The observed and expected numbers of deaths due to external causes were approximately the same, resulting in an SMR of 95.0 (95% CI = 45.6, 174.7).

Table 19: Cause-Specific SMRs for Men Only Employed in Chemical Department(s) Using the U.S. as Comparison Population (n = 485)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	32	44.1	72.5	(49.6, 102.4)
All Malignant Neoplasms	9	9.6	93.6	(42.8, 177.8)
Cancer of Buccal Cavity and Pharynx	0	0.3	—	(0.0, 1,396.4)
Cancer of Digestive Organs and Peritoneum	0	2.1	—	(0.0, 175.2)
Cancer of Esophagus	0	0.2	—	(0.0, 1,577.7)
Cancer of Stomach	0	0.3	—	(0.0, 1,195.2)
Cancer of Large Intestine	0	0.8	—	(0.0, 488.5)
Cancer of Rectum	0	0.2	—	(0.0, 2,175.6)
Cancer of Liver and Biliary Passages	0	0.2	—	(0.0, 2,072.6)
Cancer of Pancreas	0	0.5	—	(0.0, 827.2)
Cancer of All Other Digestive Organs	0	0.1	—	(0.0, 5,692.4)
Cancer of Respiratory System	5	3.6	141.0	(45.8, 329.0)
Cancer of Larynx	0	0.1	—	(0.0, 3,198.4)
Cancer of Bronchus, Trachea, and Lung	5	3.4	147.8	(48.0, 344.9)
Cancer of Other Respiratory Organs	0	0.1	—	(0.0, 7,763.4)
Cancer of Prostate	0	0.3	—	(0.0, 1,202.0)
Cancer of Testes and Other Male Genital Organs	0	0.1	—	(0.0, 3,501.4)
Cancer of Kidney	0	0.3	—	(0.0, 1,365.0)
Cancer of Bladder and Other Urinary Organs	1	0.2	669.9	(16.7, 3,732.7)

Table 19: (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Cancer of Brain and Other Central Nervous System	1	0.5	222.6	(5.6, 1,240.3)
Cancer of Thyroid and Other Endocrine Glands	0	0.0	—	(0.0, 9,544.3)
Cancer of Bone	0	0.0	—	(0.0, 8,697.5)
Cancer of All Lymphatic and Hematopoietic Tissue	2	1.2	174.0	(21.1, 628.7)
Lymphoma	0	0.2	—	(0.0, 2,466.1)
Hodgkin's Disease	0	0.2	—	(0.0, 2,346.1)
Leukemia and Aleukemia	1	0.4	226.3	(5.7, 1,260.7)
Cancer of Other Lymphatic and Hematopoietic Tissue	1	0.4	249.5	(6.2, 1,390.1)
Malignant Melanoma of Skin	0	0.3	—	(0.0, 1,200.4)
All Other Malignant Neoplasms	0	0.8	—	(0.0, 455.3)
Diabetes Mellitus	0	0.6	—	(0.0, 589.0)
Cerebrovascular Disease	0	1.3	—	(0.0, 274.4)
All Heart Disease	7	13.1	53.4	(21.5, 110.1)
Hypertension	0	0.1	—	(0.0, 5,834.4)
Respiratory Disease	0	1.9	—	(0.0, 195.3)
Ulcer of Stomach and Duodenum	0	0.1	—	(0.0, 3,083.3)
Cirrhosis of Liver	1	1.6	62.3	(1.6, 347.3)
Nephritis and Nephrosis	0	0.2	—	(0.0, 2,346.9)
External Causes	10	10.5	95.0	(45.6, 174.7)
Accidents	9	6.5	139.4	(63.7, 264.5)
Motor Vehicle Accidents	5	3.6	138.0	(44.8, 322.0)
All Other Accidents	4	2.9	140.2	(38.2, 359.0)
Suicides	1	2.5	40.0	(1.0, 223.1)

Table 19: (Continued)				
Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Homicides and Other External Causes	0	1.5	---	(0.0, 247.4)
Residual Causes*	3	4.7	63.4	(13.1, 185.3)
Unknown Causes†	2			

* all other causes of death combined

† no death certificate obtained; included only in all causes of death category

There were 37 deaths among men ever employed in film department(s) (Table 20). A statistically significant deficit was observed for all causes of death combined (SMR = 58.6; 95% CI = 41.3, 80.8). Although nonsignificant, deficits also were seen for heart disease (SMR = 69.9; 95% CI = 33.5, 128.5), all cancer (SMR = 52.9; 95% CI = 19.4, 115.2), and external causes of death (SMR = 62.9; 95% CI = 34.4, 105.5).

Table 20: Cause-Specific SMRs for Men Ever Employed in Film Department(s) Using the U.S. as Comparison Population (n = 1,116)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	37	63.1	58.6	(41.3, 80.8)
All Malignant Neoplasms	6	11.3	52.9	(19.4, 115.2)
Cancer of Buccal Cavity and Pharynx	0	0.3	—	(0.0, 1,248.9)
Cancer of Digestive Organs and Peritoneum	1	2.3	43.1	(1.1, 240.0)
Cancer of Esophagus	0	0.3	—	(0.0, 1,465.1)
Cancer of Stomach	0	0.4	—	(0.0, 1,043.7)
Cancer of Large Intestine	1	0.8	120.6	(3.0, 672.1)
Cancer of Rectum	0	0.2	—	(0.0, 2,040.6)
Cancer of Liver and Biliary Passages	0	0.2	—	(0.0, 1,798.6)
Cancer of Pancreas	0	0.5	—	(0.0, 767.6)
Cancer of All Other Digestive Organs	0	0.1	—	(0.0, 4,625.3)
Cancer of Respiratory System	2	3.7	54.1	(6.5, 195.4)
Cancer of Larynx	0	0.1	—	(0.0, 3,253.4)
Cancer of Bronchus, Trachea, and Lung	2	3.5	56.9	(6.9, 205.4)
Cancer of Other Respiratory Organs	0	0.1	—	(0.0, 5,537.8)
Cancer of Prostate	0	0.2	—	(0.0, 1,915.6)
Cancer of Testes and Other Male Genital Organs	0	0.2	—	(0.0, 1,711.8)
Cancer of Kidney	0	0.3	—	(0.0, 1,137.8)
Cancer of Bladder and Other Urinary Organs	0	0.1	—	(0.0, 2,877.2)

Table 20: (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Cancer of Brain and Other Central Nervous System	0	0.7	---	(0.0, 531.1)
Cancer of Thyroid and Other Endocrine Glands	0	0.1	---	(0.0, 6,576.8)
Cancer of Bone	0	0.1	---	(0.0, 4,865.3)
Cancer of All Lymphatic and Hematopoietic Tissue	1	1.7	58.4	(1.5, 325.5)
Lymphoma	0	0.2	---	(0.0, 1,785.5)
Hodgkin's Disease	0	0.3	---	(0.0, 1,276.9)
Leukemia and Aleukemia	0	0.7	---	(0.0, 545.8)
Cancer of Other Lymphatic and Hematopoietic Tissue	1	0.5	184.8	(4.6, 1,030.0)
Malignant Melanoma of Skin	1	0.5	191.8	(4.8, 1,058.8)
All Other Malignant Neoplasms	1	1.0	97.6	(2.4, 543.7)
Diabetes Mellitus	0	0.9	---	(0.0, 434.3)
Cerebrovascular Disease	1	1.5	66.3	(1.7, 369.6)
All Heart Disease	10	14.3	69.9	(33.5, 128.5)
Hypertension	0	0.1	---	(0.0, 5,311.6)
Respiratory Disease	0	2.0	---	(0.0, 187.3)
Ulcer of Stomach and Duodenum	0	0.1	---	(0.0, 2,826.0)
Cirrhosis of Liver	2	2.3	86.6	(10.5, 312.7)
Nephritis and Nephrosis	0	0.2	---	(0.0, 1,870.8)
External Causes	14	22.3	62.9	(34.4, 105.5)
Accidents	10	13.7	73.2	(35.1, 134.6)
Motor Vehicle Accidents	4	8.0	50.2	(13.7, 128.7)
All Other Accidents	6	5.7	104.6	(38.4, 227.6)
Suicides	2	5.2	38.6	(4.7, 139.4)

Table 20: (Continued)				
Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Homicides and Other External Causes	2	3.3	60.2	(7.3, 217.6)
Residual Causes*	4	7.7	52.0	(14.2, 133.1)

* all other causes of death combined

Eleven deaths were observed for men only employed in film department(s) (Table 21). Two statistically significant SMRs occurred for this subgroup. The all cause SMR was 41.4 (95% CI = 20.7, 74.0) and the external causes SMR was 37.3 (95% CI = 10.2, 95.6). Nonsignificant decreases were observed for all cancer (SMR = 46.9; 95% CI = 5.7, 169.5) and heart disease (SMR 96.3; 95% CI = 31.3, 224.8).

Table 21: Cause-Specific SMRs for Men Only Employed in Film Department(s) Using the U.S. as Comparison Population (n = 547)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	11	26.6	41.4	(20.7, 74.0)
All Malignant Neoplasms	2	4.3	46.9	(5.7, 169.5)
Cancer of Buccal Cavity and Pharynx	0	0.1	—	(0.0, 3,497.2)
Cancer of Digestive Organs and Peritoneum	0	0.8	—	(0.0, 438.8)
Cancer of Esophagus	0	0.1	—	(0.0, 4,205.1)
Cancer of Stomach	0	0.1	—	(0.0, 2,802.6)
Cancer of Large Intestine	0	0.3	—	(0.0, 1,223.0)
Cancer of Rectum	0	0.1	—	(0.0, 5,730.7)
Cancer of Liver and Biliary Passages	0	0.1	—	(0.0, 4,808.4)
Cancer of Pancreas	0	0.2	—	(0.0, 2,170.9)
Cancer of All Other Digestive Organs	0	0.0	—	(0.0, 11,962.2)
Cancer of Respiratory System	0	1.3	—	(0.0, 294.3)
Cancer of Larynx	0	0.0	—	(0.0, 10,015.1)
Cancer of Bronchus, Trachea, and Lung	0	1.2	—	(0.0, 310.2)
Cancer of Other Respiratory Organs	0	0.0	—	(0.0, 13,379.9)
Cancer of Prostate	0	0.1	—	0.0, 7,688.6)
Cancer of Testes and Other Male Genital Organs	0	0.1	—	(0.0, 3,598.5)
Cancer of Kidney	0	0.1	—	(0.0, 3,050.4)
Cancer of Bladder and Other Urinary Organs	0	0.0	—	(0.0, 9,158.4)

Table 21: (Continued)				
Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Cancer of Brain and Other Central Nervous System	0	0.3	---	(0.0, 1,233.1)
Cancer of Thyroid and Other Endocrine Glands	0	0.0	---	(0.0, 15,776.9)
Cancer of Bone	0	0.0	---	(0.0, 10,720.6)
Cancer of All Lymphatic and Hematopoietic Tissue	1	0.7	137.1	(3.4, 763.8)
Lymphoma	0	0.1	---	(0.0, 4,372.5)
Hodgkin's Disease	0	0.1	---	(0.0, 2,803.9)
Leukemia and Aleukemia	0	0.3	---	(0.0, 1,260.7)
Cancer of Other Lymphatic and Hematopoietic Tissue	1	0.2	452.3	(11.3, 2,520.4)
Malignant Melanoma of Skin	1	0.2	427.6	(10.7, 2,382.5)
All Other Malignant Neoplasms	0	0.4	---	(0.0, 920.0)
Diabetes Mellitus	0	0.4	---	(0.0, 1,056.3)
Cerebrovascular Disease	0	0.6	---	(0.0, 642.5)
All Heart Disease	5	5.2	96.3	(31.3, 224.8)
Hypertension	0	0.0	---	(0.0, 14,566.9)
Respiratory Disease	0	0.7	---	(0.0, 509.5)
Ulcer of Stomach and Duodenum	0	0.1	---	(0.0, 7,745.3)
Cirrhosis of Liver	0	1.0	---	(0.0, 385.9)
Nephritis and Nephrosis	0	0.1	---	(0.0, 4,857.8)
External Causes	4	10.7	37.3	(10.2, 95.6)
Accidents	3	6.6	45.8	(9.5, 133.9)
Motor Vehicle Accidents	1	3.8	26.1	(0.7, 145.4)
All Other Accidents	2	2.7	73.3	(8.9, 264.8)
Suicides	0	2.5	---	(0.0, 147.2)

Table 21: (Continued)				
Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Homicides and Other External Causes	1	1.6	60.9	(1.5, 339.6)

Discussion

A retrospective cohort mortality study was conducted of former and current employees who had worked at least one year at the 3M Plant in Decatur, Alabama and at least one day after March 1, 1961. Vital status was determined through the end of 1991.

Male and female employees had less than the expected number of deaths from all causes. For men, this decrease was statistically significant. Similar results were seen for the subgroups of men defined by department(s) worked. For each of the subgroups, the overall number of deaths was less than expected.

There were a total of 4 deaths among women and 3 of these deaths were due to external causes resulting in a nonsignificant two-fold increase in risk. Examination of the death certificates for those women with external causes of death revealed that the excess mortality was not work-related.

Follow-up of the cohort and ascertainment of causes of death for deceased cohort members was virtually complete. Vital status was determined for 1,951 (99.7%) of the 1,957 cohort members. Of the 74 deceased employees, death certificates were obtained for 72 (97.3%). Quality control measures were implemented throughout the study to ensure the integrity of the data. In addition, several comparison populations were defined and the results were compared and found to be similar.

3M provided the information used to assemble the cohort. Although every effort was made to ensure that the cohort was complete, there was no way to independently verify the completeness of the cohort. In an attempt to assess whether the cohort was complete, 3M was asked to supply year-end counts of employees at the 3M Decatur Plant for each year during the study period (i.e., 1961 - 1991). These counts were compared to the counts of employees working at the 3M Decatur Plant in December of each year as determined from

the study database. The counts from the study database were nearly the same as, or usually greater than, those reported by 3M.

Recommendation

It is recommended that the mortality experience of this group of employees be updated in 1998. At that time, the NDI will have death records available for an additional five years, 1992 - 1996. Analyses with additional years of follow-up and deaths would allow examination of whether the mortality profile is consistent over time.

to ~~update in 5yr increments, next through 1994,~~
~~[illegible] expected to be better to do in 1995~~?

References

1. Marsh GM, Preininger M. OCMAP: A user-oriented occupational cohort mortality analysis program. Amer Stat 1980;34:245.

2. Monson RR. Analysis of relative survival and proportional mortality. Comput Biomed Res 1974;7:325-332.

the study database. The counts from the study database were nearly the same as, or usually greater than, those reported by 3M.

Recommendation

It is recommended that the mortality experience of this group of employees be updated in 1998. At that time, the NDI will have death records available for an additional five years, 1992 - 1996. Analyses with additional years of follow-up and deaths would allow examination of whether the mortality profile is consistent over time.

References

1. Marsh GM, Preininger M. OCMAP: A user-oriented occupational cohort mortality analysis program. Amer Stat 1980;34:245.

2. Monson RR. Analysis of relative survival and proportional mortality. Comput Biomed Res 1974;7:325-332.

Table 22: Cause-Specific SMRs for Men Using Alabama as Comparison Population (n = 1,639)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	70	127.7	54.8	(42.7, 69.3)
All Malignant Neoplasms	15	24.6	60.9	(34.1, 100.4)
Cancer of Buccal Cavity and Pharynx	0	0.6	—	(0.0, 649.5)
Cancer of Digestive Organs and Peritoneum	1	4.3	23.1	(0.6, 128.8)
Cancer of Esophagus	0	0.5	—	(0.0, 810.9)
Cancer of Stomach	0	0.6	—	(0.0, 662.4)
Cancer of Large Intestine	1	1.6	63.8	(1.6, 355.5)
Cancer of Rectum	0	0.3	—	(0.0, 1,290.0)
Cancer of Liver and Biliary Passages	0	0.5	—	(0.0, 779.9)
Cancer of Pancreas	0	1.0	—	(0.0, 381.1)
Cancer of All Other Digestive Organs	0	0.1	—	(0.0, 2,904.8)
Cancer of Respiratory System	7	9.9	70.9	(28.5, 146.1)
Cancer of Larynx	0	0.2	—	(0.0, 1,651.8)
Cancer of Bronchus, Trachea, and Lung	7	9.5	73.4	(29.5, 151.3)
Cancer of Other Respiratory Organs	0	0.1	—	(0.0, 3,160.3)
Cancer of Prostate	0	0.5	—	(0.0, 728.3)
Cancer of Testes and Other Male Genital Organs	0	0.3	—	(0.0, 1,309.5)
Cancer of Kidney	0	0.6	—	(0.0, 657.5)
Cancer of Bladder and Other Urinary Organs	1	0.2	419.9	(10.5, 2,339.6)

Table 22: (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Cancer of Brain and Other Central Nervous System	1	1.4	70.7	(1.8, 393.8)
Cancer of Thyroid and Other Endocrine Glands	0	0.1	—	(0.0, 3,017.6)
Cancer of Bone	0	0.1	—	(0.0, 3,406.6)
Cancer of All Lymphatic and Hematopoietic Tissue	3	2.9	104.4	(21.5, 305.0)
Lymphoma	0	0.4	—	(0.0, 987.9)
Hodgkin's Disease	0	0.4	—	(0.0, 1,018.5)
Leukemia and Aleukemia	1	1.2	84.6	(2.1, 471.6)
Cancer of Other Lymphatic and Hematopoietic Tissue	2	1.0	208.7	(25.3, 754.0)
Malignant Melanoma of Skin	1	1.1	95.2	(2.4, 530.3)
All Other Malignant Neoplasms	1	2.6	38.9	(1.0, 216.7)
Diabetes Mellitus	0	1.4	—	(0.0, 259.0)
Cerebrovascular Disease	1	3.6	27.8	(0.7, 155.2)
All Heart Disease	17	32.0	53.2	(31.0, 85.1)
Hypertension	0	0.2	—	(0.0, 2,007.5)
Respiratory Disease	0	4.6	—	(0.0, 80.2)
Ulcer of Stomach and Duodenum	0	0.3	—	(0.0, 1,256.6)
Cirrhosis of Liver	3	3.4	88.9	(18.3, 259.7)
Nephritis and Nephrosis	0	0.4	—	(0.0, 917.2)
External Causes	25	42.4	59.0	(38.2, 87.1)
Accidents	19	28.2	67.3	(40.5, 105.1)
Motor Vehicle Accidents	9	17.1	52.7	(24.1, 100.0)
All Other Accidents	10	11.2	89.3	(42.8, 164.2)
Suicides	4	7.8	51.3	(14.0, 131.5)

Table 22: (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Homicides and Other External Causes	2	6.1	32.6	(3.9, 117.9)
Residual Causes*	7	13.8	50.6	(20.3, 104.2)
Unknown Causes†	2			

* all other causes of death combined

† no death certificate obtained; included only in all causes of death category

Table 23: Cause-Specific SMRs for Women Using Alabama as Comparison Population (n = 318)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	4	6.7	59.9	(16.3, 153.4)
All Malignant Neoplasms	0	2.3	—	(0.0, 162.5)
Cerebrovascular Disease	1	0.3	357.1	(8.9, 1,989.6)
All Heart Disease	0	1.0	—	(0.0, 372.0)
Respiratory Disease	0	0.3	—	(0.0, 1,391.2)
External Causes	3	1.7	179.5	(37.0, 524.7)
Accidents	2	1.1	185.8	(22.5, 671.2)
Motor Vehicle Accidents	2	0.8	251.1	(30.4, 907.2)
All Other Accidents	0	0.3	—	(0.0, 1,323.4)
Suicides	0	0.3	—	(0.0, 1,096.1)
Homicides and Other External Causes	1	0.3	392.8	(9.8, 2,188.8)

Table 24: Cause-Specific SMRs for Men Using the Alabama Counties as Comparison Population (n = 1,639)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	70	134.7	52.0	(40.5, 65.7)
All Malignant Neoplasms	15	25.1	59.9	(33.5, 98.8)
Cancer of Buccal Cavity and Pharynx	0	0.5	—	(0.0, 822.6)
Cancer of Digestive Organs and Peritoneum	1	4.2	23.7	(0.6, 132.3)
Cancer of Esophagus	0	0.4	—	(0.0, 1,048.0)
Cancer of Stomach	0	0.5	—	(0.0, 679.5)
Cancer of Large Intestine	1	1.5	64.8	(1.6, 361.3)
Cancer of Rectum	0	0.3	—	(0.0, 1,491.0)
Cancer of Liver and Biliary Passages	0	0.5	—	(0.0, 721.6)
Cancer of Pancreas	0	1.0	—	(0.0, 374.8)
Cancer of All Other Digestive Organs	0	0.1	—	(0.0, 2,730.1)
Cancer of Respiratory System	7	10.2	69.0	(27.7, 142.1)
Cancer of Larynx	0	0.2	—	(0.0, 1,623.4)
Cancer of Bronchus, Trachea, and Lung	7	9.8	71.3	(28.7, 146.9)
Cancer of Other Respiratory Organs	0	0.1	—	(0.0, 3,493.0)
Cancer of Prostate	0	0.5	—	(0.0, 711.6)
Cancer of Testes and Other Male Genital Organs	0	0.3	—	(0.0, 1,409.3)
Cancer of Kidney	0	0.6	—	(0.0, 671.1)
Cancer of Bladder and Other Urinary Organs	1	0.3	358.7	(9.0, 1,998.9)

Table 24: (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Cancer of Brain and Other Central Nervous System	1	1.3	78.0	(2.0, 434.7)
Cancer of Thyroid and Other Endocrine Glands	0	0.1	---	(0.0, 2,981.2)
Cancer of Bone	0	0.1	---	(0.0, 2,711.5)
Cancer of All Lymphatic and Hematopoietic Tissue	3	2.9	102.8	(21.2, 300.5)
Lymphoma	0	0.3	---	(0.0, 1,194.5)
Hodgkin's Disease	0	0.4	---	(0.0, 1,021.6)
Leukemia and Aleukemia	1	1.2	81.6	(2.0, 454.9)
Cancer of Other Lymphatic and Hematopoietic Tissue	2	1.0	195.5	(23.7, 706.1)
Malignant Melanoma of Skin	1	1.0	100.4	(2.5, 559.5)
All Other Malignant Neoplasms	1	3.0	33.0	(0.8, 183.9)
Diabetes Mellitus	0	1.3	---	(0.0, 275.4)
Cerebrovascular Disease	1	3.8	26.5	(0.7, 147.5)
All Heart Disease	17	34.6	49.1	(28.6, 78.6)
Hypertension	0	0.1	---	(0.0, 2,762.4)
Respiratory Disease	0	5.0	---	(0.0, 73.9)
Ulcer of Stomach and Duodenum	0	0.3	---	(0.0, 1,212.4)
Cirrhosis of Liver	3	3.2	92.8	(19.2, 271.3)
Nephritis and Nephrosis	0	0.4	---	(0.0, 925.1)
External Causes	25	45.4	55.0	(35.6, 81.3)
Accidents	19	31.2	60.9	(36.7, 95.1)
Motor Vehicle Accidents	9	19.4	46.5	(21.2, 88.2)
All Other Accidents	10	11.9	84.2	(40.4, 154.8)
Suicides	4	7.5	53.3	(14.5, 136.5)

Table 24: (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
Homicides and Other External Causes	2	6.5	30.9	(3.7, 111.8)
Residual Causes*	7	14.4	48.7	(19.6, 100.4)
Unknown Causes†	2			

* all other causes of death combined
† no death certificate obtained; included only in all causes of death category

Table 25: Cause-Specific SMRs for Women Using Alabama Counties as Comparison Population (n = 318)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% Confidence Interval
All Causes of Death	4	6.8	58.6	(16.0, 150.1)
All Malignant Neoplasms	0	2.3	—	(0.0, 162.4)
Cerebrovascular Disease	1	0.3	320.2	(8.0, 1,784.0)
All Heart Disease	0	1.1	—	(0.0, 343.9)
Respiratory Disease	0	0.3	—	(0.0, 1,381.0)
External Causes	3	1.7	172.1	(35.5, 502.9)
Accidents	2	1.2	173.1	(20.9, 625.3)
Motor Vehicle Accidents	2	0.9	230.5	(27.9, 832.6)
All Other Accidents	0	0.3	—	(0.0, 1,289.2)
Suicides	0	0.3	—	(0.0, 1,142.5)
Homicides and Other External Causes	1	0.3	382.1	(9.6, 2,129.3)

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Determination of Serum Fluorochemical Levels in Sumitomo 3M Employees

Ninety-four Sumitomo 3M employees participated in a voluntary, cross-sectional serum fluorochemical assessment. Three groups of employees were examined. Sagamihara Plant production employees (n = 32) were involved in the inspection and converting of finished goods. Sagamihara Plant management employees (n = 32) were involved in primarily non-production work activities. Tokyo Head Office employees (n = 32) are generally not occupationally-exposed to fluorochemicals. All but two of the 94 participants were male employees. Among the Sagamihara Plant production employees, the highest serum perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorohexanesulfonate (PFHS) levels were 0.628 ppm, 3.6 ppm and 0.117 ppm, respectively. Serum levels for the Sagamihara Plant Management and Tokyo Head Office were, on average, under 0.01 ppm (PFOS), 0.03 ppb (PFOA) and 0.025 ppm (PFHS)Serum levels of PFOS for Sagamihara Plant management and Tokyo Head Office employees were similar to levels seen in a small group of US 3M Center employees who were not occupationally exposed to PFOS. Serum PFOS levels of Sagamihara Plant production employees were below those of workers at the 3M Antwerp and Decatur plants.

PROTOCOL

Occupational Medicine, 220-3W-05
Medical Department
3M Company
St. Paul, MN 55144

Date: 9 January 1999

Title: DETERMINATION OF SERUM FLUOROCHEMICAL LEVELS 3M SUMITOMO EMPLOYEES

Study Protocol Number: EPI-0008
Start Date:

IRB Approval
Exempt Expedited

Estimated Date of
Final Report:

IRB Approval Date: 1/20/99

Principal Investigator: Jean Burris, R.N., M.S.[1]
Co-investigators: John C. Schumpert, M.D., M.P.H.[1]
Geary Olsen, D.V.M., Ph.D.[1]
Jeffrey Mandel, M.D., M.P.H.[1]

Study Director: Jeffrey Mandel, M.D., M.P.H.

Study Sponsor: 3M Medical Department
3M Company
220-3W-05
Saint Paul, MN 55144

1. Occupational Medicine, Medical Department, 3M Company, 220-3W-05, St. Paul, MN 55114

PROTOCOL FOR SERUM FLUOROCHEMICAL DETERMINATION IN 3M SUMITOMO EMPLOYEES

ABSTRACT

3M manufactures products which contain chemical compounds, either as intentional components or residual impurities, that have as a parent molecule, perfluorooctane sulfonyl fluoride (POSF). These compounds may be expected to transform metabolically, to an undetermined degree, to PFOS as an end-stage metabolite. Another compound manufactured by 3M includes ammonium perfluorooctanoate (PFOA). These molecules enter a number of product applications (e.g., surfactants, food packaging additives, polymers).

3M Sumitomo in Japan processes many of these compounds into products for distribution and sale. Fluorochemical applications are also investigated in 3M Sumitomo laboratories.

The purpose of this study is to measure serum fluorochemical levels in selected employees of the 3M Sumitomo population. The specific fluorochemicals to be measured include perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS).

RESEARCH METHODS

Approximately 110 employees will be invited to participate in this non-randomized cross-sectional study; all subjects are volunteers. Thirty individuals are involved in the processing and formulation various forms of pure fluorochemicals into finished products for distribution throughout Asia. Approximately 20 individuals are actively involved in laboratory fluorochemical research. Approximately 60 managerial employees, considered non-occupationally exposed to fluorochemicals, will also be invited to participate.

Information concerning respondents' age, gender, and occupation will be obtained from 3M Sumitomo Human Resources for review with the serum fluorochemical results.

The study duration will be approximately three weeks. Written informed consent will be obtained from all study subjects in Japanese (Appendix A). Each subject will be involved for about 15 – 20 minutes and will donate two10 mL blood sample. Should any employee complications arise as a consequence of phlebotomy, physicians associated with 3M Sumitomo will treat them. .

Once the blood samples are obtained, they will be frozen in dry ice and sent by 3M Center in Saint Paul via Federal Express. At the 3M Center Medical Clinic, serum will be separated by centrifugation and sent to the 3M Environmental Laboratory for analysis. Standard electrospray HPLC MS/MS will be used to determine the fluorochemical content of all serum samples. Serum will be analyzed for the presence and amount of PFOA and PFOS. Results will be reported to the Principal Investigator in parts per billion (ppb). The Principal Investigator will inform 3M Sumitomo employees

of their PFOS and PFOA levels after results and a brief explanation are be translated into Japanese.

The Principal Investigator will forward the results to the appropriate Co-investigator for statistical analysis. Descriptive statistics performed will include group mean fluorochemical level and standard deviation of the mean, and range of serum fluorochemical levels. Correlation and regression analyses will be performed utilizing information obtained concerning age, gender, and occupation.

RESULTS

A formal report of the research findings, including data analysis, will be submitted to the 3M Medical Director and Institutional Review Board at the conclusion of the study. The protocol will be amended only with approval of the 3M Institutional Review Board.

APPENDIX A – CONSENT FORM

CONSENT FORM FOR COLLECTION OF BLOOD FOR SERUM FLUOROCHEMICAL LEVEL DETERMINATION

INTRODUCTION
You and your colleagues are involved in fluorochemical production, research, or management at 3M Sumitomo. You are invited to participate in a research study being conducted by 3M Corporate Occupational Medicine regarding fluorochemicals. Your participation would involve donating two red-topped test tubes of blood. Your blood will only be tested for the amount PFOA, PFOS, and its precursors it contains; it will not be used to test for any other substances or disease. Please review this consent form carefully and be sure your questions are answered before you make a decision to participate. A 3M Sumitomo representative will be available to provide you with additional information at the time of your blood donation.

PURPOSE OF STUDY
The purpose of the study is to determine the presence and amount of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate, and its precursors in your serum.

STUDY PROCEDURES
Your blood will be drawn with one needle stick and require two test tubes. This will be a one-time blood donation.

POTENTIAL RISKS/BENEFITS
The risks of venipuncture include pain on needle insertion, bleeding under the skin, discoloration from bleeding under the skin, and discomfort from blood clotting (hematoma) if bleeding under the skin occurs.

BENEFITS
There will be no direct benefit from your participation in this study. However, the information gained from this study will further help us understand human exposures to fluorochemicals. Your individual results as well as the overall results will be communicated to you only. Your individual results will be considered confidential information and will not be disclosed to anyone outside the 3M Medical Department without your written consent.

COMPENSATION
No financial compensation will be forthcoming as a result of participating in this study. If you suffer injury or a medical condition that appears to be the result of participating in this study, you will be referred to another health care professional at no cost to you. In the event of a research related injury, compensation will be determined on a case by case basis by 3M.

CONFIDENTIALITY

The overall results collected in this study may be used in publications or public presentation. Your name will not be revealed in any publication or other documents intended for publication examination. Your individual results will be communicated to you only. Your individual results will be considered confidential information and will not be disclosed to anyone outside the 3M Medical Department without your written consent.

SUBJECT RIGHTS/AVAILABILITY OF INFORMATION

If you have any questions about the study now, or later, or in the event of a research related injury or emergency, contact Dr. Jeffrey Mandel (651-733-8670) or Jean Burris, R.N. (651-737-7867). For answers to questions about your rights in regard to this research, you may contact Dr. Larry Zobel, Chair, 3M Institutional Review Board at 651-733-5181.

VOLUNTARY PARTICIPATION AND WITHDRAWAL

Participation in this study is voluntary. Refusal to participate will involve no penalty of loss of benefits to which you are otherwise entitled. You are free to refuse to participate for any reason. The investigator may stop your participation in this study should it be determined that continued participation may be detrimental to your health.

SUBJECT CONSENT

By signing the consent form, I certify that I am at least 18 years old. I confirm that I have read this consent form, and that I have been given adequate opportunity to ask any questions I may have about this consent form or the study. I also confirm that I understand the scope of my participation in this study, and that all of my questions have been answered to my satisfaction. I am signing this consent form voluntarily, and I desire to participate in the study. I understand that I will receive a copy of this signed consent form. I understand that I will not receive any financial compensation for participating in this study.

_____ _____

Signature Date

_____

Printed Name

_____

Witness

Final Report
Epidemiology, 220-3W-05
Medical Department
3M Company
St. Paul, MN 55144

Date: September 3, 1999

Title: Determination of Serum Fluorochemical Levels in Sumitomo 3M Employees

Study Start Date: March, 1999

IRB Approval Date: January 20, 1999

Principal Investigator: Jean Burris, RN, MPH[1]

Co-Investigators: Geary W. Olsen, DVM, PhD[1]
Jeffrey H. Mandel, MD, MPH[1]
John C. Schumpert, MD, MPH[1]

Study Director: Jeffrey H. Mandel MD, MPH

Study Sponsor: 3M Medical Department
3M Company
220-3W-05
St. Paul, MN 55144

1. Occupational Medicine, Medical Department, 3M Company, 220-3W-05, St. Paul, MN 55114

Introduction

3M manufactures products, which contain chemical compounds, either as intentional components or as manufacturing residuals, which, have as a parent molecule, perfluorooctane sulfonyl fluoride (POSF). These compounds may be expected to transform metabolically, to an undetermined degree, to PFOS as an end-stage metabolite. Another compound manufactured by 3M includes ammonium perfluorooctanoate (POAA). These compounds enter a number of product applications (e.g., surfactants, polymers, and food packaging additives).

Sumitomo 3M in Japan processes many of these compounds into products such as fire protection products and surface protection products for distribution and sale. Other potential fluorochemical applications are also investigated in Sumitomo 3M laboratories.

Purpose

The purpose of this investigation was to estimate potential fluorochemical exposure to Sumitomo 3M employees, using the serum component of blood as an exposure measure.

Methods

94 Sumitomo 3M employees participated in a voluntary, cross-sectional, fluorochemical serum measurement. It is assumed that serum fluorochemicals measured represent occupational exposures, although we have not included fluorochemical air measurements from the Sagamihara plant in this assessment. Employees ranged in age from 31-67. These employees came from three job exposure groups as described in a personal communication with S. Yamana. These groups were defined as: Sagamihara Plant production employees (n=32) involved in the inspection and converting of finished goods. These workers were also involved with compounding, using chemicals made from POSF, and formulation of products containing additional fluorochemicals. The next group included Sagamihara Plant management employees (n=32) working primarily at the same site as the Sagamihara Plant production employees. There was no regular handling of fluorochemicals in their usual work capacities. The last group included management employees from the Tokyo Head Office who are generally not

occupationally exposed to fluorochemicals (n=30). All but two of the 94 participants were male employees.

Each study participant donated two 10 cc tubes of blood. Blood was collected between March 17 and 19, 1999. The blood was centrifuged twice for 15 minutes at 3000 RPM immediately after drawing to obtain serum. The serum was shipped frozen to the 3M Medical Clinic in St. Paul, Minnesota where it was distributed to the appropriate laboratories for analysis. Following analyses, individuals were notified of their results by confidential letter.

Laboratory Analysis

Individual serum was analyzed for seven fluorochemical compounds using high-pressure liquid chromatography electrospray tandem mass spectrometry measurement (LC/MS/MS). Northwest Bioanalytical (NWB) Salt Lake City, Utah analyzed five of the fluorochemical compounds. They analyzed the serum for perfluorooctane sulfonate (PFOS), perfluorooctane sulfonamide (PFOSA), n-ethyl perfluorooctanesulfonamido acetate (PFOSAA), perfluorooctanoate (POAA), and perfluorohexane sulfonate (PFHS). NWB conducts all studies within the guidelines of the US FDA Good Laboratory Practice, the OECD Principles of Good Laboratory Practice and the Japanese MHW Good Laboratory Practice Standard Ordinance for Nonclinical Laboratory Studies on the Safety of Drugs (Ordinance No. 21, PAB Notification No. 424). The 3M Environmental Technology and Services Laboratory analyzed the serum for perfluorooctanesulfonamido acetate (M556) and n-methyl perfluorooctane sulfonamido acetate (M570).

Northwest Bioanalytical's validated LC/MS/MS provided a lower limit of quantitation (LLOQ) in parts per million (ppm) of 0.0314 (PFOS), 0.001 (PFOSA), 0.00223 (PFOSAA), 0.00527 (POAA), and 0.00261 (PFHS).

3M Environmental Technology & Services Laboratory provided a lower limit of quantitation (LLOQ) in parts per million (ppm) for M556 that ranged from 0.01-0.025 ppm, and M570 that ranged from 0.01-0.05 ppm.

Data Analysis

Data were analyzed using standard statistical software programs. Comparisons of arithmetic means was done using student's t test. A statistically significant difference in one of the means was set at $p = .05$ or less.

Results

PFOS

Table 1 summarizes the serum PFOS, POAA and PFHS values for the Sumitomo 3M employees. Twenty-four PFOS values were reported below the lower limit of quantitation (0.0314 ppm). Twelve (38%) of 32 Sagamihara plant management employees had values less than the lower limit of quantitation (<LLOQ). The range of PFOS for the remaining 20 employees in this group was 0.032-0.057 ppm. Descriptive statistics and t-tests were calculated using the LLOQ value (0.0314 ppm) for those individuals who had values below the LLOQ

Forty percent of the Tokyo Head Office employees had PFOS values <LLOQ. The range of measurable PFOS was 0.033-0.0967 ppm for the remaining employees. The remaining statistics were calculated using the LLOQ value (0.0314 ppm). PFOS was quantifiable in all 32 Sagamihara Production employees. The arithmetic mean value was 0.135 ppm (range: 0.0475-0.628 ppm) which was significantly different ($p<0.05$) than the mean PFOS value for employees in both the Tokyo Head Office and Sagamihara Plant managers group.

POAA

As per Table 1 the lower limit of quantitation for POAA was 0.00527 ppm. Three of the 94 employees had a POAA value reported less than the lower limit of quantitation. One of these employees was from the Sagamihara Plant management and two were from the Tokyo Head Office. The midpoint between zero and the LLOQ was utilized in calculating the statistics for POAA. The mean POAA value for the Sagamihara Production employees was 0.201 ppm that was significantly different ($p<0.05$) than the mean POAA values for the Tokyo Head Office and Sagamihara Plant managers group.

PFHS

As per Table 1 fourteen percent of employees had PFHS values reported <LLOQ (0.00261 ppm). Ten were employees from the Sagamihara plant management group and three were from the Tokyo Head Office. The midpoint between zero and the LLOQ (0.0013 ppm) was utilized to calculate the univariate statistics for the Sagamihara plant management group. The arithmetic mean value for this group was 0.00626 ppm with a range of 0.0027-0.0241 ppm. Again, the arithmetic mean PFHS (0.020 ppm) for the Sagamihara Production employees was significantly different than the other exposure groups ($p<0.05$).

PFOSAA, PFOSA

We chose not to statistically analyze PFOSAA and PFOSA because of the large number of employees who had serum values reported below the lower limit of quantitation. Eighty-four percent of all Sumitomo 3M employees had PFOSAA values reported below the lower limit of quantitation (0.00223 ppm). Fifteen of 94 employees had a PFOSAA value reported between 0.00247 and 0.0133 ppm.

The lower limit of quantitation for PFOSA is 0.001 ppm. Four of the 94 employees had a detectable level of PFOSA. These values ranged from 0.00241-0.0068 ppm.

M556, M570

We chose not to statistically analyze M556 and M570 because of the large number of employees who had serum values reported below the lower limit of quantitation. Only one Sumitomo 3M employee had a quantifiable level of M556 (0.0032 ppm).

Ninety-three percent of all employees had M570 values reported below the lower limit of quantitation. The average M570 value for the seven employees with quantifiable levels of M570 was 0.007 ppm. The range was 0.00311-0.0144 ppm.

Discussion

Ninety-four Sumitomo 3M employees participated in a voluntary, cross-sectional serum fluorochemical assessment. Thirty-two employees were identified as Sagamihara Plant production employees involved in the inspection and converting of finished goods, doing some compounding, using chemicals made from POSF, and doing some formulation of products containing other fluorochemicals. Thirty-two employees were described as Sagamihara Plant management employees who work primarily at the same site as the Sagamihara Plant production employees. Finally, 30 management employees from the Tokyo Head Office who are not usually exposed to manufactured fluorochemicals participated in the assessment.

Employees described as having the highest exposure to POSF chemistry had PFOS levels three times higher than employees in both the Sagamihara plant management group and the Tokyo Head Office. The mean serum PFOS level for plant production employees was 0.135 ppm (range: 0.0475-0.628 ppm) compared to mean PFOS values of 0.0403 ppm (range: 0.0319-0.0566 ppm) for Sagamihara Plant management employees and mean PFOS of 0.0523 ppm (range 0.033-0.0967 ppm) for Tokyo Head Office employees.

Serum fluorochemical levels in Sumitomo 3M employees can be compared to serum levels recently measured in 3M employees occupationally exposed to fluorochemicals in facilities outside of Japan (Table 2). Serum fluorochemical levels were measured in employees at 3M Decatur Alabama as part of an on going medical monitoring program in 1997. Since 1980 the Decatur plant is the primary location of the production of certain fluorochemicals that may ultimately be measured in the serum as PFOS. Arithmetic mean serum PFOS levels in 1997 were 1.96 ppm (range 0.10-9.93 ppm).[1] Outside the United States 3M manufactures PFOS related materials at its Antwerp, Belgium facility. Arithmetic mean serum PFOS levels in Antwerp employees in 1997 were 1.5 ppm (range: 0.1-4.8 ppm).[1]

In 1998 31 3M Center employees in St. Paul, Minnesota were tested for serum PFOS. All were corporate staff or division managers. None had worked in fluorochemical

production. Employees ranged in age from 37-62 years. Arithmetic mean PFOS was 0.047 ppm (range: 0.028-0.096 ppm). Both the arithmetic mean and range for this group are similar to the arithmetic mean and range PFOS of the Sagamihara Plant management (mean=0.0403; range=0.0319-0.0566) and Tokyo Head Office (mean=0.0523; range=0.033-0.0967).

Conclusion

Ninety-four Sumitomo 3M employees participated in a voluntary, cross-sectional serum fluorochemical assessment. Serum levels were highest for Sagamihara Production employees. This is the same group where there is potential for greater occupational fluorochemical exposure, although industrial hygiene measurements were not part of this assessment. Serum levels of PFOS for Sagamihara Plant management and Tokyo Head Office employees were similar to levels seen in a small group of US 3M Center employees who were not occupationally exposed to PFOS. PFOS levels are well below levels found in workers at plant sites where POSF chemistries are produced (Decatur, Antwerp). None of these levels would be expected to be associated with health problems based on our current understanding of these compounds.

Table 1

Serum Fluorochemical Levels in Sumitomo 3M Employees

		PFOS (ppm) LLOQ=0.0314	POAA (ppm) LLOQ=0.00527	PFHS (ppm) LLOQ=0.00261
Sagamihara Plant Production Employees N=32	Mean	0.135	0.201	0.020
	Median	0.0919	0.0496	0.010
	Std Dev	0.131	0.6265	0.0283
	Range	0.0475-0.628	0.0189-3.600	0.00287-0.117
Sagamihara Plant Management N=32	Mean	0.0403	0.0117	0.00626
	Median	0.0385	0.0096	0.0047
	Std Dev	0.0071	0.0095	0.0054
	Range	0.0319-0.0566	0.00264-0.059	0.0027-0.0241
Tokyo Head Office N=30	Mean	0.0523	0.0126	0.0048
	Median	0.046	0.01195	0.0038
	Std Dev	0.0191	0.00618	0.003
	Range	0.033-0.0967	0.00264-0.029	0.0013-0.016

Table 2

Mean Serum PFOS Levels

	3M Center 1998 N=31	Decatur 1997 N=84	Antwerp 1997 N=65	Sagamihara Production N=32	Sagamihara Plant Mgmt N=32	Tokyo Head Office N=30
Mean PFOS (ppm)	0.047	1.96	1.48	0.135	0.0403	0.0523

REFERENCES

Olsen GW, Burris JM, Mandel JH, Zobel LR. Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. J Occup Env Med (1999, in press).

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

Analysis of Selected Decatur Employee Serum for Sulfonic and Carboxylic Fluorochemicals

This 3M technical report analyzed the 5 highest organic fluorine containing Decatur employee samples and analyzed the serum for perfluorooctanesulfonate, perfluorohexanesulfonate and perfluorooctanoate. In decreasing percent found, perfluorooctanesulfonate contributed between 55 - 80%, perfluorooctanoate contributed between 15 - 30% and perfluorohexanesulfonate contributed 5 - 15% of the organic fluorine. Analytical methods used were electron capture and microwave plasma detector.

3M TECHNICAL REPORT

Report Number: 7230

Date: October 4, 1979

TITLE: Analysis of Selected Decatur Employee Serum for Sulfonic and Carboxylic Fluorochemicals

AUTHOR:

ABSTRACT:

The precence of C_6 and C_8 sulfonic acids was detected in certain blood samples using both electron capture and microwave plasma detector methods.

CONFIDENTIAL

FORM 2643-... REV

CENTRAL ANALYTICAL LABORATORY

Report No. 7230

Date October 4, 1979

CONFIDENTIAL

Subject: Characterization of Decatur RF Values

Requestor:

Dept. Name Commercial Chemicals Proj. No. 91721100

Request No. A73629

Dated October 3, 1979

Report:

The serum of 10 selected 3M Decatur employees was submitted (A72754) in June, 1979 for analysis of organic fluoride levels and the total organic fluoride values were reported in Analytical Report 7194. Subsequent to that work, it became of interest to further characterize the fluoride serum value and attempt to identify the presence of 3M fluorochemicals.

Initial analysis (7/18/79) by the microwave plasma detector (MPD) established the presence of the expected perfluorooctanesulfonate and perfluorooctanoate anions in the serum extract of -25 and -22. However, the observation of perfluorohexanesulfonate anion in the above extracts was unexpected and it therefore became necessary to spend additional analytical development time in order to measure these 3 fluorochemicals in the same serum extract sample. During this time, the method was evaluated on the electron capture (EC) gas chromatograph. The EC detector would be expected to have greater sensitivity than the MPD for these fluorochemicals but with less specificity since the MPD can be operated on the fluorine channel eliminating any confusion by other EC active compounds.

We selected the 5 highest fluorine containing Decatur samples and analyzed the serum extract for perfluorohexanesulfonate, perfluorooctanesulfonate, and perfluorooctanoate anion. We used both the MPD and EC detection systems and report the following values:

		% FOUND①			
Person	RF (ppm)②	Perfluorohexanesulfonate	Perfluorooctanesulfonate	Perfluorooctanoate	Total
-22	10.1	5%	60%	30%	95%
-23	5.7	15%	70%	20%	105%
-24	9.4	5%	80%	15%	100%
-25③	11.8	5%	55%	20%	80%
-26	4.1	10%	65%	25%	100%

①The analysis was conducted on a single determination (1 ml of serum) due to limited availability of sample and time and represents our interpretation of the average of the EC and MPD values. No internal standard was used; expect ±10-20% accuracy.

②AR 6962, 7028, 7194

③Previously identified as -21(Z) due to mis-labeling by medical personnel.

CONFIDENTIAL

The above results are necessarily somewhat approximate due to the lack of an internal standard and continual refinements in the derivatization and GC of the corresponding methyl ester. Sufficient sample remains for additional analysis. Further improvements in the analysis are and will continue to be made as time permits. We thought it best to report these values now, especially since we identified the presence of perfluorohexanesulfonate in human serum extract and no toxicology studies to date have been completed on this fluorochemical.

Three years ago (10-18-76) we reported that Decatur employee . serum extract contained perfluorooctanesulfonate (based on F/NMR) and a minor fluorine containing component now identified as perfluorooctanoate. Therefore, the presence of perfluorooctanesulfonate in this individual has been known for some time. Now, with improved analytical methods, we have greater sensitivity and can determine the presence of perfluorohexanesulfonate in addition to perfluorooctanesulfonate anion.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Fluorochemical Control Study

This is a report of a cross-sectional correlation analysis of medical surveillance data collected in 1981 of Decatur chemical and film plant workers and their total organic fluorine levels. There were no statistically significant correlations reported between SGOT, SGPT, GGT, alkaline phosphatase, total bilirubin, red blood cells, hemoglobin and cholesterol for either the Decatur chemical or film plant workers. The report was reviewed by Dr. Leonard Schuman, Professor and Director of the University of Minnesota Division of Epidemiology Department (letter attached). Although this report does not specifically mention perfluorooctanesulfonate, it is submitted as it chronologically followed the 3M Technical Report that observed perfluorooctanesulfonate was a component of total organic fluorine in the sera of 5 Decatur employees.

To F. A. UBEL, M.D. - MEDICAL DEPARTMENT - 220-2E-02

From D. E. ROACH, M.D. - MEDICAL DEPARTMENT - 220-2E-02

Subject: Fluorochemical Control Study

Date: May 25, 1982

3M

The review of the 1981 Decatur fluorochemical control study has been completed. Statistical analysis was performed by Riker Research Data operations. In addition, the conclusions and statistical analyses were in turn reviewed in the Department of Epidemiology at the University of Minnesota.

The purpose of this study was to compare certain blood chemistry tests between two population groups assumed to be similar except for their work histories. The Decatur plant site was a logical selection for this review. Participation by both the Film Plant and the Chemical Plant employees was on a voluntary basis. 317 employees (out of 450) in the Chemical Plant and 306 (out of 600) in the Film plant took part in the study. Although participation was lower in the Film Plant these numbers were felt to be adequate for statistical evaluation. Any employee in the Film Plant group with more than three months history of work within the chemical plant was removed from this control group.

These populations were compared with respect to the distribution of several test results such as liver enzymes (SGOT, SGPT alkaline phosphatase, GGTP), some hematology parameters (hemoglobin and red blood cell count), cholesterol, and lastly organic fluorine levels.

In summary, we found no significant difference in the distribution of those blood test results between employees working in the Chemical Plant and those in the Film Plant control group. There was also no significant correlation between these test results and blood levels of total organic fluorine.

These results further confirm our clinical impression that to date we are unable to demonstrate evidence of illness or disease patterns among our fluorochemical exposed employees.

Efforts should continue in reducing employee exposure opportunities to help lower blood fluorine levels. Follow-up health evaluations will be continued on a regular basis. Timing and structure of this program is presently under review. If there are any questions, please call.

DER/cr

Fluorochemical Control Study

For several years health evaluations have been conducted at two major chemical plant production sites. The purpose of these evaluations was to determine whether or not any patterns of disease could be detected that might be related (1) to the exposure of employees to certain fluorochemicals and (2) to the presence of these fluorochemicals in the blood of the workers.

To date no pattern of disease has been identified. However, two additional studies had not been performed. One was the collection of total blood organic fluorine levels on chemical plant employees. The other was to develop a suitable control.

Both of these became available in 1981. The control study was undertaken at a plant site with two separate divisions represented by two manufacturing sites located approximately 500 yards apart. All were 3M production employees and the mobility of the employees between the two sites was relatively low since two separate manufacturing divisions were involved.

One objective was to compare the incidence of abnormal values of certain blood tests seen at each plant. Those tests to be compared were:

1. SGOT
2. SGPT
3. Alphaline phosphatase
4. GGPT
5. Cholesterol
6. Hemoglobin
7. Red Blood Count

"Abnormal" was to be any variation from normal despite clinical assessment.

Another objective was to see if there was a correlation between these blood test results and the levels of total blood organic fluorine.

The chemical plant employs approximately 450 people. These employees take part in a general health evaluation annually which among other things includes a chemistry profile and a CBC. Participation in the health testing is voluntary. The testing is done aboard a commercial testing van which regularly visits the joint plant site.

Total organic fluorine blood tests were drawn separately in the nursing department throughout the year at this Chemical Plant. These tests were spaced over 1981 and were sent to St. Paul for the analytical determination. The variations in organic fluorine levels changed slowly due to delayed excretion. Therefore, although not drawn simultaneously with the chemistry profile this was not thought to be a major inaccuracy in the comparison with the chemistry profile.

317 employees participated including several office personnel.

The Film plant employs approximately 600 people and had not previously been involved either in health evaluations nor in organic fluorine blood testing. Here again participation was voluntary. These employees were tested in a random manner and interspersed among the chemical plant employees on the van each day. In addition blood fluorine samples in the Film group were also drawn on the van following the same protocol as when drawn in the nurses station. The blood fluorine samples were then taken promptly to the nurses station for centrifuging, packaging and deliver to St. Paul as is the normal routine.

306 employees participated and were almost entirely production workers. Within this group any employee identified as having worked in the chemical plant for over three months were removed from this group creating a subgroup of 210 solely film plant employees.

In summary, the blood fluorine samples were handled differently between the two plants. Otherwise all blood testing was felt to be identical within both groups. The three groups to be analyzed were:

1. Chemical Plant employees (317)
2. Film Plant employees including approximately 33% of people who at one time had spent some time in the chemical plant (306).
3. Those film plant employees who had essentially no previous exposure to the chemical plant (210). The attached results and statistical analyses are attached.

PRELIMINARY DATA

ANALYSIS "ABNORMAL" LIVER ENZYMES FILM VS. CHEMICAL PLANTS

LOCATION	CHEMICAL	FILM	FILM MINUS WORKERS IN CHEM PLANT >3 MOS
NUMBER TESTED	327	306	210
EMPLOYEES WITH 1 OR MORE "ABNORMAL" ENZYMES	37 (11.3%)	26 (8.5%)	20 (9.5%)
NUMBER ABNORMAL TESTS			
GGTP	24 (65%)	12 (46%)	10 (50%)
SGPT	11 (30%)	9 (35%)	7 (35%)
ALK. P'TASE	6 (16%)	7 (27%)	3 (15%)
% OF ALL TESTED WITH ABNORMAL:			
GGTP	(7.3%)	(3.9%)	(4.8%)
SGPT	(3.4%)	(2.9%)	(3.3%)
ALK. P'TASE	(1.8%)	(2.3%)	(1.5%)
IF GGTP REMOVED FROM ALL TESTED % OF PEOPLE WITH "ABNORMAL ENZYMES"	5.8%	6.9%	7.1%

DECAT81 = 324 obs
Film 81 = 300 obs
Film81 w/o Chem = 206 obs

CORPORATE MEDICAL CORRELATIONS BETWEEN LIVER VALUES AND FLUORINE

LIVER TEST	DECATUR FILM	DECATUR FILM WITHOUT CHEMICAL WORKERS	DECATUR CHEMICAL
SGOT	.00596 .9191 293	.03647 .6109 197	-0.08539 .2328 197
SGPT	-0.02662 .6475 293	.02044 .7756 197	-0.05440 .4477 197
GGTP	-0.07622 .1940 292	-0.0575 .4800 196	-0.00369 .9589 197
ALKPHOS	-0.01766 .7630 294	.09358 .1909 197	.02925 .6632 197
TBILI	-0.05636 .3355 294	-0.05269 .4621 197	-0.03291 .6462 197
RBC	-0.09570 .1015 294	-0.03386 .6367 197	-0.01872 .7946 196
HGB	-0.08624 .1402 294	-0.01737 .8086 197	-0.01159 .8719 196
CHOLESTEROL	.03607 .5378 294	.04051 .5720 197	-0.07620 .2872 197

NOTE:
FIRST VALUE IS CORRELATION BETWEEN LIVER TEST AND BLOOD FLUORINE
SECOND VALUE IS THE SIGNIFICANCE PROBABILITY OF THE CORRELATION

CORPORATE MEDICAL CORRELATIONS BETWEEN LIVER VALUES AND FLUORINE
CALCULATION INVOLVE ONLY EMPLOYEES FROM CHEMOLITE HAVING 1981 HEP EXAMS

LIVER TEST	CHEMOLITE
SGOT	.01811 .7748
SGPT	.10291 .1031
GGTP	.06442 .3084
ALKPHOS	.12979 .0395
TBILI	-0.10845 .0858
RBC	-0.08738 .1693
HGB	-0.04083 .5214
CHOLESTEROL	.12222 .0526
AGE	.08278 .1859

NOTE:
FIRST VALUE IS CORRELATION BETWEEN LIVER TEST AND BLOOD FLUORINE
SECOND VALUE IS THE SIGNIFICANCE PROBABILITY OF THE CORRELATION

UNIVERSITY OF MINNESOTA
TWIN CITIES

Division of Epidemiology
School of Public Health
Owen Mayo Memorial Building
420 Delaware Street S.E.
Minneapolis, Minnesota 55455

REC
APR 30 1982

April 29, 1982

Dr. Frank Ubell
Medical Director
Medical Department, Medical Services
220-2E 3M Center
St. Paul, MN 55144

Dear Dr. Ubell,

I recently had the opportunity and the privilege of examining the protocol for and the data from the studies undertaken by Dr. Roach of your department on fluorochemical exposures in one of your chemical plants with a control population drawn from a film plant under your jurisdiction. I was provided with information on the size of the respective plant populations, the extent of their participation, the clinical laboratory tests on several blood parameters, the intervals of testing for blood fluorine levels, the disease patterns in these populations and the data derived therefrom.

I was impressed with the care taken to avoid bias in collection and testing of the blood samples and the insight and sophistication of the data analyses by Hawkinson and McCormack. Although participation in the health testing was voluntary the response in the chemical plant was high as would be expected (317 out of 450). (The participation in the health evaluation was even higher.) Participation in the control plant, although lower (306 out of 600), was deemed adequate for control purposes. Those controls who did have earlier exposure of 3 months or more were properly set apart as a separate subgroup.

In examining the distributions of the results on the clinical chemistry noteworthy were the similarities among all the groups. Although fluorine levels revealed increases, correlations with the clinical tests were insignificant.

In the absence of any disease patterns elicited by history and clinical examination and the general similarities in the distributions of such test results as for SGOT, SGPT, alkaline phosphatase, GGPT, cholesterol, hemoglobin and red blood cell counts, one must conclude that virtually no evidence for even pre-clinical toxicity

exists. Compartmentalization of the data on a spectrum of length of exposure and blood fluorine levels, although a refinement, would probably yield little or no additional information since the N's in the strata would be reduced to inadequate levels for significance.

Continued clinical followup on an annual basis with a view to eliciting possible chronic fluorocarbon toxicity would be feasible and worthwhile in your setting.

Thank you for permitting this appraisal of your protocol and data.

Sincerely yours,

Leonard M. Schuman, M.D.
Professor and Director

LMS:sn

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Working Memorandum on Data Quality Assessment (of the Mean and Range of PFOS (ppb) Levels in Current and Historical Human Populations)

This report documents Battelle's evaluation of the statistical quality and defensibility of 12 data sources of serum perfluorooctanesulfonate (PFOS) levels found in non-occupational human populations. These data were summarized in the 3M white paper, "Perfluorooctane Sulfonate: Current Summary of Human Sera, Health and Toxicology Data" provided to the EPA in January, 1999. The purpose of this initial collection of human non-occupational serum samples by the 3M Medical Department was to determine the presence or absence of PFOS in human blood from current and historical samples. 3M requested a review of these data by Battelle to determine what additional inferences could be obtained by analyses of these data. Battelle statisticians felt that only the pooled serum from the 18 U.S. blood banks was judged defensible for a statistical analysis. Of these data, the geometric mean PFOS blood level was 28.2 ppb (95% confidence interval 23.9-33.3). Regional differences could not be explained without additional data and analyses.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Identification of Fluorochemicals in Sera of Children in the United States

The objective of this study is to determine in a sample of children their sera levels of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexanesulfonate, N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA), N-methyl perfluorooctanesulfonamidoacetate (M570), perfluorooctanesulfonamidoacetate (M556), and perfluorooctanesulfonamide (PFOSA). In cooperation with the University of Minnesota Department of Pediatrics, samples will be obtained that represent sera from 1,131 children (ages 2 to 12 years) from 23 states in the United States. The sera were collected as part of a large clinical trial regarding group A streptococcal infections. A total of 599 samples will be tested. Sera levels will be compared to the childrens' age, gender and state of residence. The timeline for study completion, estimated at January 1, 2001, is entirely dependent upon a validated analytical method to analyze 0.1 ml of human serum for these fluorochemicals with a lower limit of quantitation of $\leq$ 10 ppb based on high performance liquid chromatography electrospray tandem mass spectrometry methods.

Corporate Occupational Medicine Section/Epidemiology Unit
Proposal
March 16, 2000

Title: Identification of Fluorochemicals in Sera of Children in the United States

Purpose: Evidence that organic compounds containing the element fluorine chemically bonded to carbon can be found in human blood was reported in the literature more than 30 years ago. Average serum perfluorooctanesulfonate (PFOS) levels may be within the 20 to 60 ppb range in the general population. This is primarily based on pooled serum samples; thus it is difficult to ascribe findings to any specific demographic group. The purpose of this study is to determine the level of several fluorochemicals from the sera of children (2 - 12 years of age) in the United States.

Significance: Determination of the serum concentrations of selected fluorochemicals will provide for a more specific understanding of the distribution of these compounds in children. These data should be helpful in the risk characterization process.

Objectives: The objective of this study is to determine in a sample of children their sera levels of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexanesulfonate, N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA), N-methyl perfluorooctanesulfonamidoacetate (M570), perfluorooctanesulfonamidoacetate (M556), and perfluorooctanesulfonamide (PFOSA).

Protocol: The Department of Pediatrics at the University of Minnesota Medical School has stored sera from 1,131 children (ages 2 to 12 years) from 23 states in the United States. The sera were collected as part of a large clinical trial regarding group A streptococccal infections. [See Pediatrics 1998;101:86-88.] Sera were obtained between January 1994 and March 1995. These children presented with signs and symptoms of acute-onset pharyngitis. All of the 1,131 children had positive throat cultures at the initial visit. Sera have been kept frozen at -20°C. At 3M's request, the Department of Pediatrics has agreed to provide 3M up to 0.1 ml per individual. An initial pilot sample was conducted to determine whether the presence or absence of fluorochemicals can be determined with 0.1 ml serum. These findings suggested that method validation should proceed. Because we are uncertain of the population distribution, it is possible to calculate sample size based on tolerance limits which are independent of the form of the distribution. However, the tolerance limits will be wider than a parametric distribution (Natrella MG. Experimental Statistics New York:Wiley & Sons, 1966). For example, the largest observation in a sample of 45 individuals of a given age and gender will be an upper limit on 95% of that specific population with confidence level 0.90. In this particular study, the sample size will be limited by the number of samples, especially in the youngest age ranges. On the other hand, the use of bootstrap methods will allow for some smoothing of data thereby increasing the precision of tolerance limits and reducing the sample size requirements (personal communication, Dr. Timothy Church, biostatistician, Univ of MN). Therefore, the following samples are proposed to be analyzed in this study:

Age Group	Total N	Total Sampled (%)
2	27	27 (100)
3	51	51 (100)
4	81	81 (100)
5	122	100 (82)
6	146	80 (55)
7	161	60 (37)
8	131	40 (31)
9	135	40 (30)
10	109	40 (37)
11	87	40 (46)
12	81	40 (49)
Total	1131	599 (53)

For those ages where the sample size is less than 100%, we will obtain equal percentages by gender. Age (birth date was not collected), gender and state of residence for each sample will be provided.

Fluorochemicals to be analyzed are PFOS, PFOA, PFHS, PFOSAA, M570, M556 and PFOSA. Prior to this study proceeding, there must be a validated method to analyze for these fluorochemicals in 0.1 ml of human serum using high performance liquid chromatography electrospray tandem mass spectrometry methods. The validated analytical method should have a lower limit of quantitation of $\leq$ 10 ppb. This validated procedure will be performed by Northwest Bioanalytical with technical assistance from the 3M Environmental Laboratory.

PI/location/cost: Geary Olsen will be the principal investigator on this project. Dr. Timothy Church (Associate Professor, University of Minnesota Division of Occupational and Environmental Health) will serve as the biostatistician. Jean Burris, Dr. Kris Hansen, Dr. Jeff Mandel and Dr. Larry Zobel will serve as 3M co-investigators. Total estimated cost of the study is $250,000.

Timeline: The timeline for study completion is entirely dependent upon a validated analytical method to analyze 0.1 ml of human serum for these fluorochemicals with an LLOQ of $\leq$ 10 ppb. If such a method is validated by June 2000, then it is estimated that the sample analyses will be completed by late summer. Statistical analyses of the data should then be completed by October 1st with a draft final report written by November 15, 2000. Review and approval may take an additional 45 days. Therefore, a final report is estimated to be available by January 1, 2001. A final report will be delayed should the validation of the analytical method take longer than expected.

WORKING MEMORANDUM
on
DATA QUALITY ASSESSMENT
by

Bruce Buxton, Warren Strauss, Owen Chang
BATTELLE
September 22, 1998

This report documents Battelle's evaluation to date of the statistical quality and defensibility of 12 data sources presented by 3M staff who suggested that these data sources might be used to estimate blood levels of perfluorooctanesulfonate (PFOS) in non-occupational human populations. According to 3M, these data were collected to help address three discovery questions posed to the 3M Medical Department: (1) Is PFOS detectable in individual human blood from a corporate-based 3M employee population known not to have worked in 3M fluorochemical manufacturing plants (but knowledgeable about fluorochemicals)? (2) Is PFOS detectable in general population pooled blood samples from different geographical locations across the United States? and (3) Is PFOS detectable in human blood which would have been collected prior to the introduction of PFOS-based chemicals into the marketplace of the general population? Subsequently, Battelle was asked whether any further statistically defensible inferences could be derived from these data; this report addresses that question. In this report we (1) list important characteristics which data sets must have in order to support statistically defensible inferences, (2) judge each of 3M's 12 data sources against these criteria, and (3) statistically analyze the available data, where appropriate.

Our findings from this initial assessment of statistical data quality can be summarized in the following points:

- We suggest 12 criteria which define statistically defensible data sources. Of the 12 data sources presented by 3M staff, no source was judged sufficient on all 12 criteria, and only one source, the 1998 pooled serum from U.S. blood banks (data source A3), was judged sufficient for subsequent statistical analysis.
- The 1998 pooled serum blood bank data indicated a national geometric mean PFOS blood level of 28.2 ppb with a 95% confidence interval of 23.9 to 33.3 ppb. Significant regional differences are apparent but can not be explained without additional data and analysis.

Data Characteristics for Statistical Defensibility

In order to make statistically defensible conclusions from a set of data, several requirements must be met regarding design of the study and implementation of the data collection effort. Each of these requirements can be associated with important quantitative assumptions underlying the numerical models which are used to make statistical inferences. In our assessment here, we considered three different types of estimation problems regarding PFOS blood levels which one might want to consider: (1) temporal changes in PFOS blood levels over the past several decades, (2) spatial trends in PFOS blood levels for various regions across the U.S., and (3) demographic trends in PFOS blood levels among various subpopulations in the U.S., or worldwide. Table 1 summarizes 12 statistical requirements for data defensibility, and associates each with the three types of estimation problems listed above. Note in this table that the first five

Table 1. Statistical Requirements for Defensible Quantitative Conclusions

Statistical Requirements	Types of Estimation Problems		
	Temporal Changes	Spatial Trends	Demographic Trends
1. Detailed documentation of the study objectives and approach, including sampling design (number and types of samples), and sampling and chemical analysis protocols	✔	✔	✔
2. Specification of a well defined target population about which inference is to be made (e.g., the entire U.S. population, or some specified subpopulation)	✔	✔	✔
3. Unbiased data collection protocols which ensure that all samples are demographically representative of the target population; generally this implies random sampling from the target population	✔	✔	✔
4. Along with PFOS blood levels, accurate collection of important predictor or explanatory information, such as demographics like age, gender, race, etc.	✔	✔	✔
5. Compositing of samples, when done, is performed only for samples within the same subpopulation so that important potential differences among subpopulations are preserved in the resulting data	✔	✔	✔
6. Sampling is performed at several (i.e., at least three) points in time which are spaced at regular time intervals (e.g., every five years)	✔		
7. At each point in time, sampling is performed cross-sectionally from the entire target population or subpopulations	✔		
8. Sampling is performed to provide uniform coverage of the entire spatial domain of interest (e.g., the entire U.S.)		✔	
9. Data is collected both for multiple locations (e.g., regions or cities) across the spatial domain and for multiple individuals within each location		✔	
10. All data are collected at approximately the same point in time to eliminate the potential for confounding due to temporal trends		✔	
11. Specification of target subpopulations which are well defined in terms of important demographic parameters (e.g., age, gender, race, etc.) and from which sampling is randomly performed			✔
12. Either fix sampling at one time and spatial location (e.g., city) or ensure that sampling for all demographic subpopulations is performed in a balanced design across the same times and locations (i.e., to eliminate potential confounding due to time or location)			✔

requirements are more general in nature and apply equally to all three types of estimation problems, while the last seven requirements apply more specifically to a different type of estimation problem.

Assessment of 12 Data Sources

On May 12, 1998, 3M staff faxed to Battelle a table listing 12 data sources they believe might be used to estimate PFOS blood levels in non-occupational human populations. This table has been retyped as shown in Figure 1. Subsequently, 3M staff sent to Battelle electronic files with the PFOS blood data they had available, as well as associated written documentation on the data sources which they had. Based on this information, Battelle attempted, and was partially successful, to obtain through literature searches more extensive documentation on these 12 studies. However, in many cases no additional written documentation could be found.

Given the information sent to Battelle by 3M and other documentation Battelle obtained from the literature search, Table 2 summarizes our assessment of how well each of the 12 data sources meets each of the 12 defensibility requirements listed in Table 1. In Table 2, a "✓" indicates that the given data source seems to meet the associated defensibility criterion, while a "X" indicates that the data source does not meet the criterion. This assessment by Battelle is based solely on the written documentation we were able to obtain, either from 3M or the literature review. In a large majority of cases, no written documentation was found and so we can not judge whether the defensibility criteria are met by a data source, or not. In these cases, a "?" is listed in Table 2. In some cases, noted as "✓?", documentation may have been found about the initial study which collected the blood samples, but no documentation was available describing how particular subsamples were selected to be sent to 3M. Also, in some cases we judged that a given data source would be appropriate for addressing just one type of estimation problem (i.e., temporal changes, spatial trends, or demographic trends), and so defensibility criteria for other problems would not be applicable. These cases are denoted by a "–" in Table 2. Table 3 lists more detailed notes about each of the data sources for those criteria where sufficient information was found in the written documentation to warrant comment.

Figure 1
Summary of Mean and Range of PFOS (ppb) Levels in Current and Historical Human Populations.
All Data Analyzed in February – April 1998

Populations	Description of Sample	Mean* (ppb)	Range* (ppb)
A. Current Populations (blood collected in 1998)			
1. Non-occupationally exposed 3M employees	31 individuals	47	28-96
2. Commercial pooled serum samples			
Intergen Laboratory	3 samples each with ≥100 donors	44	43-44
Sigman Laboratory	3 samples each with ≥100 donors	33	26-45
3. Pooled serum from 21 separate U.S. blood banks (see Figure 1 for more detail)	3 to 6 pooled samples per location with 5 to 10 donors per sample	29	9-56
B. Historical Samples (chronological order)			
1. Korean War era U.S. military recruits, 1948 to 1951	10 pooled samples with 10 donors per sample	N.D.**	N.D.
2. Swedish samples, 1957	10 individual samples	2	N.D. – 2
3. Michigan Breast Cancer Study, 1969-1971	5 individual samples	33	N.D. – 59
4. Swedish samples, 1971	10 individual samples	1	N.D. – 1
5. MRFIT pooled calibration samples, 1976	6 pooled samples with unknown number of donors per sample	31	14-56
6. MRFIT pooled calibration samples, 1980	3 pooled samples with unknown number of donors per sample	23	14-41
7. China samples (Linxian, rural province), 1984	6 individual samples	N.D.	N.D.
8. MRFIT individual samples, 1985	3 individual samples	31	N.D. – 44
9. China samples (Shandong, rural province), 1994	6 individual samples	N.D.	N.D.

* Rounded to nearest ppb
** Not detected

Note: This figure was retyped from a fax sent to Battelle on 5/12/98 without changing possible mistakes.

Table 2. Overall Assessment of Defensibility of 12 Data Sources

Statistical Requirement	Data Source											
	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
1. Detailed documentation	✓?	✓?	✓?	✓?	✓?	✓?	✓?	✓?	✓?	✓?	✓?	✓?
2. Defined target population	✓	?	?	✓	?	?	?	✓	✓	✓	✓	?
3. Unbiased sampling protocols	?	?	X	?	?	?	?	X	X	✓	X	?
4. Accurate explanatory information	✓	?	?	?	?	?	?	✓?	✓?	✓?	✓?	?
5. Appropriate compositing	–	?	?	?	–	?	–	?	?	–	–	–
6. Regular sampling in time	–	?	–	?	?	?	?	?	?	–	?	?
7. Complete sampling at each time	–	?	–	?	?	?	?	?	?	–	?	?
8. Uniform sampling across space	–	?	✓	?	?	–	?	–	–	–	–	?
9. Multiple samples locally	–	?	✓	?	?	–	?	–	–	–	–	?
10. Sampling at same time(s)	–	?	?	?	?	–	?	–	–	–	–	?
11. Defined demographic subpopulations	?	?	–	?	?	?	?	?	?	?	?	?
12. Sampling at same time(s) and location(s)	✓	?	–	?	?	?	?	?	?	?	?	?

✓ = Adequately designed and implemented
X = Inadequately designed or implemented
? = Unknown due to insufficient documentation
– = Not applicable to this data source

Table 3. Notes Regarding Defensibility of 12 Data Sources

A1. Non-occupationally exposed 3M employees
1. Detailed documentation – The only documentation is in Geary Olsen's 8/15/98 letter 2. Defined target population -- The target population was 3M employees with minimal occupational exposure 4. Accurate explanatory information – Some demographic information was collected and included, such as age and gender 12. Sampling at same time(s) and locations(s) -- The data appears to be only from employees at the corporate center
A2. Commercial pooled serum samples
1. Detailed documentation – The only documentation is in Geary Olsen's 8/15/98 letter
A3. Pooled serum from 21 separate U.S. blood banks
1. Detailed documentation – The only documentation is in Geary Olsen's 8/15/98 letter 3. Unbiased sampling protocols – There is potential bias as a result of the 50% refusal rate 8. Uniform sampling across space – The samples are geographically dispersed 9. Multiple samples locally—There are multiple regions and multiple individuals within each region
B1. Korean War era U.S. military recruits, 1948 to 1951
1. Detailed documentation – The only documentation is in Geary Olsen's 8/15/98 letter 2. Defined target population -- The target population was U.S. military recruits from the Korean War era
B2. Swedish samples, 1957
1. Detailed documentation – The only documentation is in Geary Olsen's 8/15/98 letter
B3. Michigan Breast Cancer Study, 1969-1971
1. Detailed documentation – The only documentation is in Geary Olsen's 8/15/98 letter
B4. Swedish samples, 1971
1. Detailed documentation – The only documentation is in Geary Olsen's 8/15/98 letter

Table 3. Notes Regarding Defensibility of 12 Data Sources (Continued)

B5. MRFIT pooled calibration samples, 1976
1. Detailed documentation – MRFIT is documented in Geary Olsen's 8/15/98 letter and in multiple journals including JAMA, Vol. 235, No. 6, Feb. 23, 1976, but subsampling for 3M is not documented 2. Defined target population -- The target population was men aged 35-57 with multiple risks for heart disease 3. Unbiased sampling protocols – The sampling protocol selected certain characteristics, with multiple screenings 4. Accurate explanatory information – Information about the original sample population was recorded, but no information about the pooled calibration sample is known
B6. MRFIT pooled calibration samples, 1980
1. Detailed documentation – MRFIT is documented in Geary Olsen's 8/15/98 letter and in multiple journals including JAMA, Vol. 235, No. 6, Feb. 23, 1976, but subsampling for 3M is not documented 2. Defined target population -- The target population was men aged 35-57 with multiple risks for heart disease 3. Unbiased sampling protocols – The sampling protocol selected certain characteristics, with multiple screenings 4. Accurate explanatory information – Information about the original sample population was recorded, but no information about the pooled calibration sample is known
B7. China samples (Linxian, rural province), 1984
1. Detailed documentation – Documented in Geary Olsen's 8/15/98 letter and in Journal of the National Cancer Inst., Vol. 85, No. 18, Sept 15, 1993, but subsampling for 3M is not documented 2. Defined target population -- The target population was all the residents of Linxian aged 40-69 without debilitating diseases or prior esophageal or stomach cancer 3. Unbiased sampling protocols – The sample appears to be representative of the target population 4. Accurate explanatory information -- Information about the original sample population was recorded, but no information about the individual sample is known
B8. MRFIT individual samples, 1985
1. Detailed documentation -- MRFIT is documented in Geary Olsen's 8/15/98 letter and in multiple journals including JAMA, Vol. 235, No. 6, Feb. 23, 1976, but subsampling for 3M is not documented 2. Defined target population -- The target population was men aged 35-57 with multiple risks for heart disease 3. Unbiased sampling protocols – The sampling protocol selected certain characteristics, with multiple screenings 4. Accurate explanatory information – Information about the original sample population was recorded, but no information about the pooled calibration sample is known
B9. China samples (Shandong, rural province), 1994
1. Detailed documentation -- The only documentation is in Geary Olsen's 8/15/98 letter

The main conclusion from our overall assessment of the 12 data sources is that in most cases there is simply not enough written documentation of the study design and sampling protocols to judge whether the resulting data are statistically defensible, or not. In some cases (e.g., MRFIT) documentation is available on the original study under which an entire set of blood samples was collected, but no documentation is then available on how the subsampling was performed to send samples to 3M for chemical analysis. If either the original sampling or the subsampling was biased, then the data obtained by 3M will also be biased (i.e., systematically too high or too low). Given this lack of documentation, there is currently little, if any, justification for statistically analyzing 3M's data and presenting statistical conclusions. Pooling the data across studies (e.g., to look for blood temporal, spatial, or demographic trends) is not recommended because of the widely different study objectives and target populations among the studies.

Of all the data sources provided by 3M, probably the most defensible for statistical analysis purposes is the data set of 1998 pooled serum from U.S. blood banks (data source A3). Although

detailed written documentation is lacking, it does appear that the samples may have been selected in an unbiased manner, at least within a particular blood bank. We are concerned with possible non-response bias potentially introduced by the large number of blood banks (approximately half of those contacted) which declined to send 3M the samples they requested. There are also potential biases resulting from the probable lack of control by each blood bank concerning the demographics of the donors of the samples which were sent to 3M. However, recognizing these potential serious data deficiencies which limit our ability to make valid inferences, in the next section we have subjected the resulting PFOS blood level data to a limited statistical analysis.

Statistical Analysis of Blood Bank Data

The recently collected and analyzed pooled blood samples from an independent Minnesota and 17 geographically dispersed Red Cross blood banks have been combined to estimate a nationally representative mean PFOS (ppb) level. However, a variety of assumptions are necessary, including:

1) Each pooled sample is representative of the regional population (i.e., each member of the population had an equal chance of being represented in the sample).

2) Each pooled sample has a known number of donors, and each donor is represented equally in the sample.

3) The sampling and analytical methods for obtaining serum PFOS levels is the same across all samples.

It is unlikely that the samples obtained by 3M completely satisfy all of the above assumptions. Also, the data have the following additional limitations:

1) The blood banks represent only those that donated blood – i.e., cities where the blood bank(s) refused to participate had no chance of being represented in 3M's sample. Furthermore, it is unlikely that the samples obtained from participant blood banks are representative of the general population in each geographic region. Rather, these samples are representative of people who donate blood to the Red Cross.

2) It is unknown how many donors are in each pooled sample, nor is it known how each donor was selected.

3) Most of these samples came from Red Cross blood banks, so it is likely those sampling methods are the same. The Minnesota samples probably do not have significantly different methods. As all of the analysis was performed by 3M, the analytical methods should be the same.

Although these data may not satisfy all of the assumptions detailed above, the following random effects model was used to estimate a national geometric mean PFOS level:

$$\ln(PFOS_{ij}) = \mu + B_i + \varepsilon_{ij}, \text{ where}$$

$\ln(PFOS_{ij})$ represents the natural-log transformed PFOS level associated with the jth pooled sample from the ith Blood Bank, $\exp(\mu)$ is the national geometric mean PFOS level, B_i is the difference (on the natural log scale) between the mean PFOS level at the ith blood-bank and the national mean, and ε_{ij} is the residual error left unexplained by the model (the difference between the individual sample PFOS measurements and the mean PFOS level at its corresponding blood bank). It is assumed that B_i follows a normal distribution with mean zero and variance $\sigma^2_{\text{Between Blood Banks}}$, and that ε_{ij} follows a normal distribution with mean zero and variance $\sigma^2_{\text{Within Blood Banks}}$.

Table 4 provides the estimates obtained from fitting this model to the data. Table 5 lists the individual region geometric means which were found to fall outside the 95% and 99% confidence intervals for the overall geometric mean.

Table 4. Parameter Estimates for Blood Bank Data

Parameter	Estimate	95% Confidence Interval	99% Confidence Interval
National Geometric Mean PFOS Level (e^{μ}) (ppb)	28.2	(23.9, 33.3)	(22.5, 35.4)
σ^2 Between Blood Banks $[\ln(ppb)]^2$	.095	-	-
σ^2 Within Blood Banks $[\ln(ppb)]$	.052	-	-

Table 5. Regions With Geometric Means Falling Outside Confidence Intervals for Overall Geometric Mean

Region	n	Geometric Mean (ppb)	Outside 95% CI	Outside 99% CI
Santa Barbara, CA	3	15.9	✓	✓
Omaha, NE	5	16.2	✓	✓
Billings, MT	3	22.4	✓	✓
Grand Rapids, MI	3	23.1	✓	
Cheyenne, WY	3	23.3	✓	
East Orange, NJ	3	23.5	✓	
Newark, DE	5	23.9	✓	
Anchorage, AK	3	25.3		
Las Vegas, NV	3	26.6		
Santa Rosa, CA	3	27.3		
Corpus Christi, TX	3	28.9		
Kansas City, MO	3	29.6		
Davenport, IA	3	31.9		
Scottsdale, AZ	3	36.5	✓	✓
Meridian, MS	3	37.5	✓	✓
Minneapolis, MN	6	45.6	✓	✓
Lafayette, LA	3	45.7	✓	✓
Greenville, SC	3	51.5	✓	✓

The above results demonstrate that 12 of 18 blood banks had geometric mean PFOS levels that fell outside the 95% confidence interval constructed for the estimated national geometric mean PFOS concentration. This high number of regional means falling significantly above and below the overall mean is a result of the fact that there is significant variation between the geometric

mean PFOS concentrations observed at each blood bank. It is possible that there are meaningful factors that can explain a portion of this variability. For example, the blood banks in Minneapolis, MN, Lafayette, LA and Greenville, SC may be located in close proximity to industries that apply 3M products (such as carpet manufacturers), while Santa Barbara, CA, Omaha, NE and Billings, MT may be isolated from such exposure sources. However, differences in the donor population at each blood bank may be another possible explanation for the observed variability between blood banks. To investigate the reasons for these regional differences, additional data analysis with more explanatory information would be required.

Resampling the 18 Blood Banks

3M asked Battelle their opinion of the statistical merit of resampling these 18 blood banks to help evaluate the uncertainty in PFOS blood levels within geographical locations. In the current data, there are variations of 20 ppb or more between the minimum and maximum PFOS levels measured in different pooled samples from the same geographical location.

If additional information can be collected from the blood banks, Battelle recommends that the most beneficial data would be demographic information on the pooled samples which were previously sent to 3M. We suggest that it is most important at this point in time to better understand the factors influencing the PFOS blood level variability. Collecting new blood samples without demographic information would help quantify the variability one should expect from the 18 local populations, but it would not help explain the source(s) of that variability. For designing efficient future sampling programs, we need to know how much variability to expect, but more importantly the major environmental, behavioral, nutritional, demographic, socioeconomic, etc. factors that affect that variability so that we can try to control these factors (e.g., stratify by these factors) to obtain more accurate sampling results. A second concern with resampling these 18 locations is that they may constitute a biased sampling of the general U.S. population. By taking more samples from these cities, we might be simply generating more precise but highly biased estimates.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Serum Fluorochemical trends of out-of-county residents in CLUE I (1974) and CLUE II (1989) epidemiologic investigations.

This project is a joint collaboration between 3M and Johns Hopkins University School of Public Health researchers. The objective of this study is to analyze 358 adult sera samples that were collected in 1974 and 1989 as part of the CLUE I and CLUE II studies conducted in Washington County, Maryland. The sera samples represent out-of-county residents who participated in the study. A total of 59 samples are from individuals who donated in both 1974 and 1989. An additional total of 120 samples will be analyzed in each year of different individuals. Fluorochemicals to be measured include perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexanesulfonate, N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA), N-methyl perfluorooctanesulfonamidoacetate (M570), perfluorooctanesulfonamidoacetate (M556), and perfluorooctanesulfonamide (PFOSA). The timeline for study completion, estimated at January 1, 2001, is entirely dependent upon a validated analytical method to analyze 1 ml of human serum for these fluorochemicals with a lower limit of quantitation of $\leq$ 10 ppb, based on high performance liquid chromatography electrospray tandem mass spectrometry methods.

TITLE OF RESEARCH PROPOSAL

Serum fluorochemical trends of out-of-county residents in CLUE I (1974) and CLUE II (1989) epidemiologic investigations

1. **RESEARCH QUESTION:**

Are fluorochemicals detectable in the serum samples of individuals who donated blood to a community-based specimen bank in 1974 and 1989 and to what extent do levels change over time?

2. **RATIONALE/BACKGROUND:**

The 3M Company (sponsor of this proposal) manufactures widely-used products that contain fluorochemicals. Fluorochemicals transform metabolically to perfluorooctane sulfonate (PFOS) as an endstage metabolite. PFOS is a surfactant and used in industrial and commercial processes. PFOS concentrates in the liver and undergoes enterohepatic circulation. Subchronic studies in rats and primates suggest a cumulative toxicity resulting in lowered serum total cholesterol levels and metabolic wasting. These effects may be due to effects on fatty acid transport, metabolism, membrane functions, and peroxisome proliferation.

Exposure rates to these chemicals among the general population are unknown. Occupationally exposed individuals have been monitored for the presence of fluorochemicals (Olsen et al). Among male production employees the mean levels of perfluorooctane sulfonate (PFOS) in 1995 and 1997 were 2.19 ppm and 1.75 ppm, respectively. No substantial changes in serum hepatic enzymes, cholesterol, or lipoprotein levels were noted among individuals with levels less than 6 ppm. Only 7 individuals in 1995 and 5 in 1997 had levels in excess of 6 ppm; no marked changes in lipid or hepatic enzymes were noted. It is unknown to what extent these levels can be detected in the general population. This proposal is an exploratory study to determine for the first time whether serum concentrations of fluorochemicals among a non-occupationally exposed population increase over time and whether concentrations vary by gender or age.

3. **METHODS**

From August through November, 1974, the Campaign against Cancer and Stroke was conducted in Washington County, MD. Referred to as CLUE I (from the slogan, "Give us a Clue to Cancer"), a total of 25,802 persons donated blood, of whom 20,305 were county residents. The brief history form was completed at the time of blood collection (Appendix 1). The Campaign against Cancer and Heart Disease (CLUE II) was similar to CLUE I in most respects. It was conducted from May through October, 1989. Brief histories (Appendix 1) and blood pressures were taken, and 20 ml of blood were drawn. Participants were given a food frequency questionnaire (Appendix 1) to complete at home and were asked to return it with a toenail clipping for trace metal assays. A total of 32,898 persons participated (table 1).

For this project we will use specimens from participants who are not Washington County residents and who, therefore, are not in the analytic cohort. These individuals are referred to as the "out-of-county" group. A total of 358 samples will be selected for assays (see table 1). Fifty-nine individuals in the "out-of-county" group gave blood samples in 1974 and 1989. The age distribution of these 59 individuals in 1974 was as follows: age 25-44 years - 28; age 45-64 years - 20; age 65+ years - 11. We will analyze all of this group for serum fluorochemical levels to determine change in concentrations overtime within individuals. An additional sample of 120 individuals from CLUE I and CLUE II will be selected stratified by gender and age.

Table 1. Demographic Distribution of Total Number (N = 358) of Serum Samples to be Analyzed

	Number of Subjects	
	1974	1989
Same individuals both years (n = 59/yr)		
Females*	32	32
Males*	27	27
Different individuals each year (1974, 1989)		
Females		
<40 yrs	20	20
40-60 yrs	20	20
>60 yrs	20	20
Males		
<40 yrs	20	20
40-60 yrs	20	20
>60 yrs	20	20

*Age in 1989 is provided below (1974 data and distribution by age by gender was not known at the time this proposal was written)

Assuming the correlation between the two measurements obtained in 1974 and 1989 is 0.8, the sample size of 59 provides a statistical power of 85% to detect a 10% difference between the two time periods.

Assuming the mean PFOS levels in the age group <40, 40-60 and >60 are 25, 30, and 36 ppb, respectively, and the standard deviation is 12 ppb for each group, we estimate 120 samples (40 in each age group) will provide a statistical power of more than 95% to detect a 20% difference among age groups. This sample size will also allow for a statistical power of 78% to detect a 20% difference between two groups (eg., men and women).

Assay Measurements

Northwest Bioanalytical will analyze for up to 8 serum organofluorochemicals (including PFOS) using high pressure liquid chromatography//electrospray tandem mass spectrometry (HPLC/ESMSMS) and evaluate versus an extracted curve. Because general population serum samples range in the area of the lower limit of quantitation (LLOQ) for PFOS that is currently

used by Northwest Bioanalytical, samples from this study will not be analyzed until the LLOQ for PFOS (31 ppb) at Northwest Bioanalytical can be reduced by approximately 1 order of magnitude. This validation effort is currently underway.

4. **RISKS/BENEFITS**

For the assessment of the proposed biomarkers, data and blood were collected previously in 1974 and/or in 1989 when the subjects volunteered to participate in a campaign to collect blood for a serum bank and signed an informed consent. We cannot conceive of any potential risk at this time except for possible breaches of confidentiality. The proposed substance to be measured have not been associated with adverse health outcomes in humans. This study is the first step to determine if exposure (as measured by internal dose) has even occurred among non-occupationally exposed individuals. No information will be given to individuals. Sera will be assayed and analyzed without individual identifiers. Statistical data will be presented by groups, without individual identifiers. At this point, there is no individual benefit and virtually no individual risk.

Protection against unauthorized access will be obtained by keeping the forms and computer files in rooms that are locked when not in use, and in a wing of the Health Department that is also locked when not in use. Access to these records are authorized only for students, employees of the Training Center and collaborators of approved studies who have signed a confidentiality pledge.

5. **DISCLOSURE/CONSENT**

Persons were recruited in 1974 and in 1989. At the times of blood donation, participants signed a general consent form, allowing their blood and data to be used for research purposes. For each of these campaigns, the consent form and history form were approved by the School's Committee on Human Research at its January, 2000 meeting.

6. **CONFIDENTIALITY/INSURANCES**

All persons who are permitted to have access to our records must have a legitimate purpose and must sign a pledge of confidentiality. Records for this study that contain personal identifiers are kept in rooms that are locked when not in use and in a wing of the Health Department that is also kept locked when not in use. This wing is rarely used by anyone other than employees of the Training Center or Health Department. Personal information that might be considered sensitive such as date of last menstrual period, and use of oral contraceptives or hormones is coded in a way that cannot be interpreted without a key. The key is kept in a different room from the files. When data are to be used outside of the Training Center, names and addresses are removed. In more than 36 years of the existence of the Training Center, we know of no breach of confidentiality.

The responsible persons are Dr. George W. Comstock, Director of the Training Center for Public Health Research, Ms. Sandra C. Hoffman, Asst. Director, (301-791-3230) and Dr. Kathy J. Helzlsouer, Director of the Specimen Banks, 410-955-9727.

7. **COLLABORATIVE AGREEMENTS:**

This proposal is not binding until a signed agreement is executed between the 3M Company and Johns Hopkins University.

8. OTHER IRB APPROVALS

N/A

9. SINGLE PROJECT ASSURANCE

N/A

10. PUBLICATIONS

It is 3M's intent to co-author information with Johns Hopkins University researchers derived from this study. The details of this will be outlined in the project research contract. Under this agreement Johns Hopkins University would retain the right to publish information from this study solely for its own teaching and research purposes.

11. PROPRIETARY DATA

Proprietary information will be addressed in the final agreement signed between the 3M Company and Johns Hopkins University.

12. BUDGET

The budget for the work contribution of the investigators from Johns Hopkins University is attached.

Personnel (On-campus)

Name	Role on Project	Rate/hr	Total*
Kathy Helzlsouer	Principal investigator	$1,000	$30,000
Han-Yao Huang	Co-investigator	500	45,000
Gloria Zepp	Secretary	200	18,000
Total On-Campus Personnel			**$93,000**

Personnel (Off-campus)

Sandra Hoffman	Project coordinator	$ 400	$ 4,000
Clara Krumpe	Laboratory Technician	300	18,000
Judith Bolton	Data manager	250	5,000
Total Off-Campus Personnel			**$ 27,000**

Serum Bank Maintenance Costs
(Maintenance, electricity, replacements)

Cost Per Participant Sample	$225.00 x 300 samples =	**$ 67,500**

Other Expenses

Laboratory supplies (specimen tubes, pipettes)	$225.00	
Computer Supplies	200.00	
Shipping costs (FedEx, dry ice shipping containers)	150.00	
Total Other Expenses		$ 575

Total Project Costs (Johns Hopkins University) **$188,075**

* Total is an estimate of time to complete work.

Travel costs by Johns Hopkins University investigators will be billed directly to 3M. All other research study costs (e.g., analytical costs by Northwest Bioanalytical) will be paid by 3M.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Identification of Fluorochemicals in Human Tissue

The objcetive of this study is to determine the concentration ratios, in humans, of liver to serum, liver to bile and bile to serum for perfluorooctanesulfonate (PFOS). An application for securing liver, gallbaldder, bile and serum from donors was approved by the International Institute for the Advancement of Medicine (IIAM). IIAM is a non-profit organization whose purpose is to faciliate the placement of non-transplantable human organs and tissues for biomedical research and education. All tissues are procured with the informed consent of the donor or the donor's next of kin, in accordance with the Uniform Anatomical Gift Act. Donors may be from anywhere in the United States and Canada. It is estimated to take approximately 1 year to secure tissues from 12 donors. A final report should be available by March 1, 2001.

Corporate Occupational Medicine Section/Epidemiology Unit
Proposal to EHS&R Committee
3/1/98

Title: Identification of Fluorochemicals in Human Tissue

Purpose: Evidence that organic compounds containing the element fluorine chemically bonded to carbon can be found in human blood has been known for more than 30 years. Within the past 12 months it was estimated that serum PFOS (perfluorooctane sulfonate) levels are within the 20 to 50 ppb range in the general population. This is primarily based on pooled serum samples. Although PFOS is known to concentrate in the liver and to undergo enterohepatic circulation, we do not know what tissue concentrations may be among humans. The purpose of this study is to determine the level of several fluorochemicals from the serum, liver and bile of the same individual (human).

Significance: Determination of the tissue concentrations of selected fluorochemicals will provide for a more specific understanding of the distribution of these compounds in the human. The ratio of liver to serum, liver to bile and bile to serum will be compared to data obtained from studies in nonhuman primates. Ultimately, these data will be used in defining and confirming assumptions necessary in human health risk assessment.

Objectives: The objective of this study is to determine the ratio of liver:serum, liver:bile and bile:serum to the following fluorochemicals: PFOS, PFOSA, PFOSAA, M570, M556, PFHS and POAA among 12 human donors. Total organic fluorine will also be analyzed should analytical methods permit.

Protocol: An application for securing liver, gallbladder (bile) and serum from donors will be submitted to the International Institute for the Advancement of Medicine (IIAM). IIAM is a non-profit organization whose purpose is to facilitate the placement of non-transplantable human organs and tissues for biomedical research and education. IIAM is a division of the Pennsylvania Regional Tissue and Transplant Bank, an inspected and accredited program which meets or exceeds all of the Food & Drug Administration (FDA) and the American Association of Tissue Banks standards. All tissues are procured with the informed consent of the donor or the donor's next-of-kin, in accordance with the Uniform Anatomical Gift Act. Proof of donor consent to the applicant (i.e., 3M) is not always possible because it may involve the photocopying of privileged information. However, a donor identification number is provided to the applicant. A copy of a blank consent form is available upon request. Donors may be from anywhere in the United States and Canada. All donors are tested for HIV, hepatitis B, hepatitis C, HTLV and syphilis. IIAM procures and distributes tissues on an investigator request basis. The IIAM Application Review Committee reviews each application for ethical considerations, scientific merit and feasibility. Upon approval, the 3M request is placed in the rotation for the tissue of interest. IIAM abides by U.S. legislation governing the consent process for organ tissue donation, the protection of human subjects through confidentiality

(Health and Human Services 45 CFR PART 46) and Title III of the National Organ Transplant Act, which prohibits the buying and selling of organs and tissues excluding reasonable reimbursement of expenses associated with recovery and placement

Liver and serum analyses are planned for analytical analyses using methods developed and/or approved by 3M's Environmental Laboratory. The method for fluorochemical analysis of bile is currently being developed in a European laboratory under 3M's Environmental Laboratory guidance. A total of 12 donors will be obtained. IIAM estimates that they will send 1 to 3 sets of tissue (liver, gallbladder/bile and serum) to 3M on a monthly basis. Total quantity per donation is: liver (10 grams); gallbladder/bile (intact); and serum (5 to 10 cc).

PI/location/cost: Geary Olsen will be the PI on this project. Jean Burris and Jeff Mandel are co-investigators. Kris Hansen will serve as the contact within the 3M Environmental Laboratory. It is currently expected that the serum analyses will be conducted by Northwest Laboratory. The 3M Environmental Laboratory will conduct the liver analyses. A European laboratory currently validating a method for fluorochemical determination in the bile of primates will likely extend this validation to human bile. Approximate total cost per donor is $1665. The distribution of this cost is the following: shipping of sample $50; obtaining sample from IIAM $710 (liver, 10 grams, $120; gallbladder/bile, intact, $545; serum, 5 to 10 cc, $50); tissue fluorochemical analysis $900 (liver $300; gallbladder/bile $300; serum $300). Total cost for 12 donors: 12 x $1665 = $19,980. This cost does not include the validation of fluorochemicals in human bile by the European Laboratory.

Timeline: IIAM estimates that tissue from 1 to 3 donors per month can be provided to 3M. Thus, to obtain samples from 12 donors may take between 4 and 12 months. Due to the immediate need to understand this fluorochemical distribution within human tissues, samples will be submitted for analysis upon receipt by 3M. If the last sample is obtained by the end of a six month time frame, we estimate a final report of all 12 donors can be completed within 90 days after receipt of the last donor's tissue. The final report will include the fluorochemical tissue concentrations/levels, descriptions of the age, gender, race and geographical location (state or) of the nonidentifiable donors , and pertinent medical history information that may be supplied by IIAM (e.g., cause of death, known liver disease) of the donor.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

An Epidemiologic Analysis of the Inpatient and Outpatient Claims Event and Episode Experience of 3M Decatur Employees, 1993-1998

The purpose of this research protocol is to examine the Corporate Health Strategies Ingenix (CHS) inpatient and outpatient claims databases from January 1, 1993 through December 31, 1998 of Decatur chemical and film plant employees. Claims data are not available from CHS prior to 1993. The observed claims events (individual claims) and episodes (multiple related claims grouped according to the episode they form) will be compared to an expected number calculated by indirect standardization methods to adjust for age and gender. Corrected for their different age structures, we will examine the ratio of the observed to expected chemical plant to the observed to expected film plant. The many limitations of the analysis of claims data are detailed in the study protocol. This study design was originally proposed in 1998 but was placed "on hold" to allow for the completion of the random sample assessment of serum fluorochemical measurements of Decatur chemical and film plant employees as well as the completion of the abstraction, data input and quality assurance of the Decatur work history record project. Both of these projects were required to be completed prior to the claims analysis in order to provide the necessary supporting information regarding employee serum fluorochemical levels and work history experience. Estimated date of the final report for this study is September 15, 2000.

ADDENDUM
EPIDEMIOLOGY PROTOCOL

Epidemiology, 220-3W-05
Medical Department
3M Company
St. Paul, MN 55144

Date: February 27, 2000

Title: An Epidemiologic Analysis of the Inpatient and Outpatient Claims Event and Episode Experience of 3M Decatur Employees, 1993-1998

Addendum Study
Start Date:February 27, 2000

Estimated Date of
Final Report: 9/15/2000

Protocol Number EPI-0004
IRB Approval

Exempt	Expedited
	X

Principal Investigator: Geary W. Olsen, D.V.M., Ph.D.

Co-investigators: Jean M. Burris, R.N., M.P.H
Michele M. Burlew, M.S..
Jeffrey H. Mandel, M.D., M.P.H.

Study Director: Jeffrey H. Mandel, M.D., M.P.H.

Sponsor: 3M Medical Department, 220-3W-05, St. Paul, MN

INTRODUCTION

This epidemiology study protocol (Epi-0004) was placed "on hold" in 1998 while two supplemental research activities clarified the potential differences in fluorochemical exposure among employees at the Decatur chemical and film plants. These two research endeavors were the "Fluorochemical Exposure Assessment of Decatur Chemical and Film Plant Employees (EPI-0006)" and the abstraction, data input and quality assurance of the Decatur work history record project. As a result of these two research activities, this study's methods have been modified and improved. Therefore, this Addendum supplants the original study protocol. The purpose of this revised research protocol is to examine the Corporate Health Strategies Ingenix (CHS) inpatient and outpatient claims databases from January 1, 1993 through December 31, 1998 of Decatur chemical and film plant employees. The observed claims events and episodes (as described below) will be compared to an expected number calculated by indirect standardization methods to adjust for age and gender. Provided below is a detailed description of the study methods.

METHODS

Description of CHS

CHS is located in New Haven, Connecticut. CHS creates and updates health claims and related databases for 3M and many other companies. CHS uses Parallax® software for its analytical interface. CHS also provides analytic consulting services to its member companies which will be used in this study. CHS will serve as the only claims data resource to 3M for this research study.

Description of CHS inpatient and outpatient databases

There are several CHS databases. These include: 1) the monthly cost and utilization database; 2) the focused MSR database (allows detailed investigation of trends found in monthly cost and utilization; 3) the eligibility data base; 4) the prescription drug database; 5) the inpatient database; and 6) the outpatient database. CHS can only provide claims data for Decatur employees who have chosen the 3M Medical Plan or BlueCross/Blue Shield of Alabama. Beginning in 1996, 3M Decatur employees had the option of choosing an HMO; to the best of our knowledge, few (< 50) have done so. It appears that equal percentages of chemical and film plant employees choose HMO coverage although we suspect a greater percentage of younger employees choose HMO coverage than older employees.

The formation of a complete inpatient record includes the hospital record and the physician record. There are 27 Major Diagnosis Categories (MDCs) [CHS, 1993]. There are approximately 500 DRGs which are subcategories of the 27 MDCs. DRGs were developed at Yale University and group similar patients. Diagnosis codes (ICD-9) can also be obtained from the hospital record. The MDCs that are of particular interest in this study are the following:

MDC 1	Nervous System
MDC 4	Respiratory System
MDC 5	Circulatory System
MDC 6	Digestive System
MDC 7	Hepatobiliary System and Pancreas
MDC 10	Endocrine, Nutritional and Metabolic
MDC 11	Kidney & Urinary Tract
MDC 12	Male Reproductive
MDC 13	Female Reproductive

MDC 14	Pregnancy, Childbirth & Puerperium
MDC 15	Newborns and Other Neonates
MDC 16	Blood and Immunological
MDC 17	Myeloproliferative and other neoplasms

From these MDCs more specific DRGs and even more specific ICD-9 codes can be selected for comparative analysis with the normative databases. The inpatient claims database can be analyzed for all claims from January 1, 1993 (CHS's contract with 3M began at this time) through December 31, 1998. A single hospitalization results in only one DRG and MDC code despite the possibility there could be one or more diagnoses made during hospitalization. It is also important to recognize that multiple inpatient claims of a single DRG may represent one employee or more than one employee with the same DRG. Thus, each inpatient claim cannot necessarily be considered an incident (i.e., new) or prevalence diagnosis during the study time period. Rather, this analysis should be viewed as an evaluation of the existence of inpatient claim events from January 1993 through December 31 1998. It will be possible, when necessary, to determine how many individuals contributed to the number of claim events for a MDC or a DRG. This will be helpful in data interpretation when an excess of observed claims is reported

Most physician-diagnosed illnesses do not require hospitalization. Therefore, we will also examine, for the same time period (1/1/93 - 12/31/98), the CHS outpatient claims database. As with the inpatient records, an employee may have one or more outpatient records of the same ICD-9 code. Furthermore, a claim may not represent a physician diagnosis as outpatient ICD-9 codes may be used as a suspected diagnosis code in the ordering of medical diagnostic procedures for which a claim is filed. Thus, each outpatient claim, like inpatient claims cannot necessarily be considered an incident (i.e.,

new) or prevalence diagnosis during the study time period. Rather, this analysis should be viewed as an evaluation of the existence of outpatient claim events from January 1993 through December 31 1998. It will be possible, if necessary, to identify the number of employees per each ICD-9 code. Finally, it should be noted that the same person could have several claims for the same diagnosis in both the inpatient and outpatient databases.

The three digit ICD-9 codes that will be examined for outpatient claims are the following:

ICD-9 Code	Description
140-239	Neoplasms
240-279	Endocrine, Nutritional, Metabolic, Immunity
280-289	Blood and Blood-Forming Organs
320-389	Nervous System and Sense Organs
390-459	Circulatory System
460-519	Respiratory System
520-579	Digestive System
580-629	Genitourinary System
630-676	Pregnancy, Childbirth and the Puerperium
710-739	Musculoskeletal System and Connective Tissue
740-759	Congenital Anomalies
760-779	Conditions in the Perinatal Period
780-799	Symptoms, Signs, and Ill-Defined Conditions

Should it be necessary, an analysis of the fourth digit of the ICD-9 code is feasible.

Inpatient claim events regarding newborns are assigned to the employee. Neonates (less than 30 days of age) generally, but not always, have their inpatient and outpatient claims assigned directly to the employee's record. By 30 days of age, most infants have their own separate records and are then defined as an employee dependent. Employee dependent records will not be examined.

Description of Clinical Care Groups™ (CCG) Methodology

Claims event data by itself, as described above, are not an ideal representation of clinical events when directly used. To better understand the treatment of diseases, individual services with their respective diagnosis codes should be linked together according to logic that combines clinical expertise and detailed knowledge of administrative data. When claims records are linked, they can form disease episodes, which represent all the services provided for the diagnosis, treatment, and management of a disease or condition. Thus, where individual claims data represent an "event", claims data that is grouped by diagnosis, treatment and management of a disease can represent an "episode."

Because of CHS's recent merge with Ingenix, Inc., CHS now has access to CCG software. This software groups all visits, procedures, ancillary services, and prescription drugs used in the diagnosis, treatment, and management of more than 400 diseases or conditions. An episode can be considered a constellation of one or more claims data records representing an occurrence of a disease or condition for a particular individual. An episode is assigned to a CCG Class. A CCG Class have several important qualities. It is: 1) clinically homogenous; 2) exhaustive; 3) belong to a disease hierarchy; and 4) is classified as a chronic or acute condition. Several CCG Classes constitute a CCG Category. Several CCG Categories constitute a Specialty Category. Altogether, there are 442 CCG Classes that fall into 103 CCG Categories and 20 Specialty Categories. The CCG software provides a summary output file with one record per episode. The methodology on how the CCG software works is described elsewhere [Valdivia and Van

Vorst, 2000]. For the purpose of this study, the CCG software will be utilized to describe the episode experience of the study cohorts (described below).

Decatur Study Population

The initial study population consists of all full-time and inactive employees at the Decatur plant as identified in the 3M epidemiology's Decatur work history database as of January 1, 1993. Employees hired, transferred to a non-Decatur site or terminated during the interval of time from 1/1/93 through 12/31/98 will have their claim event analyses limited to their time of employment. Full-time active employees who retired or went on long-term disability (LTD) within the six year interval of time (1993 - 1998) will be followed through 12/31/98. However, it should be recognized that employees placed on LTD are eligible for Medicare after 18 months; thereafter 3M becomes the secondary provider. Employees who retire prior to age 65 may have 3M as the primary insurance provider. Medicare becomes the employee's primary provider upon reaching the employee's 65th birthday.

Cohort Size

Based on a review of the recently computerized epidemiology Decatur work history database, the number of employees eligible for this study are the following (percents in parentheses):

Category	Chemical	Film
All Employees	652	659
Gender		
Male	530 (81)	558 (85)
Female	122 (19)	101 (15)
Age		
<30	59 (9)	36 (6)
30-39	124 (19)	82 (12)
40-49	238 (37)	194 (29)
50-59	182 (28)	267 (41)
>=60	49 (7)	80 (12)
Average age	45 years	49 years
"High" exposure job#	498 (76)	490 (77)*
Only worked in chemical (film) plant#	388 (60)	424 (64)
Worked only in chemical (or film) plant and, at least, from 1983-1998	176 (27)	269 (41)

*refers to comparable non-exposed jobs in film (i.e., operators, maintenance and supervisors) similar to those jobs in chemical which were considered "high" exposure (see text for definition of "high").
#refers to chemical employees with no work experience in film after 1/1/83 and film employees with no work experience in chemical after 1/1/83.

Data Analysis

Claims Events

For each year from 1993-1998, the chemical and film plant cohorts will be identified in order to calculate the observed and expected inpatient (MDC, DRG) and outpatient (ICD-9) claims experience. The overall claim events experience, for each MDC, DRG and ICD-9 code, is the sum of the individual six years of observed and

expected experience. Employees who chose an HMO in 1996, 1997 and 1998 will be excluded from the analyses for the year(s) they have HMO coverage.

Analysis of the claim events experience will be examined by whether the employee is considered a chemical plant or film plant employee. Each employee's work history record has been examined by the investigators (JMB, GWO, MMB) to determine whether the employee: 1) ever worked in chemical, film or both; 2) worked in chemical, film or both during the claims analysis time period of January 1, 1993 through December 31, 1998; and 3) worked continuously in chemical or film for the entire 10 years prior to the beginning of the claims analysis time period. For employees who have worked in both plants, their records were reviewed to identify which plant each employee primarily worked at in his/her career at Decatur. Both chemical and film plant employees will be subdivided in the analyses (see below) as to whether they have, or have not, worked in the other plant. The majority of film plant employees with prior work experience in the chemical plant worked at the chemical plant for a short period of time (1 to 3 months) and this usually occurred during the first year of their employment. Film plant workers were employed, on average, 25 years. Like film plant employees, many chemical plant employees have worked at the film plant at the beginning of their career. In addition, employees who have site-wide responsibilities (those who may work in both chemical and film such as environmental health and safety specialists) were assigned to the chemical plant.

Each employee was assigned a job title which described the person's usual job activity while a Decatur employee. These job titles were: boiler operator, environmental health and safety specialist, engineer, mill operator, maintenance, office worker, operator,

quality control worker, shipping clerk and supervisor. Based on the findings from the Decatur exposure assessment study (Olsen et al., 1999), maintenance workers, operators (includes cell, chemical, mill and waste) and supervisors in the chemical plant had significantly higher serum fluorochemical levels [e.g., approximate geometric mean of 1 to 2 ppm of PFOS (perfluorooctanesulfonate) and/or PFOA (perfluorooctanoic acid)] than the other job categories (approximate geometric mean levels of 0.2 to 0.5 ppm for PFOS and/or PFOA). Therefore, these two job groups will be the high and low fluorochemical exposure categories in the chemical plant analyses. All chemical plant workers, regardless of job title, were found to have, on average, significantly higher serum fluorochemical levels than employees assigned to the film plant. The geometric mean for film plant employees approximated 0.1 ppm PFOS and less than 0.1 ppm for PFOA. Thus, the film plant workers are considered to be the least exposed and will be considered the comparison population.

The observed number of claims based on MDC's, DRG's and ICD-9 codes will be compared to an expected value using indirect standardization methods [Tsai and Wen, 1986] to adjust for age (<40, 40-49 and >=50) and gender (male, female). Two normative databases will be used to calculate the expected number of claims: 1) the entire U.S. 3M population excluding the Cottage Grove (location code = 064) and Cordova (location code = 034) sites due to their fluorochemical production activities; and 2) the U.S. 3M population excluding employees at the St. Paul (location code = 089), Woodbury (location code = 343), Cottage Grove (location code = 064) and Cordova (location code = 034) sites. The latter database, hereafter called the 3M manufacturing plant normative database, will be developed to provide a normative database that would likely be more representative of 3M manufacturing

plant employees as it excludes the St. Paul 3M Center (corporate and research employees). It also excludes the downtown St. Paul manufacturing plant as the 3M St. Paul sites use the same location code (089). A third normative database, which consists of other companies that belong to the CHS aggregate database, was not considered appropriate to use by CHS staff because the different health insurance plans that these other companies use may greatly influence the likelihood of hospitalization. This is especially true when studying small populations such as those identified in this Decatur study.

Standardized Claims Ratios (SCR) for MDCs (inpatient), DRGs (inpatient) and ICD-9 codes (outpatient) will be calculated. The $SCR_{chemical}$ is the ratio of the observed to expected claims experience for the chemical plant. The SCR_{film} is the ratio of the observed to expected claims experience for the film plant. Ninety-five percent Confidence Intervals (95% CI) of the $SCR_{chemical}$ and SCR_{film} will be calculated using methods described by Rothman and Boice [1979]. However, the estimate that is most important is the ratio of these two indirect standardized ratios which we will define as the Claims Event Ratio:

$$CER = SCR_{chemical} / SCR_{film}$$

The direct comparison of two indirect standardized ratios (e.g., Standardized Mortality Ratios) can be made only if the age structures are similar or age-specific mortality ratios are homogeneous. Because the chemical and film plant populations have somewhat different age structures used for indirect standardization (see cohort size above which shows chemical plant employees are somewhat younger than film plant employees), the SCRs for the chemical and film plant populations are not necessarily directly comparable. We will use the methods by Tsai and Wen (1986) to compare whether the ratio of two indirect standardized ratios, corrected for their age structure, is similar to an unadjusted ratio. The ratio of two

indirect standardized ratios, upon correction for their age structure differences, can be considered an unbiased estimator of a risk ratio [Tsai and Wen, 1986].

$$CER_{corrected} = SCR_{chemical\text{-}corrected} / SCR_{film\text{-}corrected}$$

Each age corrected SCR is not meaningful by itself, only the ratio, $CER_{corrected}$, of two corrected SCRs. Although Tsai and Wen suggest that the confidence interval of $CER_{corrected}$ can be calculated using the procedures developed by Ederer and Mantel (1974) for the ratio of two Poisson variables, such methods, in fact, cannot be used. Unlike CER which is a Poisson variable (sum $Obs_{chem\text{-}3\ age\ groups}$ /sum $Obs_{film\text{-}3\ age\ groups}$) multiplied by a constant (sum $Exp_{film\text{-}3\ age\ groups}$ /sum $Exp_{chem\text{-}3\ age\ groups}$), the calculation of a confidence interval for $CER_{corrected}$ is not readily apparent as each $Obs_{corrected\text{-}chem}/Obs_{corrected\text{-}film}$ subgroup (n = 3 age groups) is not a Poisson variable and neither is the sum (personal communication, Dr. Tim Church, biostatistician, University of Minnesota). Therefore, our analysis strategy will be to calculate both CER and a $CER_{corrected}$. If the two ratios are relatively similar, which we suspect they will be given the fact that their age structures are not substantially different, then we will only calculate the confidence limit of CER ($SCR_{chemical}/SCR_{film}$) using the methods proposed by Ederer and Mantel (197). We will calculate CER and $CER_{corrected}$ for those inpatient (MDC and DRG) and outpatient (ICD-9) claim categories where there are two or more observed claims in either the chemical or film plant populations.

Because of confidentiality concerns regarding employee medical information, the identification of the inpatient and outpatient claims experience is known only by CHS. Therefore, the cohorts must be established by 3M prior to the determination of the claims data. Record linkage with the CHS claims data will include the employee's name, gender, social security number, employee number and date of birth.

The observed and expected inpatient and outpatient claims experience (and their SCRs, CERs and $CERs_{corrected}$) will be determined for several cohorts (excluding those individuals who have HMO coverage). These will include the following comparisons:

1) all chemical plant workers (n = 652) compared to all film plant workers (n = 659);

2) all chemical plant workers who never worked in film (n = 388) versus all film plant workers who never worked in chemical (n = 424);

3) all 'high' (defined above) exposure chemical plant workers who have not worked in film for at least since January 1, 1983, if ever (n = 498), compared to their job counterparts (considered 'least exposed') in the film plant who have not worked in chemical for at least since January 1, 1983, if ever (n = 490, this would include operators, supervisors and maintenance workers in the film plant); and

4) all long-term 'high' exposure chemical plant workers who have not worked in film since at least January 1, 1983, if ever (n = 211), compared to their job counterparts of 'least exposed' workers in the film plant who have not worked in chemical since at least January 1, 1983, if ever (n = 345);

Claims Episodes

Comparable indirect standardization techniques, as described above for the claims events data, will be used to analyze the claims episode data derived from the CCG software. We will utilize the same two 3M normative populations for calculation of expected values. Basically, instead of calculating an observed to expected ratio of claim 'events', we will calculate an observed to expected ratio of episodes based on the CCG software. In other words: $CEpR = SC_{Ep}R_{chemical}/SC_{Ep}R_{film}$) where CE_pR = Claims Episodes Ratio; $SC_{Ep}R_{chemical}$ = Standardized Episode Claims Ratio for chemical; and $SC_{Ep}R_{film}$ = Standardized Episode

Claims Ratio for film. The CE_pR will include the CCG Class, CCG Category and Specialty Category episodes.

Additional Study Logistics

Jennifer Bilodeau and Doug Bates will coordinate the CHS aspects of this project. Geary Olsen will serve as the 3M principal investigator. Jean Burris, Michele Burlew and Jeff Mandel will serve as 3M co-investigators. For each cohort that 3M provides, CHS will determine the observed and calculate the expected number of claims by MDC (inpatient), DRG (inpatient), ICD-9 (outpatient) codes as well as the CCG Classes, CCG Categories and CCG Specialty Categories (as discussed above) per each age group and gender. Calculation of the SCR_{chem}, SCR_{film} and CER (and their associated 95% confidence intervals) will be performed by 3M investigators as well as the calculation of the $SCR_{chem\text{-}corrected}$, $SCR_{film\text{-}corrected}$ and $CER_{corrected}$. The title of the revised study protocol is changed (from the original protocol) to include outpatient claims, claim episodes and extend the study time period one additional year (through 1998).

Quality Assurance

This study protocol, any addenda to the protocol, data analyses, and a copy of the final report will undergo a Quality Assurance audit. Permanent records of all other data generated during the course of this study are subject to privacy and confidentiality considerations. All data gathered or generated including protocol addendum and the final report will be archived by the Medical Department, 3M Company, St. Paul,

Minnesota. Upon review and approval of the final report, study results will be communicated to the Decatur employees.

DISCUSSION

The purpose of this study is to examine the inpatient and outpatient medical claim event experience of Decatur chemical and film employees from January 1, 1993 through December 31, 1998. Since the writing of the original study protocol, software became available to also analyze claims data into episodes. The overall hypothesis is that the inpatient and outpatient claims event experience as well as the episode experience is comparable between employees of the 3M Decatur chemical and film plants who differ in their serum fluorochemical measurements by, on average, one order of magnitude, but do not differ in their access to the same medical care delivery system (i.e., the Decatur medical practices available to them). With the computerization of the Decatur work history database, we can also restrict the claims analysis to those chemical plant employees who have worked solely in the chemical plant for at least 10 years prior (starting 1/1/83) to the claims analysis time period in jobs that are considered to have the highest potential of exposure.

This claim event analysis can provide a much broader perspective of possible employee health outcomes than a retrospective cohort mortality study, especially in instances, such as this, where employees' serum fluorochemical exposure levels, based on findings from toxicology studies, would not suggest an increased risk for mortality. We can also address some questions of female chemical employees about the potential effect, if any, of placental transfer of their occupational-derived fluorochemical serum levels.

The CHS claims event data will allow for an evaluation of the claim events related to childbirth and the neonatal period. We are not certain whether the CCG episodes data will provide data regarding the neonatal period. The analysis of claims data is less subjective than direct employee interviews that would lack the extremely time-consuming validation of self-reports through a medical record review. The study design also maintains the confidentiality of employees' medical claims data.

There are several limitations that must be understood. First and foremost, an analysis of claims data should not be considered a study of risk (i.e., the probability of disease in an 'exposed' group compared to the probability of disease in an 'unexposed' group). Claims data should not be considered incidence or prevalence data. Rather, the study should be viewed as an analysis of the comparison of medical claim events between two populations (chemical and film). A claim does not mean that it represents an accurate diagnostic description of the reason for the hospitalization or the outpatient visit. As previously discussed, ICD-9 codes for outpatient claims may be related to the reason why a procedure was done, not because the diagnosis was made as the result of the procedure. The claims episodes data also does not provide information regarding incident risk. It may, however, provide a reasonable prevalence description during the six year time period. The employees' claim event and episode experience will only be examined for a recent 6 year time period at a chemical plant that began fluorochemical production in the early 1960's. It is not possible to conduct a comprehensive longitudinal analysis of claim events and episodes data. The study is also limited within the 6 year time frame as we can examine only for the existence of claims data for individuals who remain full-time active employees or are inactive employees between 1/1/93 and

12/31/98. The claim event and episode experience of employees who subsequently quit, terminated or transferred during the six years are not considered. Employees who are placed on LTD will likely only have claim events reported by CHS for the first 18 months as Medicare would then take over as the primary provider. Likewise claim event and episode experience of those individuals who retire and reach the age of 65 will likely not be observed as these individuals are primarily covered by Medicare. Part-time and summer employees as well as contractors' employees are not included in the analysis. Another limitation is the small size of the study population (total of 652 chemical and 659 film plant employees). The person-years will be further reduced by the exclusion of those employees who have chosen HMO coverage.

Despite the above shortcomings, this claim event and episode analysis can provide 3M Decatur employees a better perspective as to whether the health experience of chemical plant employees, as measured by the existence of CHS claims information, is significantly different than the claims experience of film plant employees. Additional investigations can be designed should there be significantly more observed than expected claims events and/or episodes for chemical than film plant employees for specific MDC, DRG, ICD-9 and CCG codes.

REFERENCES

CHS (1993). Parallax analyst interface 1997 codes catalog. New Haven:Corporate Health Strategies, Inc.

Ederer F, Mantel N (1974). Confidence limits on the ratio of two Poisson variables. Am J Epidemiol 100:165-167.

Olsen GW, Logan PW, Simpson CA, Hansen KJ, Burris JM, Burlew MM, Schumpert JC, Mandel JH (1999). Fluorochemical exposure assessment of Decatur chemical and film plant employees. St. Paul:3M Company.

Rothman KJ, Boice JD (1979). Epidemiologic Analysis with a Programmable Calculator. U.S. DHEW NIH Publication No. 79-1649.

Tsai SP, Wen CP (1986). A review of methodological issues of the standardized mortality ratio (SMR) in occupational cohort studies. Int J Epidemiol 15:8-21.

Valdivia T, Van Vorst K (2000). Clinical Care Groups™ Methodology. Ingenix Inc., San Francisco.

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

3M

Deterimination of Serum Elimination Half-Lives of Ammonium Perfluorooctanoate, Perfluorooctane Sulfonic Acid, Perfluoroheaxane sulfonic acid and Total Organic Fluorine in Decatur Chemical Plant Retirees

The objective of this study is quantitate the human serum elimination half life of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA) , perfluoroohexanesulfonate (PFOS) and total organic fluorine (TOF). Blood will be collected biannually from approximately 25 retireed 3M Decatur employees for a period of 5 years. Upon collection and analysis of the third sample (baseline, 6 months and then 12 months) and thereafter, an interim draft half-life analysis report will be prepared.

PROTOCOL

Epidemiology,220-3W-05
Medical Department
3M Company
St. Paul, MN 55144

Date: October 14, 1998

Title: DETERMINATION OF SERUM ELIMINATION HALF-LIVES OF AMMONIUM PERFLUOROOCTANOATE, PERFLUOROOCTANE SULFONIC ACID, PERFLUOROHEXANE SULFONIC ACID AND TOTAL ORGANIC FLUORINE IN 3M DECATUR CHEMICAL PLANT RETIREES.

Study Start Date:	Protocol Number # 0007 IRB Approval # 98095 Exempt XX Expedited
Estimated Date of Final Report:	June, 2004
IRB Approval Date:	14 October 1998
Principal Investigator:	Jean M. Burris RN, MPH
Co-investigators:	Geary Olsen, DVM, PhD[1] John C. Schumpert MD Cathy Simpson, RN[2] Jeffrey Mandel, MD, MPH[1]
Study Director::	Jeffrey Mandel, MD, MPH[1]
Study Sponsor:	Medical Department 3M Company 220-3W-05 Saint Paul, MN 55144

1. Occupational Medicine, Medical Department, 3M Company, 220-3W-05, St. Paul, MN 55114
2. 3M Decatur Specialty Adhesives and Chemicals Plant, P.O. Box 2206, Decatur, AL 35609-2206

ABSTRACT

3M manufactures products which contain chemical compounds, either as intentional components or residual impurities, that have as a parent molecule, perfluorooctane sulfonyl fluoride (POSF). These compounds may be expected to transform metabolically, to an undetermined degree, to PFOS as an end-stage metabolite. Other compounds manufactured by 3M include ammonium perfluorooctanoate (PFOA) and perfluorohexane sulfonic acid (PFHS). These molecules enter a number of product applications (e.g., surfactants, food packaging additives, polymers).

The biological elimination rates of fluorochemicals have been studied in rats, pregnant rats, female rabbits and hamsters. Biological half-lives have been calculated from the studies in rats and dogs. In addition, the half-life of serum total organic fluorine in a fluorochemical worker was estimated using serial collections of serum total organic fluorine. These studies reveal that PFOS and PFOA may persist in the body for prolonged periods, and with continuing exposure, accumulate over time in biological systems. To date, the actual elimination half-life of perfluorooctane sulfonate (PFOS), ammonium perfluorooctanoate (PFOA), perfluorohexane sulfonic acid (PFHS), and total organic fluorine (TOF) in human serum is not completely understood. The objectives of this study are to quantitate the elimination half life in human serum of PFOA, PFOS, PFHS, and TOF. These half -lives will be calculated from declines in serum fluorochemical levels in 3M Decatur Chemical Plant Retirees.

INTRODUCTION

3M manufactures products which contain chemical compounds, either as intentional components or residual impurities, that have as a parent molecule, perfluorooctane sulfonyl fluoride (POSF). These chemicals include: perfluorooctane sulfonate (PFOS), N-ethyl perfluorooctanesulfonamide, N-ethyl perfluorooctanesulfonamido ethanol, N-methyl perfluorooctanesulfonamido ethanol and chemicals derived from it, and the mixture of mono-, di- and tri [N-ethyl perfluorooctane sulfonamidoethyl] phosphates. There may be other precursors in the workplace, including perfluorohexanesulfonyl fluoride (PHSF). These molecules enter a number of product applications (e.g., surfactants, food packaging additives, polymers). These compounds may be expected to transform metabolically, to an undetermined degree, to PFOS (and PFHS) as an end-stage metabolite. Potassium perfluorooctane sulfonate ($C_8F_{17}OSO_2K^+$) is itself, a surfactant used as a wetting and foaming agent in industrial and commercial processes. (Olsen, et al., 1998)

Subchronic studies in rats and primates suggest there may be a potential for cumulative toxicity with PFOS over time with the primary effect related to metabolic wasting. These molecules have long elimination half lives in biological systems. Although the mechanism of toxicity is not fully understood, toxicity may be due to an effect on peroxisome proliferation, fatty acid metabolism, membrane function, protein synthesis and/or mitochondrial bioenergetics. (Olsen, et al., 1998)

The biological elimination rates of fluorochemicals have been studied in rats, pregnant rats, female rabbits and hamsters. Biological half-lives have been calculated from the studies in rats and dogs. In addition, the half-life of serum total organic fluorine in a fluorochemical worker

was estimated using serial collections of serum total organic fluorine. These studies reveal that PFOS and PFOA may persist in the body for prolonged periods, and with continuing exposure, accumulate over time in biological systems. To date, the actual elimination half-life of perfluorooctane sulfonate (PFOS), ammonium perfluorooctanoate (PFOA), perfluorohexane sulfonic acid (PFHS), and total organic fluorine (TOF) in human serum is not completely understood.

The objectives of this study are to quantitate the elimination half life in human serum of PFOA, PFOS, PFHS, and TOF; these half lives will be calculated from declines in serum fluorochemical levels in 3M Decatur Chemical Plant Retirees. This information is necessary in order to: 1) plan the most appropriate periodicity of medical surveillance and 2) guide medical decision making of workers from fluorochemical production facilities when those workers' serum fluorochemical levels are determined to be unsafely elevated. A benefit of this research will be to understand the natural history of fluorochemical excretion.

LITERATURE REVIEW

AMMONIUM PERFLUOROOCTANOATE (PFOA or FC-143)

Excretion rates of PFOA have been observed in rats, and found to be different by gender and route of excretion. Following single i.v. doses of ^{14}C-FC-143 (carbonyl carbon labeled) in rats, Johnson et al, found that females excreted virtually all the administered ^{14}C within 1 day. Urinary excretion for males was about 50% of the dose by day 6 and 83% by day 36. Fecal ^{14}C excretion for females was 1.5% by 3 days and for males was 5.4% by 36 days. (Johnson, et al.,

1980) Rapid urinary excretion of ^{14}C following oral doses of ^{14}C-FC-143 was also shown to occur in pregnant rats. (Johnson, et al., 1983)

Excretion rates vary by species studied. Excretion of radiolabeled PFOA was studied in four species by Dupont in 1988. Excretion as a percentage of administered dose 120 hours after dosing was in the following order; female rat, male and female rabbit and male hamster (> 99%); female hamster (60%); male rat (39%); male and female mice (21%). (Dupont, 1988) Of note, the administered dose and routes of exposure and excretion were not specified in this study report.

Rats and dogs respond to pharmacologic interventions that change PFOA excretion rate. In male rats administered single i.v. doses of ^{14}C-FC-143 (carbonyl carbon labeled) cholestyramine (4% w/w in feed) increased cumulative 15 day fecal ^{14}C excretion 9.8-fold versus controls. Total ^{14}C excretion (feces plus urine) was also enhanced, although less dramatically (84.3% of dose vs. 71.8% for controls). (Johnson, et al., 1980a; Johnson JD, et al., 1984) There is no difference between the renal clearances of ^{14}C in male and female dogs either before or after probenecid. Probenecid significantly reduces PFOA clearance in dogs. Glomerular filtration rates of PFOA were similar in rats and dogs. (Hanhijarvi, et al., 1988)

Elimination half-life has been calculated under numerous conditions in rats and dogs. The results show the same differential elimination rate in rats by gender (i.e., female > male), as well as half-life differences by route of exposure and species. Following a single oral dose of ^{14}C-FC-143 (carbonyl carbon labeled) in male rats, the plasma half-life was 4.8 days. (Johnson, et al., 1979)

In female rats administered oral or i.v. doses of ^{14}C-FC-143 (carbonyl carbon labeled), over 90% of the administered dose was recovered in the urine within the first 12 hours. The whole body elimination half-life of PFOA in male and female rats was 15 days and less than one day, respectively, following a single 4-mg/kg intraperineal dose. (Vanden Heuval, et al., 1991) (Johnson, et al., 1983; Johnson, et al., 1980) The half-life of PFOA in the liver was 60 hours for females and 210 hours for male rats. (Ylinen, et al., 1990) The decreased excretion rate (i.e., increased elimination half-life) in males is seen across at least two species: rats (as above) and dogs. The plasma half-life of PFOA was longer in male dogs (473 to 541 hours) than in females (202 to 305 hours). (Hanhijarvi, et al., 1988)

The elimination half-life appears to be similar in male rats exposed to either inhalational or dermal exposure. Following repeated inhalation exposures to FC-143 over a two-week period, blood organic fluoride levels in male rats showed a half-life of five to seven days. (Kennedy, et al., 1986) A blood half-life of five to seven days was seen following repeated dermal exposures in male rats. (Kennedy, 1985)

A calculation of the elimination half-life of an organic fluorine species has been reported in the human. The half-life of total serum organic fluorine in a fluorochemical worker who was removed from further exposure was greater than 18 months. This worker had a blood organic fluorine level of 40 parts per million over the period of one year. The worker's organic fluorine level rose to 70 parts per million for no apparent reason. The worker was then removed from the fluorochemical production area and his blood and urine samples were periodically checked for organic fluorine and PFOA over an 18-month period. A total of 18 months transpired before the

worker's blood organic fluorine level had returned to its base line of 40 parts per million. (Ubel, et al. 1980) Other estimates of serum fluorochemical half-lives have been calculated in the one thousand day range. Ψ This anecdotal account does not actually track the serum PFOA level, but rather the serum total organic fluorine (TOF) level. It was found through empirical means that 90% of the blood organic fluorine existed as PFOA. (Ubel, et al. 1980)

PERFLUOROOCTANE SULFONIC ACID (PFOS or FC-95)

Perfluorooctane sulfonic acid appears to persist in biological systems, being excreted more slowly than PFOA. Single i.v. doses (mean 4.2 mg/kg) of ^{14}C-FC-95 and 0.9% NACL were administered to male rats. By 89 days after dosing, 30.2% of the administered ^{14}C had been excreted in the urine and 12.6% had been excreted in the feces. (Johnson, et al., 1979)

Pharmacologic interventions do appear to increase the excretory rate of PFOS. Fecal and total excretion of ^{14}C were markedly increased in male rats administered Cholestyramine (about 2.7 gm/kg per day) and their diet following single i.v. doses of ^{14}C-FC-95. The results suggest that there was significant enterohepatic circulation of FC-95. (Johnson, et al., 1980a; Johnson, et al., 1984)

Much less is known concerning elimination half-life of PFOS than PFOA; only one calculation of the elimination half-life of PFOS has been attempted. The plasma elimination half-life of ^{14}C following single oral administration of ^{14}C-FC-95 (mean dose 4.2 mg/kg) to male rats was 7.5 days. (Johnson, et al. 1979a)

Ψ Mandel, J. Personal communication.

RESEARCH METHODS

The overall research design is prospective in nature, obtaining multiple serial blood samples from retirees throughout the course of a five-year period. We will track the decline in several serum fluorochemical levels in humans. The health outcomes that will be documented in this study are the half-life determinations of serum ammonium perfluorooctanoate (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHS), and serum total organic fluorine (TOF).

High-performance liquid chromatography mass spectrometry/mass spectrometry will be utilized to analyze all serum samples. The accuracy and reliability of this device will be the state of the art at the time of analysis. Periodically, split samples from one or two subjects will be utilized to assess reliability of the analysis.

Only individuals who have retired from 3M Decatur chemical plant from the 1st of January 1995 through the 1st of January 1998, will be included in the study. Using information received from the Human Resources Department of 3M Decatur, 34 individuals were identified to have retired during this time period. Of the 34 individuals identified, 7 have previously been enrolled in the fluorochemical medical surveillance program. Four of these individuals participated in the medical surveillance program in 1994 but not 1997; 3 individuals participated in 1997 only. We will attempt to enroll all 34 retirees into the study. Future retirees will not be enrolled.
Serum fluorochemical levels will be drawn every six months. All serum fluorochemical levels will be drawn within a one-month time frame (i.e., within one month of March and September).

Retirees will be recruited to the study through first, an introductory letter and second, a phone conversation from the principle investigator or his designee. Subsequent to the follow-up telephone conversation and if the retiree is willing to enter the study, a written informed consent form (Appendix A) and a medical questionnaire (Appendix B) will be sent to the retiree for his review. Once the retiree signs the consent form, completes the questionnaire, and returns the documents to the principle investigator, he will be entered into the study and scheduled for blood collection. Participation in the study is voluntary and retirees may drop out at any time.

Blood collection will be scheduled biannually, in April and October (to not conflict with Holiday and summer vacation travel). Letters announcing blood collection dates and times will be sent out to each study subject one month prior to each blood collection month. Retirees will update their medical questionnaire at each blood collection. All blood collections will be performed under the supervision of the 3M Decatur plant nurse, Cathy Simpson, RN. Two red-topped tubes will be obtained from each retiree; the 3M Occupational Medicine Service methodology for handling and shipping of fluorochemical blood samples will be applied to all blood samples. Samples will be analyzed by the 3M Environmental Laboratory or its designated contract laboratory. Retirees will receive written reports of their serum fluorochemical levels following each blood collection cycle.

Two methods of calculating the serum fluorochemical half life will be utilized. In the first method, a one compartment model will be assumed and the formula

$$q_t = q_0 (0.5)^{t/t_{1/2}}$$

will be utilized to estimate the half life of elimination for PFOS, PFOA, PFHS, and TOF. The terms q_t *and* q_0 refer to the serum concentrations at time t and time 0, t is the elapsed time; $t_{1/2}$ is what we seek to solve.

The second method will be used to verify the first. It also assumes a one compartment model. Each retiree's fluorochemical levels will be graphed on a log-linear scale as serum fluorochemical vs. elapsed time from t_0 (in months) and entered into a database. Statistical analysis will be performed to determine the best-fit model that describes the slope of the log-linear line. (Appendix C)

The slope of the log-linear line is related to the elimination constant ($-k_{el}$) through the equation

$$Slope = -k_{el}(2.303)$$

Once the elimination constant is calculated, the half life can be calculated using the relationship

$$t_{1/2} = 0.693/k_{el}$$

Elimination half lives for TOF, PFOS, PFHS, and PFOA will be determined. (Benez, et al. 1985, Medinsky, et al. 1996.)

DISCUSSION

There are a number of limitations associated with the study, including:

1. The lack of pre-study serum fluorochemical levels in the majority of retirees, making it impossible to develop baseline levels for the majority of the study group. The $t0$ values will therefore need to be their first fluorochemical level *post-retirement*.

2. Previous studies have performed serum total organic fluorine levels and not serum testing specific for serum PFOS or PFOA; comparability with previous work is thereby compromised.
3. Retirees on medications such as colestipol or cholestyramine, which can artificially increase the rate of elimination of fluorochemicals from the blood will artificially increase the elimination in unpredictable ways, thereby causing an underestimation of the elimination half life in the entire study population.
4. Retirees may unknowingly have medical conditions that will decrease their toxicant elimination rate unpredictably (e.g., renal failure, congestive heart failure, cholelithiasis, etc.), thereby causing an inaccurate estimation of the elimination half life in the entire study population.

For these reasons, the study will occur over the protracted period of five years instead of a shorter time period (i.e., three years). As the only human data plotting total organic fluorine suggest plasma elimination half-life of 18 months, a three year study would allow for verification of that estimate since three years would be equal to two half-lives. A five-year study, therefore, should include over three half-lives of elimination, and make it possible to use several years' data to calculate the elimination half life.

The overall results collected in this study may be used in publications or in public presentations. Retiree names or other individual data will not be revealed in any publication or other documentations intended for public examination. Individual results will be communicated only to the retiree. Individual results will be considered confidential information and will not be disclosed to anyone outside of the 3M Medical Department without the retiree's written consent.

Quality assurance will be performed per the guidelines set forth in the standard operating procedure for the creation, review, and approval of 3M epidemiology study protocols.

Archiving of study materials, upon completion of the study, will be performed per the standard operating procedure for the creation, auditing, review and approval of 3M epidemiology final reports.

The study will be communicated to the retirees, management, and the technical community. Communications to the retirees will be in the form of a letter, which states the retiree's various blood fluorochemical levels. These letters will be sent out following each blood draw. Communication to management will be in the form of a written document which will undergo the review process as set forth in the standard operating procedure for the creation, auditing, review and approval of 3M epidemiology final reports. The communication to the technical community will be in the form of a scientific paper, which will be submitted to a relevant peer reviewed scientific publication.

REFERENCES

1. Olsen GW, Burris JM, Mandel JH, and LR Zobel. An epidemiologic investigation of clinical chemistries, hematology and hormones in relation to serum levels of perfluorooctane sulfonate in male fluorochemical production employees. J Occup Environ Med 1998;

2. Johnson JD and Ober RE. Extent and route of excretion and tissue distribution of total carbon-14 in male and female rats after a single IV dose of FC-143-^{14}C. Riker Laboratories, Inc. St., Paul, MN; 1980.

3. Johnson JD and Conard GJ. Extent and route of excretion of total carbon-14 in pregnant rats after a single oral dose of ammonium ^{14}C-perfluorooctanoate. Riker Laboratories, Inc., St. Paul, MN; 1983.

4. Vanden Heuval J, Kuslikis B, Van Refelghem M, and Peterson R. Tissue distribution, metabolism, and elimination of perfluorooctanoic acid. *J Biochem Toxicol* 1991; 6:83-92.

5. DuPont. Ammonium perfluorooctanoate. Toxicity Hazard Information from MSDS; 1988.

6. Johnson JD and Ober RE. Enhanced elimination of FC-95-^{14}C and FC-143-^{14}C in rats with cholestyramine treatment. Riker Laboratories, Inc., St. Paul, MN, 1980a.

7. Johnson JD, Gibson SJ, and Ober RE. Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C]perfluorooctanoate or potassium[^{14}C]perfluorooctanesulfonate. *Fund Appl Toxicol* 1984; 4:972-976.

8. Griffith FD and Long JE. Animal toxicity studies with ammonium perfluorooctanoate. *Am Ind Hyg Assoc J* 1980; 41:576-583.

9. Hanhijarvi H, Ylinen M, Haaranen T and Nevalainen T. A proposed species difference in the renal excretion of perfluorooctanoic acid in the beagle dog and the rat. In: Beynen AC and Solleveld HA, eds. *New Developments in Biosciences: Their Implications for Laborotory Animal Science*. Martinus Nijhoff, Dordrecht, 1988.

10. Gilliland FD. Fluorocarbons and human health: studies in an occupational cohort. Ph.D. Dissertation, U. of Minnesota, Minneapolis, MN; 1992.

11. Johnson JD and Ober RE. Absorption of FC-143-^{14}C in rats after a single oral dose. Riker Laboratories, Inc., St. Paul, MN; 1979.

12. Johnson JD and Ober RE. Extent and route of excretion of total carbon-14 pregnant rats after a single oral dose of ammonium ^{14}-C perfluorooctanoate. Riker Laboratories, Inc., St. Paul, MN; 1983.

13. Kennedy GL, Jr., Hall GT, Brittelli MR, Barnes JR, and Chen HC. Inhalational toxicity of ammonium perfluorooctanoate. *Fund Chem Toxicol* 1986; 24:1325-1329.

14. Kennedy GL, Jr. Dermal toxicity of ammonium perfluorooctanoate. *Toxicol Appl Pharmacol* 1985; 81:348-355.

15. Ylinen M, Kojo A, Hanhijarvi H, and Peura P. Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. *Bull Environ Contam Toxicol* 1987; 44:46-53.

16. Ubel FA, Sorenson SD, and Roach DE. Health status of plant workers exposed to fluorochemicals, a preliminary report. *Am Ind Hyg Assoc J* 1980; 41:584-589.

17. Johnson JD, Gibson SJ, and Ober RE. Extent and route of excretion and tissue distribution of total carbon-14 in rats after a single i.v. dose of FC-95-^{14}C. Riker Laboratories, Inc., St. Paul, MN; 1979.

18. Johnson JD and Ober RE. Absorption of FC-95-14C after a single oral dose. Riker Laboratories, Inc., St. Paul, MN; 1979a.

19. Benet LZ and Sheiner LB. Pharmacokinetics: The dynamics of drug absorption. Distribution, and elimination. In: Goodman Gilman A, Goodman LS, Rall TW, and Murad F, ed. Goodman and Gilman's The Pharmacologic Basis of Theapeutics, Seventh Edition. Macmillan Publishing Company, New York, 1985.

20. Medinsky MA and Klaasen CD. Toxicokenetics. In: Carasett and Doull's Toxicology: The Basic Science of Poisons, Fifth Edition. Klaasen CD, Amdur MO, and Doull J, editors. New York, McGraw-Hill, 1996.

Appendix A

CONSENT FORM FOR COLLECTION OF BLOOD FOR SERUM FLUOROCHEMICAL LEVEL HALF-LIFE DETERMINATION

INTRODUCTION

You and your colleagues were involved in fluorochemical production at 3M Decatur prior to your retirement. You are invited to participate in a research study being conducted by 3M Occupational Medicine Services regarding fluorochemicals. Your participation would involve donating one red-topped test tube of blood approximately every six months for the next five years. Your blood will only be tested for the amount and kind of fluorochemicals it contains; it will not be used to test for any other substances or disease. Please review this consent form carefully and be sure your questions are answered before you make a decision to participate. The plant nurse will be available to provide you with additional information at the time of your medical surveillance blood donation.

PURPOSE OF STUDY

The purpose of the study is to determine how much and what types of fluorochemicals are eliminated from the human body over time.

STUDY PROCEDURES

Your blood will be drawn with one needle stick and require two test tubes. This will occur about every six months for the next five years. A letter will be sent to you in advance informing you of the proper date, time, and place to report to for blood collection.

POTENTIAL RISKS/BENEFITS

The only discomfort you may feel is from the needle stick. You may also have some temporary redness/bruising/swelling in this area after blood collection.

BENEFITS

There will be no direct benefit from your participation in this study. However, the information gained from this study will further help us understand human exposures to fluorochemicals. Your individual results as well as the overall results will be communicated to you only. Your individual results will be considered confidential information and will not be disclosed to anyone outside the 3M Medical Department without your written consent.

COMPENSATION

If you suffer injury or a medical condition that appears to be the result of participating in this study, you will be referred to another health care professional at no cost to you. In the event of a research related injury, compensation will be determined on a case by case basis by 3M.

CONFIDENTIALITY

The overall results collected in this study may be used in publications or public presentation. Your name will not be revealed in any publication or other documents intended for publication examination. Your individual results will be communicated to you only. Your individual results will be considered confidential information and will not be disclosed to anyone outside the 3M Medical Department without your written consent.

SUBJECT RIGHTS/AVAILABILITY OF INFORMATION

If you have any questions about the study now, or later, or in the event of a research related injury or emergency, contact Dr. Jeffrey Mandel (651-733-8670) or Jean Burris, R.N. (651-737-7867). For answers to questions about your rights in regard to this research, you may contact Dr. Larry Zobel, Chair, 3M Institutional Review Board at 651-733-5181.

VOLUNTARY PARTICIPATION AND WITHDRAWAL

Participation in this study is voluntary. Refusal to participate will involve no penalty of loss of benefits to which you are otherwise entitled. You are free to withdraw from the study at any time for any reason. The investigator may stop your participation in this study should it be determined that continued participation may be detrimental to your health. Following each semi-annual blood collection, you will receive a check for $50.00 as compensation for your time and effort.

SUBJECT CONSENT

By signing the consent form, I certify that I am at least 18 years old. I confirm that I have read this consent form, and that I have been given adequate opportunity to ask any questions I may have about this consent form or the study. I also confirm that I understand the scope of my participation in this study, and that all of my questions have been answered to my satisfaction. I am signing this consent form voluntarily, and I desire to participate in the study. I understand that I will receive a copy of this signed consent form. I understand that I will receive a check for $50.00 immediately following each semi-annual collection of my blood.

_____ _____

Signature Date

_____

Printed Name

_____

Witness

Appendix B

MEDICAL HISTORY QUESTIONNAIRE

Please list the names of the **ALL** the medications you take at least once a day.

Please circle the conditions you now suffer from or have suffered from in the last three years.

Kidney Failure

Gall Bladder Disease

Hepatitis/Jaundice

Congestive Heart Failure

Liver Disease

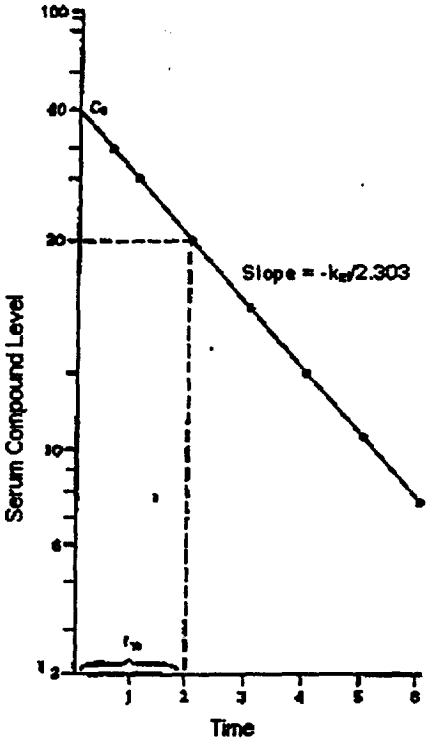
Inflammatory Bowel Disease

Crohn's Disease

Pernicious Anemia

Appendix C

Example of a log-linear plot that will be used to estimate the serum fluorochemical elimination half life in Decatur retirees.



3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICAL COMPOUNDS FROM SERUM FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-4.1 **Adoption Date:** 03/01/99

Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager	4/27/99 Date
Group Leader	4/26/99 Date
Technical Reviewer	04/26/99 Date

1.0 SCOPE AND APPLICATION

1.1 **Scope:** This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from serum.

1.2 **Applicable compounds:** Fluorochemical surfactants or other fluorinated compounds.

1.3 **Matrices:** Rabbit, rat, bovine, monkey, and human serum or other fluids as designated in the validation report.

2.0 Summary of Method

2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from serum, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard (see *3.0* ***Definitions***). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 μm nylon filter into glass autovials.

2.2 These sample extracts are analyzed following method ETS-8-5.1 or other appropriate methods.

3.0 Definitions

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$

3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 Warnings and Cautions

4.1 Health and safety warnings

4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 Interferences

5.1 There are no interferences known at this time.

6.0 Equipment

6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 Supplies and Materials

7.1 Gloves

7.2 Eppendorf or disposable pipettes

7.3 Nalgene bottles, capable of holding 250 mL and 1 L

7.4 Volumetric flasks, glass, type A

7.5 I-CHEM vials, glass, 40 mL glass

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Oxford Dispenser – 3.0 to 10.0 mL

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 Reagents and Standards

8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system

8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent

8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 **Fluorochemical standards**

8.9.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

8.9.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.9.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

8.9.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.9.6 M556 (3M Specialty Chemical Division), molecular weight = 557

8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.10.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.

8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.11 Standards preparation

8.11.1 Prepare PFOS standards for the standard curve.

8.11.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.11.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).

8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.11.7 Dilute working standard 1 with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.12 Surrogate stock standard preparation

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 mL volumetric flask and record the actual weight.

8.12.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.12.3 Prepare a surrogate working standard. Transfer approximately 1 mL of surrogate stock to a 10 mL volumetric flask and bring to volume with methanol for a working standard of 100 ppm. Record the actual volume transferred.

9.0 SAMPLE HANDLING

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

9.2 Allow samples to thaw to room temperature prior to extraction.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method blanks and matrix blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of the serum following this procedure and use as matrix blanks. See **11.1.4**.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration checks

10.3.1 Prepare continuing calibration check samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing check per group of 10 samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.3.3 Prepare each continuing calibration check from the same matrix used to prepare the initial curve.

10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than
5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

11.1.1 Transfer 1 mL of serum to a 15 mL centrifuge tube.

11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.

11.1.5 Refer to validation report **ETS-8-4.0 & ETS-8-5.0-V-1,** which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb - 1000 ppb.

11.3 Extract spiked matrix standards following **12.6-12.16** of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1 Approximate spiking amounts for standards and spikes Using 1.0 mL of matrix		
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen samples and allow to thaw at room temperature or in a lukewarm waterbath.

12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube.

12.3 Return unused samples to freezer after extraction amounts have been removed.

12.4 Record the initial volume on the extraction worksheet.

12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.

12.6 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in **11.2**.

12.7 Spike each matrix with the appropriate amount of standard as described in **11.1**, or **Table 1** in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.

12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.

12.10 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.

12.11 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.

12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.

12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

12.14 Label a fresh 15 mL centrifuge tube with the same information as in **12.5**.

12.15 Remove 4.0 mL of the organic layer to this clean 15 mL centrifuge tube.

12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.

12.17 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.

12.18 Vortex mix for 30 seconds.

12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.20 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.21 Cap and store extracts at room temperature or at approximately 4 °C until analysis.

12.22 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 Data Analysis and Calculations

13.1 Calculations

13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of standard x concentration of standard (µg /mL)}}{\text{mL of standard + mL of surrogate standard + initial matrix volume (mL)}} =$$

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.

14.3 Refer to section 14 of ETS-8-5.1 for method performance criteria.

15.0 Pollution Prevention and Waste Management

15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

18.1 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1.**

18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-5.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date
1	Section 12.21 Changed to include sample storage at room temperature. Section 12.13 Added the shaker speed. Section 12.17 Final volume is 1.0 mL; not adjusted for initial volumes less than 1.0 mL.	04/02/99

Extraction Worksheet ETS-8-4.1

Study #_____ Matrix_____ Box #_____ Wk/Day_____ DateSpiked/Analyst	Surrogate Std approx. ppm actual ppm #_____	FC-Mix approx. 0.5 pm actual ppm #_____	FC-Mix approx. 5 ppm actual ppm #_____	FC-Mix approx. 50 ppm actual ppm #_____	Comments
CCV					
MS					
MSD					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

Blank	Std #	amount =	mL
Serum Extraction Method	:		**Date & Initials**
Vortex 15 sec.			
Pipette Matrix	Volume	mL	
Pipette 1 mL of 0.5 M TBA, pH 10. pH = _____	Std. #_____		
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer	Std. #_____		
Dispense 5 mL of methyl-t-butyl ether	TN-A-_____		
Shake 20 min.	Shaker speed:		
Centrifuge 20-25 min.	Centrifuge speed:		
Remove a 4 mL aliquot of organic layer			
Put on Nitrogen Evaporator to dryness	Temperature:		
Add methanol Volume_____ mL	TN-A-_____		
Vortex 30 sec.			
Filter using a 3cc B-D syringe with a 0.2µm filter into a 1.5 mL autosample vial			

Cont. Cal. Verifications used same matrix as for std curve.

MDL/LOQ values for rabbit serum

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.74	5.55	5 ppb - 1000 ppb
PFOSA	1.51	4.79	5 ppb - 1000 ppb
PFOSAA	3.46	20.5	5 ppb - 1000 ppb
EtFOSE-OH	11.4	36.2	5 ppb - 1000 ppb
M556	6.03	19.2	5 ppb - 1000 ppb
PFOSEA	5.71	18.2	5 ppb - 1000 ppb

MDL/LOQ values in rat, bovine, monkey, and human serum, and monkey plasma were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit serum curves to determine equivalence. Responses in the rat, bovine, monkey, and human were equivalent to the rabbit responses, therefore, their MDL and LOQ will be the same values as determined in rabbit serum.

Please see LOQ Summary and MDL study in ETS-8-4.0 & 5.0-V-1 for further information.

Compound: PFOS

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.995 - 978	24.8 - 978	83-108	4.67-11.0
Low Curve	4.94 - 248	4.94 - 248	85-104	5.34-12.0
High curve	97.8 - 978	97.8 – 978	85-106	4.84-9.80
1/X	0.995 - 978	4.94 - 978	94-111	4.60-10.5

Compound: PFOSA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	4.93 - 976	88-103	5.10-14.7
Low Curve	4.93 - 97.6	4.93 - 97.6	87-105	9.85-14.7
High curve	24.8 - 976	24.8 - 978	93-102	5.08-13.9
1/X	0.993 - 976	4.93 - 976	94-103	5.10-14.5

Compound: PFOSAA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.991 - 974	24.7 - 974	81-111	4.18-10.6
Low Curve	4.92 - 247	9.74 - 247	97-107	6.38-21.8
High curve	49.2 - 974	97.4 - 974	85-108	4.33-12.5
1/X	0.991 - 974	9.74 - 974	95-115	4.11-23.2

Compound: EtFOSE-OH

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	49.3 - 976	77-110	11.2-25.5
Low Curve	4.93 – 97.6	9.76 – 97.6	97-107	14.1-21.3
High curve	49.3 - 976	97.6 - 976	90-109	11.5-19.6
1/X	0.993 - 493	9.76 - 976	86-111	11.1-21.2

Compound: PFOSEA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	24.8 - 976	96-106	10.1-16.2
Low Curve	4.93 - 248	9.76 - 248	91-110	11.8-19.5
High curve	49.3 - 976	49.3 - 976	86-106	10.2-18.2
1/X	0.993 - 976	9.76 - 976	95-117	10.1-19.1

Compound: M556

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	24.8 - 976	88-106	4.82-17.9
Low Curve	4.93 - 97.6	9.76 – 97.6	100-105	5.95-18.2
High curve	97.6 - 976	97.6 - 976	81-111	5.11-9.74
1/X	0.993 - 976	9.76 - 976	97-110	4.77-19.5

Ion Pair Standard Curves – Fluids

Prep date(s):
Analyte(s):
Sample matrix:

Standard number:
Equipment number:
Final solvent and TN:
Blank fluid/identifier:

Method/revision:
Target analyte(s):
FC mix std approx. 0.500 ppm:
FC mix std approx. 5.00 ppm:
FC mix std approx. 50.0 ppm:
Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	All Am't spiked mL	All Final vol mL
0.500	0.507	0.532	0.501	0.521	0.501	0.010	1.015
0.500	0.507	0.532	0.501	0.521	0.501	0.020	1.025
5.00	5.07	5.32	5.01	5.21	5.01	0.005	1.010
5.00	5.07	5.32	5.01	5.21	5.01	0.010	1.015
5.00	5.07	5.32	5.01	5.21	5.01	0.020	1.025
50.0	50.1	53.2	50.1	52.1	50.1	0.005	1.010
50.0	50.1	53.2	50.1	52.1	50.1	0.010	1.015
50.0	50.1	53.2	50.1	52.1	50.1	0.015	1.020
50.0	50.1	53.2	50.1	52.1	50.1	0.020	1.025

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/mL	PFOSA Final conc ng/mL	PFOSAA Final conc ng/mL	EtFOSE Final conc ng/mL	PFOSEA Final conc ng/mL	M556 Final conc ng/mL	Surrogate Std conc ng/mL	All Am't spiked mL
4.93	5.00	5.24	4.94	5.01	5.13	100	0.005
9.76	9.89	10.4	9.78	9.93	10.2		
24.8	25.1	26.3	24.8	25.2	25.8	**Surrogate**	
49.3	50.0	52.4	49.4	50.1	51.3	Final conc	
97.6	98.9	104	97.8	99.3	102	ng/mL	
248	251	263	248	252	258	500	
493	500	524	494	501	513		
735	746	782	737	749	766		
976	989	1038	978	993	1017		

Validated ranges – approximate concentrations

Serum	PFOS	PFOSA	PFOSAA	EtFOSE-OH	PFOSEA	M556
Rabbit	5.00-1000	5.00-1000	5.00-1000	5.00-1000	5.00-1000	5.00-1000
Bovine	Estimates only. Use values for rabbit.					
Rat	Estimates only. Use values for rabbit.					
Monkey & Plasma	Estimates only. Use values for rabbit.					
Human	Estimates only. Use values for rabbit.					

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-5.1 **Adoption Date:** 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

[signature]	4/26/
Laboratory Manager	Date
Kirsten [signature]	4/26/99
Group Leader	Date
Lisa A Clemen	04/26/99
Technical Reviewer	Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method describes the analysis of serum extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey, and human serum, or other fluids as designated in the validation report.

2.0 SUMMARY OF METHOD

2.1 This method describes the analysis of fluorochemical surfactants extracted from serum or other fluids, using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, m/z= 499. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the parent ion.

3.0 DEFINITIONS

3.1 **Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e., not under a vacuum).

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole systems are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.

3.4 **Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e., Z-spray components are compatible with some other Z-spray systems, etc.)

3.5 **Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 To minimize interferences when analyzing samples, teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (House air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water or equivalent, and may be provided by a Milli-Q TOC Plus system or other vendor

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. See **ETS-8-4.1**.

9.0 SAMPLE HANDLING

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C, or at room temperature, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Verifications

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ($y = my + b$), weighted $1/x$, not forced through zero, using MassLynx or other suitable software.

11.2 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.

12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording) or MRM. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).

12.1.3 Typically the analytical batch run sequence begins with a set of extracted matrix standards and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in Section **12.1.1**.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
8.50 min.	90%	10%
11.0 min.	90%	10%
12.0 min.	40%	60%

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to **ETS-9-24.0** for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.

12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.

12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 HPLC constant flow mode, flow rate 10 – 500 µL/min

12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result - Background Result}}{\text{Expected Result}} \times 100$$

13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc. - Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/mL):

$$\frac{\text{(ng of PFOS calc. from std. Curve x Dilution Factor)}}{\text{(Initial Volume of matrix (mL) + mL of Surrogate Standard)}} \times \frac{1\ \mu g}{1000\ ng}$$

Final Volume (mL)

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see **ETS-8-4.1, Attachment B,** for a listing of current validated MDL and LOQ values.

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

14.2.1 Solvent blanks, method blanks, and matrix blanks values are must be below the lowest standard in the calibration curve

14.3 Calibration Curves

14.3.1 The r^2 value for the calibration curve must be 0.980 or better.

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries are must be within ± 30% of the spiked concentration.

14.5 Continuing Calibration Verifications

14.5.1 Continuing calibration verification percent recoveries must be ± 30% of the spiked concentration.

14.6 If criteria listed in this method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.

16.3 Plot the calibration curve by linear regression, weighted 1/x, then print these graphs and store in the study folder.

16.4 Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.

16.5 Summarize data using suitable software (Excel 5.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.

16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: ETS-8-5.1 Data summary spreadsheet.

18.0 References

18.1 FACT-M-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"

18.3 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1**.

19.0 Affected Documents

19.1 ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 Revisions

Revision Number.	Reason For Revision	Revision Date
1	Section 6.1.2 Clarification of HP1100 system components. Section 11.1 Average of two curves, not standard values, are used for plotting linear regression and added the 1/x weighting of the curve. Section 12.2.2.4 Clarification of solvent ramp. Section 17.1 Changed from attachment B to A.	04/02/99

Laboratory Study

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICAL COMPOUNDS FROM LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-6.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager — Date: 7/22/99

Group Leader — Date: 7/14/99

Technical Reviewer — Date: 07/14/99

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from liver.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver, or other tissues, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals can be extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard. An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

2.2 These sample extracts are analyzed following method ETS-8-7.0 or other appropriate methods.

3.0 DEFINITIONS

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$

3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 INTERFERENCES

5.1 There are no interferences known at this time.

6.0 EQUIPMENT

6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.

6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples

6.1.2 Vortex mixer, VWR, Vortex Genie 2

6.1.3 Centrifuge, Mistral 1000 or IEC

6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 Supplies and Materials

7.1 Gloves

7.2 Dissecting scalpels

7.3 Eppendorf or disposable pipettes

7.4 Nalgene bottles, capable of holding 250 mL and 1 L

7.5 Volumetric flasks, glass, type A

7.6 I-CHEM vials, 40 mL glass

7.7 Plastic sampule vials, Wheaton, 6 mL (or appropriate size)

7.8 Centrifuge tubes, polypropylene, 15 mL

7.9 Labels

7.10 Oxford Dispensor – 3.0 to 10.0 ml

7.11 Syringes, capable of measuring 5 µL to 50 µL

7.12 Graduated pipettes

7.13 Syringes, disposable plastic, 3 cc

7.14 Syringe filters, nylon, 0.2 µm, 25 mm

7.15 Timer

7.16 Crimp cap autovials and caps

7.17 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 Reagents and Standards

8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and be provided by a Milli-Q TOC Plus™ system

8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent

8.6 Methyl-*tert*-butyl ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Liver, frozen from supplier

8.9 Dry ice from supplier

8.10 **Fluorochemical standards**

8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

8.10.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.10.6 M556 (3M Specialty Chemical Division), molecular weight = 557

8.10.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.10.8 Other fluorochemicals, as appropriate

8.11 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.11.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.

8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.12 Standards preparation

8.12.1 Prepare PFOS standards for the standard curve.

8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.12.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).

8.12.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.13 Surrogate stock standard preparation

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 ml volumetric flask and record the actual weight.

8.13.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.13.3 Prepare a surrogate working standard. Transfer approximately 1.0 ml of surrogate stock to a 10 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 ppm. Record the actual volume transferred.

9.0 SAMPLE HANDLING

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

10.0 QUALITY CONTROL

10.1 Matrix blanks and method blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of liver homogenate following this procedure and use as matrix blanks. Refer to **11.1.6**.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually a control liver received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration verifications

10.3.1 Prepare continuing calibration verification samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing calibration verification sample per group of 10 samples. For example, if a sample set = 34, four verifications are prepared and extracted.

10.3.3 Prepare each continuing calibration verification from the same matrix used to prepare the initial curve.

10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.

11.1.2 If 40 g is not available, use appropriate amounts of liver and water to ensure a 1:5 ratio.

11.1.3 Refer to **13.0** to calculate the actual density of liver homogenate and the concentration of solid liver tissue dispersed in 1.0 mL of homogenate solution.

11.1.5 Add 1 mL of homogenate to a 15 mL centrifuge tube. Re-suspend solution by shaking between aliquots while preparing a total of eighteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.

11.1.6 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks.

11.1.7 Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen samples, two matrix blanks, and two method blanks.

11.1.8 Refer to validation reports **ETS-8-6.0 and ETS-8-7.0-V-1** or **Attachment B,** which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.9 Use **Attachment C** as an aid in calculating the concentrations of the working standards. Refer to **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each working standard, blank, or continuing verification, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb – 1000ppb.

11.3 Extract spiked liver homogenates following **12.14-12.25** of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1 **Approximate Spiking Amounts for Calibration Standards**		
Working Standard (Approx. Conc.)	µl	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	2	0.005 ppm
0.50 ppm	4	0.010 ppm
0.50 ppm	10	0.025 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen liver samples.

12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.

12.3 Weigh the sample directly into a tared plastic sampule vial.

12.4 Record the liver weight in the study notebook.

12.5 Return unused liver portions to freezer.

12.6 Add 2.5 mLs of water to sampule vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds. Label the sampule vial with the study number, weight, liver ID, date and analyst initials.

12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sampule vial. Refer to attached worksheet for documenting the remaining steps.

12.12 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as method blanks.

12.13 Spike all samples, including blanks and standards ready for extraction with surrogate standard as described in section **11.2**.

12.14 Spike each matirx with the appropriate amount of standard as described in 11.1, or Table 1 of that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.15 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.

12.16 Check to ensure 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.

12.17 To each sample, add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.

12.18 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.

12.19 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.

12.20 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

12.21 Label a fresh 15 mL centrifuge tube with the same information as in **12.10**.

12.22 Remove 4.0 mL of the organic layer to the fresh 15 mL centrifuge tube.

12.23 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.

12.24 Add 1.0 mL to each centrifuge tube using a graduated pipette.

12.25 Vortex mix for 30 seconds.

12.26 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.27 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.28 Cap and store extracts at room temperature or at approximately 4 °C until analysis.

12.29 Complete the extraction worksheet, attached to this document, and tape in study notebook or include in study binder, as appropriate.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.1 Calculate the average density of the liver homogenate by recording each mass of ten separate 1.0 mL aliquots of homogenate.

$$\text{Average density (mg/mL)} = \frac{\text{Average mass (mg) of the aliquots}}{\text{1.0 mL aliquot}}$$

13.1.2 Calculate the amount of liver (mg) per 1.0 mL homogenate (or concentration of dispersed solid tissue per mL of homogenate suspension) using the following equation:

$$\frac{\text{g of Liver} \times \text{Average density* of homogenate (mg/mL)}}{\text{(g of Liver + g of Water)}}$$

* refer to **13.1.1** for details.

13.1.3 Calculate actual concentrations of PFOS and other fluorochemicals in calibration standards using the following equation:

$$\frac{\mu\text{L of Standard} \times \text{Concentration } (\mu\text{g /mL})}{\text{mg Liver / 1 mL homogenate*}} = \text{Final Concentration } (\mu\text{g/g or mg/kg}) \text{ of PFOS in Liver}$$

*refer to **13.1.2** for details.

14.0 METHOD PERFORMANCE

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (refer to **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

14.2.3 Continuing calibration verification samples to determine the continued accuracy of the initial calibration curve.

14.3 Refer to section **14** of **ETS-8-7.0** for method performance criteria.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard calculation and concentration worksheet

18.0 REFERENCES

18.1 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1**.

18.2 AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"

18.3 FACT-M-1.1, "Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-7.0, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date

Study #_____ Matrix_____ Box #_____ Wk/Day_____ Date Spiked/Analyst	Surrogate Std approx. ppm actual ppm #_____	FC Mix Std approx. 0.5 ppm actual ppm #_____	FC Mix Std approx. 5 ppm actual ppm #_____	FC Mix Std approx. 50 ppm actual ppm #_____	Comments
CCV					
MS					
MSD					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

Blank	Liver Homogenate: Std #	Liver amount =	g
Liver Extraction Method	:		**Date & Initials**
Spike surrogate and Standard mix, Vortex 15 sec.			
Pipette 1 mL of Liver Solution			
Pipette 1 mL of †0.5 M TBA, pH 10. pH =	Std. #		
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	Std. #		
Dispense 5ml of Methyl-t-Butyl Ether	TN-A-		
Shake 20 min.	Shaker Speed		
Centrifuge 20-25 min.	Centrifuge Speed		
Remove a 4 mL aliquot of organic layer			
Put on Nitrogen Evaporator to dryness	Evaporator Temperature		
Add 1.0 mL of Methanol	TN-A-		
Vortex 30 sec.			
Filter using a 3cc B-D syringe with a 0.2µm SRI filter into autosample vial			

Cont. Cal. Verifications used the same matrix as for the standard curve.

MDL/LOQ values for rabbit liver

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	8.45	26.9	30 ppb – 1200 ppb
PFOSA	3.50	11.1	12 ppb – 1200 ppb
PFOSAA	24.6	78.3	30 ppb – 1200 ppb
EtFOSE-OH	108	345	60 ppb – 900 ppb*
M556	82.3	262	60 ppb – 1200 ppb
PFOSEA	33.9	108	30 ppb- 1200 ppb

MDL/LOQ values in rat, bovine, and monkey liver were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit liver curves to determine equivalence. Responses in the rat, bovine, and monkey liver curves were equivalent to the rabbit responses, therefore, their MDL and LOQ will be assumed to be equivalent to those values as determined for the rabbit liver.

Refer to LOQ Summary and MDL study in ETS-8-6.0 & 7.0-V-1 for further information
* EtFOSE-OH estimates only for MDL and LOQ. Did not meet criteria for validation.

Compound: PFOS

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	6 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	12 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSAA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.16 - 1232	12 - 1200	30 - 1200	30 - 900	60 - 900	N/A	N/A

Compound: EtFOSE-OH

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 900	31 - 900	N/A	N/A	N/A	N/A

Compound: PFOSEA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	31 - 1200	N/A	N/A	N/A	N/A

Compound: M556

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	60 - 1200	N/A	N/A	N/A	N/A

Ion Pair Standard Curves – Tissue

Prep date(s):
Analyte(s):
Sample matrix:

Standard number:
Equipment number:
Final solvent and TN:
Blank liver/identifier:

Method/revision:
Target analyte(s):
FC mix std approx. 0.500 ppm:
FC mix std approx. 5.00 ppm:
FC mix std approx. 50.0 ppm:
Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	Std conc ug/mL	All Am't spiked mL	All Density g
0.500	0.500	0.500	0.500	0.500	0.500		0.002	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.004	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.010	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.020	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.040	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.010	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.020	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.030	0.167
50.0	50.0	50.0	50.0	50.0	50.0		0.004	0.167

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/g	PFOSA Final conc ng/g	PFOSAA Final conc ng/g	EtFOSE Final conc ng/g	PFOSEA Final conc ng/g	M556 Final conc ng/g	Std conc ng/g	Surrogate Std conc ng/mL	All Am't spiked mL
5.99	5.99	5.99	5.99	5.99	5.99		100	0.005
12.0	12.0	12.0	12.0	12.0	12.0			
29.9	29.9	29.9	29.9	29.9	29.9		**Surrogate**	
59.9	59.9	59.9	59.9	59.9	59.9		Final conc	
120	120	120	120	120	120		ng/mL	
299	299	299	299	299	299		0.500	
599	599	599	599	599	599			
898	898	898	898	898	898			
1198	1198	1198	1198	1198	1198			

Validated ranges – approximate concentrations

Liver	PFOS	PFOSA	PFOSAA	EtFOSE-OH	POAA	PFOSEA
Rabbit	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb
Bovine	Estimates only, use rabbit values.					
Rat	Estimates only, use rabbit values.					
Monkey	Estimates only, use rabbit values.					

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-7.0 **Adoption Date:** 07/22/99

Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager — Date: 7/22/99

Group Leader — Date: 7/14/99

Technical Reviewer — Date: 07/14/99

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of liver extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey liver, or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

2.1 This method describes the analysis of fluorochemical surfactants extracted from liver using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, m/z = 499. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the selected parent ion.

3.0 DEFINITIONS

3.1 **Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.

3.4 **Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)

3.5 **Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details refer to the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 **Health and Safety Warnings:**

4.1.1 Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 To minimize interferences when analyzing samples, Teflon shall not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source.

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade air regulated to approximately 100 psi (house air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data

7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor

8.1.3 Ammonium acetate, reagent grade or equivalent

8.1.3.1 When preparing different amounts than those listed, adjust accordingly.

8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. Refer to **ETS-8-6.0**.

9.0 Sample Handling

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples may be stored at room temperature, or refrigerated at approximately 4° C, until analysis can be performed.

10.0 Quality Control

10.1 Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks, and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Checks

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard every tenth sample, with a minimum of one per batch.

11.0 Calibration and Standardization

11.1 Analyze the extracted matrix standards prior to and following each set of sample extracts. The average of two standard curves will be plotted by linear regression ($y = mx + b$), weighted 1/x, not forced through the origin, using MassLynx or other suitable software.

11.2 If the curve does not meet requirements perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Set up the sample list.

12.1.1.1 Assign a sample list filename using MO-DAY-last digit of year-increasing letter of the alphabet starting with a

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

12.1.2 To create a method click on method in the Acquisition control panel then mass spectrometer headings and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. Refer to Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM.

12.1.3 Typically the analytical batch run sequence begins and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration verification injected standard after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in Section **12.1.1**.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
1.0 min.	40%	60%
4.5 min.	95%	5%
6.5 min.	95%	5%
7.0 min.	40%	60%
9.0 mi.	40%	60%

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to **ETS-9-24.0**, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Turn on the nitrogen.

12.3.5 Open the tune page. Clicks on operate to initiate source block and desolvation heaters.

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

12.3.6.2 Set the flow to 10 - 500 uL/min or as appropriate

12.3.6.3 Observe droplets coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

12.3.7 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

12.3.7.3 HPLC constant flow mode flow rate 10 – 500 μL/min

12.3.7.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.

12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result - Background Result}}{\text{Expected Result}} \times 100$$

13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc. - Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.6 Calculate actual concentrations in matrix (µg/g):

$$\frac{\text{(ng of PFOS calc. from std. Curve x Dilution Factor)}}{\frac{\text{(Initial Weight of Liver (g)}}{\text{Final Volume (mL)}}} \times \frac{1\ \mu g}{1000\ ng}$$

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Refer to **ETS-8-6.0, Attachment B** for a listing of current validated MDL and LOQ values.

14.2 Solvent Blanks, Method Blanks and Matrix Blanks

14.2.1 Solvent blanks, method blanks, and matrix blanks must be below the lowest standard in the calibration curve.

14.3 Calibration Curves

14.3.1 The r^2 value for the calibration must be 0.980 or better.

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries must be within ± 30% of the spiked concentration.

14.5 Continuing Calibration Verification

14.5.1 Continuing calibration verification percent recoveries must be within ± 30% of the spiked concentration.

14.6 If criteria listed in the method performance section are not met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 Pollution Prevention and Waste Management

15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 Records

16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.

16.3 Plot the calibration curve by linear regression, weighted 1/x, then print these graphs and store in the study folder.

16.4 Print data integration summary, integration method, and chromatograms from MassLynx and store in the study folder.

16.5 Summarize data using suitable software (Excel 5.0+) and store in the study folder, refer to **Attachment A** for an example of a summary spreadsheet.

16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: ETS-8-7.0 Data summary spreadsheet

18.0 References

18.1 FACT-M-2.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"

18.3 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1**

19.0 Affected Documents

19.1 ETS-8-6.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date

Laboratory Study

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ng/g	Initial Wt. g	Dilution Factor	Final Conc. ug/g

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ng/g): Taken from the MassLynx integration summary.
Initial Wt. (g): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/g): Calculated by dividing the initial volume from the concentration