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April 20, 2000

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Dr. Oscar Hernandez
US EPA/OPPT/CCD (7405)
401 M Street, SW
Room E615B
Washington, DC 20460

Dear Dr. Hernandez:

I am writing to clarify information contained in the two-generation reproduction study on potassium perfluorooctanesulfonate. A report of this study was submitted on February 15, 2000 as a supplement to the TSCA Section 8(e) Notice of 9/14/98. That notice had been filed midway through the study when we became aware of F1 pup mortality at the highest two doses in the study (1.6 and 3.2 mg/kg/d). The study was conducted with perfluorooctanesulfonate given by oral gavage at four doses, 0.1, 0.4, 1.6 and 3.2 mg/kg/d. Following the pup mortality seen in the 1.6 and 3.2 mg/kg/day dose groups, only the 0.1 and 0.4 mg/kg/day doses groups were continued into the second generation.

Language in the report indicated that F2 pup viability was affected in the 0.4 mg/kg/day dose group. This is not the case. Attached is an amended report from the laboratory performing the study, Primedica, Argus Division. The amended language clearly states that there were no toxicologically important effects on pup survival or growth at the highest dosage tested 0.4 mg/kg/day. I have attached table E20 for your reference. It shows no significant differences between the vehicle control group and the 0.4 mg/kg/day dose group for number of stillborn (0.3+/- 0.6 and 0.5 +/- 0.9), pup viability (97.1 % and 96.2%) or number of pups surviving per litter, preculling, on day 4 (13.7 +/- 3.3 and 13.8 +/- 2.4).

Dr. Marv Case, Director of Toxicology at 3M and Dr. Mildred Christian and Dr. Raymond York of Primedica Argus prepared a paper for publication that reflects this interpretation. This paper is still under internal review. Dr. John (Jack) Moore, formerly of EPA and now a consultant in Toxicology, has also reviewed the full report and agrees with this interpretation.

I request that you treat the attached report as superceding the earlier report. You will note that the study report submitted as follow up to the 8e had only summary data tables. The enclosed report includes all of the data tables, individual as well as summary.

Also enclosed is an analytic report that gives the perfluorooctanesulfonate concentrations in liver and serum samples collected from selected animals in the two-generation reproduction study. The serum and liver analytical data included in the report offer a limited understanding, because of the timing and limited number of samples, of the pharmacokinetics of perfluorooctanesulfonate.

Two additional studies have been undertaken to develop an understanding of the disposition of perfluorooctanesulfonate during gestation and lactation. In the first of these studies, potassium perfluorooctanesulfonate was administered by gavage up to the point of mating, and disposition of the body burden in dams to pups was followed during gestation. In the second study, dosing continued through gestation and lactation, and disposition was followed through lactation. The in-life aspects of these studies are complete, and reports are enclosed. Protocols for the analytical work associated with these two studies is enclosed. Final analytical reports should be available within three months.

A complete cross fostering study was also done. This study was done with only two groups, a control and a group dosed at 1.6 mg/kg/day. This dose level had minimal effect on the dams but produced neonatal mortality in the original two generation study. The cross-fostering study shows that neonatal pup mortality is related to exposure of pups *in utero* during gestation, although there appears to be a contribution to the mortality from the treated dams if pups were also exposed *in utero*. A copy of the final report is enclosed.

Two ancillary reports are associated with the cross-fostering study. In the first, serum perfluorooctanesulfonate levels in dams and pups were monitored at lactation days 14 and 21 or 22. This report demonstrates that pups are exposed *in utero* and can be exposed via milk. Litters with exposure *in utero* and via milk from treated foster-dams have serum values approximating foster-dam serum values on days 21 or 22 of lactation. Litters which were born of untreated dams but cross-fostered to treated dams have serum concentrations roughly one-third of foster-dam values at the end of lactation. Litters born of treated dams but cross-fostered to untreated dams have serum values of approximately half their birth dams at the end of lactation. The untreated foster dams are found to acquire single-digit ppm levels of perfluorooctanesulfonate in their serum, presumably from exposure to pup urine and feces.

The second ancillary report to the cross-fostering study relates to electron microscopy conducted on lung and liver tissue of pups culled shortly after birth. Lung tissue was examined for possible effects of perfluorooctanesulfonate on pulmonary surfactant production. There was no difference between control and exposed pups with regard to lung architecture and the presence of lamellar bodies in Type II pneumocytes. Surfactant production appeared normal. Since perfluorooctanesulfonate causes peroxisome proliferation in rodents, liver tissue was examined for the presence of peroxisomes. Pups exposed *in utero* were found to have a two to three fold increase in peroxisomes. Copies of both reports are enclosed.

An additional follow-up study is being planned to explore the possible role of decreases in cholesterol at birth as a mechanism for reduced pup viability. It has been shown (Levin et. al., 1989) that rat pups undergo a burst of steroid synthesis activity prior to birth followed by a period of much reduced synthesis following birth (perhaps due to the presence of exogenous cholesterol during lactation). HMG CoA reductase and HMG CoA synthase mRNAs are highest during late fetal life and undergo a precipitous reduction immediately after parturition. Harb et. al. showed that fluvastatin, an HMG CoA reductase inhibitor marketed as Lescol for hypercholesterolemia, if administered prior to and during gestation, causes neonatal mortality. The mortality was prevented by co-administration of mevalonate, the product of the reaction catalyzed by HMG CoA reductase.
Perfluorooctanesulfonate reduces serum cholesterol in laboratory animals and is reported to reduce activity of HMG CoA reductase (Haughom and Spydevold, 1992). Our planned study would test the hypothesis that the neonatal mortality observed in the high dose groups of the two-generation perfluorooctanesulfonate study is due to the reduction in cholesterol synthesis due to reduced HMG CoA reductase activity. This would be done by attempting mevalonate rescue, similar to the fluvastatin study. We will also attempt rescue with cholesterol.

Two additional objectives are being designed into the planned study. The first would be to more precisely define a NOEL and LOEL for pup mortality by testing doses between 0.4 and 1.6 mg/kg/day. The second is to obtain additional pharmacokinetic data.

Human infants do not undergo a similar precipitous drop in cholesterol synthesis at birth (Levin et. al., 1989). Establishing reduced cholesterol synthesis as a mechanism of action would have significant implications when assessing risk associated with the findings in the two-generation study. The planned study is currently under negotiation with a contract laboratory. We will share the protocol and expected completion date with you when they are available.

We are looking forward to discussions between our scientists and agency scientists about the information we have submitted and continue to submit on perfluorooctanesulfonate.

Sincerely,

Larry R. Zobel, MD MPH
Staff Vice President & Medical Director

c: Dr. Charles Auer, Director Chemical Control Division (no attachments)

References:

Harb, R.V., Hartman, H.A., and Cox, R.H. (1994), Prevention of fluvastatin induced toxicity, mortality, and cardiac myopathy in pregnant rats by mevalonic acid supplementation. *Teratology,* **50,** 19-26

Haughom, B. and Spydevold, O. (1992), The mechanism underlying the hyperlipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOS) and clofibric acid. *Biochimica et Biophysica Acta,* **1128**, 65-72.

Levin, M.S., Pitt, A.J.A., Schwartz, A.L., Edwards, P.A. and Gordon, J.I. (1989), Developmental changes in the expression of genes involved in cholesterol biosynthesis and lipid transport in human and rat fetal and neonatal livers. *Biochimica et Biophysica Acta,* **1003**, 293-300.

Attachments

Protocol 418-008 Combined oral (gavage) fertility, developmental and perinatal/postnatal reproduction toxicity study of PFOS in rats (Sponsor's study number: 6295.9), Table E20, 1-3.

Harb, R.V., Hartman, H.A., and Cox, R.H. (1994), Prevention of fluvastatin induced toxicity, mortality, and cardiac myopathy in pregnant rats by mevalonic acid supplementation. *Teratology*, **50**, 19-26

Haughom, B. and Spydevold, O. (1992), The mechanism underlying the hyperlipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOS) and clofibric acid. *Biochimica et Biophysica Acta*, **1128**, 65-72.

Levin, M.S., Pitt, A.J.A., Schwartz, A.L., Edwards, P.A. and Gordon, J.I. (1989), Developmental changes in the expression of genes involved in cholesterol biosynthesis and lipid transport in human and rat fetal and neonatal livers. *Biochimica et Biophysica Acta*, **1003**, 293-300.

Final Report Protocol 418-013 Oral (Gavage) Pharmacokinetic Study of PFOS in Rats (Sponsor's study number: T-6295.12), June 24, 1999

Protocol FACT-TOX-110 Oral (Gavage) Pharmacokinetic Study of PFOS in Rats, Protocol, June 8, 1999

Final Report Protocol 418-015 Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats (Sponsor's study number: T-6295.14), July 23, 1999

Protocol FACT-TOX-111 Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats, Protocol, June 8, 1999

Summary PFOS Rat Two-Generation Reproduction Study (Study numbers: 3M T-6295.9, Argus 418-008 [in-life], FACT-TOX-012 [analytical])

Report Amendment I, 13 April 2000, Protocol 418-008 (Sponsor's study number: 6295.9) Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats, Final Report

Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number: 2759-39-3) in the Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage, Laboratory Report No. <U2006>, Requestor Project No. <3M TOX 6295.9>, October 27, 1999

Interoffice Memo from Tom Kestner to Leo Gehlhoff, "Fluorochemical Isomer Distribution by ^{19}F-NMR Spectroscopy, December 1, 1997

Final Report Protocol 418-008 Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats (Sponsor's study number 6295.9, June 10, 1999

Summary PFOS Rat Cross-Fostering (Study numbers: 3M T-6295.13, Argus 418-014)

Final Report Protocol 418-014, Oral (Gavage) Cross-Fostering Study of PFOS in Rats (Sponsor's study number: T-6295.13), July 23, 1999

Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) (CAS Number: 2759-39-3) in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage, Laboratory Report No. U2779, Requestor Project No. 3M Tox 6295.13, June 10, 1999

Perluorooctanesulfonate:

PK During Pregnancy and Lactation + Analytical Protocol

PROTOCOL 418-008: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: 6295.9)

TABLE E20 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	22	21	24
PUPS DELIVERED (TOTAL)	N	316	325	357
	MEAN±S.D.	14.4 ± 3.5	15.5 ± 2.5	14.9 ± 2.6
LIVEBORN	MEAN±S.D.	14.1 ± 3.5	15.1 ± 2.4	14.3 ± 2.5
	N(%)	310(98.1)	318(97.8)	344(96.4)
STILLBORN	MEAN±S.D.	0.3 ± 0.6	0.3 ± 1.1	0.5 ± 0.9
	N(%)	6(1.9)	7(2.2)	13(3.6)
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED				
DAY 1	N/N(%)	2/310(0.6)	2/318(0.6)	2/344(0.6)
DAYS 2- 4	N/N(%)	7/308(2.3)	2/316(0.6)	11/342(3.2)
DAYS 5- 7	N/N(%)	0/171(0.0)	2/167(1.2)	5/191(2.6)
DAYS 8-14	N/N(%)	0/171(0.0)	1/165(0.6)	1/178(0.6)a
DAYS 15-21	N/N(%)	0/171(0.0)	0/164(0.0)	0/177(0.0)a
VIABILITY INDEX b	%	97.1	98.7	96.2
	N/N	301/310	314/318	331/344
LACTATION INDEX c	%	100.0	98.2	96.7
	N/N	171/171	164/167	177/183

DAY(S) = DAY(S) POSTPARTUM

a. Excludes values for litter 13257; the dam was found dead on day 10 of lactation; eight pups were sacrificed.

b. Number of live pups on day 4 (preculling) postpartum/number of liveborn pups on day 1 postpartum.

c. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 4 (postculling) postpartum.

PROTOCOL 418-008: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: 6295.9)

TABLE E20 (PAGE 2): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	22	21	24
SURVIVING PUPS/LITTER a				
DAY 1 b	MEAN±S.D.	14.1 ± 3.5	15.1 ± 2.4	14.3 ± 2.5
DAY 4 PRECULLING	MEAN±S.D.	13.7 ± 3.3	15.0 ± 2.8	13.8 ± 2.4
DAY 4 POSTCULLING	MEAN±S.D.	7.8 ± 0.8	8.0 ± 0.2	8.0 ± 0.2
DAY 7	MEAN±S.D.	7.8 ± 0.8	7.8 ± 0.5	7.8 ± 0.7
DAY 14	MEAN±S.D.	7.8 ± 0.8	7.8 ± 0.7	7.7 ± 0.8 [24]c
DAY 21	MEAN±S.D.	7.8 ± 0.8	7.8 ± 0.7	7.7 ± 0.8 [24]c
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED				
DAY 1 b	MEAN±S.D.	47.9 ± 15.2	52.1 ± 10.9	47.0 ± 13.3
DAY 4 PRECULLING	MEAN±S.D.	47.9 ± 15.1	51.5 ± 11.1	46.6 ± 13.4
DAY 4 POSTCULLING	MEAN±S.D.	47.0 ± 10.0	49.6 ± 1.6	49.1 ± 5.7
DAY 7	MEAN±S.D.	47.0 ± 10.0	48.9 ± 3.9	48.7 ± 6.4
DAY 14	MEAN±S.D.	47.0 ± 10.0	49.2 ± 2.6	48.3 ± 6.2 [24]c
DAY 21	MEAN±S.D.	47.0 ± 10.0	49.2 ± 2.6	48.3 ± 6.2 [24]c

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Average number of live pups per litter, including litters with no surviving pups.
b. Includes pups born alive, found dead day 1 postpartum.
c. Excludes values for litter 13257; the dam was found dead on day 10 of lactation; eight pups were sacrificed.

PROTOCOL 418-008: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: 6295.9)

TABLE E20 (PAGE 3): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	22	21	24
LIVE LITTER SIZE AT WEIGHING				
DAY 1	MEAN±S.D.	14.0 ± 3.5	15.0 ± 2.6	14.2 ± 2.5
DAY 4 PRECULLING	MEAN±S.D.	13.7 ± 3.3	15.0 ± 2.8	13.8 ± 2.4
DAY 4 POSTCULLING	MEAN±S.D.	7.8 ± 0.8	8.0 ± 0.2	7.9 ± 0.3
DAY 7	MEAN±S.D.	7.8 ± 0.8	7.8 ± 0.5	7.8 ± 0.7
DAY 14	MEAN±S.D.	7.8 ± 0.8	7.8 ± 0.7	7.7 ± 0.8 [23]a
DAY 21	MEAN±S.D.	7.8 ± 0.8	7.8 ± 0.7	7.7 ± 0.8 [23]a
PUP WEIGHT/LITTER (GRAMS)				
DAY 1	MEAN±S.D.	6.3 ± 0.8	6.1 ± 0.5	6.2 ± 0.5
DAY 4 PRECULLING	MEAN±S.D.	8.7 ± 1.6	8.2 ± 1.0	8.0 ± 1.3
DAY 4 POSTCULLING	MEAN±S.D.	8.8 ± 1.6	8.3 ± 1.0	8.0 ± 1.3
DAY 7	MEAN±S.D.	14.7 ± 2.4	13.9 ± 2.2	12.8 ± 2.6*
DAY 14	MEAN±S.D.	32.0 ± 3.5	31.8 ± 3.1	28.9 ± 4.7** [23]a
DAY 21	MEAN±S.D.	50.1 ± 5.1	49.2 ± 5.0	46.5 ± 6.3 [23]a

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for litter 13257; the dam was found dead on day 10 of lactation; eight pups were sacrificed.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

From Dianna 12/1/99 ** my copy *

TERATOLOGY 50:19–26 (1994)

Prevention of Fluvastatin-Induced Toxicity, Mortality, and Cardiac Myopathy in Pregnant Rats by Mevalonic Acid Supplementation

ROMAN V. HRAB, HOWARD A. HARTMAN, AND RAYMOND H. COX, JR.
Regulatory Toxicology, Drug Safety Department, Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, East Hanover, New Jersey 07936

ABSTRACT Mevalonic acid is a product of the enzyme HMG-CoA reductase which is essential for cholesterol biosynthesis. Fluvastatin (Sandoz compound XU 62-320) is a potent inhibitor of this enzyme and, hence, mevalonic acid production. In three separate studies, oral administration of fluvastatin at 12 and 24 mg/kg/day to mated rats from day 15 of gestation through weaning resulted in unanticipated maternal mortality at the time of parturition and during lactation. Microscopic evaluations performed in two studies revealed significant cardiac myopathy in the dying animals. Drug-related clinical signs, significant maternal body weight loss, and an increase in stillborn pups and neonatal mortality were also noted at one or both dose levels. Supplementation of fluvastatin administration with 500 mg/kg b.i.d. of mevalonic acid completely blocked and/or ameliorated the mortality, cardiac myopathy, and other adverse effects. These studies indicate that the adverse maternal effects observed with fluvastatin before or following parturition resulted from exaggerated pharmacologic activity at the dose levels administered, i.e., inhibition of the enzyme HMG-CoA reductase, its immediate product mevalonic acid, and cholesterol biosynthesis.
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? cardiac myopathy

Fluvastatin (Sandoz compound XU 62-320) is a synthetic potent inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (Engstrom et al., '88; Kathawala et al., '88: Kathawala, '91). Administration of fluvastatin in rats, dogs, and monkeys induces significant reductions in serum total cholesterol, low-density lipoprotein cholesterol, and serum triglyceride levels (Engstrom et al., '88). During the safety assessment of fluvastatin, nonclinical studies were performed to evaluate the effect of this compound on fertility, reproductive performance, and teratogenicity in rats and rabbits. There were no adverse effects on fertility or reproductive performance up to the highest dose levels tested in male (20 mg/kg/day) and female (6 mg/kg/day) rats, and no evidence of teratogenic activity in rats at doses up to 36 mg/kg/day or rabbits at doses up to 10 mg/kg/day (R.V. Hrab, unpublished data). However, in a perinatal/postnatal study (Segment III), 12 and 24 mg/kg/day of fluvastatin administered to pregnant rats from day 15 pc (post coitus) through weaning resulted in maternal mortality at or near term and during the postpartum period. This mortality was replicated in a subsequent follow-up study, and microscopic tissue evaluation revealed the occurrence of cardiomyopathy only in the dying animals. No cardiac pathology was detected in nonpregnant animals in these studies with fluvastatin. As in previous rat studies (Stoll et al., '88), forestomach epithelial hyperplasia and hyperkeratosis were found. These have been shown to be specific to rodents and to be a result of contact irritation by fluvastatin (Robison et al., '94).

Coadministration of mevalonic acid, the immediate product of the enzyme HMG-CoA reductase, has been shown to prevent or antagonize various organ toxicities induced in rats and rabbits with other HMG-CoA reductase inhibitors (MacDonald et al., '88; Kornbrust et al., '89). In pregnant rats, it has been shown that 500 mg/kg b.i.d. of mevalonic acid suppressed the teratogenicity of mevinolinic acid, an inhibitor of HMG-CoA reductase, when administered prior to and approximately 5 hr after administration of mevinolinic acid (Minsker et al., '83). Since fluvastatin is an inhibitor of the enzyme HMG-CoA reductase and therefore of mevalonic acid production, a relationship was considered to exist between the exaggerated pharmacologic activity associated with HMG-CoA reductase inhibition by fluvastatin and the occurrence of maternal mortality and/or cardiac lesions. The study reported herein was designed to determine whether coadministration of me-

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Address reprint requests to Roman V. Hrab, Regulatory Toxicology, Drug Safety Department, Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, East Hanover, NJ 07936.

This paper was submitted for publication shortly after the untimely death of Dr. Cox in August 1993.

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TABLE 1. Daily dosing schedule

Group	First dose administration	Second dose administration (approx. 1/2 hr. after first dose)	Third dose administration (approx. 5 hr. after second dose)
I	Deionized H_2O	1% CMC	Deionized H_2O
II	Mevalonic acid 500 mg/kg	1% CMC	Mevalonic acid 500 mg/kg
III	Mevalonic acid 500 mg/kg	Fluvastatin 12 mg/kg/day	Mevalonic acid 500 mg/kg
IV	Mevalonic acid 500 mg/kg	Fluvastatin 24 mg/kg/day	Mevalonic acid 500 mg/kg
V	—	Fluvastatin 12 mg/kg/day	—
VI	—	Fluvastatin 24 mg/kg/day	—

valonic acid with fluvastatin from day 15 of gestation through day 21 postpartum could inhibit or block the adverse effects noted with fluvastatin.

MATERIALS AND METHODS

Animals

One hundred twenty female rats (Charles River CD®-Sprague-Dawley-derived) obtained from Charles River Breeding Laboratories, Inc., Kingston, New York, were approximately 14 wk old at the start of the study. A group of males of the same strain and age, and from the same supplier, was used for mating, during which each female was housed with one male. Observation of vaginal plugs the following morning was considered evidence of mating and day 0 of gestation or day 0 post coitus (pc). The day on which pups were born was designated day 0 postpartum (pp), while day 21 pp was the day of weaning. As the females were mated, they were randomly assigned to six groups until 20 rats per group were mated. After mating, females were housed individually in suspended stainless steel wire-bottom cages in a temperature and humidity controlled room with a 12-hr light and 12-hr dark cycle. On day 20 of gestation, the rats were transferred to transparent plastic cages with bedding consisting of hardwood chips. Certified Purina rodent chow (pellets) and water (provided via automatic watering device) were supplied ad libitum.

Compounds administered

Lot 28 of fluvastatin (XU 62-320) was used in this experiment. Purity was determined by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to be 99+%. DL-mevalonic acid lactone was obtained from Fluka Chemical Corp. (Ronkonkoma, NY), and purity was found to be 96+% as determined by HPLC.

where get Fluvastatin?

Dosage administration

Dosing solutions of fluvastatin were prepared fresh daily in 1% carboxymethylcellulose (CMC). Mevalonic acid was prepared fresh twice daily in deionized water. Individual animal doses were calculated and administered daily (to females only) via gavage from day 15 of gestation through day 21 postpartum at a volume of 10 ml/kg (Table 1). Dose calculations were based on the daily body weight from day 15 of gestation until delivery (or day 28 pc for those animals which did not deliver). Following delivery (day 0 pp) through weaning (day 21 pp), the dose calculations were based on the most recently recorded body weight. Control rats received three separate doses: 10 ml/kg b.i.d. of deionized water and 10 ml/kg/day of 1% CMC, also based on the same body weight schedules as in the dose groups. Concentrations of the dosing solutions were assayed and verified four times during the study.

Examinations

Individual clinical observations were recorded at least once daily. Body weights were recorded on days 0, 7, and daily from days 15 through 20 of pregnancy. After each animal delivered, maternal body weights were recorded on days 0, 7, 14, and 21 pp. Food consumption was recorded only during gestation on days 0, 7, 15, and 20 pc.

Following delivery of each litter, viability, clinical signs, and external examinations of pups were recorded on days 0 pp through 21 pp. The sex and individual weights of all viable pups were recorded on days 0, 1, 4, 7, 14, and 21 pp. Pups stillborn or found dead during the postpartum period and not autolyzed or cannibalized were examined, identified, and preserved in neutral buffered 10% formalin. Dams dying spontaneously or sacrificed moribund were necropsied. Animals which did not deliver were necropsied on day 28 pc. On day 21 pp, all remaining dams and pups were euthanized, necropsied, and examined. Pups with lesions as well as several control group pups were identified and saved in neutral buffered 10% formalin. All other pups were discarded.

Post mortem examinations

Following induction of deep surgical anesthesia using excess CO_2, animals were euthanized by severing the axillary vessels for exsanguination. Terminal body weight was recorded for each dam and a thorough dissection was performed on all surviving dams at weaning (day 21 pp). All gross lesions and approximately 40 representative tissue specimens were collected and preserved in neutral buffered 10% formalin.

Spontaneously dying animals were dissected and a complete set of tissue specimens, including the gravid reproductive tracts, were collected and preserved. Nonpregnant animals were sacrificed on day 28 pc. Since the treatment-related cardiac findings occurred only in

TABLE 2. Cause of death of spontaneously dying animals

Dose group	Total number of females that died on study	Pregnant females that died before delivery on day 22 pc	Pregnant females that died postpartum	Nonpregnant females dying during experiment
I Control	0	0	0	0
II Mevalonic acid 500 mg/kg	2	0	1 (sac. moribund day 1 pp)[4]	1 (day 26 pc)[5]
III Fluvastatin 12 mg/kg, mevalonic acid 500 mg/kg	1	0	0	1 (day 18 pc)[4]
IV Fluvastatin 24 mg/kg, mevalonic acid 500 mg/kg	2	0	0	1 (day 19 pc)[3] 1 (elective sac. day 15 pc)[7]
V Fluvastatin 12 mg/kg	3	1[1]	1 (day 4 pp)[1] 1 (day 13 pp)[6]	0
VI Fluvastatin 24 mg/kg	8	1[2]	1 (day 2 pp)[2] 1 (day 5 pp)[2] 1 (day 6 pp)[2] 2 (sac. moribund day 10 pp)[2] 1 (day 12 pp)[2] 1 (day 14 pp)[2]	0

[1]Cause could not be determined.
[2]Heart lesion (vacuolar degeneration, and/or myocarditis probably related to death).
[3]Intubation accident, lesions seen by gross or microscopic examination.
[4]Intubation accident based on clinical observations and gross or microscopic examination.
[5]Possible intubation accident, although microscopic findings were not conclusive.
[6]Intubation accident based on clinical observations, although microscopic findings were not conclusive.
[7]Broken, malaligned incisors; animal in poor health.

pregnant animals, data from the nonpregnant animals were considered separately.

Based on results from previous studies, microscopic evaluations were limited to the hearts and stomachs from all animals. For spontaneously dying or animals sacrificed moribund, the tissue evaluation included gross lesions, lungs, trachea, thymus with mediastinum, and liver.

Statistical analysis

All statistical evaluations of data compared treatment groups with controls at $P \leq 0.05$ and $P \leq 0.01$ levels of significance, with a two-tailed analysis. Maternal body weight, duration of gestation, number of pups delivered, live pups per litter, pup weight, and implantation sites were evaluated by analysis of variance (ANOVA) followed by Dunnett's Test. Fisher's Exact Test was used to evaluate maternal fertility and reproductive performance, numbers of dead pups, and sex ratio, as well as neonatal necropsy examination data. Maternal terminal body weights were evaluated by a one-way ANOVA followed by Duncan's Multiple Range Test.

RESULTS

Maternal mortality and clinical observations

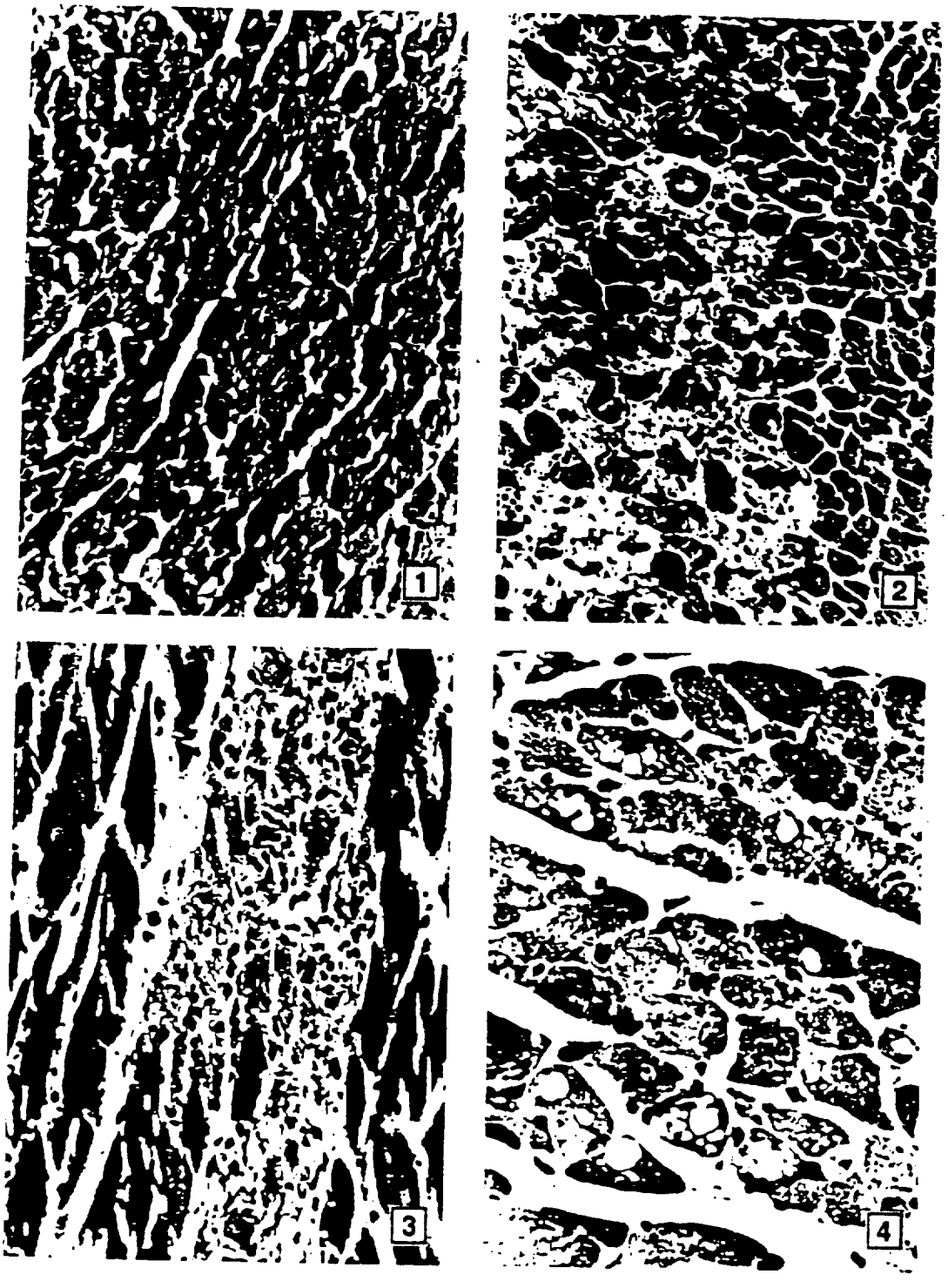
Drug-related maternal mortality occurred in animals receiving 12 and 24 mg/kg/day of fluvastatin where 2 out of 17 and 8 out of 17 pregnant animals, respectively, died or were sacrificed moribund (Table 2). One animal from each of these groups died on day 22 of gestation without completing delivery but had full-term fetuses in utero. Others died within the first 2 wk postpartum. The cause of death of the 8 animals administered fluvastatin at 24 mg/kg/day was related to the presence of cardiomyopathy characterized by hyaline, granular or vacuolar degeneration, and/or myocarditis (Fig. 2–5). The cause of death of the 2 rats administered fluvastatin at 12 mg/kg/day could not be determined.

Necropsy examination of the 2 animals that died on day 22 of gestation revealed dead, morphologically normal full-term fetuses in the uterus, indicating that they were probably alive up to the time of the dam's death. Furthermore, drug-related clinical observations were seen in the 24 mg/kg/day fluvastatin animal which included decreased locomotor activity (on days 20 and 21 pc), splayed (extended) hindlimbs, tremors, ataxia, labored breathing, salivation, lacrimation, and disorientation on day 21 pc. Similar signs were seen in the 12 mg/kg/day animal on day 22 pc prior to death.

Other animals from the 12 and/or 24 mg/kg/day fluvastatin groups also exhibited various drug-related clinical signs including ataxia, impairment/loss of righting reflex, decreased locomotor activity, hunched or flattened body position, splayed (extended) hind limbs, ptosis, tremors, disorientation, labored breathing, skin pallor, and dehydration.

Maternal body weight and food consumption

No treatment-related differences in maternal body weight gain were noted during gestation days 15–20, the initial period of administration of fluvastatin and/or mevalonic acid (Table 3). During the first 7 days after delivery, a transient, statistically significant ma-



Figs. 1–4.

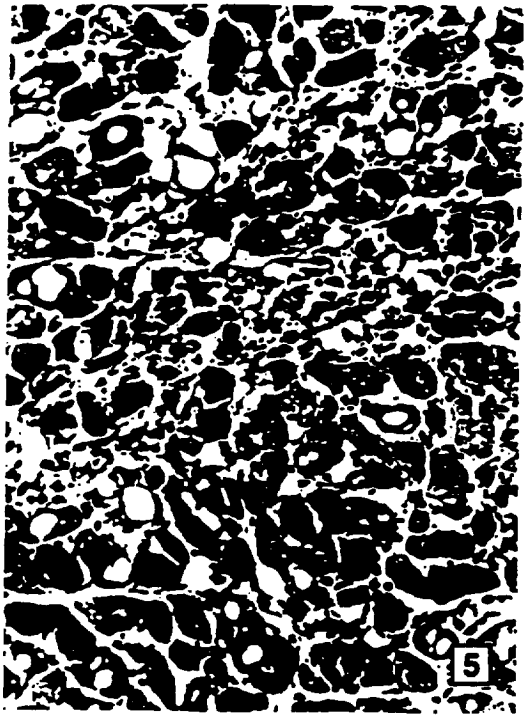


Fig. 5. 24 mg/kg: death on day 14 pp. In some high-dose animals dying in the later postpartum period, myocardial lesions encountered were often more complex, frequently characterized by areas of myofiber disintegration accompanied by inflammatory infiltrates and interstitial edema. The affected areas ranged from limited to widespread. Not seen in earlier myocardial lesions was the occurrence of conspicuous cytoplasmic vacuolization illustrated in this figure (× 400).

ternal body weight *loss* of 5% and 9% was apparent in the 12 and 24 mg/kg/day fluvastatin dose groups, respectively, compared to a 4% *gain* in controls. A 2% body weight *loss* occurred in animals receiving the 24 mg/kg/day dose level of fluvastatin supplemented with mevalonic acid. During the next 7 days (days 7–14 pp), a body weight gain was evident in all groups. However, considering the first 2 wk postpartum (days 0–14), an overall body weight loss was nevertheless apparent in the 2 fluvastatin groups (Table 3).

Subsequently, during days 14–21 pp, a slight maternal body weight loss was observed in all groups including controls. Maternal weight loss is not uncommon during this time period and is probably due to a greater nutritional demand on the dams resulting from increased lactation, combined with more ingestion of feed by the pups and therefore less by the dams. Although not statistically significant, a slight decrease in food consumption of approximately 11% was evident during the treatment period from days 15–20 of gestation in both groups administered 24 mg/kg/day of fluvastatin, with and without mevalonic acid (Table 3).

Fertility and reproductive performance

No remarkable variation in pregnancy rate occurred among the groups. Considering the mean length of the gestation period of those animals completing delivery, there was no treatment-related variation among groups (Table 4). A slightly lower gestation index in Group VI (fluvastain at 24 mg/kg/day) reflects the one animal which had no viable pups, 5 stillborn pups, and 7 dead pups of unknown viability status at birth.

Postnatal litter data

The mean number of liveborn pups was lower in both groups administered fluvastatin alone (Groups V and VI) when compared to concurrent controls (Table 5). An increased incidence of stillborn pups occurred in the 12 and 24 mg/kg/day fluvastatin Group V (7%) and Group VI (22%), compared to a range of 2–3% in controls and Groups II, III, and IV. There was no evidence to suggest any remarkable increase in in utero postimplantation embryo/fetal mortality (i.e., early or late resorptions) in any of the groups, either before or after initiation of fluvastatin and/or mevalonic acid administration (Table 5). Neonatal mortality of 15% and 56% was observed through weaning in both 12 and 24 mg/kg/day fluvastatin Groups V and VI, respectively (Table 5), with a statistically significant increase noted at 8–14 days postpartum in Group V and as early as 1–4 days postpartum in Group VI. Although the overall neonatal mortality in Group IV (24 mg/kg/day fluvastatin supplemented with mevalonic acid) was also statistically significant, it was minimal.

Drug-related lower birth weights as well as lower neonatal body weights during the lactation period were evident in both 24 mg/kg/day fluvastatin dose groups, with and without mevalonic acid supplementation.

The most prominent neonatal clinical observations occurred in Group VI (24 mg/kg/day of fluvastatin) where several litters, whose dams were adversely af-

Fig. 1. Control animal: typical appearance of nontreated control myocardial tissue (× 200).

Fig. 2. 24 mg/kg: death on day 21 pc before delivery. An example illustrating the significant but focal nature of myocardial hyaline degeneration, interstitial edema, and evidence of actual myofiber loss encountered in dying high-dose animals. Lesions of this type (left half of illustration) were detected only in animals dying during delivery or immediately postpartum. The myocardium in the right half of this illustration is unaffected (× 200).

Fig. 3. 24 mg/kg: death on day 22 pc before delivery. Discrete area of significant myocardial necrosis with mononuclear phagocytic infiltration. Affected myofibers have undergone granular degeneration. Adjacent myofibers are normal. Multiple foci of this type were seen elsewhere in the myocardium of this animal (× 200).

Fig. 4. 24 mg/kg: sacrificed moribund on day 10 pp. Conspicuous myofiber cytoplasmic vacuolization was frequently noted in animals dying later in the postpartum period. These cytoplasmic alterations were unaccompanied by inflammation or other evidence of myofiber degeneration. The distribution of affected fibers ranged from limited areas to widespread regions of myocardium. The vacuoles themselves were found to be devoid of fat or glycogen (× 400).

TABLE 3. Percent body weight change and relative food consumption compared to controls

Group	I Control	II Mevalonic Acid	III Fluvastatin + Mevalonic Acid	IV Fluvastatin + Mevalonic Acid	V Fluvastatin	VI Fluvastatin
	0 mg/kg/day	500 mg/kg b.i.d.	12 mg/kg/day + 500 mg/kg b.i.d.	24 mg/kg/day + 500 mg/kg b.i.d.	12 mg/kg/day	24 mg/kg/day
% body weight change:						
days 15–20 pc	18	18	18	16	16	16
0–7 pp	4	3	2	−2	−5*	−9*
0–14 pp	8	4	4	2	−1**	−3*
7–14 pp	4	1	2	4	4	1
14–21 pp	−5	−3	−4	−3	−3	−1
Relative food consumption:						
days 15–20 pc	100%	100%	96%	89%	93%	89%

*P ≤ 0.01.
**P ≤ 0.05.

TABLE 4. Fertility and reproductive performance

Group	I Control	II Mevalonic Acid	III Fluvastatin + Mevalonic Acid	IV Fluvastatin + Mevalonic Acid	V Fluvastatin	VI Fluvastatin
	0 mg/kg/day	500 mg/kg b.i.d.	12 mg/kg/day + 500 mg/kg b.i.d.	24 mg/kg/day + 500 mg/kg b.i.d.	12 mg/kg/day	24 mg/kg/day
No. of animals mated/group	20	20	20	20	20	20
# Pregnant	18	17	15	18	17	17
Pregnancy rate[1]	90%	85%	75%	90%	85%	85%
Gestation index[2]	100%	100%	100%	100%	100%	94%
No. of animals completing delivery	18	16[3]	15	18	16	16
Mean length gestation of animals completing delivery (days)	21.9	21.8	22.0	21.7	22.1	22.2
Range (days)	21–22	21–22	22	21–22	22–23	21–23

[1]Percent matings resulting in pregnancy; includes animals which died.
[2]Percent pregnancies resulting in litters with one or more viable offspring; does not include animals which died.
[3]Does not include one animal which delivered 4 stillborn, 4 dead, and 6 viable pups, but was sacrificed in a moribund condition on day 1 pp following a probable intubation accident on day 22 pc.

TABLE 5. Neonatal litter size and mortality

Group	Control	II	III	IV	V	VI
Mean no. of pups delivered	15.4	16.1	17.1	15.6	13.6	14.9
Mean no. of liveborn pups	15.1	15.9	16.7	15.2	12.6	11.1*
Mean no. of implantation sites	16.4	17.3	18.2	16.7	15.2	16.3
In utero post implantation loss	6.1%	6.9%	6.0%	6.6%	10.5%	8.6%
Overall neonatal mortality through weaning	3%	5%	6%	7%**	15%*	56%*

*P ≤ 0.01.
**P ≤ 0.05.

fected, had pups which appeared pale, thin, weak, and/or dehydrated. Necropsy examination of pups which were found dead during the lactation period, or were culled prior to weaning, showed that both groups administered fluvastatin alone (Group V and VI) had a higher incidence of pups without milk visible in the stomach.

Macroscopic and microscopic evaluation of spontaneously dying animals

No macroscopic evidence of treatment-related effects was noted other than the anticipated forestomach thickening present in 2 of 3 and 7 of 8 animals from the 12 and 24 mg/kg/day fluvastatin groups, respectively, which died or were sacrificed moribund.

TABLE 6. Time and incidence of treatment-related maternal mortality[1]

Study	Segment III			Segment III follow-up				Segment III mevalonate supplementation				
	Fluvastatin			Fluvastatin				MEV	Fluvastatin + MEV	Fluvastatin + MEV	Fluvastatin	
Dose (mg/kg)	2	12	24	2	6	12	24	500	12 + 500	24 + 500	12	24
Day 15–21 pc		2					2					
0 pp = 22 pc		2	4				4				1	1
23 pc		1	1									
1–4 pp			1				2				1	1
5–15 pp		1	6			1	3					6

[1]To facilitate simultaneous presentation of data, the control groups (all negative) were omitted.

Microscopic findings consisted of a variety of myocardial lesions in all dying Group VI (fluvastatin at 24 mg/kg/day) animals. The cardiomyopathy was characterized by focal myocarditis and lesions of myofiber hyaline, granular and vacuolar degeneration (Table 2). These findings were considered treatment-related and similar to those seen in a previous study in pregnant or lactating animals dying during or after parturition.

Two types of myocardial lesions were detected microscopically. In animals dying at and within a few days after parturition, the lesions were characterized by hyaline degeneration of isolated groups of myofibers accompanied by slight interstitial edema and hemorrhage (Fig. 2 and 3). The lesions typical of animals dying 3–4 days after parturition or later were characterized by widespread cytoplasmic vacuolar changes in the myofibers, i.e., the occurrence of large and small clearly defined round empty vacuoles (Fig. 4 and 5). Occasionally these were accompanied by a sarcolemmal response and inflammatory cell infiltrates, which suggested a myofiber proliferative or reparative response to the induction of the myocardial injury.

Cardiomyopathy was not present in the spontaneously dying animals which had been given a lower dose (12 mg/kg/day) of fluvastatin alone. All animals dying in the 24 mg/kg/day fluvastatin group had cardiac lesions. These lesions were considered related to the cause of death in these animals.

Terminal sacrifice animals

The only treatment-related effect noted occurred in all the groups which received fluvastatin. Most of the animals in Group III (14 out of 19), Group IV (17 out of 18), Group V (14 out of 17), and Group VI (11 out of 12) had varying degrees of forestomach thickening. No evidence of this effect was detected in Group II (mevalonic acid alone) or the controls. In Groups III and IV, supplementation with mevalonate did not influence the incidence of fluvastatin-induced forestomach thickening.

Microscopically, the forestomach thickening was characterized by epithelial hyperplasia/hyperkeratosis in all groups which received fluvastatin, including those receiving the mevalonic acid supplementation. No evidence of ulceration accompanied these hyperplastic lesions. The hyperplasia ranged from mild to moderate in degree. In the absence of specific quantitative measurements, there appeared to be correlation, with greater degrees of hyperplasia seen at the higher doses of fluvastatin in Groups IV and VI (24 mg/kg/day) compared to the response seen in Groups III and V (12 mg/kg/day). There did appear to be a lesser degree of hyperplasia/hyperkeratosis in the animals administered mevalonate and 12 mg/kg/day fluvastatin (Group III) compared to the low dose of fluvastatin alone (Group V).

Histological evidence of fluvastatin-induced myocardial fiber injury and/or inflammation was not present in the mevalonate-supplemented Groups III and IV. Likewise, in the surviving Group V and VI (fluvastatin at 12 and 24 mg/kg/day) animals, myocardial lesions were not detected.

DISCUSSION

The maternal toxic effects induced by fluvastatin reported herein had been encountered in two previous studies at similar dose levels, and consisted of maternal mortality at delivery and during the postpartum period (Table 6). Microscopic evidence of associated myocardial lesions was detected in the follow-up study. An increase in stillborn pups was observed with no evidence of earlier in utero fetal mortality, suggesting a toxic effect on the dams immediately prior to, or during, parturition. The absorption and disposition of fluvastatin have been studied in nonpregnant rats (Tse et al., '90) and in pregnant and lactating rats, and in suckling pups (A. Schweitzer, unpublished data). In all cases fluvastatin was rapidly eliminated with little or no accumulation in the tissues. Limited placental transfer resulted in very low levels in embryos or fetuses. Fluvastatin was detected in maternal milk and subsequently at measurable levels in suckling pups. However, the neonatal mortality observed soon after parturition, as well as during the lactation period, may be a reflection of adverse effects relative to deficient maternal lactation and lack of litter care as influenced by maternal toxicity.

The results indicate that fluvastatin-induced maternal and neonatal mortality could be completely blocked

and/or ameliorated by coadministration of mevalonic acid. Adverse effects on maternal body weight were nonexistent with mevalonic acid supplemented fluvastatin at 12 mg/kg/day and markedly improved at 24 mg/kg/day, although food consumption remained slightly decreased at 24 mg/kg/day despite supplementation with mevalonic acid. The decreased neonatal body weight gain during lactation at 24 mg/kg/day may be related to the reduced maternal food intake noted at this dose level.

While mevalonate supplementation prevented mortality and the development of heart lesions in Groups III and IV (fluvastatin at 12 and 24 mg/kg/day), it did not reduce the direct effect of fluvastatin on the gastric forestomach squamous epithelium, although the degree did appear somewhat lower in Group III. This is considered further evidence that hyperplasia/hyperkeratosis is the result of direct or contact irritation by fluvastatin on the squamous forestomach epithelium.

The fluvastatin-induced early cardiac lesions characterized by hyaline degeneration (before or at parturition) resembled those induced by isoproterenol (pregnant rats are uniquely sensitive)[1]. The postnatal lesions (vacuolar sarcoplasmic changes and myocarditis) appeared to be the result of sarcoplasmic fluid accumulation possibly related to alteration of cellular membrane structures.[1] The relationship or progression between pre- and postpartum lesions, if any, is not clear. Likewise, it is not known at what point in their development either would be reversible.

Several factors existed which probably contributed to the development of myocardial damage and/or death of the fluvastatin-treated animals. In addition to the concurrent stresses of pregnancy, parturition, and lactation, the exaggerated pharmacologic activity of fluvastatin, i.e., inhibition of mevalonate and cholesterol biosynthesis, was a significant factor. HMG-CoA reductase and its immediate product mevalonic acid play a key role in cholesterol biosynthesis and are essential for the synthesis of steroid hormones, cell membranes, cell growth, and normal cellular metabolism (Brown and Goldstein, '80; Alberts, '88; MacDonald et al., '88). It would not be unexpected, therefore, that inhibition of this critical enzyme would influence or disturb the dynamic events occurring in late gestation and at parturition. The data from Groups III and IV clearly indicate that mevalonate supplementation prevents mortality and the development of heart lesions. It is not clear from these studies exactly what the effect is in the myocardial tissues which allows development of the heart lesions, and why the restored or maintained levels of mevalonate suppress or block the development of these lesions.

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The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid

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The influence of the peroxisomal proliferators perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid on lipid metabolism in rats was studied. Dietary treatment of male Wistar rats with these three compounds resulted in rapid and pronounced reduction in both cholesterol and triacylglycerols in serum. The concentration of liver triacylglycerols was increased by about 300% by PFOSA. Free cholesterol was increased by both perfluoro compounds. Cholesteryl ester was reduced to 50% by PFOSA as well by clofibrate. In hepatocytes from fed rats, all the compounds resulted in reduced cholesterol synthesis from acetate, pyruvate and hydroxymethyl glutarate, but there was no reduction of synthesis from mevalonic acid. The oxidation of palmitate was also increased in all groups. The perfluoro compounds, but not clofibrate, caused some reduction in fatty acid synthesis. The activity of liver HMG-CoA reductase was reduced to 50% or less in all treatment groups and all three compounds led to lower activity of acyl-CoA : cholesterol acyltransferase (ACAT). Changes in other enzymes related to lipid metabolism were inconsistent. The present data suggest that the hypolipemic effect of these compounds may, at least partly, be mediated via a common mechanism: impaired production of lipoprotein particles due to reduced synthesis and esterification of cholesterol together with enhanced oxidation of fatty acids in the liver.

Introduction

Many hypolipemic drugs cause proliferation of peroxisomes and increase the activity of the peroxisomal β-oxidation in rats [1–3]. Chemically, these drugs constitute a heterogeneous group including clofibrate, tibric acid, niadenate and long-chain acylthioacetic acids, tiadenol, long-chain thia acids and MEDICA 16 [1–6]. It has been suggested that the increase in the fatty acyl-CoA oxidizing system contributes to the hypolipemic effect of these drugs [7,8]. The dominating mechanism underlying reduction of serum triacylglycerols and cholesterol by these drugs is, however, uncertain. Other mechanisms which may be important for the hypolipemic effect include: reduced hepatic synthesis of fatty acids and cholesterol [9–12]; reduced triacylglycerol release by the liver [13,14]; increased rate of VLDL degradation [6]; increased uptake or reduced release of fatty acids by adipose tissue [15,16] and increased excretion of cholesterol into bile and feces [17].

Ikeda et al. [18,19] observed that perfluorooctanoic acid (PFOA) and perfluorooctane sulphonic acid (PFOSA) efficiently induced the peroxisomal β-oxidation in rats that were fed these compounds (0.02% in the diet). Just et al. [20] reported that the perfluorocarboxylic acids alter hepatic lipid metabolism and reduce serum lipid levels showing that these compounds also belong to the group of peroxisomal proliferators with hypolipemic effect. The perfluorinated compounds are particularly interesting since they obviously are not subject to ordinary metabolic modifications. The effects of these compounds must, therefore, be due to effects of the compounds per se.

Peroxisomal inducers may bring about the hypolipemic effect by affecting different steps in lipid metabolism. However, it seems likely that some important steps in lipid metabolism are common targets for these compounds. The unphysiological character of the perfluoro compounds makes it possible that their effect

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Abbreviations: PFOA, perfluorooctanoic acid; PFOSA, perfluorooctane sulphonic acid; ACAT, acyl-CoA: cholesterol acyltransferase; HMG, hydroxymethyl glutaric acid; TTA, tetra-decylthioacetic acid; VLDL, very-low-density lipoproteins.

on lipid metabolism follows a pattern that would make it easier to understand possible mechanism for the lipid reduction effect of peroxisomal proliferators.

In the search for a possible common mechanism underlying the hypolipemic effect of peroxisomal inducers, we have compared hepatic fatty acid metabolism, cholesterol synthesis and the activities of enzymes related to these metabolic processes in the liver of rats fed perfluorooctane sulphonic acid (PFOSA), perfluorooctanoic acid (PFOA) and clofibrate.

Methods and Materials

Animals

Male Wistar rats were used. They were divided into five groups. One group was allowed normal food ad libitum (control rats). Three other groups were given food which contained 0.3% clofibric acid, 0.02% perfluorooctanoic acid or 0.02% perfluorooctane sulphonic acid, respectively. The diets were prepared by soaking standard food (in pellet form) in diethylether in which the compounds had been dissolved. The fifth group was restricted in food intake to that consumed by the PFOSA group. The stock diet was used for these paired feeding experiments. The average body weight when the experiments started was 269 g and the average daily food consumption per rat in the different groups were; control: 23.7 g, clofibrate: 23.1 g, PFOA: 22.7 g, PFOSA: 20.0 g.

Materials

[2-^{14}C]Mevalonic acid, [1-^{14}C]pyruvic acid and [2-^{14}C]pyruvic acid were from New England Nuclear. [1-^{14}C]acetic acid was obtained from Amersham (UK). Clofibrate was from Fluka (Buchs, Switzerland). Perfluorooctanoic acid was purchased from Aldrich-Chemie (Steinheim, Germany) and Perfluoro-octane sulphonic acid was from Fluorochem (Old Glossop, UK). Other chemicals were from Sigma (St. Louis, MO, USA).

Preparation of hepatocytes

The effects of dietary treatment were studied in hepatocytes isolated from rats fed the diets for 1 week and for the study of direct effects of the compounds, hepatocytes were isolated from rats fed the standard diet. Isolation of hepatocytes was performed by perfusion with collagenase, according to Berry and Friend [21], with the modifications described by Seglen [22].

Oxidation of palmitate and conversion of labelled substrate into lipids

Fatty acid oxidation was measured according to Christiansen et al. [23] with 0.5 mM palmitate as substrate. Fatty acids and cholesterol synthesized from radioactive precursors were extracted from the cell suspension after 90 min incubation. The reaction was stopped by the addition of 5% saturated KOH in ethanol and the mixture was heated at 90°C for 1 h. The nonsaponifiable lipids were extracted with petroleum ether. The suspension was then acidified with HCl. The extracts were evaporated to dryness and the radioactive lipid residue was dissolved in 100 μl hexane. The lipid extracts were chromatographed with hexane/diethyl ether/acetic acid (80:20:1) on silica gel thin-layer plates. The spots corresponding to cholesterol and fatty acids were identified by standards and were then isolated for measurement of radioactivity.

Enzyme assays and measurements of DNA, protein and lipids

Pyruvate dehydrogenase was estimated by measuring the $^{14}CO_2$ liberated when hepatocytes were incubated with 5 mM [1-^{14}C]pyruvate for 30 min at 37°C. Acetate thiokinase was measured as described by Jones and Lipman [24]. Liver microsomes were prepared according to Easom and Zammit [25], and HMG-CoA reductase was measured according to Drevon et al. [26]. CDPcholine : 1,2-diacylglycerol cholinephosphotransferase, EC 2.7.8.2 and lysolecithin acyltransferase

TABLE I

Body and liver weights and liver lipid content in rats fed different diets for 7 days

Clofibrate was given as 0.3% (w/w) and PFOA and PFOSA as 0.02% in the diet. Values are given as means ± S.E. There were four observations in each group. Fisher's *P*-values are given; * $P < 0.05$; ** $P < 0.01$ vs. control group.

	Control	Clofibrate	PFOA	PFOSA	Stock diet limited fed
Body weight (g)	305 ± 2	298 ± 3	288 ± 6 *	275 ± 6 **	282 ± 7 **
Liver weight (g)	11.3 ± 0.4	16.1 ± 0.8 **	18.8 ± 0.7 **	15.9 ± 0.7 **	
Liver triacylglycerols (μmol/g liver)	4.1 ± 0.5	4.0 ± 0.7	3.9 ± 0.3	13.8 ± 18 **	4.1 ± 0.6
Liver non-esterified cholesterol (μmol/g liver)	4.4 ± 0.4	4.5 ± 0.3	6.5 ± 0.4 *	7.6 ± 0.3 **	
Liver cholesterol ester (μmol/g liver)	0.58 ± 0.08	0.24 ± 0.02 **		0.27 ± 0.03 *	

(EC 2.3.1.23) was measured as described by Parthasarathy et al. [27]. Acyl-CoA:cholesterol acyltransferase (ACAT) was measured according to Rustan et al. [28] and the synthesis of phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine was measured as described by Vance [29]. Other enzymes were measured as described earlier [30]. DNA was measured by the method of Labarca and Paigen [31] and protein was estimated by the biuret method or by the method of Lowry et al. [32].

Liver lipids were extracted with chloroform/methanol (2:1, v/v). Triacylglycerols was determined directly on the dried extract with a kit method (Nyco, Oslo, Norway). Free and esterified cholesterol was determined by Nycotest kit method for cholesterol (Nyco) after separation of the extract on thin-layer chromatography. Serum cholesterol and triacylglycerols was measured directly by the kit methods.

Results

Table I shows that 0.02% PFOA or PFOSA in the diet resulted in a lower body weight after 7 days of feeding as compared with the control group. The clofibrate diet (0.3%) did not affect the rat weight. The group with restricted food intake to that of the PFOSA group had about the same weight as the PFOSA group. All the compounds resulted in a 40–60% increased liver weight. Similar effect on the liver weight has been observed earlier in rats fed clofibrate [9,10,33].

Changes in serum and liver lipids by clofibrate, PFOA and PFOSA

Fig. 1A shows that all three diets significantly reduced serum cholesterol. In all treatment groups, cholesterol was significantly reduced (to 50–70% of control) after 24 h. Dietary treatment for 2 weeks resulted in further cholesterol reductions by 70% or more. In agreement with other observations, fasting for 2 days did not bring about significant cholesterol changes.

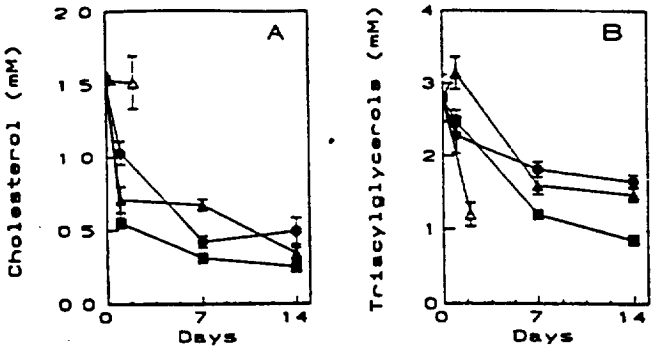

Fig. 1. Effect of clofibrate, PFOA and PFOSA on the serum lipids. (A) Cholesterol; (B) triacylglycerols; △, fasted; ●, 0.3% clofibrate; ▲, 0.02% PFOA; ■, 0.02% PFOSA. The data represent mean ± S.E. of four observations.

Fig. 1B shows the effects of clofibrate, PFOA and PFOSA on triacylglycerols in the rat serum. None of the compounds resulted in significant changes after 1 day of treatment. After 7 days, clofibrate and PFOA resulted in reduction to about 60% of control value and no further reduction was obtained with further treatment. PFOSA reduced triacylglycerols to about 50% and 30% of control value after 1 and 2 weeks of treatment. The significant reduction obtained by fasting for 2 days was as expected. In the rats fed stock diet but restricted to that consumed by the PFOSA group, the serum triacylglycerols was 2.43 ± 0.13 mM after 1 week of treatment (not shown) which is not significantly lower than observed in the control group (fed stock diet ad libitum).

Table I shows that PFOSA increased the liver triacylglycerols content to more than 3-times the control value. This effect of PFOSA on triacylglycerols was clearly in contrast to the effect of clofibrate and PFOA which did not affect the content of triacylglycerols and clearly indicates that PFOSA inhibits the excretion of triacylglycerols from the liver. Both PFOA and in particular PFOSA increased the liver content of non-esterified cholesterol. In contrast to this, a significant

TABLE II

Conversion of labelled substrates into ^{14}C-labelled cholesterol by hepatocytes of rats fed different diets for 7 days

Clofibrate was given as 0.3% (w/w) and PFOA and PFOSA as 0.02% in the diet. The concentration of [2-^{14}C]mevalonate and [3-^{14}C]hydroxymethylglutarate was 0.5 mM. Incubations with [1-^{14}C]acetate and [2-^{14}C]pyruvate were conducted with 5 mM of the labelled substrates and included also unlabelled glucose (10 mM). Values are means ± S.E. There were four observations in each group. Fisher's P values are given * $P < 0.05$; ** $P < 0.01$.

Substrate	Formation of ^{14}C-labelled cholesterol (nmol substrate carbon · mg DNA^{-1}·h^{-1})			
	control	clofibrate	PFOA	PFOSA
[1-^{14}C]Acetate	147.6 ± 21.4	59 ± 13 **	37.6 ± 16.5 *	21.2 ± 7.4 **
[2-^{14}C]Pyruvate	78.2 ± 14.6	46 ± 9.5 **	35.2 ± 9.7 *	26.5 ± 5.4 **
[3-^{14}C]Hydroxy-methylglutarate	17.9 ± 3.3	3.6 ± 0.9 **	3.6 ± 1.3 **	5.0 ± 1.1 **
[2-^{14}C]Mevalonate	2280 ± 150	2160 ± 280	1730 ± 240	2030 ± 310

TABLE III

Conversion of labelled substrates into ^{14}C-labelled fatty acids and oxidation of [U-^{14}C]palmitate by hepatocytes of rats fed different diets for 7 days

The concentrations of compounds in the diet and the concentrations of [1-^{14}C]acetate and [2-^{14}C]pyruvate were as described in Table II. The concentration of [U-^{14}C]palmitate was 0.5 mM. Values are means ± S.E. There were 4 observations in each group. Fisher's P values are given: * $P < 0.05$; ** $P < 0.01$ vs. control group.

Substrate	^{14}C-Labelled fatty acid formation (nmol substrate carbon·mgDNA^{-1}·h^{-1})			
	control	clofibrate	PFOA	PFOSA
[1-^{14}C]Acetate	252 ± 28	180 ± 28	90 ± 54 *	104 ± 28 **
[2-^{14}C]Pyruvate	221 ± 21	322 ± 23 *	182 ± 55	128 ± 21 *
	[U-^{14}C]Palmitate oxidation (nmol substrate oxidized·mgDNA^{-1}·h^{-1})			
	control	clofibrate	PFOA	PFOSA
	502 ± 50	726 ± 36 *	564 ± 63	861 ± 102 *

TABLE IV

Conversion of [1-^{14}C]acetate into ^{14}C-labelled fatty acids and cholesterol by hepatocytes in the presence of hypolipemic compounds

The concentration of [1-^{14}C]acetate was 5 mM and the incubations also included 10 mM glucose. Each incubation flask contained 4 mg cell protein and 1.5% albumin in 3 ml Krebs-Henseleit bicarbonate buffer. The concentration of hypolipemic compounds was 1 mM. Values are means ± S.E. There were four observations in each group. Fisher's P values are given ** $P < 0.01$ vs. control group.

Addition	Formation of ^{14}C-labelled cholesterol	Formation of ^{14}C-labelled fatty acid
	(nmol substrate carbon mg DNA^{-1}·h^{-1})	
None	114 ± 8	313 ± 14
TTA	1.4 ± 0.07 **	7.5 ± 1.3 **
Clofibric acid	57 ± 0.7 **	243 ± 8 **
PFOA	63 ± 11 **	611 ± 20 **
PFOSA	65 ± 7 **	1908 ± 29 **

reduction (to approx. 50%) in esterified cholesterol in the treated rats (PFOA-treated rats not measured) was found.

Conversion of labelled substrates into cholesterol by hepatocytes of rats fed different diets

Table II shows the incorporation of labelled carbon atoms from differently labelled acetate, pyruvate, mevalonate and hydroxymethylglutaric acid (HMG). The major point emerging from this table is that the rate of cholesterol synthesis was significantly reduced from all substrates which are proximal to the HMG-CoA dehydrogenase step in all treatment groups. In contrast, cholesterol synthesis from mevalonate was not reduced in any of the groups.

Table III shows the synthesis of fatty acids from pyruvate and acetate. Both PFOA and PFOSA treatment reduced lipid synthesis (not significant from pyruvate in the PFOA group). No reduction in fatty acid synthesis was found in hepatocytes from clofibrate-treated animals. The table further shows that the peroxisomal inducers, as expected, resulted in an increased rate of palmitate oxidation (although PFOA did not result in a significant increase). The experiments do not exclude the possibility that intracellular content of the tested compounds might have a direct (reversible) effect on fatty acid and cholesterol synthesis in vivo. Such effects could have escaped our detection since the compounds might have been washed out during the cell isolation procedure. To test possible direct inhibitory effects of these drugs, the synthesis of fatty acids and cholesterol in hepatocytes with additions of the compounds to the incubation medium were performed (Table IV). The table shows that 1 mM of clofibric acid, PFOA and FPOSA inhibited the choles-

TABLE V

Activity of enzymes related to the synthesis of cholesterol and fatty acids in liver of rats fed different diets for 7 days

The concentrations of compounds in the diet were as described in Table I. Values are given as means ± S.E. There were four observations in each group. * $P < 0.05$; ** $P < 0.05$; ** $P < 0.01$ vs. control group.

Enzyme	Activity (μmol·(mg DNA^{-1}·min^{-1})			
	control	clofibrate	PFOA	PFOSA
Pyruvat dehydrogenase	0.59 ± 0.05	0.58 ± 0.04	0.42 ± 0.08	0.36 ± 0.04 *
Citrate synthase	0.97 ± 0.12	0.94 ± 0.06	0.80 ± 0.05	1.10 ± 29
ATP-citrate lyase	1.54 ± 0.34	0.78 ± 0.38	0.75 ± 17 *	0.24 ± 0.06 **
Acetate thiokinase	8.7 ± 0.64	5.7 ± 0.85	3.5 ± 0.5 **	5.7 ± 0.28 **
Malate dehydrogenase (NADP) (decarboxylating)	0.36 ± 0.05	0.83 ± 0.05 **	1.34 ± 0.05 **	0.21 ± 0.01
Malate dehydrogenase	27.1 ± 3.2	18.4 ± 2.4 *	20.7 ± 2.6	19.6 ± 3.0
Glucose-6-phosphate dehydrogenase	0.50 ± 0.10	0.17 ± 0.03 *	0.23 ± 0.04 *	0.11 ± 0.01 **
Isocitrate dehydrogenase (NADP)	2.44 ± 0.35	2.71 ± 0.56	2.73 ± 0.19	3.23 ± 0.13
	(nmol·μg protein^{-1}·min^{-1})			
HMG-CoA reductase	0.31 ± 0.03	0.16 ± 0.04 *	0.15 ± 0.05 *	0.11 ± 0.01 **

terol synthesis to the same extent (approx. 50%). Surprisingly, tetradecylthioacetic acid (TTA), another inducer of peroxisomal β-oxidation with hypolipemic effect in rats [3,4], almost completely inhibited cholesterol synthesis. The pronounced inhibitory effect of TTA on the fatty acid synthesis observed by Skrede et al. [34] was also confirmed. Clofibric acid reduced the fatty acid production by about 20%. However, a direct inhibitory effect on the fatty acid synthesis is not a property shared by all the tested compounds. Both perfluorinated compounds unexpectedly stimulated the rate of fatty acid synthesis strongly. It is unlikely that this was due to an inhibition of Krebs cycle with a concomitant increase in lipogenic precursors, since these compounds inhibited the cholesterol synthesis. It seems more likely that the compounds stimulate a rate-limiting enzyme, e.g., acetyl-CoA carboxylase. At lower concentrations (0.5 and 0.1 mM), there were very small inhibitory effects of clofibric acid, PFOA and PFOSA (data not shown).

Enzymes related to the synthesis of cholesterol and fatty acids from pyruvate and acetate

Table V shows that pyruvate dehydrogenase, citrate synthase and acetate thiokinase were only slightly affected by treatment with the three compounds. ATP-citrate lyase activity was reduced to about 15% of control values by PFOSA. PFOA reduced the activity of this enzyme significantly to 50%. The effect of the compounds on three NADPH-generating enzymes shows a remarkable pattern. All three compounds significantly reduced the activity of glucose-6-phosphate dehydrogenase. PFOSA reduced the activity to 20% of control values. In contrast, isocitrate dehydrogenase was unaffected by all three compounds. The activity of malic enzyme was increased 2- and 3.5-fold by clofibrate and PFOA, respectively. Malate dehydrogenase, which is not specifically involved in lipid synthesis, was virtually unchanged by any of the compounds. The activity of HMG-CoA reductase, the rate-limiting and regulated step in cholesterol synthesis, was reduced to 50% or less in all three treatment groups.

Enzymes related to the synthesis of cholesteryl ester and phospholipids in rats fed different diets

Table VI shows that acyl-CoA: cholesterol acyltransferase (ACAT), was significantly downregulated by all three compounds. PFOSA, which had the strongest effect, reduced the activity to one third of control. The downregulation of this enzyme is in keeping with the reduction of liver cholesterol ester (Table I). The table further shows that the activities of two enzymes important in the phopholipid turnover, acyl-CoA : 1-acylglycero-3-phosphocholine acyltransferase and CDP-choline : 1.2-diacylglycerol cholinephosphotransferase, were not significantly altered in the rats fed any of the hypolipemic drugs. The activity of phosphatidylserine synthase, phosphatidylethanomaline synthase and phosphatidylcholine synthase were also unaffected by any of the dietary regimes utilized (not shown).

Activities of enzymes related to phospholipid synthesis in the presence of hypolipemic drugs

Table VII shows that at 0.5 mM clofibric acid had little effect on activities of the enzymes listed in the table. The five enzymes were all inhibited by both perfluorinated. compounds. PFOA had a particular inhibitory effect on phosphatidylserine synthase activity which was reduced to 18% of normal with 0.5 mM PFOA. PFOSA had strongest inhibitory effect on the activity of CDP-choline : 1,2-diacylglycerol cholinephosphotransferase, phosphatidylserine synthase and phosphatidylethanolamine synthase, which were reduced to 14%, 13% and 28% of control, respectively with 0.5 mM PFOSA. At lower concentration (0.1 mM) the inhibitory effects of the perfluorinated compounds were very moderate (data not shown). Clofibric acid had no significant effect at 1 mM concentration.

TABLE VI

Activity of enzymes related to synthesis of cholesteryl esters and phospholipids in livers of rats fed hypolipemic drugs for 7 days

The concentrations in the diet were as described in Table I. Values are given as means ± S.E. There were four observations in each group. ** $P < 0.01$ vs. control group.

Enzyme	Control	Clofibrate	PFOA	PFOSA
Acyl-CoA Cholesterol acyltransferase (ACAT) (nmol/mg protein per min)	660 ± 51	427 ± 18 **	323 ± 39 **	237 ± 41 **
Acyl-CoA : 1-acylglycero-3-phosphocholine acyltransferase (nmol/mg protein per min)	14.0 ± 1.3	12.4 ± 1.0	12.7 ± 1.3	11.8 ± 1.8
CDP-choline : 1.2-diacylglycerol cholinephosphotransferase (nmol/mg protein per min)	10.4 ± 1.3	9.1 ± 1.2	7.6 ± 0.7	8.6 ± 1.0

TABLE VII

Activities of enzymes related to phospholipid synthesis in the presence of hypolipemic drugs

Concentrated solutions (10 mM) of clofibric acid, PFOA and PFOSA in DMSO were diluted to 0.5 mM final concentration in assay system (The controls were added the same amount of pure DMSO.) The effect of the drugs on each of the enzymes were tested four times and in each of the experiments, the activities were calculated as per cent of the control value. Values are given as means ± S.E.

	Control	Clofibric acid	PFOA	PFOSA
Acyl-CoA lysophosphatidyl transferase	100	90 ± 6	56 ± 7	61 ± 5
CDP-choline : 1,2-diacylglycerol cholinephosphotransferase	100	95 ± 5	71 ± 2	14 ± 0.6
Phosphatidylserine synthase	100	101 ± 9	18 ± 4	13 ± 1.4
Phosphatidylethanolamine synthase	100	101 ± 3	47 ± 5	28 ± 2
Phosphatidylcholine synthase	100	89 ± 3	47 ± 5	48 ± 3

The effect of the compounds on the activity of ACAT was tested only at 100 μM and with this concentration the ACAT activity was unaffected by the compounds (not shown).

Discussion

Reduction of the steady-state levels of serum lipids may be visualized as the result of downregulation of lipid synthesis or increased clearance from plasma. The aim of this study was to evaluate the effects of three different peroxisomal proliferators on a series of enzymes involved in hepatic lipid synthesis. The most important observations with all three compounds was downregulation of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis and of cholesterol esterification enzyme (ACAT). The agreement between the observed alterations in the enzyme activities and production of cholesterol and lipids in intact liver cells provides some support for the assumption that such enzyme measurements do reflect real changes in metabolic activity in the intact organ and shed light on the mechanism of the hypolipemic effects of the agents investigated in this study. These compounds do not show a correlated effect on the lipogenic and cholesterogenic pathways in the in vivo experiments. The in vitro experiments indicate that there are direct, presumably reversible, effects on these pathways. These effects are different than those observed after dietary manipulation. The latter probably represent changes in enzyme concentrations since, at least, the changes in the cholesterogenic pathway are correlated with the changes in HMG-CoA reductase.

The perfluorinated compounds utilized in this study are strong local irritants. The observed actions should not be regarded as mere unspecific toxic effects since, in addition to reduction of the activity of some enzyme systems, these compounds increase the activity of other enzymes, e.g., enzymes of the peroxisomal fatty acid β-oxidation system [18,19]. However, local irritation may explain the reduction of food intake and slower weight increase observed after feeding perfluorinated compounds. However, this probably contributes little to the reduction in serum triacylglycerols, since no significant reduction was observed in rats with restricted food intake. Increased liver weight correlates well with earlier studies on peroxisomal proliferators [35,36].

Cholesterol and fatty acid synthesis

The reduction of cholesterol synthesis from different labelled substrates fits well with the reduced activity of HMG-CoA reductase observed in this study. Three substrates, proximal to the reductase step, were incorporated into cholesterol at a reduced rate whereas no reduction from mevalonate was observed in any of the treatment groups. Lowering of HMG-CoA reductase levels by clofibrate is in accord with other observations [9,37,38]. Even though the three compounds tested in this study reduce the HMG-CoA reductase activity, this effect may not be a characteristic of all peroxisomal inducers. MEDICA 16, another compound in this group, does not act via an effect on this reductase [12,39], but rather inhibits the synthesis of cholesterol at a step distal to HMG-CoA reductase. Our observations with clofibrate are at variance with the data obtained by Azarnoff et al. [10] who found that cholesterol synthesis from mevalonic acid was reduced in livers of rats fed clofibrate.

In contrast to the inhibitory effect of MEDICA 16 on ATP-citrate lyase [39], clofibric acid, PFOA and PFOSA had essentially no direct effect on ATP-citrate lyase at 1 mM (data not shown), but the drugs downregulated the enzyme after dietary administration. This effect may contribute to reduced synthesis of cholesterol in vivo, since the enzyme is important in the main pathyway for cholesterol precursor synthesis. It is interesting that all three compounds reduced one of the NADPH generating enzymes (glucose-6-phosphate dehydrogenase), while isocitrate dehydrogenase (NADP) was unchanged. Reduced capacity for NADPH generation can therefore hardly contribute to reduction in serum lipid levels.

The synthesis of fatty acids from acetate or pyruvate was not reduced in hepatocytes from rats fed clofibrate (Table III), but was significantly reduced in the hepatocytes from rats fed PFOSA. The reduced rate of fatty acid synthesis in the PFOA and PFOSA groups is probably not related to reduction in serum triacylglycerols. In rats fed PFOSA, there was accumulation of liver triacylglycerols. The lack of reduction of fatty acid synthesis by clofibrate suggests that other pro-

cesses in lipid metabolism must be more essential for the hypolipemia. Tomarelli et al. [40] found increased synthesis of lipids from acetate in rats fed clofibric acid and also in rats fed the very potent hypolipemic compound WY-14,643.

Synthesis of cholesteryl ester and phospholipids

Liver exports lipids to other organs mainly as VLDL particles. In addition to apolipoproteins, triacylglycerols and free cholesterol, these particles consist mainly of esterified cholesterol and phospholipids. Hence, reduced hepatic synthesis of these components might lead to reduced transport of lipids from the liver.

The hepatic content of cholesteryl ester was reduced both in clofibrate and PFOSA fed rats (PFOA fed rats were not tested) even where free cholesterol was not reduced (Table I). Similar effect was obtained by Avignan et al. [41] with the hypolipemic drug MER-29. Reduced cholesteryl ester production is most likely a result of downregulation of the ACAT activity which was observed in all treatment groups. A direct inhibitory effect on the enzyme by the compounds is probably of less importance since 100 μM of the compounds did not affect the enzyme activity (higher concentrations were not tested). Vance [29] argued that synthesis of phospholipids may be limiting for lipoprotein synthesis. In this study, we have not observed any downregulation of enzymes involved in the synthesis or metabolism of phospholipids. We have observed only small direct effects of clofibric acid on these enzymes. It seems likely, however, that reduced phospholipid synthesis plays a role in the lipid-reducing effect of the perfluorinated compounds, since these had direct inhibitory effect on several important enzymes. Parthasarathy et al. [27] suggested from their studies that inhibition of phosphatidyl-choline synthesis, particularly by the lysolecithin acyltransferase pathway, may be related to a drug's effectiveness in decreasing serum lipids. We found that the transferase was moderately inhibited by the perfluorinated compounds. We also observed that this enzyme was virtually unaffected by 0.5 mM (Table VII) and also by 1 mM of clofibric acid. This agrees with the data reported by Parthasarathy et al. [27]. Our data do not support the hypothesis that reduced activity of lysolecothine acyltransferase plays a central role in the hypolipemic effect of the compounds we have tested. Inhibition of other enzymes of phospholipid synthesis may play a role.

Reduced release of lipids from the liver

In earlier studies, it has been reported that clofibrate reduces the release of lipids from the liver [13,14]. A direct effect on the excretion process by clofibrate was not confirmed by accumulation of liver triacylglycerols in clofibrate-fed, nor in PFOA-fed animals. A reduced release of lipids from liver was probably an effect of PFOSA, however, since the compound increased liver triacylglycerols by about 200% in spite of reduced rate of fatty acid synthesis (Table IV).

Since interference with the synthesis of cholesteryl ester may cause reduced hepatic lipid output [42], it may be concluded that reduction in cholesterol synthesis and esterification due to downregulation of HMG-CoA reductase and ACAT together with enhanced fatty acid oxidation in the liver, are effects caused by clofibric acid as well as by the perfluorinated compounds. This may reduce VLDL production by liver which plays a central role after the postprandial chylomicronemic period during which the liver is the dominating organ for delivery of lipids to serum.

In addition, the different hypolipemic drug may act by inhibiting the synthesis of other lipoprotein components such as phosphatidylcholine [27].

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Developmental changes in the expression of genes involved in cholesterol biosynthesis and lipid transport in human and rat fetal and neonatal livers

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Cloned cDNAs encoding a number of enzymes involved in cholesterol biosynthesis as well as extracellular and intracellular lipid transport were used to compare the developmental maturation of these biologic functions in the fetal and neonatal rat and human liver. The results of RNA blot hybridization analyses indicate that steady-state levels of rat HMG-CoA synthase, HMG-CoA reductase and prenyl transferase mRNAs are highest in late fetal life and undergo precipitous (up to 80-fold) co-ordinate reductions immediately after parturition. These changes reflect the ability of the fetal rat liver to produce large quantities of cholesterol as well as the repression of this function during the suckling period in response to exogenous dietary cholesterol. Striking co-ordinate patterns of HMG-CoA synthase, reductase and prenyl-transferase mRNA accumulation were also observed in four extrahepatic rat tissues (brain, lung, intestine and kidney) during the perinatal period. The concentrations of all three mRNAs in the 8-week-old human fetal liver are similar to those observed throughout subsequent intrauterine development with less than 2-fold changes noted between the 8th through 25th weeks of gestation. Analysis of the levels of human apo AI, apo AII, apo B and liver fatty acid binding protein mRNAs during this period and in newborn liver specimens also indicated less than 2–3-fold changes. These observations suggest that the 8-week human liver has achieved a high degree of biochemical differentiation with respect to functions involved in lipid metabolism/transport which may be comparable to that present in 19–21 day fetal rat liver. Further analysis of human and rat fetal liver RNAs using cloned cDNAs should permit construction of a developmental time scale correlating hepatic biochemical differentiation to be constructed between these two mammalian species.

Introduction

Marked changes in the requirements for products derived from isoprene (e.g. cholesterol) occur during development. The enzyme which catalyzes the key, rate-limiting step in cholesterol biosynthesis–microsomal 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase)–undergoes large fluctuations during rat liver ontogeny [1,2]. Enzyme activity is high prior to birth, declines to low levels during the suckling period (postnatal days 1–13) and demonstrates a transient increase at weaning. Changes in its activity parallel developmental alterations in the rate of incorporation of radiolabeled acetate into sterols [2]. Bruenger and Rilling [3] documented changes in the activities of two other cholesterogenic enzymes in the developing rat liver: squalene synthetase and prenyl transferase (or farnesyl/pyrophosphate synthetase). Prenyl transferase is one of five enzymes that participate in the conversion of mevalonate to squalene, the precursor of sterols. Both squalene synthetase and prenyl transferase display similar developmental activity profiles in rat liver: they fall following birth, rise to a peak during the mid to late suckling period (postnatal days 10–12), fall once more during the suckling-weaning transition reaching a nadir by postnatal day 20, only to rise one final time during weaning. The mechanisms which result in these alterations in enzyme activity may be quite complex as illustrated by the fact that the mevalonate-mediated

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decrease in HMG-CoA reductase levels observed in adult animals reflects decreases in gene transcription as well as increased rates of protein degradation [4,5].

Little is known about the developmental history of the activities of these enzymes in human fetal and neonatal liver or about the ontogeny of expression of genes involved in the transport and metabolic processing of lipids in this tissue. The rat liver provides a convenient reference for a comparative study of such developmental changes. For example in addition to the information about expression of cholesterogenic enzyme activities, recent analyses of apolipoprotein gene expression in the developing rat liver indicate that a complex pattern of activation occurs during late fetal and early neonatal life. Apolipoprotein AI and E mRNAs begin to accumulate in this tissue plus its embryonic homologue (the fetal yolk sac endoderm) between days 15–21 of the 21-day gestation period [6–9]. Remarkable increases in the levels of these mRNAs occur during the suckling period as the animals adapt to the high fat (principally triacylglycerol) diet of mothers milk [10,11]. By contrast, rat liver apo B mRNA levels reach a peak by the 18th fetal day that is not exceeded at any time during subsequent development [12]. Following birth, hepatic apo B mRNA concentrations progressively fall during the suckling and weaning periods [12]. This fall appears to be mediated by thyroxine [13]. A third pattern of activation is exhibited by the apo AIV gene which remains dormant until the suckling weaning transition (days 13–14) when the rat liver begins to export large amounts of triacylglycerol-rich lipoproteins [6].

We have begun a comparative analysis of the accumulation of mRNAs encoding proteins involved in lipid metabolism in the fetal and neonatal human and rat liver. A panel of cloned cDNAs encoding apolipoproteins AI, AII, and B, an intracellular fatty acid binding protein as well as HMG-CoA reductase, HMG-CoA synthase and prenyl transferase were used to characterize the state of enzymatic differentiation of the human fetal liver from weeks 8 to 25 of development and in the newborn. The results indicate that these mRNAs appear at an early phase of human fetal life (by week 8) and undergo only minimal (less than 3-fold changes) in their concentration in total liver RNA during the rest of intrauterine as well as early postnatal life. This early expression of lipid metabolic capability contrasts with the more marked changes in mRNA levels observed in the perinatal rat liver.

Materials and Methods

Preparation of RNA from rat and human liver

Timed-pregnant, neonatal and young adult Sprague-Dawley rats were obtained from Sasco (St. Louis, MO). Weaned animals were maintained on a standard chow diet ad libitum and a fixed 12 h (6:00 a.m. to 6:00 p.m.) light cycle. Animals (n = 10–40 for each time point) were killed between 1200 and 1400 h and their livers, lungs, brains, kidneys and small intestines immediately frozen in liquid nitrogen. Human fetal liver samples from first and second trimester aborted fetuses were obtained by Schwartz et al. [14] and maintained at −90°C for 15–20 years. Fetal age was estimated from crown-rump lengths using nomograms developed by Tanimura et al. [15]. Breitfeld and Schwartz [16] and Michaelson and Orkin [17] have used these human fetal liver samples previously to successfully prepare RNA for in vitro translation. Additional human liver samples were obtained from a preterm newborn and a full term newborn, both of whom died of acute respiratory failure. These were stored at −90°C for about 15 years [14]. A single adult liver specimen was procured from a male organ donor who died of trauma and had no history or clinical evidence of hepatic dysfunction [18].

Total cellular RNA was extracted from frozen pulverized tissues using the guanidine thiocyanate/cesium chloride procedure [19]. RNA integrity was assessed by denaturing, methylmercury agarose gel electrophoresis [20].

RNA blot hybridization studies

Dot blots were prepared by applying four amounts of each tissue RNA sample (0.5, 1, 2, and 3 μg) to nitrocellulose filters as described in a previous publication [6]. Yeast tRNA was added to each tissue RNA sample prior to denaturation so that the total RNA input per dot was always 3 μg. Blots containing samples of rat liver, intestine, kidney, brain and lung RNA were probed with ^{32}P-labeled, double-stranded cDNAs encoding hamster HMG-CoA reductase [22], rat HMG-CoA synthase [23], and rat prenyl transferase [24]. Dot blots of human liver RNA samples were probed with ^{32}P-labeled cDNAs specifying human preproapo A [25], human preproapo AII [26], human preapo B [27], human liver fatty acid binding protein [28], human α-fetoprotein [29], human HMG-CoA reductase [30], rat prenyl transferase and rat HMG-CoA synthase. Conditions selected for filter hybridization and washing are listed in Ref. 12. These stringencies were equivalent to those used by others to produce specific interactions between these cDNAs and their respective mRNAs. The relative abundance of each mRNA in the tissue RNA preparations was determined by quantitative scanning laser densitometry of filter autoradiographs using a LKB XL Ultroscan densitometer. Only signals in the linear range of film sensitivity were utilized for calculations of relative mRNA concentration.

Northern blots of total cellular RNA were produced following electrophoretic fractionation through agarose gels containing formaldehyde [31].

Results and Discussion

Accumulation of HMG-CoA synthase, HMG-CoA reductase and prenyl transferase mRNAs in the developing rat liver

Cloned cDNAs encoding HMG-CoA synthase, HMG-CoA reductase, and prenyl transferase were used to probe dot blots of total cellular RNA prepared from rat livers harvested during fetal days 16–21, the suckling period (defined as the first 13 postnatal days, Ref. 32), the weaning phase (days 14–28) and from animals proceeding through sexual maturation (postnatal days 35–70). Material was collected from 10 to 40 male and female animals representing 1–4 litters at each developmental stage studied. The results of our dot blot hybridization analyses are presented in Fig. 1 and show

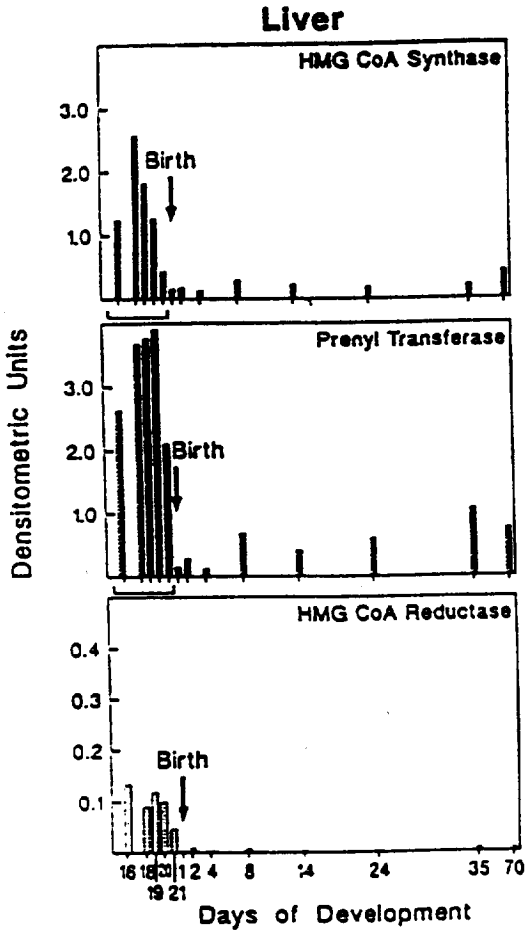

Fig. 1. Developmental changes in rat liver HMG-CoA Synthase, HMG-CoA Reductase and Prenyl Transferase mRNA levels. Total cellular RNA was prepared from pooled rat livers (n = 10–40 animals per time point). Cloned rat cDNAs encoding the three enzymes were used to probe dot blots containing four concentrations of each RNA. The relative concentration of each mRNA was calculated based on scanning laser densitometry of filter autoradiographs and expressed in arbitrary densitometric units. Note that the specific activities of the probes were not identical and therefore no comparisons can be made about the relative levels of each mRNA at a particular stage of liver development.

a remarkable simila n the patterns of change of each mRNA during rat . al and neonatal development. High levels are noted during late gestation. These fall abruptly within 24 h after parturition reaching concentrations that are as much as 80-fold lower than those peak levels encountered prior to birth (e.g., see the middle panel of Fig. 1 which shows changes in prenyl transferase mRNA concentration). A transient 2–4-fold rise in HMG-CoA synthase and prenyl transferase but not HMG-CoA reductase mRNA accumulation occurs during the mid-suckling period (day 8) followed by yet another rise during early adulthood. It is important to note that this pattern of change is not a general phenomenon in the developing rat liver. For example, when the developmental profiles of apo AI, apo AIV and L-FABP mRNAs were studied using these RNAs, quite different ontologic changes were noted (see, for example, Ref. 6).

The developmental profile of rat liver HMG-CoA reductase and prenyl transferase mRNAs parallel the changes in the activities of these enzymes which have been previously documented by several groups [3,33–35]. The corresponding activities of HMG-CoA synthase have not been reported. The rat fetus obtains only about 10% of the sterol required for growth and development from its mother [36]. Therefore, it is not surprising that very high levels of these mRNAs are observed in rat fetal liver during the period surveyed. The highest levels of apo B mRNA occur during the same phase of rat liver development (i.e., in late gestation) only to be followed by an abrupt, early postnatal drop [12]. This provision for expressing the principal apolipoprotein involved in cholesterol transport at a time of maximal endogenous production emphasizes the co-ordinate nature of these developmental changes in the fetal rat liver.

The precise signals for the rapid postnatal fall in the capacity of the livers of suckling rats to synthesize cholesterol are not known. It may represent an adaptive response to adequate supplies of exogenous cholesterol delivered via mother's milk [3,33,37]. Based on the RNA blot hybridization data, it appears that the mechanism involves at least in part a reduction in the steady-state levels of mRNAs encoding these key enzymes in cholesterol biosynthesis. The data do not allow us to say to what extent such alterations reflect a change in gene transcription or mRNA stability.

The changes observed in the levels of prenyl transferase mRNA during the late suckling through weaning phases can be directly correlated with changes in the levels of this enzyme activity [3]. By the 14th postnatal day, HMG-CoA reductase activity in rat liver has fallen to nearly undetectable levels [36]. However, during this second postnatal week, rises in prenyl transferase occur. This rise coincides with that exhibited by another enzyme involved in the mevalonate to squalene pathway.

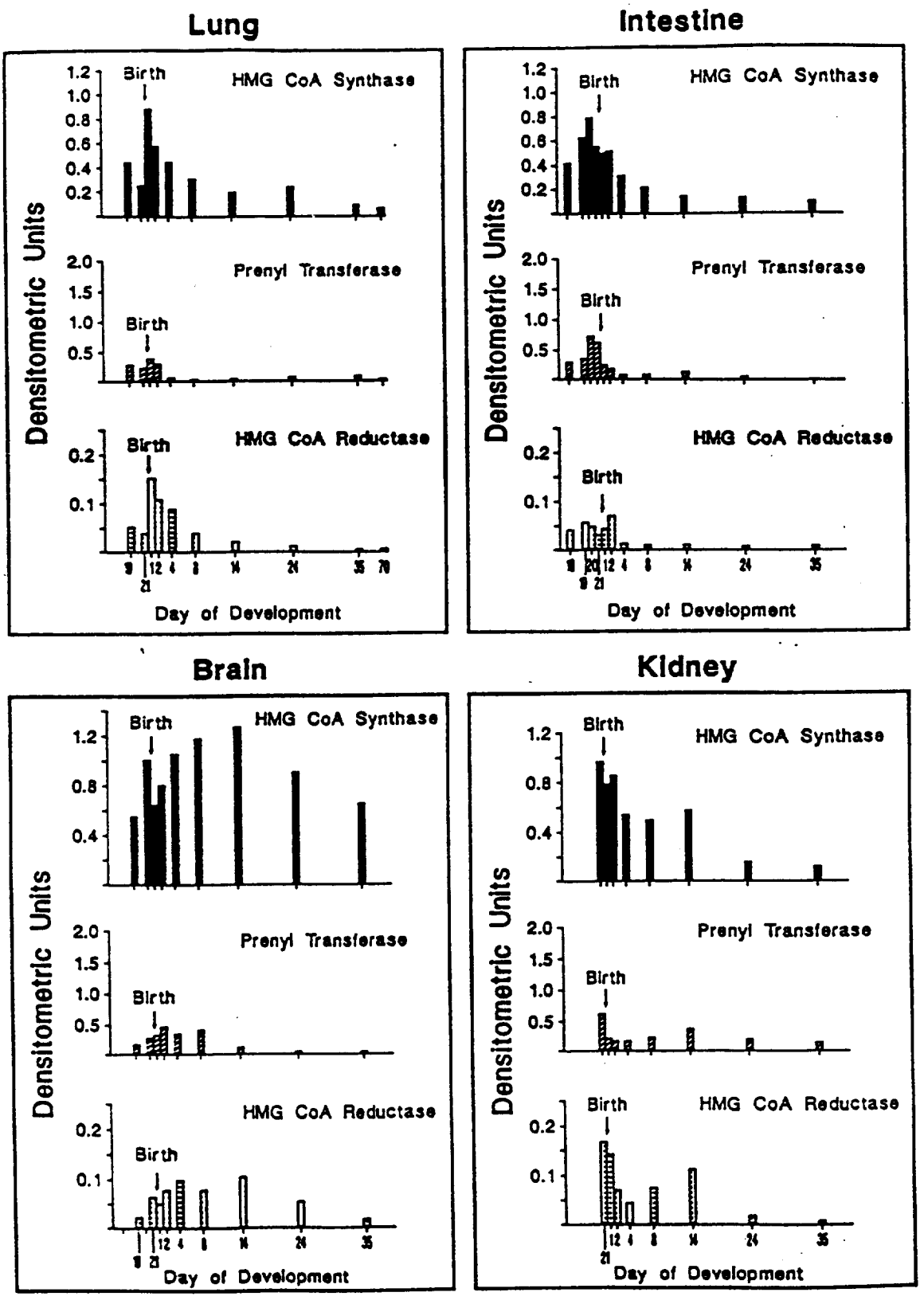

Fig. 2. Coordinate patterns of accumulation of HMG-CoA synthase, prenyl transferase, and HMG-CoA reductase mRNAs during lung, intestinal, brain and kidney development. Total cellular RNA was isolated from pooled tissues harvested from 10–40 rats at each day of fetal and postnatal life noted on the *x*-axis. Relative mRNA concentrations were determined by scanning dot blot autoradiographs and expressed in arbitrary densitometric units. The only comparison which is permitted by this form of data expression is that which involves the same mRNA within a given tissue.

squalene synthetase. These changes in prenyl transferase and squalene synthetase activity do not correspond to any known change in cholesterol synthesis. Bruenger and Rilling [3] noted that since the primary metabolic destination of isoprenoid precursors is cholesterol, changes in the activities of these enzymes may reflect as yet unknown developmental alterations in the metabolic targeting of mevalonate to other compounds (e.g., dolichol and its derivatives, ubiquinones, or isopentenyl tRNAs [38] and prenylated proteins [39]). Although information about the activity profile of HMG-CoA synthase during rat liver development has not been documented in the literature, based on the mRNA data presented in Fig. 1, its developmental profile would be predicted to more closely resemble that of prenyl transferase than HMG-CoA reductase. Moreover, the blot hybridization studies demonstrate for the first time that there are co-ordinate developmental changes in the levels of these three mRNAs encoding enzymes involved in cholesterol biosynthesis.

Expression of the HMG-CoA synthase, HMG-CoA reductase and prenyl transferase genes in extrahepatic tissues during rat development

The remarkable similarity in the accumulation 'profiles' of these three mRNAs observed during the perinatal period of rat liver differentiation was not unique to this organ. This is illustrated in Fig. 2 which summarizes results obtained from probing dot blots of lung, small intestinal, brain and kidney RNAs prepared from 10–40 animals at each of as many as ten different stages of development. Two obvious conclusions can be made after inspection of the data. First, the timing and direction of developmental variation in relative mRNA concentration was virtually identical for each species within each tissue. Second, as in the liver, the highest concentration of each mRNA during lung, intestinal and kidney development was encountered in the late and early postpartum period with subsequent declines occurring in the suckling and/or weaning phases. The notable exception was brain where a progressive postnatal rise in the concentration of each mRNA occurred that generally reached a peak in the mid to late suckling period. Thus expression of the rat HMG-CoA synthase, HMG-CoA reductase and prenyl transferase genes appears to be elaborately programmed to respond in a similar temporal fashion both within and between different tissues from late fetal life through the end of weaning.

Accumulation of mRNAs encoding cholesterol biosynthetic enzymes and lipid transport proteins in fetal, neonatal and adult human liver

Fig. 3 provides the results of our analysis of developmental changes in the concentration of the three cholesterol biosynthetic enzyme mRNAs in human liver. A cloned human HMG-CoA reductase cDNA plus cDNAs encoding rat HMG-CoA synthase and rat prenyl transferase were used for these studies. Since the last two represent heterologous cDNAs, a preliminary experiment was performed. Northern blots of RNA prepared from a human hepatocellular carcinoma cell line (Hep G2) were probed with the rat cDNAs employing the same hybridization stringencies listed in Ref. 12 but the final wash temperature was reduced from 55°C to 45°C. The results indicated that each rat probe reacted with a unique human mRNA species–3.1 and 2.2 kb HMG-CoA synthase mRNA and a 1.2 kb prenyl transferase mRNA (data not shown). These sizes are comparable to those previously reported for the corresponding rat mRNAs [23,40]. Dot blots of total cellular RNA isolated from 8.5–25-week fetal livers ($n = 1$–3 individuals per time point) plus two newborns and one adult without evidence of liver dysfunction were then prepared and probed with the three cDNAs using hybridization and washing conditions established above. As in the case of the rat liver RNA dot blots, multiple concentrations (0.5–2 μg) of each human liver RNA sample were included in the dot blots (see panel A of Fig. 3).

Inspection of Fig. 3B reveals a relatively monotonous developmental profile for all three mRNAs. By 8 weeks of gestation these mRNAs have achieved steady-state levels which do not change more than 2-fold during the remaining 17 weeks of fetal life that were surveyed and less than 3–5-fold when compared to the two newborn and single adult RNA preparations. When there was an opportunity to compare more than one sample at a given stage of human fetal development, remarkably little individual variation was noted in the relative level of a given mRNA.

This less than 2-fold change in cholesterogenic mRNA levels during fetal life is not a general phenomena. It contrasts with results obtained when these same RNA preparations were probed with other cDNAs encoding proteins not involved in lipid metabolism. For example, a control experiment which examined the levels of α-fetoprotein mRNA disclosed the approx. 10-fold reduction expected between first trimester liver and liver harvested at the beginning of the last trimester (see panels A and B of Fig. 3) [41]. In addition, a recent study revealed marked changes in the levels of epsilon, gamma and theta globin mRNAs as well as ceruloplasmin mRNA in the 10–25-week human fetal liver RNA samples [42]. Moreover, the patterns of change of these five different mRNAs were quite distinct from one another.

There is little information about the activities of these cholesterol biosynthetic enzymes during human fetal development. However, the relatively monotonous HMG-CoA reductase, HMG-CoA synthase and prenyl transferase mRNA levels are compatible with observa-

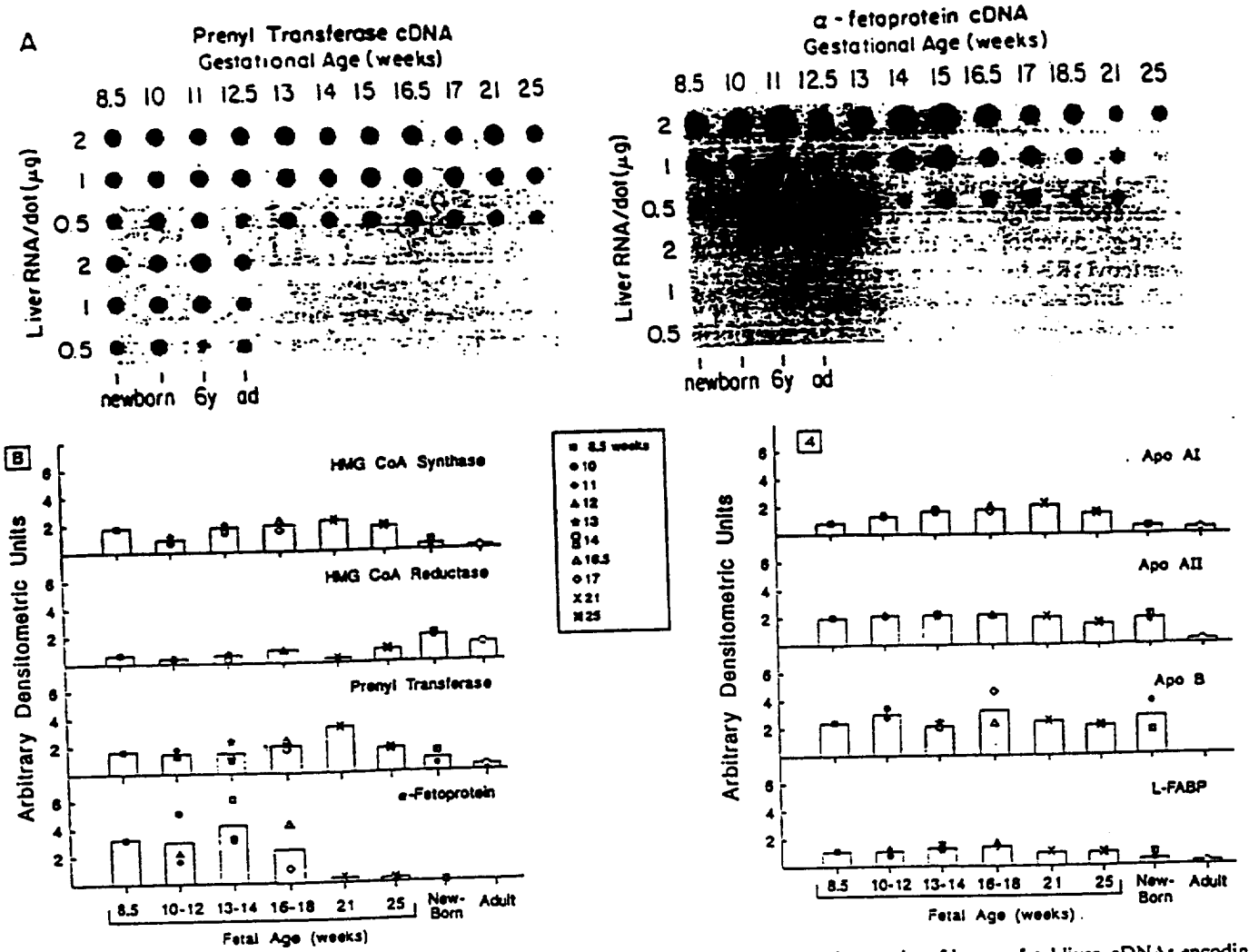

Fig. 3. Developmental changes in the concentration of mRNAs encoding cholesterogenic enzymes in samples of human fetal liver. cDNAs encoding human HMG-CoA reductase plus rat HMG-CoA synthase, rat prenyl transferase (plus human α-fetoprotein) were used to probe dot blots containing several concentrations (0.5–2 μg) of each human liver total cellular RNA sample. Fetal age is based on crown-rump lengths [15]. Fetal age is based on crown-rump lengths [15]. Note that the two 14-week samples came from separate fetuses (see the key defining the symbol ascribed to each RNA preparation). Panel A displays representative dot blots probed with ^{32}P-labeled prenyl transferase and α-fetoprotein cDNAs. Panel B presents developmental profiles of mRNAs encoding the three cholesterol biosynthetic enzymes and α-fetoprotein. As in Fig. 1 arbitrary densitometric units were used to express mRNA levels. No conclusions can be made about the relative concentrations of each of the four mRNAs in a liver RNA sample prepared at a particular developmental stage.

Fig. 4. Developmental profile of human liver mRNAs specifying proteins involved in lipid transport. Blots prepared with the same fetal, neonatal and adult human liver RNA samples as those used to generate the data shown in Fig. 3, were probed with ^{32}P-labeled cloned cDNA specifying human apolipoproteins apo AI, apo AII, apo B, and liver fatty acid binding protein (L-FABP).

tions made by Carr and Simpson [43] that only slight alterations occur in the rate of cholesterol synthesis in the developing human fetal liver. (This rate was estimated to be approx. 9 mg/day during the 18th week of gestation.)

When the levels of other mRNAs encoding proteins involved in lipid transport were examined in human fetal liver, similar 'flat' developmental profiles were observed (Fig. 4). mRNAs specifying the two principal human HDL apolipoproteins, AI and AII, plus the principal protein component of LDL, apo B, achieve steady-state concentrations by 8 weeks of gestation that are similar to those documented during weeks 10–25 and in term infants. Variations were less than 2-fold in all cases. Moreover, the mRNA encoding liver fatty acid binding protein (L-FABP), a small cytoplasmic protein believed to participate in the uptake and/or metabolic processing of exogenous fatty acids (reviewed in Ref. 44), displays a similar lack of change in its concentration during this period (Fig. 4). Together these data

suggest an early biochemical maturation of the human fetal liver with respect to its capacity for cholesterol synthesis and lipid transport.

Differentiation of the human and rat fetal liver

There are very few studies which have provided information about the biochemical maturity of human liver from midgestation to term, so it is very difficult to put our data concerning expression of genes involved in lipid metabolism in any sort of comparative metabolic context at the present time. Greengard has conducted an extensive analysis of the literature dealing with enzymatic differentiation of human and rat liver [45]. Although the data for human are both limited in the number of enzymes examined and the scope of the analyses through fetal life, based on published reports concerning approx. 30 enzymes it appears that most liver enzymes exhibit significant quantitative differences in their activities between the second trimester and adulthood. For example, enzymes involved in synthesis of DNA, pentoses, nonessential amino acids, as well as glycolysis have activities in the actively growing fetal liver which are different from those in the adult tissue. In the few examples where developmental profiles are available in fetal and newborn human and rat liver, it appears that enzyme activities change in the same direction in both mammalian species [45] although the timing of such changes has not been well enough characterized to provide a comparative time scale for human and rat liver differentiation. Comparison of our observations concerning human apo AI, apo B and L-FABP mRNA levels in total human fetal liver RNA with previously published studies in rat liver [6,12,46] suggests that the 8–12-week human fetal liver is at least as well 'differentiated' as the 19–21-day fetal rat liver with respect to expression of these mRNAs. Further studies, based on RNA hybridization techniques, should prove valuable in establishing this comparative developmental time scale for a variety of differentiated functions.

One important caveat needs to be mentioned concerning interpretation of these data. Total cellular RNA prepared from fetal rat and human liver by definition includes mRNAs from all cellular constituents in the liver. These cellular populations undergo remarkable changes during development. A limited number of quantitative morphometric studies of human fetal liver indicate that hepatic parenchymal cells account for approx. 50–70% of its cellular population between the 8th to 28th weeks of gestation with erythroid cells representing 40–50% of fetal liver nuclei and granulocytic precursors less than 10% [47]. In the fetal rat liver, approx. 50% of liver cells are involved in hematopoiesis just prior to parturition with this number rapidly decreasing to less than 5% by the end of the suckling period [48]. The consequences of these shifts in cell populations are obviously important when considering regulation of gene expression in this tissue. For example, the relatively constant levels of mRNAs encoding proteins involved in lipid metabolism that were documented in total human fetal liver RNA during gestation may 'mask' relatively dramatic changes in their concentrations within a given cell type and/or changes in their expression in different cell types. Therefore, it will be very important to extend these analyses using in situ hybridization and immunocytochemical techniques to examine changes in the cell specific expression of these genes in the fetal human and rat liver.

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FINAL REPORT

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ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: T-6295.12

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PROTOCOL 418-013

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TITLE: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS

ARGUS RESEARCH LABORATORIES, INC.
PROTOCOL NUMBER: 418-013
SPONSOR'S STUDY NUMBER: T-6295.12

I. SUMMARY AND CONCLUSION

A. Methods[a]

Eighty virgin female Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats were assigned to five dosage groups (Groups I through V), 16 rats per dosage group. The rats were administered the test article, PFOS (FC-95), or the vehicle, 0.5% Tween® 80 in Reverse Osmosis Membrane Processed Deionized Water (R.0. Deionized Water), orally (via gavage), once daily beginning 42 days prior to cohabitation through either day 14 or 20 of presumed gestation (DG 14 or DG 20). Dosages were 0 (Vehicle), 0.1, 0.4, 1.6 and 3.2 mg/kg/day. The dosage volume was 5 mL/kg.

The rats were observed for viability at least twice each day of the study and for clinical signs of effects of the test article before and approximately one hour after dosage and on the day sacrificed. Body weights were recorded daily during the dosage period and at sacrifice. Feed consumption values were recorded weekly to cohabitation and daily during presumed gestation.

Urine and fecal samples were collected one day prior to initiation of cohabitation to the following morning and DGs 6 to 7, 14 to 15 and 20 to 21. Samples were frozen and shipped to the Sponsor for analysis. Blood samples were collected on the day cohabitation was initiated and on DGs 7, 15 and 21. Blood was centrifuged and the serum was frozen and shipped to the Sponsor for analysis.

Rats were sacrificed on DG 15 or 21, as described below, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. A liver section and the milk-secreting glands from the axillary, thoracic, abdominal and inguinal

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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).

regions of each dam were collected, frozen and shipped to the Sponsor for analysis.

Eight randomly selected female rats, with a confirmed date of mating, per dosage group were Caesarean-sectioned on DG 15. The gravid uterus was excised and weighed. Rats were examined for number and distribution of corpora lutea, implantation sites, viable and nonviable embryos. A sample of the amniotic fluid of each viable embryo was collected and pooled by litter. Each viable embryo was removed from the uterus with the attached placenta and pooled by litter. These samples were frozen shipped to the Sponsor for analysis.

All remaining female rats in each dosage group were Caesarean-sectioned on DG 21 or estimated DG 21. The gravid uterus was excised and weighed. Individual placental weights were recorded. Rats were examined for number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions. Each fetus was removed from the uterus, weighed and examined for sex and gross external alterations. Samples of the amniotic fluid were collected when possible, and pooled by litter. Placentae were collected and pooled by litter. Blood samples were collected from each fetus, pooled by litter and centrifuged. All samples were frozen and shipped to the Sponsor for analysis.

Lung and liver samples were collected from three fetuses per litter from two to five litters per dosage group. Sections from the half of the lungs and one lobe of the liver were shipped to Pathology Associates International, Durham, North Carolina, for possible future evaluation. The other half of the lungs and the other lateral lobe of the liver were frozen and shipped to the Sponsor for analysis.

The livers of the remaining fetuses, plus any remaining liver from the three selected fetuses, were collected, pooled by litter and frozen. The remaining carcass (and head) of each fetus was frozen and shipped to the Sponsor for analysis.

B. Results

No deaths or premature deliveries were attributed to PFOS treatment. Three rats (one each in the 0.1, 0.4 and 1.6 mg/kg/day dosage groups) were sacrificed because the eye was injured during orbital sinus bleeding. One rat in the vehicle control group did not have a confirmed mating date and delivered on day 66 of the study. Two rats in the 3.2 mg/kg/day dosage group delivered on DG 21. All other rats survived to scheduled sacrifice.

All adverse clinical observations during the precohabitation and gestation periods were considered unrelated to the test article and no gross lesions were revealed by necropsy.

Body weight gains for the entire precohabitation period (DSs 1 to 42) were 88.8%, 80.8%, 66.3% and 17.4% of the control group value in the 0.1, 0.4, 1.6 and 3.2 mg/kg/day dosage groups, respectively. Body weights were 98.0%, 96.3%, 93.6% and 85.3% of the control group value in these four respective dosage groups on DS 42.

As the result of random selection for assignment to Caesarean-sectioning on either DG 15 or DG 21, groups assigned to Caesarean-sectioning on DG 15 weighed less on DG 0 than groups assigned to Caesarean-sectioning on DG 21. Reflecting the random selection and differences in average body weights of the two subsets, as well as the relatively small group sizes, body weight gains differed in the two subsets. In both subsets, body weight gains were reduced on DGs 0 to 7 in groups administered 0.4 mg/kg/day and higher dosages of PFOS, after which body weight changes did not demonstrate a clear dosage-dependent pattern.

Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced in the 0.4, 1.6 and 3.2 mg/kg/day dosage groups in each week of the precohabitation period. Reflecting these effects of the test article, absolute and relative feed consumption values were reduced for the entire precohabitation period (DSs 1 to 42) in the 0.4, 1.6 and 3.2 mg/kg/day dosage groups.

Groups administered 0.4 mg/kg/day and higher dosages of the test article had reduced absolute and relative feed consumption values during the first week of the gestation period. Absolute feed consumption values continued to be reduced for the remainder of the dosage period in the 0.4 and 1.6 mg/kg/day dosage groups assigned to DG 15 Caesarean-sectioning and in the 3.2 mg/kg/day dosage group for rats assigned to either DG 15 or DG 21 Caesarean-sectioning.

No Caesarean-sectioning or litter parameters were affected by dosages of the test article as high as 3.2 mg/kg/day administered to rats Caesarean-sectioned on DG 15.

The litter averages for implantations, litter sizes and live fetuses were reduced in the 3.2 mg/kg/day dosage group Caesarean-sectioned on DG 21. Values were below the ranges observed historically at the Testing Facility. Fetal body weights were also reduced in the 3.2 mg/kg/day dosage group. No other Caesarean-sectioning or litter parameters were affected by dosages of the test article as

high as 3.2 mg/kg/day administered to rats Caesarean-sectioned on DG 21. No fetal gross external alterations were observed.

C. Conclusion

The purpose of this study, as stated in the protocol, was to evaluate the pharmacokinetics of PFOS in Fo generation dams and F1 generation fetuses following PFOS treatment to female rats during premating and gestation. This reports includes study data from the in-life portion which was conducted at Argus Research Laboratories, Inc. The PK samples that were collected during the in-life portion of the study were sent to the Sponsor for analysis and will be reported separately. It is the responsibility of the Sponsor, 3M Corporate Toxicology, to combine the in-life results with the analytical results into a final pharmacokinetic report. The results for this study mimic those reported in the Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats (Argus Research Laboratories, Inc., Protocol 418-008), reported 10 June 1999.

24 JUN 99

Mildred S. Christian, Ph.D., Fellow, ATS Date
Executive Director of Research

24-JUN 99

Alan M. Hoberman, Ph.D., DABT Date
Director of Research

24-JUN-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:

418-013

A.4. Sponsor's Study Number:

T-6295.12

A.5. Purpose of the Study:

The purpose of this study was to evaluate the pharmacokinetics of PFOS in Fo generation dams and F1 generation fetuses following PFOS treatment of Crl:CD®BR VAF/Plus® female rats during premating and gestation.

A.6. Study Design:

The requirements of the U.S. Food and Drug Administration (FDA)[1] were used as the basis of study design.

A.7. Regulatory Compliance:

The study was conducted in compliance with 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 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)
Margaret M. Martin (Research Associate)
Corrie L. Pabst (Quality Control Associate)

A.13. Report Preparation:

Raymond G. York, Ph.D., DABT
Jo Ann Frazee, M.S. (Study Coordinator)
Susan K. Bradshaw, B.S. (Data Management Specialist)
Karen G. Parker, A.A. (Report Administrator)

A.14. Report Review:

Mildred S. Christian, Ph.D., Fellow, ATS (Executive Director of Research)
Allan M. Hoberman, Ph.D, DABT (Director of Research)

A.15. Date Protocol Signed:

9 November 1998

A.16. Dates of Technical Performance:

Rat Arrival Date	10 NOV 98
Dosage Period (42 days prior to cohabitation until DG[a] 14 or 20)	16 NOV 98 - 21 JAN 99
Cohabitation Period	27 DEC 98 PM - 01 JAN 99 AM
Caesarean-Sectioning Period (DG 15)	12 JAN 99 - 13 JAN 99
Caesarean-Sectioning Period (DG 21)	18 JAN 99 - 22 JAN 99

A.17. Records Maintained:

The original report, raw data and reserve samples of the bulk 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 prepared formulations were discarded at the Testing Facility. Unused bulk test article will be returned to the Study Monitor upon completion of all work with the test article.

B. Test Article Information:

B.1. Description:

PFOS (FC-95) – an off-white powder

B.2. Lot/Batch Number:

217 (Expiration date: May 2000)

B.3. Date Received and Storage Conditions:

The test article was received on 21 October 1998, and stored at room temperature.

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.

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a. DG is used as an abbreviation for day of (presumed) gestation.

B.5. Analysis of Purity:

Information regarding the identity, composition, strength and purity of the test article is on file with the Sponsor.

C. Vehicle Information:

C.1. Description:

0.5% Tween® 80 in Reverse Osmosis Membrane Processed Deionized Water (R.O. Deionized Water).

C.2. Lot Numbers:

Tween® 80 - M29477 and M03H05

C.3. Date Received and Storage Conditions:

The Tween® 80 was received from J.T. Baker, Phillipsburg, New Jersey, on 17 September 1998 (Lot Number M29477) and 3 December 1998 (Lot Number M03H05), and stored at room temperature. The 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 components and prepared 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 and Storage Conditions:

Suspensions of PFOS were prepared daily at concentrations of 0, 0.02, 0.08, 0.32 and 0.64 mg/mL. Prepared formulations were stored at room temperature.

D.1. Sample Information:

Sample Type	Size	Date Retained	Storage/Shipping Conditions	Shipped To	Date Shipped
Concentration (all levels)	2 mL[a]	15 NOV 98 20 JAN 99	Frozen	Sponsor	16 NOV 98 20 JAN 99
Bulk Test Article Reserve	1 g	12 NOV 98	Room temperature	Testing Facility Archives	25 NOV 98
Vehicle Components Reserve					
Tween® 80	5 mL	12 NOV 98[b] 10 DEC 98[c]	Room temperature	Testing Facility Archives	25 NOV 98 27 JAN 99
R.O. deionized water	5 mL	12 NOV 98	Room temperature	Testing Facility Archives	25 NOV 98

a. Duplicate samples were taken from the first and last preparation on the day prepared. One sample of each set was shipped for analysis. The remaining samples were retained at the Testing Facility as backups. Backup samples are stored frozen (-70°C or below) and will be discarded at the request of the Sponsor.
b. Lot Number M29477.
c. Lot Number M03H05.

D.2. Analytical Results:

Data verifying the stability of the test article in the vehicle for 48 hours under the conditions of administration and the stability of the bulk test article are on file with the Sponsor. Homogeneity of prepared formulations is on file with the Sponsor.

Results of the concentration analysis were not available at the time of the writing of this report.

E. Test System:

E.1. Species:

Rat

E.2. Strain:

Crl:CD®BR VAF/Plus® (Sprague-Dawley)

E.3. Supplier (Source):

Charles River Laboratories, Inc., Raleigh, North Carolina

E.4. Sex:

Female (Note: Male rats were used only for the purposes of breeding and are not considered part of the Test System.)

E.5. Rationale for Test System:

The Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat was selected as the Test System because: 1) this strain of rat was used in the reproductive and developmental toxicity studies; 2) historical data and experience exist at the Testing Facility[5-7]; and 3) the test article is pharmacologically active in the species and strain.

E.6. Test System Data:

Number of Rats	122
Approximate Date of Birth	07 SEP 98
Approximate Age at Arrival	65 days
Weight (g) on the Day After Arrival	193 - 223
Weight (g) at Study Assignment	208 - 224

E.7. Breeder Male Rat Data:

Number of Rats	112
Approximate Date of Birth	13 JAN 98
Approximate Age at Arrival	78 days
Weight (g) on the Day After Arrival	300 - 356
Weight (g) at Cohabitation	558 - 871

E.8. Method of Randomization:

Upon arrival, rats were assigned to individual housing on the basis of computer-generated random units. After acclimation, virgin female rats were selected for study on the basis of physical appearance and body weight recorded during acclimation. Female rats were assigned to five dosage groups (Groups I through V), 16 rats per dosage group, using a computer-generated (weight-ordered) randomization procedure. Within each dosage group, consecutive order was used to assign female rats to cohabitation with breeder male rats, one male rat per female rat. A table of random units was used to select eight female rats with confirmed mating dates per dosage group for Caesarean-section examinations on DG 15. The remaining female rats in each dosage group were Caesarean-sectioned on DG 21[a].

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a. See APPENDIX D (DEVIATIONS FROM THE PROTOCOL AND THE STANDARD OPERATING PROCEDURES OF THE TESTING FACILITY), item 1.

E.9. System of Identification:

Male rats were given unique permanent identification numbers upon assignment to the Testing Facility's breeder male rat population. Female rats were assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study. Each rat was individually identified with a Monel® self-piercing ear tag (Gey Band and Tag Co., Inc., No. MSPT 20101) inscribed with the rat'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. Room temperature and humidity were monitored constantly throughout the study. Room temperature was targeted at 64°F to 79°F (18°C to 26°C); relative humidity was targeted at 30% to 70%. See APPENDIX E (TEMPERATURE AND RELATIVE HUMIDITY REPORTS.)

F.3. Housing:

All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals*[8]. Female rats were individually housed in stainless steel, wire-bottomed cages except during the cohabitation period and during collection intervals for urine and fecal samples. During cohabitation, each pair of rats was housed in the male rat's cage. During collection intervals for urine and fecal samples the female rats were individually housed in metabolism cages. No nesting materials were supplied because the female rats were sacrificed before parturition was expected.

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:

Rats were given *ad libitum* access to Certified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, Missouri) in individual feeders.

F.7. Feed Analysis:

Analyses were routinely performed by the feed supplier. No contaminants at levels exceeding the maximum concentration limits 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 Study Director nor the Sponsor 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 rats *ad libitum* from individual water bottles and/or from an automatic watering access system. 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 Study Director nor the Sponsor 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	Dosage (mg/kg/day)[a]	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Female Rats	Assigned Rat Numbers
I	0 (Vehicle)	0	5	16	19501 - 19516
II	0.1	0.02	5	16	19517 - 19532
III	0.4	0.08	5	16	19533 - 19548
IV	1.6	0.32	5	16	19549 - 19564
V	3.2	0.64	5	16	19565 - 19580

a. The test article was considered 100% pure for the purpose of dosage calculations.

G.2. Rationale for Dosage Selection:

Dosages were selected on the basis of a previous study conducted with the test article (Argus Research Laboratories, Inc., Protocol 418-008).

G.3. Route of Administration:

Oral (gavage)

G.4. Rationale for Route of Administration:

The oral (gavage) route was selected for use because: 1) this was the route of administration in the developmental and reproductive toxicology studies; and 2) it is one of the possible routes of human exposure.

G.5. Frequency of Administration:

Appropriate dosages of the test article or vehicle were administered orally (via gavage) once daily to female rats beginning 42 days prior to cohabitation through either DG 14 or DG 20. The dosage volume was 5 mL/kg, adjusted daily on the basis of the individual body weights recorded before intubation. The rats were intubated once daily at approximately the same time each day.

G.6. Length of Study:

Approximately 11 weeks

G.7. Method of Study Performance:

The female rats were observed for viability at least twice each day of the study and for general appearance at least once during acclimation. The rats were also examined for clinical observations of effects of the test article, abortions, premature deliveries and deaths before and approximately one hour after dosage and once prior to sacrifice.

Body weights were recorded at least once during acclimation, daily during the dosage period and at sacrifice. Feed consumption values were recorded at least once during the acclimation period, weekly to cohabitation and daily during presumed gestation.

After 42 days of test article administration, 80 healthy virgin female rats were placed into cohabitation with 80 breeder male rats (one male rat per female rat in the male rat's cage)[a]. Mating was evaluated daily during the cohabitation period and confirmed by the observation of spermatozoa in a smear of the vaginal contents and/or a copulatory plug *in situ*. Female rats considered to be at DG 0 were returned to individual housing.

Urine and fecal samples were collected from female rats at the following intervals: one day prior to initiation of cohabitation to the following morning and from each of the mated female rats on DGs 6 to 7, 14 to 15 and 20 to 21. Following each 24-hour collection interval, samples were collected into centrifuge tubes, placed on dry ice and maintained frozen (-70°C or below) until shipment (on dry ice) to the Sponsor for analysis.

Blood samples were collected from each of the female rats following removal from metabolism caging (prior to test article or vehicle administration) on the day cohabitation was initiated (prior to cohabitation) and from each of the mated female rats on DGs 7, 15 and 21. On all days of collection except DGs 15 and 21 (dams at their terminal collection interval), blood samples (approximately 1 mL each) were collected from the orbital sinus. On DG 21, blood samples (approximately 4 mL each) were collected via the inferior vena cava. When blood collection on DG 15 was a final bleed for the respective dam (i.e., the dam was Caesarean-sectioned that day), the sample was approximately 4 mL and was collected via the inferior vena cava. Blood was transferred into serum separator tubes and spun in a refrigerated centrifuge. The serum was transferred into polypropylene tubes labeled with the study number, rat identification, date of collection, study day and collection timepoint. All samples were immediately frozen on dry ice and stored (-70°C or below) until shipment (on dry ice) to the Sponsor for analysis.

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a. See APPENDIX D, item 1.

G.8. Gross Necropsy[a]:

Rats were sacrificed by carbon dioxide asphyxiation on DG 15 or 21, as described below. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. A liver section (right lateral lobe) and the milk-secreting glands from the axillary, thoracic, abdominal and inguinal regions (left side only) of each dam (except rats without confirmed dates of mating) were collected, frozen and stored (-70°C or below) until shipment (on dry ice) to the Sponsor for analysis. To confirm the pregnancy status, uteri from rats that appeared nonpregnant were stained with 10% ammonium sulfide[9]. Tissues with gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation; all other maternal tissues were discarded. Representative photographs of maternal lesions are available in the raw data.

G.8.a. DG 15 Caesarean-Sectioning

Eight randomly selected female rats, with a confirmed date of mating, per dosage group were Caesarean-sectioned on DG 15. The gravid uterus was excised and weighed. Rats were examined for number and distribution of corpora lutea, implantation sites, viable and nonviable embryos. A viable embryo was defined as oval or crescent shaped, pink, firm and enclosed in an amniotic sac filled with clear fluid. A nonviable embryo is amorphous, small, pale pink to tan or deep red to black, soft and enclosed in an amniotic sac filled with clear, cloudy or opaque fluid.

A sample of the amniotic fluid of each viable embryo was collected and pooled per litter, frozen and stored (-70°C or below) until shipment, on dry ice, to the Sponsor for analysis. Each viable embryo was removed from the uterus with the attached placenta, pooled per litter frozen and stored (-70°C or below) until shipment, on dry ice, to the Sponsor for analysis.

G.8.b. DG 21 Caesarean-Sectioning

All remaining female rats in each dosage group were Caesarean-sectioned on DG 21 (rats with confirmed dates of mating) or estimated DG 21 (rats without confirmed dates of mating). The gravid uterus was excised and weighed. Individual placental weights were recorded. Rats were examined for number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in

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a. A table of random units was used to select one control group rat from which all tissues examined at necropsy were retained, in order to provide control tissues for any possible histopathological evaluations of gross lesions.

which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to 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 fetus was removed from the uterus, placed in an individual container and identified with a tag noting the study number, litter number, and uterine distribution. Each fetus was subsequently weighed and examined for sex and gross external alterations. Live fetuses were sacrificed via decapitation.

Samples of the amniotic fluid (when possible) and the placenta of each fetus were collected, individually pooled per litter, frozen and stored (-70°C or below) until shipment to the Sponsor for analysis. Blood samples were collected from each fetus via decapitation, pooled per litter and transferred into serum separator tubes. The samples were spun in a refrigerated centrifuge. The serum was transferred into polypropylene tubes labeled with the study number, litter identification, date of collection, study day and collection timepoint. All samples were immediately frozen on dry ice and stored (-70°C or below) until shipment (on dry ice) to the Sponsor for analysis.

Lung and liver samples were collected from three fetuses per litter from two to five litters per dosage group. One half of the lungs and one lateral lobe of the liver were individually retained in neutral buffered 10% formalin in scintillation vials. Prior to fixation, several (minimum of two) sections (approximately 1 mm thick) of this half of the lungs and this lobe of the liver were removed using a scalpel and retained in McDowell-Trump's Fixative (stored under refrigeration in scintillation vials). The lung and liver sections in McDowell-Trump's Fixative and the lung and liver samples in neutral buffered 10% formalin were shipped (on cold packs), for possible future evaluation by electron microscopy or light microscopy, respectively, to Pathology Associates International, Durham, North Carolina. The other half of the lungs and the other lateral lobe of the liver were flash frozen in liquid nitrogen and stored in heat-sealable pouches on dry ice. These flash frozen lung and liver samples were maintained frozen (-70°C or below) until shipment (on dry ice) to the Sponsor for analysis.

The livers of the remaining fetuses, plus any remaining liver from the three selected fetuses, were collected, pooled by litter and frozen. The remaining carcass (and head) of each fetus was frozen. These samples were maintained frozen (-70°C or below) until shipment (on dry ice) to the Sponsor for analysis.

G.8.c. Moribund Sacrifice or Premature Delivery

Rats that were sacrificed because of moribund condition or premature delivery were examined for the cause on the day the observation was made. The rats

were examined for gross lesions. A liver section (right lateral lobe) and the milk-secreting glands from the axillary, thoracic, abdominal and inguinal regions of each dam (left side only) were collected, frozen and stored (-70°C or below) until shipment (on dry ice) to the Sponsor for analysis. Pregnancy status and uterine contents were recorded. Delivered pups were examined to the extent possible, using the same methods described for term fetuses. Uteri of apparently nonpregnant rats were stained with 10% ammonium sulfide to confirm the absence of implantation sites[9].

G.9. Statistical Analyses:

Averages and percentages were calculated. Litter values were used where appropriate.

III. RESULTS

A. Mortality, Premature Delivery, Clinical and Necropsy Observations (Summary - Table 1; Individual Data - Tables 12 and 13)

A.1. Mortality and Premature Delivery

No deaths or premature deliveries were attributed to PFOS treatment. Three rats (one each in the 0.1, 0.4 and 1.6 mg/kg/day dosage groups) were sacrificed because the eye was injured during orbital sinus bleeding. One rat in the vehicle control group did not have a confirmed mating date and delivered on day 66 of the study. Two rats in the 3.2 mg/kg/day dosage group delivered on DG 21. All other rats survived to scheduled sacrifice.

A.2. Clinical Observations

All adverse clinical observations during the precohabitation and gestation periods were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; and/or 2) the observation occurred in only one or two rats. These observations included missing or swollen forepaw digit, scabs on forepaw, swollen forepaw, localized alopecia on the limbs, head, back and/or underside, chromorhinorrhea, chromodacryorrhea, swollen ears, dental problems (missing, broken and/or misaligned incisors), swollen snout, corneal hemorrhage, corneal opacity, axillary mass, exophthalmos, enophthalmos, lenticular opacity, lacrimation, ungroomed coat, traumatized cornea, cloudy, white film on eye, missing right eye, large eye, dark red eye, and lids unable to close.

A.3. Necropsy Observations

No gross lesions were revealed by necropsy.

B. Body Weights, Uterine Weights and Body Weight Changes (Figures 1 through 3; Summaries - Tables 2 through 5; Individual Data – Tables 14 and 15)

B.1. Precohabitation

Body weight gains for the entire precohabitation period (DSs 1 to 42) were 88.8%, 80.8%, 66.3% and 17.4% of the control group value in the 0.1, 0.4, 1.6 and 3.2 mg/kg/day dosage groups, respectively. Body weights were 98.0%, 96.3%, 93.6% and 85.3 % of the control group value in these four respective dosage groups on DS 42.

B.2. Gestation

As the result of random selection for assignment to Caesarean-sectioning on either DG 15 or DG 21, groups assigned to Caesarean-sectioning on DG 15 weighed less on DG 0 than groups assigned to Caesarean-sectioning on DG 21. Reflecting the random selection and differences in average body weights of the two subsets, as well as the relatively small group sizes, body weight gains differed in the two subsets. In both subsets, body weight gains were reduced on DGs 0 to 7 in groups administered 0.4 mg/kg/day and higher dosages of PFOS, after which body weight changes did not demonstrate a clear dosage-dependent pattern.

Gestation body weights and body weight gains were unaffected by the 0.1 mg/kg/day dosage of the test article. Gravid uterine weights were unaffected by dosages of the test article as high as 3.2 mg/kg/day.

C. Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables 6 through 9; Individual Data – Tables 16 and 17)

C.1. Precohabitation

Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced in the 0.4, 1.6 and 3.2 mg/kg/day dosage groups in each week of the precohabitation period. Reflecting these effects of the test article, absolute feed consumption values were 94.8%, 92.2% and 83.8% of the control group value, and relative feed consumption values were 95.1%, 94.3% and 90.7% of the control group value for the entire precohabitation period (DSs 1 to 42) in the 0.4, 1.6 and 3.2 mg/kg/day dosage groups.

Absolute and relative feed consumption values during the precohabitation period were unaffected by the 0.1 mg/kg/day dosage of the test article.

C.2. Gestation

Groups administered 0.4 mg/kg/day and higher dosages of the test article had reduced absolute and relative feed consumption values during the first week of the gestation period (no values were available for evaluation for the 1.6 mg/kg/day dosage group assigned to Caesarean-sectioning on DG 15). Absolute feed consumption values continued to be reduced for the remainder of the dosage period in the 0.4 and 1.6 mg/kg/day dosage groups assigned to DG 15 Caesarean-sectioning and in the 3.2 mg/kg/day dosage group for rats assigned to either DG 15 or DG 21 Caesarean-sectioning. Relative feed consumption values tended to be increased over the control group values for the 1.6 and 3.2 mg/kg/day dosage groups after DG 10.

Absolute and relative feed consumption values during gestation were unaffected by the 0.1 mg/kg/day dosage of the test article.

D. Caesarean-Sectioning, Litter and Fetal Gross External Observations (Summaries –Tables 10 and 11; Individual Data - Tables 18 through 21)

D.1. Day 15 of Gestation

Pregnancy occurred in 6 (75.0%), 7 (87.5%), 8 (100.0%), 6 (85.7%) and 8 (100.0%) of the rats in each dosage group.

No Caesarean-sectioning or litter parameters were affected by dosages of the test article as high as 3.2 mg/kg/day administered to rats Caesarean-sectioned on DG 15. The litter averages for corpora lutea, implantations, viable and nonviable embryos and percent nonviable embryos per litter were comparable among the five dosage groups. No dam had a litter consisting of only nonviable embryos.

D.2. Day 21 of Gestation

Pregnancy occurred in 7 (100.0%), 7 (100.0%), 5 (83.3%), 2 (50.0%) and 6 (100.0%) rats with a confirmed mating date in each dosage group. One rat in each of the 0.1 and 0.4 mg/kg/day dosage groups was moribund sacrificed on DG 0 and the pregnancy status could not be determined and two rats in the 3.2 mg/kg/day dosage group delivered before Caesarean-sectioning on DG 21, as previously described. As a result, Caesarean-sectioning observations were based on 7, 7, 5, 2 and 4 pregnant rats with one or more live fetuses in Groups I through V, respectively.

The litter averages for implantations, litter sizes and live fetuses were reduced in the 3.2 mg/kg/day dosage group. Values were below the ranges observed historically at the Testing Facility[a]. Fetal body weights were also reduced in the 3.2 mg/kg/day dosage group. No other Caesarean-sectioning or litter parameters were affected by dosages of the test article as high as 3.2 mg/kg/day administered to rats Caesarean-sectioned on DG 21. The litter averages for corpora lutea, early and late resorptions, placental weights, percent live male fetuses and percent resorbed conceptuses were comparable among the five dosage groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. No fetal alterations were identified at gross external examination.

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a. See APPENDIX F (HISTORICAL CONTROL DATA).

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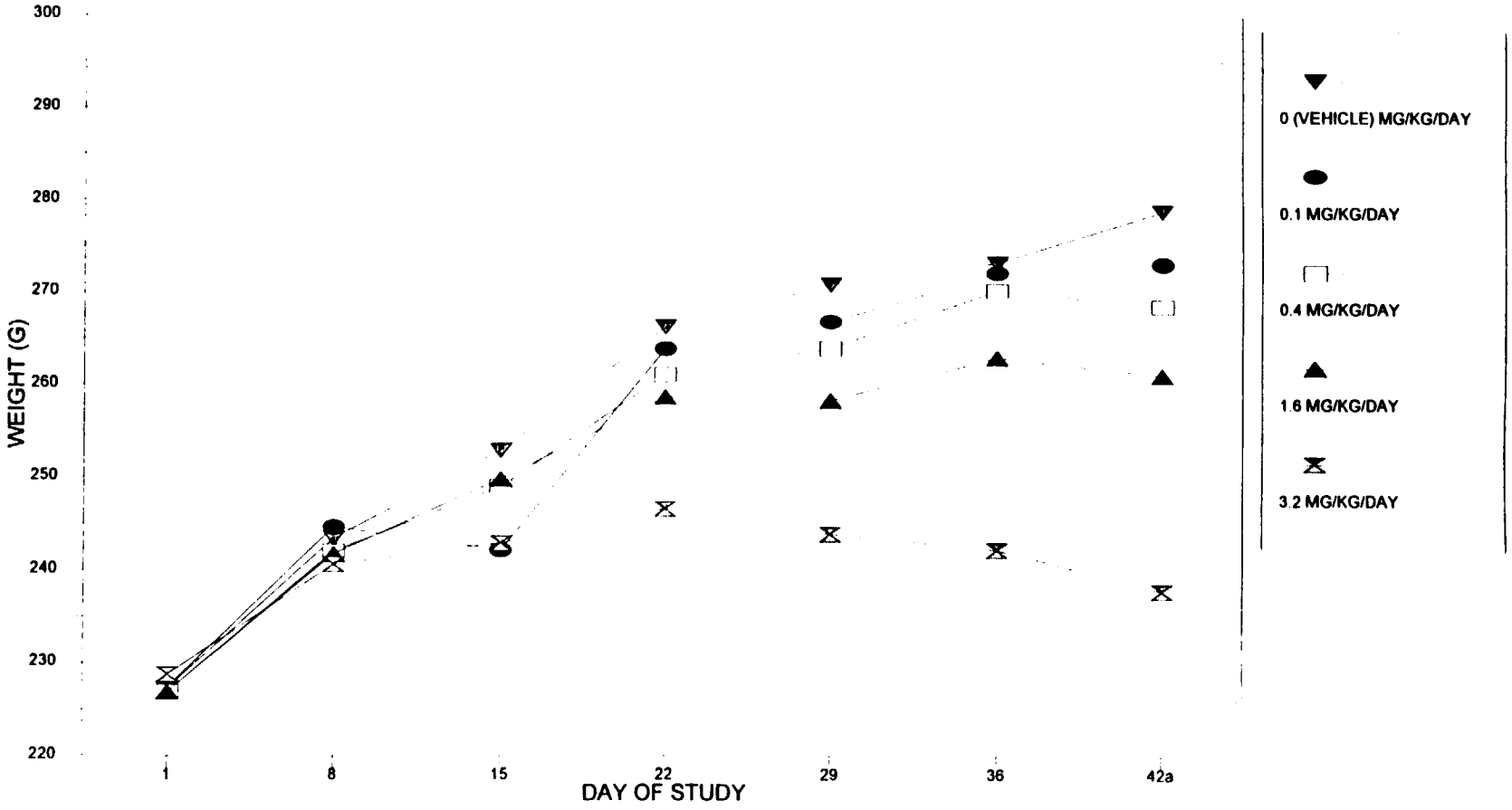
APPENDIX A

REPORT FIGURES

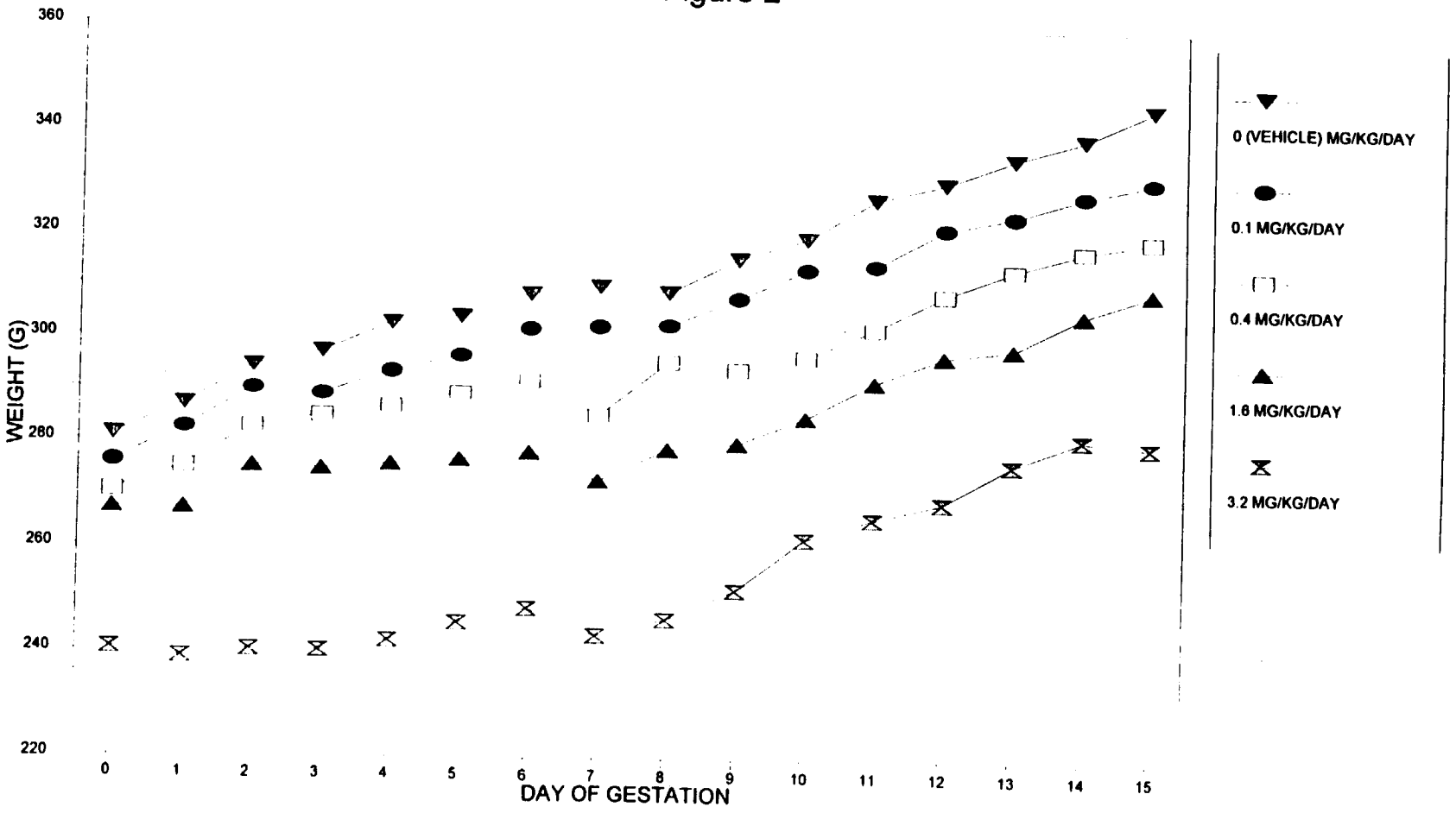
BODY WEIGHTS
PRECOHABITATION
Figure 1



a. Last value recorded before cohabitation

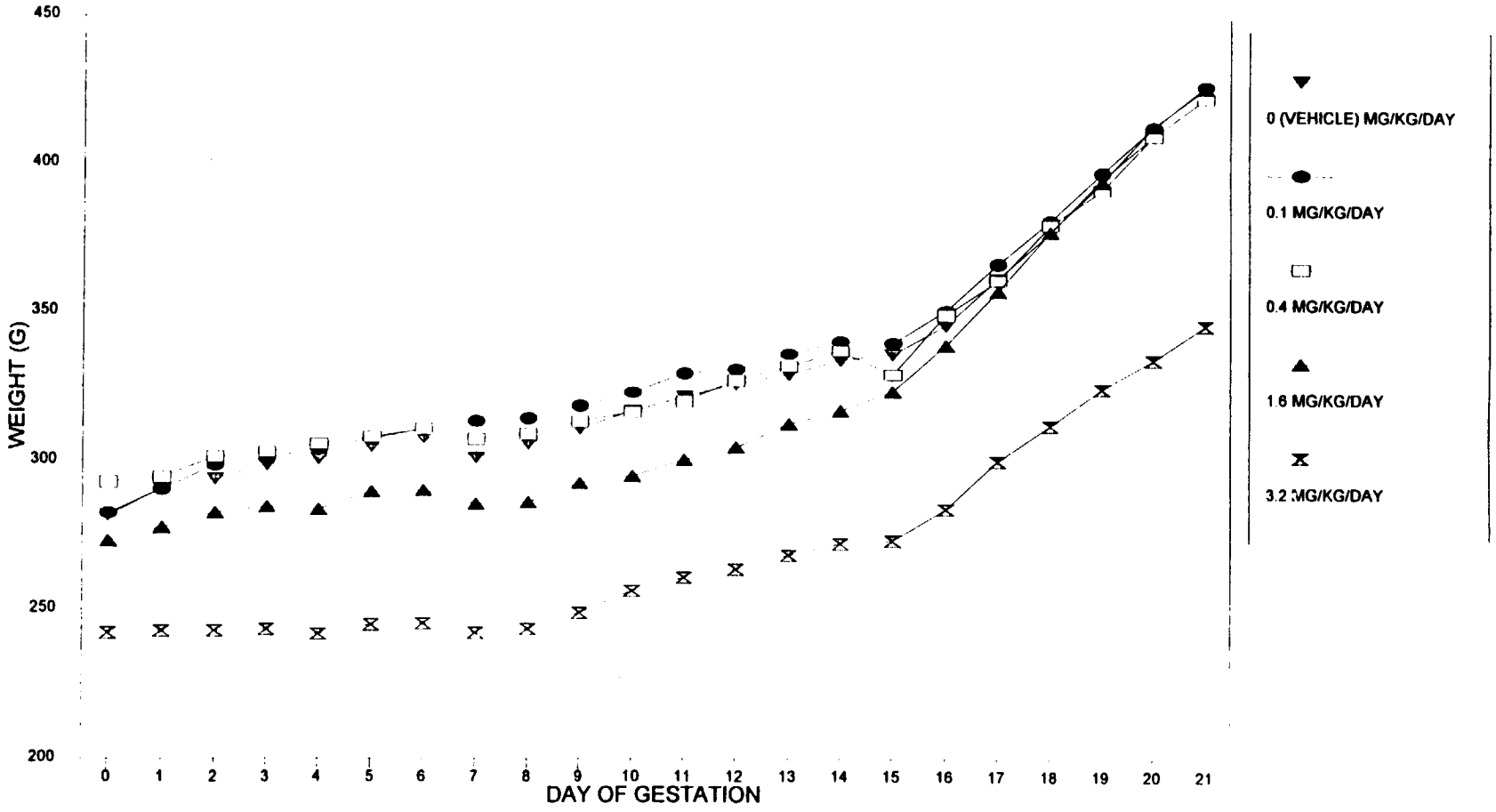
MATERNAL BODY WEIGHTS
RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

Figure 2



MATERNAL BODY WEIGHTS
RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

Figure 3



APPENDIX B

REPORT TABLES

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 1 (PAGE 1): CLINICAL AND NECROPSY OBSERVATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP	I	II	III	IV	V
DOSAGE (MG/KG/DAY)	0 (VEHICLE)	0.1	0.4	1.6	3.2
MORTALITY					
DELIVERED AND SACRIFICED	1	1	1	1	2
MORIBUND SACRIFICED	1a	0	0	0	2b,c
	0	1d	1e	1f	0
PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION):					
MAXIMUM POSSIBLE INCIDENCE	672/ 16	672/ 16	672/ 16	672/ 16	672/ 16
FOREPAW: THIRD DIGIT MISSING	0/ 0	0/ 0	0/ 0	0/ 0	34/ 1
LOCALIZED ALOPECIA: TOTAL	40/ 1	43/ 2	0/ 0	26/ 2	9/ 1
LIMBS	40/ 1	43/ 2	0/ 0	14/ 1	9/ 1
UNDERSIDE	0/ 0	0/ 0	0/ 0	12/ 1	0/ 0
FOREPAW: THIRD DIGIT SWOLLEN	0/ 0	0/ 0	0/ 0	0/ 0	3/ 1
FOREPAWS: SCAB(S)	0/ 0	0/ 0	0/ 0	14/ 1	3/ 1
CHROMORHINORRHEA	0/ 0	0/ 0	1/ 1	5/ 4	1/ 1
CHROMODACRYORRHEA	0/ 0	5/ 1	0/ 0	0/ 0	1/ 1
EARS: SWOLLEN	0/ 0	0/ 0	0/ 0	3/ 1	0/ 0
FOREPAWS: SWOLLEN	0/ 0	0/ 0	0/ 0	2/ 1	0/ 0
INCISORS: TOTAL	0/ 0	21/ 1	0/ 0	0/ 0	0/ 0
MISSING/BROKEN	0/ 0	12/ 1	0/ 0	0/ 0	0/ 0
MISALIGNED	0/ 0	9/ 1	0/ 0	0/ 0	0/ 0
SNOUT: SWOLLEN	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 1 (PAGE 2): CLINICAL AND NECROPSY OBSERVATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
PRESUMED GESTATION: g					
MAXIMUM POSSIBLE INCIDENCE	282/ 15	283/ 16	261/ 15	216/ 12	260/ 14
LOCALIZED ALOPECIA: TOTAL	3/ 1	37/ 3	3/ 1	45/ 3	46/ 4
LIMBS	3/ 1	23/ 2d	0/ 0	37/ 2	43/ 3c
UNDERSIDE	0/ 0	0/ 0	3/ 1	16/ 1	3/ 1
HEAD	0/ 0	14/ 1	0/ 0	0/ 0	0/ 0
BACK	0/ 0	0/ 0	0/ 0	7/ 1	0/ 0
FOREPAW: THIRD DIGIT MISSING	0/ 0	0/ 0	0/ 0	0/ 0	22/ 1
CORNEAL HEMORRHAGE	0/ 0	0/ 0	0/ 0	6/ 1	16/ 1
CORNEAL OPACITY	0/ 0	27/ 3d	20/ 2e	0/ 0	16/ 1
RIGHT AXILLA: MASS	0/ 0	0/ 0	0/ 0	0/ 0	11/ 1
EXOPHTHALMOS	0/ 0	4/ 2d	4/ 2e	1/ 1	2/ 1
CHROMODACRYORRHEA	11/ 1	1/ 1d	1/ 1e	0/ 0	1/ 1
FOREPAWS: SCAB(S)	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
ENOPHTHALMOS	0/ 0	0/ 0	8/ 1	0/ 0	0/ 0
LENTICULAR OPACITY	16/ 1a	0/ 0	3/ 1	0/ 0	0/ 0
LACRIMATION	0/ 0	0/ 0	2/ 1	0/ 0	0/ 0
RIGHT EYE: LARGE	0/ 0	1/ 1d	1/ 1e	0/ 0	0/ 0
RIGHT EYE: DARK RED	0/ 0	1/ 1d	1/ 1e	0/ 0	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 1 (PAGE 3): CLINICAL AND NECROPSY OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
PRESUMED GESTATION: g					
MAXIMUM POSSIBLE INCIDENCE	282/ 15	283/ 16	261/ 15	216/ 12	260/ 14
RIGHT EYE: LIDS UNABLE TO CLOSE	0/ 0	1/ 1d	1/ 1e	0/ 0	0/ 0
CHROMORHINORRHEA	7/ 2	0/ 0	1/ 1	0/ 0	0/ 0
SNOUT: SWOLLEN	0/ 0	3/ 2d	1/ 1e	0/ 0	0/ 0
UNGROOMED COAT	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
TRAUMATIZED CORNEA	0/ 0	33/ 3	0/ 0	0/ 0	0/ 0
RIGHT EYE: CLOUDY, WHITE FILM	0/ 0	11/ 1	0/ 0	0/ 0	0/ 0
RIGHT EYE: MISSING	0/ 0	11/ 1	0/ 0	0/ 0	0/ 0
INCISORS: MISSING/BROKEN	0/ 0	7/ 1	0/ 0	0/ 0	0/ 0

PERSISTENT ADVERSE CLINICAL OBSERVATIONS WERE CONFIRMED AT NECROPSY, NO ADDITIONAL GROSS LESIONS WERE IDENTIFIED

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.

a. Dam 19503 did not have a confirmed mating date and delivered on day 66 of study.
b. Dam 19571 delivered on day 21 of gestation.
c. Dam 19579 delivered on day 21 of gestation.
d. Rat 19520 was moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.
e. Rat 19541 was moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.
f. Rat 19557 was moribund sacrificed on day 43 of study.
g. Restricted to rats with a confirmed mating date.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 2 (PAGE 1): BODY WEIGHTS - PRECOHABITATION - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	16	16	16	16	16
BODY WEIGHT (G)						
DAY 1	MEAN±S.D	227.3 ± 7.2	227.4 ± 4.3	226.9 ± 6.2	226.9 ± 6.9	228.8 ± 7.0
DAY 8	MEAN±S.D	243.4 ± 10.2	244.6 ± 6.8	242.0 ± 9.4	241.7 ± 10.1	240.6 ± 10.3
DAY 15	MEAN±S.D	252.9 ± 11.6	252.1 ± 8.6	249.0 ± 11.7	249.8 ± 11.2	242.9 ± 10.7
DAY 22	MEAN±S.D	266.3 ± 12.8	263.9 ± 10.5	261.1 ± 15.1	258.7 ± 12.3	246.6 ± 12.4
DAY 29	MEAN±S.D	270.9 ± 16.0	266.9 ± 11.5	264.0 ± 17.1	258.4 ± 14.4	243.9 ± 13.7
DAY 36	MEAN±S.D	273.3 ± 18.6	272.2 ± 12.7	270.3 ± 17.9	263.0 ± 15.0	242.2 ± 15.1
DAY 42a	MEAN±S.D	278.9 ± 18.2	273.2 ± 15.4	268.6 ± 23.4	261.1 ± 15.9	237.8 ± 14.2

DAY = DAY OF STUDY
a. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 3 (PAGE 1): BODY WEIGHT CHANGES - PRECOHABITATION - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	16	16	16	16	16
BODY WEIGHT CHANGE (G)						
DAYS 1 - 8	MEAN±S.D.	+16.1 ± 5.6	+17.2 ± 5.1	+15.1 ± 5.1	+14.8 ± 5.7	+11.9 ± 6.2
DAYS 8 - 15	MEAN±S.D.	+9.5 ± 5.6	+7.5 ± 4.7	+7.0 ± 4.4	+8.1 ± 4.7	+2.3 ± 4.6
DAYS 15 - 22	MEAN±S.D.	+13.4 ± 5.8	+11.8 ± 4.8	+12.1 ± 5.2	+8.9 ± 4.2	+3.6 ± 4.9
DAYS 22 - 29	MEAN±S.D.	+4.6 ± 6.6	+3.0 ± 5.7	+2.9 ± 6.1	-0.2 ± 4.8	-2.6 ± 6.0
DAYS 29 - 36	MEAN±S.D.	+2.4 ± 7.1	+5.3 ± 5.4	+6.3 ± 6.4	+4.6 ± 4.4	-1.7 ± 6.4
DAYS 36 - 42a	MEAN±S.D.	+5.6 ± 5.2	+0.9 ± 6.1	-1.7 ± 23.6	-1.9 ± 6.0	-4.5 ± 5.7
DAYS 1 - 42a	MEAN±S.D.	+51.6 ± 16.9	+45.8 ± 14.6	+41.7 ± 22.6	+34.2 ± 13.2	+9.0 ± 11.3

DAYS = DAYS OF STUDY

a. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 4 (PAGE 1): MATERNAL BODY WEIGHTS AND GRAVID UTERINE WEIGHTS - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	8	8	8	7a	8
PREGNANT	N	6	7	8	6	8
MATERNAL BODY WEIGHT (G)						
DAY 0	MEAN±S.D.	281.0 ± 28.9	276.0 ± 10.5	270.4 ± 14.4	267.2 ± 16.4	240.5 ± 10.5
DAY 1	MEAN±S.D.	287.0 ± 26.0	282.4 ± 13.0	275.1 ± 20.8	267.2 ± 15.1	238.9 ± 13.6
DAY 2	MEAN±S.D.	294.3 ± 25.1	290.0 ± 11.2	282.8 ± 19.3	275.3 ± 17.0	240.2 ± 13.4
DAY 3	MEAN±S.D.	297.2 ± 27.5	289.1 ± 11.2	285.0 ± 16.9	274.8 ± 17.4	240.0 ± 14.8
DAY 4	MEAN±S.D.	302.8 ± 28.4	293.6 ± 10.5	286.8 ± 17.4	275.8 ± 15.8	242.1 ± 10.8
DAY 5	MEAN±S.D.	304.2 ± 26.2	296.6 ± 12.4	289.4 ± 16.3	276.8 ± 19.0	245.6 ± 12.2
DAY 6	MEAN±S.D.	308.7 ± 27.1	302.0 ± 14.2	291.9 ± 17.3	278.3 ± 16.3	248.4 ± 11.7
DAY 7	MEAN±S.D.	310.2 ± 28.2	302.6 ± 12.2	285.5 ± 20.6	273.0 ± 14.5	243.4 ± 13.0
DAY 8	MEAN±S.D.	309.2 ± 25.0	302.8 ± 12.0	295.8 ± 21.3	279.0 ± 17.7	246.5 ± 9.7
DAY 9	MEAN±S.D.	315.7 ± 23.1	308.0 ± 13.7	294.4 ± 17.9	280.3 ± 16.1	252.2 ± 10.2
DAY 10	MEAN±S.D.	319.7 ± 25.0	313.7 ± 17.9	296.9 ± 19.0	285.3 ± 15.6	262.0 ± 10.5
DAY 11	MEAN±S.D.	327.3 ± 24.5	314.6 ± 13.9	302.4 ± 18.6	292.2 ± 16.8	265.9 ± 9.9
DAY 12	MEAN±S.D.	330.5 ± 23.9	321.7 ± 15.0	309.1 ± 18.7	297.2 ± 18.9	269.1 ± 9.5

DAY = DAY OF GESTATION

a. Excludes values for rat 19561; necropsy and Caesarean-section observations were not recorded.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 4 (PAGE 2): MATERNAL BODY WEIGHTS AND GRAVID UTERINE WEIGHTS - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	8	8	8	7a	8
PREGNANT	N	6	7	8	6	8
MATERNAL BODY WEIGHT (G)						
DAY 13	MEAN±S.D.	335.3 ± 24.6	324.3 ± 15.6	314.0 ± 19.8	298.8 ± 17.8	276.6 ± 13.3
DAY 14	MEAN±S.D.	339.2 ± 25.8	328.4 ± 15.5	317.8 ± 19.2	305.5 ± 20.8	281.6 ± 12.6
DAY 15	MEAN±S.D.	345.2 ± 27.8	331.1 ± 18.6	319.9 ± 22.4	309.8 ± 17.7	280.4 ± 13.3
GRAVID UTERINE WEIGHTS (G)	MEAN±S.D.	19.8 ± 4.3	17.5 ± 5.2	16.3 ± 5.6	17.6 ± 1.3	17.1 ± 4.0
DAY 15C b	MEAN±S.D.	325.3 ± 25.7	313.7 ± 15.7	303.6 ± 19.5	292.2 ± 16.9	263.3 ± 11.3

DAY = DAY OF GESTATION

a. Excludes values for rat 19561; necropsy and Caesarean-section observations were not recorded.

b. 15C = Corrected maternal body weight (day 15 of gestation body weight minus the gravid uterine weight).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 4 (PAGE 3): MATERNAL BODY WEIGHTS AND GRAVID UTERINE WEIGHTS - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	7a	7b	6a,b	4a,c	6a
PREGNANT	N	7	7	5	2	6
MATERNAL BODY WEIGHT (G)						
DAY 0	MEAN±S.D.	281.6 ± 14.5	282.3 ± 19.1	292.8 ± 22.7	273.0 ± 1.4	242.0 ± 14.7
DAY 1	MEAN±S.D.	290.3 ± 15.6	290.4 ± 17.9	294.4 ± 24.7	277.5 ± 0.7	242.8 ± 17.3
DAY 2	MEAN±S.D.	293.8 ± 14.8	298.3 ± 16.5	301.2 ± 24.8	282.5 ± 0.7	242.7 ± 18.2
DAY 3	MEAN±S.D.	298.4 ± 12.6	300.4 ± 19.3	302.6 ± 23.3	284.5 ± 2.1	243.2 ± 20.3
DAY 4	MEAN±S.D.	300.4 ± 13.4	304.0 ± 14.2	305.4 ± 24.5	283.5 ± 0.7	241.8 ± 21.3
DAY 5	MEAN±S.D.	304.8 ± 9.6	307.4 ± 16.8	307.8 ± 26.1	289.5 ± 2.1	244.8 ± 22.8
DAY 6	MEAN±S.D.	307.6 ± 10.7	310.6 ± 17.4	310.6 ± 26.6	290.0 ± 2.8	245.2 ± 24.2
DAY 7	MEAN±S.D.	301.0 ± 12.8	313.3 ± 18.3	307.4 ± 25.9	285.5 ± 0.7	242.0 ± 19.8
DAY 8	MEAN±S.D.	305.8 ± 12.8	314.4 ± 16.6	309.0 ± 26.1	286.0 ± 1.4	243.5 ± 21.0
DAY 9	MEAN±S.D.	311.0 ± 10.4	318.7 ± 15.4	313.4 ± 27.3	292.5 ± 2.1	249.0 ± 20.5
DAY 10	MEAN±S.D.	317.0 ± 8.8	323.1 ± 16.9	316.6 ± 26.3	295.0 ± 7.1	256.3 ± 18.1
DAY 11	MEAN±S.D.	321.7 ± 10.1	329.6 ± 15.2	320.2 ± 23.7	300.5 ± 7.8	260.7 ± 21.2
DAY 12	MEAN±S.D.	326.1 ± 11.6	330.8 ± 16.8	327.2 ± 28.2	304.5 ± 12.0	263.5 ± 21.0

DAY = DAY OF GESTATION

a. Excludes values for rats that did not have a confirmed mating date.

b. Excludes values for rats 19520 and 19541, which were moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.

c. Excludes values for rat 19557, which was moribund sacrificed on day 43 of study (day 2 of cohabitation).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 4 (PAGE 4): MATERNAL BODY WEIGHTS AND GRAVID UTERINE WEIGHTS - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	7a	7b	6a,b	4a,c	6a
PREGNANT	N	7	7	5	2	6
MATERNAL BODY WEIGHT (G)						
DAY 13	MEAN±S.D.	329.1 ± 10.1	336.0 ± 14.9	331.8 ± 28.0	312.5 ± 4.9	268.2 ± 21.2
DAY 14	MEAN±S.D.	334.0 ± 12.1	340.3 ± 17.4	337.0 ± 28.7	317.0 ± 12.7	272.2 ± 22.2
DAY 15	MEAN±S.D.	336.0 ± 16.0	339.7 ± 17.5	329.2 ± 24.3	323.5 ± 6.4	273.2 ± 20.3
DAY 16	MEAN±S.D.	345.7 ± 13.8	350.7 ± 20.9	349.4 ± 29.5	339.0 ± 15.6	283.8 ± 20.8
DAY 17	MEAN±S.D.	361.8 ± 16.2	366.8 ± 22.8	361.0 ± 28.1	357.5 ± 12.0	300.0 ± 27.4
DAY 18	MEAN±S.D.	377.4 ± 15.5	381.4 ± 23.2	379.8 ± 31.0	377.5 ± 20.5	312.0 ± 26.7
DAY 19	MEAN±S.D.	395.6 ± 18.5	397.6 ± 24.9	391.8 ± 29.7	394.5 ± 24.7	324.5 ± 29.6
DAY 20	MEAN±S.D.	410.1 ± 20.1	413.0 ± 25.2	410.2 ± 34.7	413.5 ± 34.6	334.3 ± 32.5
DAY 21	MEAN±S.D.	423.0 ± 27.4	427.0 ± 29.0	423.0 ± 30.7	426.5 ± 23.3	346.0 ± 33.8
GRAVID UTERINE WEIGHTS (G)	MEAN±S.D.	96.3 ± 12.0	92.0 ± 27.0	91.3 ± 34.0	112.7 ± 37.0	59.5 ± 26.8
DAY 21C d	MEAN±S.D.	326.7 ± 22.6	335.0 ± 22.4	331.7 ± 26.2	313.8 ± 13.6	283.2 ± 23.8

DAY = DAY OF GESTATION

a. Excludes values for rats that did not have a confirmed mating date.

b. Excludes values for rats 19520 and 19541, which were moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.

c. Excludes values for rat 19557, which was moribund sacrificed on day 43 of study (day 2 of cohabitation).

d. 21C = Corrected maternal body weight (day 21 of gestation body weight minus the gravid uterine weight).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	8	8	8	7a	8
PREGNANT	N	6	7	8	6	8
MATERNAL BODY WEIGHT CHANGE (G)						
DAYS 0 - 7	MEAN±S.D.	+29.2 ± 7.7	+26.6 ± 5.0	+15.1 ± 7.6	+5.8 ± 9.0	+2.9 ± 11.3
DAYS 7 - 8	MEAN±S.D.	-1.0 ± 6.4	+0.3 ± 3.9	+10.2 ± 7.8	+6.0 ± 6.1	+3.1 ± 8.0
DAYS 8 - 9	MEAN±S.D.	+6.5 ± 3.9	+5.1 ± 2.5	-1.4 ± 10.6	+1.3 ± 5.5	+5.8 ± 2.0
DAYS 9 - 10	MEAN±S.D.	+4.0 ± 3.5	+5.7 ± 11.6	+2.5 ± 3.2	+5.0 ± 2.4	+9.8 ± 5.5
DAYS 7 - 10	MEAN±S.D.	+9.5 ± 5.8	+11.1 ± 8.9	+11.4 ± 5.0	+12.3 ± 8.8	+18.6 ± 8.5
DAYS 10 - 13	MEAN±S.D.	+15.7 ± 2.1	+10.6 ± 11.0	+17.1 ± 7.6	+13.5 ± 4.3	+14.6 ± 5.3
DAYS 13 - 15	MEAN±S.D.	+9.8 ± 3.9	+6.8 ± 12.2	+5.9 ± 9.6	+11.0 ± 4.6	+3.8 ± 4.6
DAYS 0 - 15	MEAN±S.D.	+64.2 ± 9.9	+55.1 ± 10.2	+49.5 ± 12.8	+42.7 ± 4.2	+39.9 ± 8.5
DAYS 0 - 15C b	MEAN±S.D.	+44.3 ± 11.7	+37.7 ± 8.0	+33.2 ± 11.6	+25.1 ± 3.2	+22.8 ± 7.2

DAYS = DAYS OF GESTATION
a. Excludes values for rat 19561; necropsy and Caesarean-section observation were not recorded.
b. 15C = Corrected maternal body weight (day 15 of gestation body weight minus the gravid uterine weight).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 5 (PAGE 2): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	7a	7b	6a,b	4a,c	6a
PREGNANT	N	7	7	5	2	6
MATERNAL BODY WEIGHT CHANGE (G)						
DAYS 0 - 7	MEAN±S.D.	+19.4 ± 9.6	+31.0 ± 11.7	+14.6 ± 7.6	+12.5 ± 0.7	+0.0 ± 6.8
DAYS 7 - 8	MEAN±S.D.	+4.8 ± 10.2	+1.1 ± 7.6	+1.6 ± 4.8	+0.5 ± 2.1	+1.5 ± 3.7
DAYS 8 - 9	MEAN±S.D.	+5.1 ± 3.1	+4.3 ± 3.4	+4.4 ± 8.0	+6.5 ± 0.7	+5.5 ± 4.9
DAYS 9 - 10	MEAN±S.D.	+6.0 ± 2.9	+4.4 ± 4.9	+3.2 ± 5.2	+2.5 ± 4.9	+7.3 ± 4.4
DAYS 7 - 10	MEAN±S.D.	+16.0 ± 11.0	+9.8 ± 6.9	+9.2 ± 7.2	+9.5 ± 7.8	+14.3 ± 6.7
DAYS 10 - 13	MEAN±S.D.	+12.1 ± 4.0	+12.8 ± 3.7	+15.2 ± 5.5	+17.5 ± 2.1	+11.8 ± 8.1
DAYS 13 - 15	MEAN±S.D.	+6.8 ± 7.4	+3.7 ± 9.6	-2.6 ± 15.4	+11.0 ± 1.4	+5.0 ± 7.3
DAYS 15 - 18	MEAN±S.D.	+41.4 ± 4.5	+41.7 ± 14.8	+50.6 ± 11.6	+54.0 ± 14.1	+38.8 ± 11.8
DAYS 18 - 21	MEAN±S.D.	+45.6 ± 15.7	+45.6 ± 12.2	+43.2 ± 12.3	+49.0 ± 2.8	+34.0 ± 8.5
DAYS 0 - 21	MEAN±S.D.	+141.4 ± 25.0	+144.7 ± 20.3	+130.2 ± 23.8	+153.5 ± 24.7	+104.0 ± 26.3
DAYS 0 - 21C d	MEAN±S.D.	+45.2 ± 18.5	+52.7 ± 16.4	+38.9 ± 14.0	+40.8 ± 12.2	+36.2 ± 16.7

DAYS = DAYS OF GESTATION

a. Excludes values for rats that did not have a confirmed mating date.

b. Excludes values for rats 19520 and 19541, which were moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.

c. Excludes values for rat 19557, which was moribund sacrificed on day 43 of study (day 2 of cohabitation).

d. 21C = Corrected maternal body weight (day 21 of gestation body weight minus the gravid uterine weight).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 6 (PAGE 1): ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - PRECOHABITATION - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	16	16	16	16	16
FEED CONSUMPTION (G)						
DAYS 1 - 8	MEAN±S.D.	19.7 ± 1.8 [15]a	19.4 ± 1.5	18.8 ± 1.8	18.9 ± 1.5 [15]a	19.1 ± 1.5
DAYS 8 - 15	MEAN±S.D.	18.8 ± 1.8 [11]a	18.8 ± 1.6 [15]a	17.4 ± 1.6 [15]a	18.2 ± 1.9 [15]a	16.6 ± 1.6 [15]a
DAYS 15 - 22	MEAN±S.D.	20.0 ± 1.7 [14]a	19.5 ± 1.6	18.5 ± 2.1 [15]a	17.9 ± 1.7 [13]a	16.4 ± 1.5 [14]a
DAYS 22 - 29	MEAN±S.D.	18.6 ± 1.8 [12]a	18.9 ± 1.9 [15]a	18.1 ± 2.3 [14]a	17.2 ± 1.7 [13]a	15.3 ± 1.7
DAYS 29 - 36	MEAN±S.D.	18.7 ± 2.0	18.0 ± 1.8	18.0 ± 1.5	17.1 ± 1.8	15.1 ± 1.5 [15]a
DAYS 36 - 42b	MEAN±S.D.	19.5 ± 2.2 [13]a	18.0 ± 1.8	17.9 ± 1.7 [13]a	16.7 ± 1.8 [13]a	14.8 ± 1.3 [13]a
DAYS 1 - 42b	MEAN±S.D.	19.2 ± 1.3 [13]a	18.8 ± 1.4	18.2 ± 1.8 [13]a	17.7 ± 1.4 [13]a	16.1 ± 1.1 [13]a

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values that were incorrectly recorded, as well as those associated with spillage.
b. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 7 (PAGE 1): RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - PRECOHABITATION - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	16	16	16	16	16
FEED CONSUMPTION (g)						
DAYS 1 - 8	MEAN±S.D.	83.8 ± 6.1 [15]a	82.1 ± 6.1	80.0 ± 5.7	80.6 ± 5.9 [15]a	81.4 ± 4.9
DAYS 8 - 15	MEAN±S.D.	75.8 ± 5.4 [11]a	75.6 ± 6.0 [15]a	70.8 ± 4.2 [15]a	74.3 ± 7.6 [15]a	68.7 ± 5.0 [15]a
DAYS 15 - 22	MEAN±S.D.	77.0 ± 5.1 [14]a	75.5 ± 5.8	72.8 ± 4.8 [15]a	70.4 ± 5.6 [13]a	66.6 ± 5.2 [14]a
DAYS 22 - 29	MEAN±S.D.	69.4 ± 5.2 [12]a	71.4 ± 6.4 [15]a	68.8 ± 4.8 [14]a	66.1 ± 5.8 [13]a	62.4 ± 5.1
DAYS 29 - 36	MEAN±S.D.	68.8 ± 6.2	67.0 ± 5.8	67.6 ± 4.7	65.6 ± 6.5	61.4 ± 4.9 [15]a
DAYS 36 - 42b	MEAN±S.D.	71.5 ± 9.4 [13]a	65.6 ± 5.4	65.9 ± 4.8 [13]a	64.0 ± 7.9 [13]a	61.9 ± 5.0 [13]a
DAYS 1 - 42b	MEAN±S.D.	74.2 ± 4.9 [13]a	72.4 ± 4.7	70.6 ± 4.3 [13]a	70.0 ± 6.0 [13]a	67.3 ± 2.6 [13]a

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values that were incorrectly recorded, as well as those associated with spillage.
b. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 8 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	8	8	8	7a	8
PREGNANT	N	6	7	8	6	8
MATERNAL FEED CONSUMPTION (G/DAY)						
DAYS 0 - 7	MEAN±S.D.	24.0 ± 2.2	22.5 ± 2.0	20.4 ± 2.8 [2]b	b	16.5 ± 1.7 [3]b
DAYS 7 - 8	MEAN±S.D.	22.7 ± 1.2	21.4 ± 2.7	21.0 ± 2.5	19.5 ± 2.8	16.8 ± 4.3
DAYS 8 - 9	MEAN±S.D.	24.2 ± 1.9	24.7 ± 3.4	22.1 ± 2.4	22.3 ± 1.6	20.1 ± 1.4
DAYS 9 - 10	MEAN±S.D.	25.0 ± 2.9	23.0 ± 3.5	22.6 ± 3.5	21.7 ± 1.4	20.5 ± 2.3
DAYS 7 - 10	MEAN±S.D.	24.0 ± 1.7	23.0 ± 3.0	21.9 ± 2.3	21.2 ± 1.7	19.1 ± 1.8
DAYS 10 - 13	MEAN±S.D.	24.8 ± 2.6	24.3 ± 2.2	23.2 ± 1.9	23.0 ± 2.0	22.6 ± 1.7
DAYS 13 - 15	MEAN±S.D.	21.9 ± 2.1	20.7 ± 1.4 [6]b	18.9 ± 3.0 [7]b	18.8 ± 1.1	18.7 ± 2.6 [5]b
DAYS 0 - 15	MEAN±S.D.	23.9 ± 2.0	23.0 ± 1.8 [6]b	21.0 ± 2.2 [7]b	20.1 ± 1.2	18.0 ± 1.8 [5]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 19561; necropsy and Caesarean-section observations were not recorded.
b. Excludes values that were not recorded, as well as those associated with spillage.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 8 (PAGE 2): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	7a	7b	6a,b	4a,c	6a
PREGNANT	N	7	7	5	2	6
MATERNAL FEED CONSUMPTION (G/DAY)						
DAYS 0 - 7	MEAN±S.D.	23.6 ± 1.4	23.7 ± 2.3	21.0 ± 3.3 [3]d	20.3 ± 1.0	17.0 ± 1.8 [4]d
DAYS 7 - 8	MEAN±S.D.	21.8 ± 2.6	23.0 ± 3.1	22.2 ± 2.4	20.5 ± 0.7	15.2 ± 3.4 [5]d
DAYS 8 - 9	MEAN±S.D.	23.6 ± 2.4	25.6 ± 1.8	22.8 ± 4.5	20.0 ± 4.2	18.2 ± 2.7
DAYS 9 - 10	MEAN±S.D.	24.3 ± 1.5	24.8 ± 3.4	22.4 ± 4.5	22.5 ± 0.7	20.0 ± 2.1
DAYS 7 - 10	MEAN±S.D.	23.2 ± 1.4	24.4 ± 2.3	22.5 ± 3.6	21.0 ± 1.4	18.0 ± 2.1
DAYS 10 - 13	MEAN±S.D.	24.4 ± 1.8	25.3 ± 2.0	24.5 ± 2.8	23.3 ± 1.4	20.9 ± 1.9
DAYS 13 - 15	MEAN±S.D.	22.1 ± 2.5	20.9 ± 1.6 [6]d	19.1 ± 2.9	21.8 ± 1.8	17.6 ± 2.0
DAYS 15 - 18	MEAN±S.D.	25.3 ± 2.1	24.3 ± 5.6	24.9 ± 4.7	26.8 ± 0.2	23.6 ± 2.4
DAYS 18 - 21	MEAN±S.D.	23.9 ± 3.2	25.1 ± 2.6	24.9 ± 2.4	25.5 ± 0.7	21.9 ± 4.2
DAYS 0 - 21	MEAN±S.D.	23.8 ± 1.1	24.1 ± 1.6	23.0 ± 2.6	22.6 ± 0.1	19.0 ± 1.9

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that did not have a confirmed mating date.
b. Excludes values for rats 19520 and 19541, which were moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.
c. Excludes values for rat 19557, which was moribund sacrificed on day 43 of study (day 2 of cohabitation).
d. Excludes values that were not recorded, as well as those associated with spillage.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 9 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	8	8	8	7a	8
PREGNANT	N	6	7	8	6	8
MATERNAL FEED CONSUMPTION (G/KG/DAY)						
DAYS 0 - 7	MEAN±S.D.	80.6 ± 8.2	77.2 ± 6.8	71.6 ± 5.2 [2]b	b	67.0 ± 6.6 [3]b
DAYS 7 - 8	MEAN±S.D.	73.6 ± 7.3	70.9 ± 9.1	72.2 ± 6.8	70.5 ± 7.6	69.0 ± 20.7
DAYS 8 - 9	MEAN±S.D.	77.6 ± 7.0	80.8 ± 9.8	75.0 ± 5.9	79.9 ± 4.5	80.8 ± 6.1
DAYS 9 - 10	MEAN±S.D.	78.8 ± 8.1	74.0 ± 11.1	76.3 ± 8.6	76.7 ± 4.3	79.7 ± 8.0
DAYS 7 - 10	MEAN±S.D.	76.6 ± 6.2	75.2 ± 9.4	74.7 ± 4.2	75.8 ± 4.0	76.4 ± 9.0
DAYS 10 - 13	MEAN±S.D.	75.8 ± 6.5	76.3 ± 6.3	75.7 ± 3.3	78.6 ± 7.5	84.3 ± 5.6
DAYS 13 - 15	MEAN±S.D.	64.6 ± 5.2	63.3 ± 4.2 [6]b	60.2 ± 8.6 [7]b	61.7 ± 5.2	67.2 ± 6.4 [5]b
DAYS 0 - 15	MEAN±S.D.	76.5 ± 6.2	75.8 ± 5.1 [6]b	71.8 ± 3.8 [7]b	70.9 ± 4.7	71.3 ± 4.6 [5]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 19561; necropsy and Caesarean-section observations were not recorded.
b. Excludes values that were not recorded, as well as those associated with spillage.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 9 (PAGE 2): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - GESTATION - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	7a	7b	6a,b	4a,c	6a
PREGNANT	N	7	7	5	2	6
MATERNAL FEED CONSUMPTION (G/KG/DAY)						
DAYS 0 - 7	MEAN±S.D.	79.6 ± 7.2	79.0 ± 7.5	71.6 ± 5.0 [3]d	71.6 ± 3.3	70.2 ± 5.7 [4]d
DAYS 7 - 8	MEAN±S.D.	72.0 ± 7.7	73.1 ± 7.6	72.2 ± 7.9	71.8 ± 2.6	61.6 ± 14.6 [5]d
DAYS 8 - 9	MEAN±S.D.	76.7 ± 10.0	80.8 ± 5.7	73.2 ± 12.7	69.1 ± 14.3	73.8 ± 9.7
DAYS 9 - 10	MEAN±S.D.	77.4 ± 6.1	77.3 ± 8.2	70.6 ± 9.6	76.6 ± 1.2	79.8 ± 12.4
DAYS 7 - 10	MEAN±S.D.	75.3 ± 5.4	77.0 ± 5.2	71.9 ± 8.7	72.4 ± 4.3	73.2 ± 11.1
DAYS 10 - 13	MEAN±S.D.	75.3 ± 5.3	76.6 ± 4.6	75.4 ± 4.2	76.8 ± 2.6	80.1 ± 8.9
DAYS 13 - 15	MEAN±S.D.	66.4 ± 6.7	62.3 ± 4.1 [6]d	57.4 ± 7.6	68.4 ± 3.8	65.0 ± 7.5
DAYS 15 - 18	MEAN±S.D.	71.4 ± 5.2	67.1 ± 14.1	70.1 ± 12.2	76.9 ± 3.5	80.6 ± 4.5
DAYS 18 - 21	MEAN±S.D.	59.4 ± 6.2	62.3 ± 7.0	62.1 ± 6.7	63.5 ± 5.8	66.4 ± 11.2
DAYS 0 - 21	MEAN±S.D.	72.1 ± 4.0	72.0 ± 3.2	69.1 ± 5.4	71.2 ± 1.6	70.4 ± 4.1

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that did not have a confirmed mating date.
b. Excludes values for rats 19520 and 19541, which were moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.
c. Excludes values for rat 19557, which was moribund sacrificed on day 43 of study (day 2 of cohabitation).
d. Excludes values that were not recorded, as well as those associated with spillage.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 10 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	8	8	8	7a	8
PREGNANT	N(%)	6(75.0)	7(87.5)	8(100.0)	6(85.7)	8(100.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 15 OF GESTATION	N	6	7	8	6	8
CORPORA LUTEA	MEAN±S.D.	17.3 ± 3.4	18.0 ± 1.7	17.1 ± 3.0	19.8 ± 3.2	17.4 ± 2.6
IMPLANTATIONS	MEAN±S.D.	15.8 ± 1.9	13.4 ± 3.8	13.0 ± 3.7	14.2 ± 1.3	13.6 ± 3.5
VIABLE EMBRYOS	N	89	86	94	77	101
	MEAN±S.D.	14.8 ± 2.8	12.3 ± 3.7	11.8 ± 4.7	12.8 ± 1.2	12.6 ± 2.9
NONVIABLE EMBRYOS	N	6	8	10	8	8
	MEAN±S.D.	1.0 ± 1.3	1.1 ± 1.3	1.2 ± 1.4	1.3 ± 1.0	1.0 ± 1.4
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	3(50.0)	5(71.4)	4(50.0)	5(83.3)	4(50.0)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	6(100.0)	7(100.0)	8(100.0)	6(100.0)	8(100.0)
% NONVIABLE EMBRYOS/LITTER	MEAN±S.D.	6.8 ± 8.4	8.6 ± 9.3	13.4 ± 17.7	9.2 ± 6.5	6.2 ± 8.3

a. Excludes values for rat 19561; necropsy and Caesarean-section observations were not recorded.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 10 (PAGE 2): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	7a	7b	6a,b	4a,c	6a
PREGNANT	N(%)	7(100.0)	7(100.0)	5(83.3)	2(50.0)	6(100.0)
DELIVERED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(33.3)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	7	7	5	2	4
CORPORA LUTEA	MEAN±S.D.	18.6 ± 4.2	16.1 ± 2.3	21.8 ± 4.8	20.5 ± 2.1	16.2 ± 2.5
IMPLANTATIONS	MEAN±S.D.	15.1 ± 2.1	14.0 ± 2.9	14.4 ± 4.2	17.0 ± 7.1	10.5 ± 4.4
LITTER SIZES	MEAN±S.D.	13.7 ± 1.7	12.4 ± 4.0	13.2 ± 5.2	16.0 ± 5.6	8.8 ± 4.4
LIVE FETUSES	N	96	87	66	32	35
	MEAN±S.D.	13.7 ± 1.7	12.4 ± 4.0	13.2 ± 5.2	16.0 ± 5.6	8.8 ± 4.4
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	1.4 ± 1.7	1.6 ± 1.5	1.2 ± 1.8	1.0 ± 1.4	1.8 ± 1.5
EARLY RESORPTIONS	N	10	9	6	2	7
	MEAN±S.D.	1.4 ± 1.7	1.3 ± 1.1	1.2 ± 1.8	1.0 ± 1.4	1.8 ± 1.5
LATE RESORPTIONS	N	0	2	0	0	0
	MEAN±S.D.	0.0 ± 0.0	0.3 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

a. Excludes values for rats that did not have a confirmed mating date.
b. Excludes values for rats 19520 and 19541, which were moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.
c. Excludes values for rat 19557, which was moribund sacrificed on day 43 of study (day 2 of cohabitation).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 10 (PAGE 3): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	8	8	8	8	8
INCLUDED IN ANALYSES	N	7a	7b	6a,b	4a,c	6a
PREGNANT	N(%)	7(100.0)	7(100.0)	5(83.3)	2(50.0)	6(100.0)
DELIVERED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(33.3)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	7	7	5	2	4
DAMS WITH ANY RESORPTIONS	N(%)	5(71.4)	5(71.4)	2(40.0)	1(50.0)	3(75.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	7(100.0)	7(100.0)	5(100.0)	2(100.0)	4(100.0)
PLACENTAE APPEARED NORMAL	N(%)	7(100.0)	7(100.0)	5(100.0)	2(100.0)	4(100.0)

a. Excludes values for rats that did not have a confirmed mating date.
b. Excludes values for rats 19520 and 19541, which were moribund sacrificed on day 0 of presumed gestation; pregnancy status could not be determined.
c. Excludes values for rat 19557, which was moribund sacrificed on day 43 of study (day 2 of cohabitation).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 11 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED EMBRYOS OR FETUSES) - SUMMARY

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
LITTERS WITH ONE OR MORE LIVE FETUSES	N	7	7	5	2	4
IMPLANTATIONS	MEAN±S.D.	15.1 ± 2.1	14.0 ± 2.9	14.4 ± 4.2	17.0 ± 7.1	10.5 ± 4.4
LIVE FETUSES	N	96	87	66	32	35
	MEAN±S.D.	13.7 ± 1.7	12.4 ± 4.0	13.2 ± 5.2	16.0 ± 5.6	8.8 ± 4.4
LIVE MALE FETUSES	N	44	46	36	15	19
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	46.0 ± 11.7	52.2 ± 14.2	59.3 ± 19.4	45.8 ± 5.9	55.3 ± 31.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.12 ± 0.29	5.31 ± 0.42	5.06 ± 0.09	5.02 ± 0.16	4.72 ± 0.23
MALE FETUSES	MEAN±S.D.	5.24 ± 0.29	5.50 ± 0.54	5.19 ± 0.12	5.12 ± 0.20	4.82 ± 0.28
FEMALE FETUSES	MEAN±S.D.	5.02 ± 0.33	5.06 ± 0.33	4.84 ± 0.14	4.95 ± 0.16	4.69 ± 0.27 [3]a
PLACENTAL WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	0.50 ± 0.06	0.54 ± 0.08	0.48 ± 0.06	0.50 ± 0.06	0.55 ± 0.11
MALE FETUSES	MEAN±S.D.	0.50 ± 0.06	0.55 ± 0.09	0.49 ± 0.07	0.52 ± 0.09	0.56 ± 0.11
FEMALE FETUSES	MEAN±S.D.	0.49 ± 0.05	0.52 ± 0.07	0.44 ± 0.08	0.47 ± 0.04	0.53 ± 0.13 [3]a
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	8.8 ± 9.9	13.4 ± 14.6	11.0 ± 19.2	4.6 ± 6.4	18.3 ± 13.7

NO FETAL ALTERATIONS WERE IDENTIFIED AT GROSS EXTERNAL EXAMINATION

[ ] = NUMBER OF VALUES AVERAGED

a. Litter 19568 had no female fetuses.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 12 (PAGE 1): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP I		0 (VEHICLE) MG/KG/DAY
RAT #		DESCRIPTION
19501		NO ADVERSE FINDINGS
19502		NO ADVERSE FINDINGS
19503	DS(43- 66)	LENTICULAR OPACITY
	DS(66)	DELIVERED AND SACRIFICED
19504		NO ADVERSE FINDINGS
19505		NO ADVERSE FINDINGS
19506		NO ADVERSE FINDINGS
19507		NO ADVERSE FINDINGS
19508		NO ADVERSE FINDINGS
19509	DG(17- 21)	CHROMORHINORRHEA a
19510		NO ADVERSE FINDINGS
19511	DS(3- 42)	LOCALIZED ALOPECIA: LIMBS
	DG(0- 2)	LOCALIZED ALOPECIA: LIMBS
	DG(3)	ALOPECIA NO LONGER APPARENT
	DG(0- 15)	LENTICULAR OPACITY
	DG(14- 15)	CHROMORHINORRHEA a
19512	DG(11- 21)	CHROMODACRYORRHEA a
19513		NO ADVERSE FINDINGS
19514		NO ADVERSE FINDINGS
19515		NO ADVERSE FINDINGS
19516		NO ADVERSE FINDINGS

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION

a. Observation confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 12 (PAGE 2): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP II		0.1 MG/KG/DAY
RAT #		DESCRIPTION
19517	DG(7- 15)	TRAUMATIZED CORNEA a
19518		NO ADVERSE FINDINGS
19519	DS(13- 42)	LOCALIZED ALOPECIA: LIMBS
	DG(0- 21)	LOCALIZED ALOPECIA: LIMBS a
19520	DS(10- 14)	CHROMODACRYORRHEA
	DS(10- 21)	INCISORS: MISSING/BROKEN
	DS(22)	INCISORS: GROWN IN
	DS(22- 30)	INCISORS: MISALIGNED
	DS(31)	INCISORS: REALIGNED
	DS(30- 42)	LOCALIZED ALOPECIA: LIMBS
	DS(42)	SNOUT: SWOLLEN
	DG(0)	CHROMODACRYORRHEA a
	DG(0)	EXOPHTHALMOS a
	DG(0)	SNOUT: SWOLLEN a
	DG(0)	LOCALIZED ALOPECIA: LIMBS a
	DG(0)	CORNEAL OPACITY
	DG(0)	RIGHT EYE: LARGE
	DG(0)	RIGHT EYE: DARK RED
	DG(0)	RIGHT EYE: LIDS UNABLE TO CLOSE
	DG(0)	MORIBUND SACRIFICED
19521	DS(44- 45)	EXOPHTHALMOS
	DG(0)	EXOPHTHALMOS NO LONGER APPARENT
	DG(4- 5)	SNOUT: SWOLLEN
	DG(6)	SWELLING RESOLVED
	DG(11- 21)	CORNEAL OPACITY
19522		NO ADVERSE FINDINGS
19523		NO ADVERSE FINDINGS
19524	DS(43- 45)	RIGHT EYE: CLOUDY, WHITE FILM
	DS(43- 45)	SNOUT: SWOLLEN
	DG(0)	SWELLING RESOLVED
	DS(44- 45)	EXOPHTHALMOS
	DG(0)	EXOPHTHALMOS NO LONGER APPARENT
	DG(0- 10)	TRAUMATIZED CORNEA
	DG(0- 10)	RIGHT EYE: CLOUDY, WHITE FILM
	DG(0- 13)	LOCALIZED ALOPECIA: HEAD
	DG(14)	ALOPECIA NO LONGER APPARENT
	DG(11- 21)	RIGHT EYE: MISSING a

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 12 (PAGE 3): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP II		0.1 MG/KG/DAY
RAT #		DESCRIPTION
19525		NO ADVERSE FINDINGS
19526		NO ADVERSE FINDINGS
19527		NO ADVERSE FINDINGS
19528	DG(2- 8)	INCISORS: MISSING/BROKEN
	DG(9)	INCISORS: GROWN IN
19529		NO ADVERSE FINDINGS
19530		NO ADVERSE FINDINGS
19531	DG(0- 2)	EXOPHTHALMOS
	DG(3)	EXOPHTHALMOS NO LONGER APPARENT
	DG(1- 15)	CORNEAL OPACITY
	DG(3- 15)	TRAUMATIZED CORNEA a
19532		NO ADVERSE FINDINGS

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION

a. Observation confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 12 (PAGE 4): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP III		0.4 MG/KG/DAY
RAT #		DESCRIPTION
19533	DG(0- 1)	LACRIMATION
	DG(0- 2)	LENTICULAR OPACITY
	DG(0- 2)	EXOPHTHALMOS
	DG(3)	EXOPHTHALMOS NO LONGER APPARENT
	DG(3- 21)	CORNEAL OPACITY
	DG(14- 21)	ENOPHTHALMOS a
19534		NO ADVERSE FINDINGS
19535	DS(25)	CHROMORHINORRHEA
19536		NO ADVERSE FINDINGS
19537		NO ADVERSE FINDINGS
19538		NO ADVERSE FINDINGS
19539		NO ADVERSE FINDINGS
19540		NO ADVERSE FINDINGS
19541	DG(0)	CHROMODACRYORRHEA a
	DG(0)	EXOPHTHALMOS a
	DG(0)	SNOUT: SWOLLEN a
	DG(0)	CORNEAL OPACITY
	DG(0)	RIGHT EYE: LARGE
	DG(0)	RIGHT EYE: DARK RED
	DG(0)	RIGHT EYE: LIDS UNABLE TO CLOSE
	DG(0)	MORIBUND SACRIFICED
19542	DG(10- 12)	LOCALIZED ALOPECIA: UNDERSIDE
	DG(13)	ALOPECIA NO LONGER APPARENT
19543		NO ADVERSE FINDINGS
19544		NO ADVERSE FINDINGS
19545		NO ADVERSE FINDINGS
19546	DG(1)	CHROMORHINORRHEA
	DG(1)	UNGROOMED COAT
19547		NO ADVERSE FINDINGS
19548		NO ADVERSE FINDINGS

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION

a. Observation confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 12 (PAGE 5): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP IV		1.6 MG/KG/DAY
RAT #		DESCRIPTION
19549	DS(57- 59)	LOCALIZED ALOPECIA: LIMBS
	DS(60)	ALOPECIA NO LONGER APPARENT
	DS(62- 63)	LOCALIZED ALOPECIA: LIMBS
	DS(64)	ALOPECIA NO LONGER APPARENT
19550		NO ADVERSE FINDINGS
19551		NO ADVERSE FINDINGS
19552	DS(44- 50)	CORNEAL OPACITY
	DS(50- 68)	CORNEAL HEMORRHAGE a
	DS(62- 68)	LOCALIZED ALOPECIA: LIMBS a
19553		NO ADVERSE FINDINGS
19554	DS(1)	CHROMORHINORRHEA
19555		NO ADVERSE FINDINGS
19556	DS(29- 43)	LOCALIZED ALOPECIA: LIMBS
	DG(0- 21)	LOCALIZED ALOPECIA: LIMBS a
19557	DS(43)	CHROMODACRYORRHEA a
	DS(43)	EXOPHTHALMOS a
	DS(43)	SNOUT: SWOLLEN a
	DS(43)	CORNEAL OPACITY
	DS(43)	RIGHT EYE: LARGE
	DS(43)	RIGHT EYE: DARK RED
	DS(43)	RIGHT EYE: LIDS UNABLE TO CLOSE
	DS(43)	MORIBUND SACRIFICED
19558	DS(36- 38)	EARS: SWOLLEN
	DS(39)	SWELLING RESOLVED
19559	DG(15)	EXOPHTHALMOS
	DG(16)	EXOPHTHALMOS NO LONGER APPARENT
	DG(16- 21)	CORNEAL HEMORRHAGE a
19560	DS(2)	CHROMORHINORRHEA
	DG(15- 21)	LOCALIZED ALOPECIA: BACK a
19561		NO ADVERSE FINDINGS
19562	DS(23)	CHROMORHINORRHEA
19563		NO ADVERSE FINDINGS

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION

a. Observation confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 12 (PAGE 6): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP IV		1.6 MG/KG/DAY
RAT #		DESCRIPTION
19564	DS(1)	CHROMORHINORRHEA
	DS(6)	CHROMORHINORRHEA
	DS(29- 42)	LEFT FOREPAW: SCAB (DID NOT EXCEED 0.5 CM X 0.3 CM)
	DS(34- 35)	RIGHT FOREPAW: SCAB (0.4 CM X 0.3 CM)
	DS(36- 42)	RIGHT FOREPAW: TWO SCABS (DID NOT EXCEED 0.5 CM X 0.3 CM)
	DS(31- 42)	LOCALIZED ALOPECIA: UNDERSIDE
	DS(36- 37)	FOREPAWS: SWOLLEN
	DS(38)	SWELLING RESOLVED
	DG(0)	LEFT FOREPAW: SCAB (DID NOT EXCEED 0.5 CM X 0.3 CM)
	DG(0)	RIGHT FOREPAW: TWO SCABS (DID NOT EXCEED 0.5 CM X 0.3 CM)
	DG(1)	SCABS HEALED
	DG(0- 15)	LOCALIZED ALOPECIA: UNDERSIDE a
	DG(1- 15)	LOCALIZED ALOPECIA: LIMBS a

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION

a. Observation confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 12 (PAGE 7): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP V		3.2 MG/KG/DAY
RAT #		DESCRIPTION
19565	DG(0- 15)	CORNEAL HEMORRHAGE a
	DG(1- 2)	EXOPHTHALMOS
	DG(3)	EXOPHTHALMOS NO LONGER APPARENT
19566	DS(13)	CHROMODACRYORRHEA
	DG(0- 15)	CORNEAL OPACITY
	DG(13- 15)	LOCALIZED ALOPECIA: UNDERSIDE a
19567		NO ADVERSE FINDINGS
19568	DS(9- 11)	RIGHT FOREPAW: THIRD DIGIT SWOLLEN
	DS(12)	SWELLING RESOLVED
	DS(9- 42)	RIGHT FOREPAW: THIRD DIGIT MISSING
	DG(0- 21)	RIGHT FOREPAW: THIRD DIGIT MISSING a
19569		NO ADVERSE FINDINGS
19570	DS(34- 43)	LOCALIZED ALOPECIA: LIMBS
	DS(36- 38)	RIGHT FOREPAW: SCAB (0.2 CM IN DIAMETER)
	DS(39)	SCAB HEALED
	DG(0- 15)	LOCALIZED ALOPECIA: LIMBS a
19571	DG(21)	DELIVERED AND SACRIFICED
19572	DG(11- 21)	LEFT AXILLA: MASS (1.5 CM X 1.0 CM X 1.0 CM)a
19573		NO ADVERSE FINDINGS
19574	DS(1)	CHROMORHINORRHEA
19575	DG(1)	CHROMODACRYORRHEA
19576		NO ADVERSE FINDINGS
19577		NO ADVERSE FINDINGS
19578	DS(54- 68)	LOCALIZED ALOPECIA: BACK a
19579	DG(0- 21)	LOCALIZED ALOPECIA: LIMBS a
	DG(21)	DELIVERED AND SACRIFICED
19580	DG(11- 15)	LOCALIZED ALOPECIA: LIMBS a

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
a. Observation confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 13 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
I					
0 (VEHICLE)	19501	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19502	DG 15	NP	58	ALL TISSUES APPEARED NORMAL.
	19503	DS 66	P	66	DELIVERED AND SACRIFICED ON DAY 66 OF STUDY. ALL TISSUES APPEARED NORMAL. UTERINE CONTENTS: 14 IMPLANTATION SITES (14 DELIVERED PUPS).b
	19504	DG 21	P	65	ALL TISSUES APPEARED NORMAL.
	19505	DG 21	P	64	ALL TISSUES APPEARED NORMAL.
	19506	DG 21	P	64	ALL TISSUES APPEARED NORMAL.
	19507	DG 21	P	67	ALL TISSUES APPEARED NORMAL.
	19508	DG 15	P	58	ALL TISSUES APPEARED NORMAL.
	19509	DG 21	P	64	ALL TISSUES APPEARED NORMAL.
	19510	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19511	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19512	DG 21	P	66	ALL TISSUES APPEARED NORMAL.
	19513	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19514	DG 15	NP	57	ALL TISSUES APPEARED NORMAL.
	19515	DG 15	P	58	ALL TISSUES APPEARED NORMAL.
	19516	DG 21	P	65	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 12) for external observations confirmed at necropsy.
b. Appeared normal for their developmental ages at the time maternal death occurred.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 13 (PAGE 2): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
II 0.1	19517	DG 15	NP	58	ALL TISSUES APPEARED NORMAL.
	19518	DG 21	P	64	ALL TISSUES APPEARED NORMAL.
	19519	DG 21	P	63	ALL TISSUES APPEARED NORMAL.
	19520	DG 0	-	43	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED. ALL TISSUES APPEARED NORMAL.
	19521	DG 21	P	66	ALL TISSUES APPEARED NORMAL.
	19522	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19523	DG 21	P	67	ALL TISSUES APPEARED NORMAL.
	19524	DG 21	P	66	ALL TISSUES APPEARED NORMAL.
	19525	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19526	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19527	DG 15	P	58	ALL TISSUES APPEARED NORMAL.
	19528	DG 21	P	63	ALL TISSUES APPEARED NORMAL.
	19529	DG 21	P	63	ALL TISSUES APPEARED NORMAL.
	19530	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19531	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19532	DG 15	P	57	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 12) for external observations confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 13 (PAGE 3): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
III					
0.4	19533	DG 21	P	63	ALL TISSUES APPEARED NORMAL.
	19534	DG 21	P	63	ALL TISSUES APPEARED NORMAL.
	19535	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19536	DG 21	P	65	ALL TISSUES APPEARED NORMAL.
	19537	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19538	DG 15	P	58	ALL TISSUES APPEARED NORMAL.
	19539	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19540	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19541	DG 0	-	43	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED. ALL TISSUES APPEARED NORMAL.
	19542	DG 15	P	58	ALL TISSUES APPEARED NORMAL.
	19543	DG 21	P	66	ALL TISSUES APPEARED NORMAL.
	19544	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19545	DS 68	NP	67	ALL TISSUES APPEARED NORMAL.
	19546	DG 21	P	63	ALL TISSUES APPEARED NORMAL.
	19547	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19548	DG 21	NP	63	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 12) for external observations confirmed at necropsy.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 13 (PAGE 4): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
IV 1.6	19549	DS 68	NP	67	ALL TISSUES APPEARED NORMAL.
	19550	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19551	DS 68	NP	67	ALL TISSUES APPEARED NORMAL.
	19552	DS 68	NP	67	ALL TISSUES APPEARED NORMAL.
	19553	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19554	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19555	DG 15	NP	57	ALL TISSUES APPEARED NORMAL.
	19556	DG 21	NP	64	ALL TISSUES APPEARED NORMAL.
	19557	DS 43	-	43	MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION). ALL TISSUES APPEARED NORMAL.
	19558	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19559	DG 21	NP	64	ALL TISSUES APPEARED NORMAL.
	19560	DG 21	P	66	ALL TISSUES APPEARED NORMAL.
	19561b	DG 15	-	57	-
	19562	DG 21	P	65	ALL TISSUES APPEARED NORMAL.
	19563	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19564	DG 15	P	57	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 12) for external observations confirmed at necropsy.
b. Necropsy and Caesarean-section observations were not recorded for rat 19561.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 13 (PAGE 5): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
V 3.2	19565	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19566	DG 15	P	58	ALL TISSUES APPEARED NORMAL.
	19567	DG 15	P	58	ALL TISSUES APPEARED NORMAL.
	19568	DG 21	P	63	ALL TISSUES APPEARED NORMAL.
	19569	DG 21	P	66	ALL TISSUES APPEARED NORMAL.
	19570	DG 15	P	58	ALL TISSUES APPEARED NORMAL.
	19571	DG 21	P	66	DELIVERED ON DAY 21 OF GESTATION. ALL TISSUES APPEARED NORMAL. UTERINE CONTENTS: 15 IMPLANTATION SITES (SEVEN DELIVERED PUPS, SIX LIVE FETUSES AND TWO EARLY RESORPTIONS).b
	19572	DG 21	P	64	ALL TISSUES APPEARED NORMAL.
	19573	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19574	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19575	DG 15	P	57	ALL TISSUES APPEARED NORMAL.
	19576	DS 68	NP	67	ALL TISSUES APPEARED NORMAL.
	19577	DG 21	P	63	ALL TISSUES APPEARED NORMAL.
	19578	DS 68	NP	67	ALL TISSUES APPEARED NORMAL.
	19579	DG 21	P	65	DELIVERED ON DAY 21 OF GESTATION. ALL TISSUES APPEARED NORMAL. UTERINE CONTENTS: 11 IMPLANTATION SITES (THREE DELIVERED PUPS AND EIGHT LIVE FETUSES).b
	19580	DG 15	P	57	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
a. Refer to the individual clinical observations table (Table 12) for external observations confirmed at necropsy.
b. Appeared normal for their developmental ages at the time maternal death occurred.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 1): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP I 0 (VEHICLE) MG/KG/DAY

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
19501	215.	218.	222.	219.	220.	226.	232.	231.	228.	223.	227.	232.	234.	231.	230.	234.
19502	221.	231.	234.	240.	230.	240.	243.	243.	240.	244.	248.	247.	249.	246.	251.	254.
19503	227.	224.	224.	226.	234.	235.	233.	238.	237.	240.	236.	241.	247.	248.	245.	241.
19504	231.	233.	235.	239.	242.	240.	251.	252.	252.	254.	256.	259.	264.	263.	266.	271.
19505	225.	224.	225.	232.	238.	242.	243.	242.	242.	245.	246.	247.	250.	254.	256.	254.
19506	240.	236.	240.	243.	248.	253.	251.	254.	255.	255.	253.	260.	263.	261.	263.	261.
19507	219.	222.	224.	223.	221.	221.	226.	231.	234.	229.	234.	238.	242.	240.	240.	246.
19508	225.	227.	228.	226.	232.	238.	240.	238.	241.	247.	249.	244.	252.	256.	258.	252.
19509	221.	219.	218.	222.	226.	227.	227.	227.	230.	233.	236.	231.	238.	242.	245.	243.
19510	236.	234.	234.	238.	243.	238.	243.	250.	250.	252.	245.	253.	258.	258.	251.	258.
19511	229.	229.	230.	236.	242.	243.	241.	251.	256.	251.	248.	258.	262.	258.	256.	268.
19512	235.	232.	240.	243.	241.	240.	245.	251.	248.	249.	254.	259.	255.	256.	261.	263.
19513	235.	238.	240.	246.	252.	254.	252.	261.	264.	266.	264.	267.	274.	276.	274.	281.
19514	234.	228.	238.	240.	244.	240.	250.	252.	257.	247.	249.	256.	259.	260.	260.	257.
19515	223.	224.	227.	222.	223.	229.	233.	230.	229.	230.	235.	235.	235.	234.	235.	241.
19516	221.	226.	230.	234.	231.	237.	239.	243.	238.	249.	246.	248.	248.	250.	255.	254.

DAY	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
19501	237.	237.	236.	239.	244.	244.	238.	244.	246.	246.	245.	247.	250.	248.	244.	250.
19502	256.	257.	254.	260.	266.	264.	263.	262.	268.	273.	273.	268.	268.	270.	268.	266.
19503	246.	254.	253.	252.	253.	260.	259.	252.	257.	259.	261.	256.	260.	263.	263.	259.
19504	272.	271.	273.	272.	281.	280.	282.	277.	279.	285.	286.	284.	281.	287.	291.	287.
19505	253.	257.	261.	260.	260.	257.	257.	262.	264.	268.	272.	271.	275.	272.	273.	271.
19506	268.	267.	264.	268.	268.	266.	262.	265.	268.	267.	265.	269.	276.	272.	265.	271.
19507	250.	251.	251.	251.	259.	261.	262.	255.	257.	263.	263.	264.	260.	268.	264.	267.
19508	258.	265.	264.	260.	267.	272.	268.	267.	270.	273.	271.	268.	274.	274.	276.	271.
19509	246.	249.	249.	245.	253.	257.	256.	251.	256.	257.	257.	252.	258.	258.	260.	256.
19510	261.	261.	256.	262.	264.	265.	255.	262.	264.	258.	259.	262.	261.	259.	257.	264.
19511	269.	271.	266.	272.	277.	273.	267.	280.	280.	274.	274.	285.	286.	281.	277.	288.
19512	267.	264.	270.	274.	272.	273.	275.	282.	280.	275.	284.	284.	282.	279.	280.	283.
19513	284.	288.	284.	294.	300.	299.	292.	297.	304.	303.	305.	310.	311.	308.	302.	313.
19514	264.	269.	268.	269.	270.	275.	274.	278.	276.	273.	282.	287.	284.	277.	285.	290.
19515	244.	244.	245.	249.	252.	251.	247.	248.	253.	246.	242.	245.	248.	248.	244.	240.
19516	251.	258.	265.	260.	256.	264.	266.	265.	265.	268.	272.	270.	261.	268.	275.	274.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 2): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP I				0 (VEHICLE) MG/KG/DAY					
DAY	33	34	35	36	37	38	39	40	41	42a
19501	251.	252.	249.	250.	252.	252.	249.	251.	256.	255.
19502	268.	276.	270.	266.	271.	274.	271.	270.	273.	280.
19503	263.	266.	267.	263.	265.	268.	268.	269.	270.	277.
19504	288.	293.	295.	292.	292.	295.	296.	292.	293.	298.
19505	281.	279.	280.	278.	280.	285.	283.	282.	280.	285.
19506	273.	266.	264.	260.	256.	257.	257.	254.	261.	261.
19507	262.	266.	270.	262.	262.	268.	268.	267.	263.	266.
19508	278.	285.	283.	277.	283.	288.	283.	279.	288.	289.
19509	258.	262.	260.	259.	258.	260.	258.	260.	262.	266.
19510	268.	265.	257.	259.	254.	260.	260.	263.	261.	260.
19511	290.	287.	282.	291.	292.	284.	284.	292.	296.	292.
19512	285.	281.	284.	286.	286.	284.	287.	292.	288.	288.
19513	317.	314.	308.	317.	318.	317.	312.	322.	325.	324.
19514	288.	283.	286.	288.	287.	286.	290.	298.	293.	290.
19515	250.	248.	245.	246.	251.	255.	252.	251.	257.	257.
19516	271.	277.	278.	279.	274.	278.	282.	280.	274.	275.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 3): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP II 0.1 MG/KG/DAY

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
19517	221.	219.	225.	228.	230.	227.	233.	237.	235.	232.	238.	244.	244.	240.	242.	249.
19518	234.	238.	237.	249.	250.	250.	252.	261.	264.	264.	265.	262.	271.	273.	272.	271.
19519	225.	230.	236.	237.	237.	243.	247.	244.	248.	252.	254.	252.	251.	258.	260.	258.
19520	230.	227.	232.	237.	240.	238.	243.	242.	239.	240.	240.	246.	241.	240.	246.	250.
19521	220.	222.	229.	235.	239.	235.	242.	245.	244.	242.	248.	254.	251.	250.	257.	259.
19522	234.	236.	237.	242.	247.	250.	245.	256.	255.	255.	250.	258.	263.	258.	257.	263.
19523	228.	227.	228.	232.	238.	238.	234.	239.	240.	243.	243.	245.	248.	251.	250.	254.
19524	233.	231.	235.	239.	239.	236.	242.	250.	249.	249.	254.	257.	256.	256.	262.	265.
19525	224.	218.	225.	229.	231.	229.	233.	235.	232.	234.	239.	240.	240.	242.	240.	246.
19526	227.	230.	229.	228.	234.	238.	240.	241.	239.	246.	249.	251.	247.	243.	250.	258.
19527	227.	233.	231.	233.	237.	239.	244.	248.	244.	252.	254.	260.	258.	255.	258.	262.
19528	229.	231.	237.	238.	240.	239.	245.	248.	242.	243.	248.	253.	252.	251.	250.	256.
19529	226.	229.	227.	234.	235.	234.	234.	239.	238.	243.	238.	241.	244.	243.	242.	243.
19530	228.	229.	230.	238.	239.	242.	234.	240.	244.	245.	243.	251.	255.	252.	250.	257.
19531	222.	220.	230.	233.	236.	235.	242.	244.	247.	242.	241.	243.	249.	248.	244.	253.
19532	230.	230.	230.	228.	234.	239.	244.	245.	239.	246.	248.	253.	250.	246.	254.	256.

DAY	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
19517	247.	245.	250.	249.	253.	247.	254.	256.	257.	256.	261.	260.	256.	249.	258.	261.
19518	275.	276.	279.	278.	280.	288.	286.	284.	289.	290.	289.	285.	293.	291.	289.	289.
19519	264.	267.	270.	265.	266.	271.	274.	274.	269.	273.	278.	276.	270.	277.	280.	278.
19520	248.	246.	254.	250.	254.	253.	258.	258.	257.	260.	260.	260.	263.	255.	259.	261.
19521	259.	258.	264.	269.	267.	264.	268.	272.	272.	266.	275.	275.	271.	268.	276.	280.
19522	268.	266.	257.	265.	269.	270.	267.	275.	282.	278.	272.	278.	281.	277.	274.	280.
19523	260.	259.	259.	262.	268.	266.	262.	263.	267.	269.	269.	267.	268.	271.	268.	264.
19524	264.	264.	266.	272.	270.	268.	275.	277.	276.	276.	280.	285.	276.	273.	278.	282.
19525	245.	249.	247.	247.	251.	255.	253.	251.	250.	247.	251.	253.	252.	257.	252.	258.
19526	256.	252.	250.	256.	262.	265.	260.	260.	263.	266.	268.	264.	260.	268.	271.	270.
19527	267.	264.	264.	269.	270.	275.	273.	270.	272.	277.	278.	277.	270.	275.	280.	278.
19528	255.	254.	260.	260.	256.	256.	256.	266.	261.	259.	266.	264.	256.	252.	261.	263.
19529	244.	243.	242.	245.	246.	248.	241.	246.	247.	246.	240.	251.	246.	247.	244.	248.
19530	259.	261.	254.	264.	269.	267.	264.	264.	270.	267.	265.	271.	271.	266.	263.	265.
19531	255.	255.	253.	258.	264.	261.	255.	262.	265.	265.	262.	270.	271.	270.	262.	270.
19532	257.	256.	255.	262.	268.	269.	265.	265.	270.	270.	274.	271.	267.	270.	276.	273.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 4): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP II				0.1 MG/KG/DAY					
DAY	33	34	35	36	37	38	39	40	41	42a
19517	262.	256.	259.	262.	260.	255.	262.	266.	265.	257.
19518	294.	298.	295.	296.	301.	303.	300.	298.	303.	310.
19519	273.	277.	280.	276.	273.	269.	276.	278.	278.	276.
19520	265.	265.	269.	272.	267.	264.	265.	268.	273.	263.
19521	279.	274.	281.	284.	280.	279.	288.	288.	285.	288.
19522	285.	286.	276.	280.	286.	285.	278.	282.	287.	282.
19523	271.	273.	274.	266.	266.	268.	273.	274.	275.	274.
19524	282.	276.	280.	279.	278.	276.	281.	284.	280.	279.
19525	253.	254.	254.	251.	257.	252.	254.	255.	252.	255.
19526	262.	262.	268.	270.	269.	268.	271.	270.	272.	272.
19527	278.	276.	282.	286.	286.	284.	279.	283.	285.	293.
19528	257.	253.	260.	261.	258.	256.	262.	266.	259.	257.
19529	251.	247.	242.	247.	249.	250.	244.	254.	253.	251.
19530	274.	270.	267.	271.	274.	272.	267.	270.	277.	269.
19531	273.	269.	266.	277.	283.	276.	273.	280.	282.	276.
19532	272.	277.	281.	278.	271.	271.	282.	278.	280.	269.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 5): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP III 0.4 MG/KG/DAY

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
19533	221.	224.	222.	220.	226.	236.	228.	229.	223.	227.	233.	231.	232.	230.	235.	236.
19534	234.	241.	244.	251.	256.	258.	253.	262.	266.	263.	259.	264.	266.	269.	267.	276.
19535	221.	220.	226.	223.	226.	230.	227.	229.	230.	230.	233.	230.	230.	232.	235.	234.
19536	225.	225.	230.	233.	235.	230.	230.	242.	242.	243.	240.	243.	246.	246.	245.	246.
19537	217.	219.	223.	221.	222.	224.	227.	233.	227.	229.	235.	233.	231.	231.	234.	237.
19538	230.	227.	229.	233.	237.	237.	241.	242.	243.	243.	244.	248.	246.	247.	248.	250.
19539	226.	227.	225.	230.	231.	230.	234.	238.	233.	230.	233.	236.	239.	238.	236.	240.
19540	230.	229.	230.	234.	236.	236.	233.	241.	243.	243.	241.	246.	250.	250.	249.	251.
19541	218.	218.	216.	220.	225.	226.	227.	229.	230.	230.	228.	234.	236.	236.	236.	231.
19542	237.	236.	231.	240.	245.	244.	240.	250.	252.	253.	248.	250.	257.	261.	258.	255.
19543	234.	233.	242.	244.	249.	245.	252.	254.	254.	253.	257.	257.	263.	258.	266.	268.
19544	231.	231.	232.	235.	241.	245.	246.	248.	245.	254.	253.	258.	257.	253.	259.	262.
19545	222.	220.	232.	231.	231.	229.	234.	238.	239.	240.	235.	239.	241.	245.	248.	246.
19546	234.	235.	231.	239.	240.	239.	242.	248.	248.	248.	250.	258.	263.	265.	263.	267.
19547	223.	228.	228.	230.	235.	232.	234.	243.	242.	240.	239.	247.	248.	246.	247.	251.
19548	228.	231.	233.	234.	239.	242.	246.	246.	243.	247.	249.	250.	253.	254.	258.	259.

DAY	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
19533	240.	237.	238.	244.	246.	245.	246.	243.	245.	248.	252.	250.	244.	247.	253.	257.
19534	282.	280.	281.	286.	291.	288.	281.	293.	296.	297.	295.	303.	298.	296.	291.	302.
19535	239.	238.	242.	243.	244.	248.	244.	247.	245.	247.	251.	251.	254.	255.	258.	256.
19536	246.	250.	253.	255.	252.	255.	259.	255.	255.	258.	261.	260.	256.	262.	265.	261.
19537	238.	234.	235.	237.	244.	243.	236.	239.	238.	238.	244.	243.	240.	240.	240.	245.
19538	252.	253.	252.	255.	254.	252.	253.	255.	255.	256.	256.	256.	254.	254.	256.	259.
19539	242.	242.	244.	246.	248.	248.	246.	248.	253.	250.	247.	250.	253.	249.	251.	253.
19540	256.	256.	253.	264.	263.	263.	260.	266.	274.	270.	269.	275.	280.	274.	273.	277.
19541	235.	240.	239.	238.	239.	244.	241.	242.	236.	242.	244.	246.	239.	243.	248.	247.
19542	261.	265.	264.	259.	266.	269.	268.	266.	266.	272.	272.	264.	272.	274.	272.	265.
19543	266.	265.	276.	281.	274.	277.	281.	285.	287.	284.	289.	290.	284.	278.	282.	290.
19544	265.	265.	262.	269.	273.	276.	274.	271.	284.	278.	280.	275.	270.	278.	281.	276.
19545	244.	247.	253.	253.	254.	255.	257.	259.	260.	261.	260.	263.	262.	265.	260.	260.
19546	269.	274.	278.	274.	283.	288.	286.	285.	288.	292.	295.	293.	286.	294.	297.	293.
19547	249.	252.	249.	251.	258.	259.	254.	257.	263.	262.	259.	263.	267.	265.	267.	269.
19548	261.	260.	261.	266.	268.	267.	264.	266.	269.	268.	268.	266.	265.	268.	264.	263.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 6): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP III				0.4 MG/KG/DAY					
DAY	33	34	35	36	37	38	39	40	41	42a
19533	254.	252.	256.	259.	260.	257.	256.	261.	264.	259.
19534	302.	300.	299.	308.	308.	305.	302.	308.	307.	311.
19535	254.	254.	258.	257.	262.	263.	261.	262.	259.	259.
19536	262.	264.	264.	262.	259.	266.	266.	264.	262.	268.
19537	246.	247.	247.	253.	252.	250.	248.	255.	253.	254.
19538	258.	258.	259.	259.	261.	260.	258.	256.	260.	260.
19539	256.	254.	252.	258.	258.	259.	257.	257.	257.	255.
19540	280.	281.	280.	282.	289.	281.	278.	284.	289.	286.
19541	242.	243.	244.	246.	243.	248.	244.	249.	247.	246.
19542	271.	278.	274.	267.	274.	276.	274.	269.	273.	276.
19543	285.	282.	289.	291.	294.	288.	293.	296.	291.	286.
19544	274.	276.	283.	289.	282.	284.	275.	290.	295.	211.
19545	268.	267.	264.	260.	264.	272.	272.	270.	270.	290.
19546	291.	299.	300.	298.	295.	299.	308.	305.	300.	274.
19547	269.	268.	273.	272.	274.	271.	271.	275.	275.	297.
19548	264.	268.	266.	264.	261.	260.	261.	264.	267.	266.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 7): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP IV 1.6 MG/KG/DAY

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
19549	220.	220.	219.	226.	229.	227.	228.	231.	233.	233.	230.	235.	237.	238.	238.	241.
19550	239.	236.	247.	250.	251.	251.	253.	264.	260.	263.	256.	264.	270.	269.	263.	269.
19551	228.	230.	230.	234.	240.	242.	242.	252.	250.	249.	250.	251.	261.	263.	264.	263.
19552	230.	230.	235.	242.	236.	232.	237.	240.	239.	231.	239.	243.	242.	238.	242.	245.
19553	218.	216.	215.	221.	228.	226.	223.	224.	233.	230.	232.	230.	233.	236.	236.	232.
19554	232.	236.	234.	239.	247.	248.	246.	250.	254.	254.	251.	258.	260.	263.	259.	264.
19555	231.	234.	237.	234.	240.	249.	248.	248.	246.	250.	253.	258.	253.	256.	260.	264.
19556	230.	229.	232.	240.	238.	233.	240.	246.	243.	240.	247.	253.	251.	246.	254.	257.
19557	221.	225.	224.	223.	226.	230.	234.	236.	231.	238.	239.	243.	243.	241.	244.	247.
19558	234.	239.	239.	244.	247.	250.	246.	249.	252.	256.	256.	258.	259.	263.	267.	263.
19559	220.	227.	228.	230.	227.	235.	237.	234.	235.	239.	237.	233.	238.	242.	242.	238.
19560	225.	229.	232.	230.	232.	238.	243.	246.	240.	246.	247.	252.	248.	248.	255.	255.
19561	220.	220.	222.	228.	232.	224.	218.	230.	237.	235.	233.	234.	242.	239.	231.	239.
19562	235.	239.	242.	243.	239.	236.	241.	241.	240.	240.	245.	249.	247.	250.	250.	253.
19563	216.	220.	221.	225.	229.	230.	229.	234.	233.	235.	229.	233.	239.	240.	239.	240.
19564	231.	230.	229.	230.	240.	240.	242.	242.	241.	247.	245.	250.	251.	252.	252.	252.

DAY	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
19549	248.	246.	248.	250.	252.	252.	248.	253.	254.	253.	251.	254.	249.	251.	250.	256.
19550	271.	267.	265.	271.	273.	275.	268.	274.	275.	272.	266.	272.	275.	272.	265.	271.
19551	265.	270.	272.	273.	273.	275.	279.	284.	280.	278.	279.	281.	274.	279.	283.	284.
19552	245.	240.	244.	247.	246.	243.	244.	249.	249.	246.	248.	252.	247.	241.	243.	249.
19553	232.	238.	237.	236.	235.	240.	236.	238.	233.	234.	239.	238.	232.	234.	241.	239.
19554	266.	267.	263.	267.	271.	271.	266.	269.	271.	269.	267.	271.	271.	266.	265.	271.
19555	266.	263.	268.	272.	272.	270.	275.	276.	277.	273.	282.	284.	282.	275.	283.	285.
19556	258.	251.	260.	263.	266.	264.	264.	268.	261.	262.	272.	267.	261.	257.	260.	263.
19557	251.	247.	246.	251.	253.	254.	252.	254.	256.	255.	257.	256.	249.	252.	251.	251.
19558	266.	266.	268.	271.	269.	276.	273.	273.	276.	277.	280.	282.	280.	284.	283.	280.
19559	242.	244.	245.	242.	246.	250.	248.	244.	248.	247.	248.	242.	246.	248.	246.	243.
19560	257.	253.	255.	260.	260.	256.	259.	264.	262.	256.	260.	262.	257.	250.	256.	266.
19561	242.	239.	236.	241.	244.	245.	241.	247.	249.	244.	242.	245.	247.	242.	240.	243.
19562	258.	256.	259.	256.	264.	264.	267.	266.	262.	266.	266.	267.	262.	262.	268.	269.
19563	243.	247.	246.	243.	242.	245.	248.	246.	247.	246.	246.	249.	249.	253.	252.	253.
19564	255.	253.	254.	257.	260.	259.	258.	256.	261.	261.	259.	259.	254.	258.	257.	254.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 8): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP IV				1.6 MG/KG/DAY					
DAY	33	34	35	36	37	38	39	40	41	42a
19549	256.	255.	252.	258.	259.	263.	266.	264.	269.	272.
19550	272.	274.	266.	272.	272.	270.	266.	269.	271.	270.
19551	280.	284.	284.	284.	280.	278.	283.	283.	283.	282.
19552	250.	252.	254.	257.	257.	260.	262.	262.	261.	263.
19553	237.	228.	231.	235.	231.	226.	227.	232.	233.	230.
19554	273.	272.	267.	272.	272.	268.	268.	271.	273.	270.
19555	284.	280.	287.	287.	290.	282.	288.	288.	286.	280.
19556	257.	254.	261.	261.	258.	250.	258.	258.	256.	253.
19557	252.	252.	255.	255.	259.	256.	252.	254.	255.	251.
19558	282.	281.	284.	288.	288.	290.	291.	294.	291.	290.
19559	248.	250.	249.	247.	252.	254.	255.	252.	254.	252.
19560	261.	259.	265.	266.	265.	260.	266.	264.	259.	258.
19561	246.	246.	242.	246.	249.	252.	244.	249.	246.	239.
19562	266.	263.	266.	268.	269.	271.	268.	268.	273.	263.
19563	254.	251.	253.	258.	253.	257.	260.	258.	260.	255.
19564	249.	256.	256.	254.	253.	250.	257.	256.	256.	250.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 9): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP V 3.2 MG/KG/DAY

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
19565	217.	222.	214.	221.	225.	226.	224.	222.	224.	224.	232.	229.	224.	229.	228.	231.
19566	238.	234.	240.	246.	245.	240.	243.	249.	251.	242.	240.	245.	252.	247.	243.	250.
19567	234.	237.	239.	241.	245.	240.	231.	236.	237.	232.	230.	234.	235.	230.	229.	230.
19568	240.	246.	247.	249.	248.	247.	250.	247.	248.	250.	251.	256.	255.	253.	250.	253.
19569	220.	221.	224.	226.	229.	236.	233.	238.	236.	234.	236.	238.	236.	238.	238.	239.
19570	228.	234.	234.	234.	233.	237.	238.	243.	242.	238.	243.	246.	247.	247.	247.	247.
19571	235.	230.	228.	243.	241.	245.	249.	252.	252.	248.	253.	255.	252.	250.	256.	256.
19572	227.	227.	226.	226.	228.	230.	231.	234.	233.	235.	238.	241.	240.	242.	242.	242.
19573	221.	224.	221.	221.	226.	231.	233.	231.	230.	236.	234.	233.	232.	230.	238.	235.
19574	236.	237.	237.	244.	249.	249.	245.	254.	255.	255.	253.	254.	258.	260.	257.	256.
19575	230.	233.	230.	237.	238.	245.	242.	248.	247.	248.	245.	245.	249.	252.	252.	245.
19576	230.	233.	234.	236.	242.	237.	243.	249.	242.	238.	247.	248.	247.	247.	250.	254.
19577	222.	224.	225.	226.	226.	229.	229.	231.	235.	236.	238.	240.	239.	242.	240.	241.
19578	232.	232.	235.	242.	245.	244.	250.	254.	252.	252.	256.	258.	258.	257.	258.	261.
19579	220.	221.	223.	224.	221.	224.	227.	225.	222.	224.	217.	215.	215.	223.	223.	215.
19580	230.	230.	228.	238.	242.	240.	237.	237.	243.	242.	234.	241.	238.	237.	236.	238.

DAY	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
19565	230.	227.	236.	230.	232.	230.	227.	229.	232.	231.	227.	231.	236.	235.	232.	231.
19566	255.	253.	246.	252.	257.	253.	241.	249.	251.	251.	243.	252.	249.	249.	239.	245.
19567	234.	230.	227.	233.	235.	229.	226.	232.	230.	228.	223.	230.	231.	226.	220.	225.
19568	258.	262.	258.	264.	265.	266.	262.	261.	263.	267.	264.	263.	264.	264.	265.	264.
19569	237.	241.	241.	241.	243.	245.	241.	237.	246.	247.	242.	238.	240.	242.	239.	243.
19570	247.	248.	251.	250.	254.	255.	249.	252.	249.	250.	254.	251.	251.	249.	249.	254.
19571	256.	252.	257.	255.	258.	254.	257.	256.	258.	252.	256.	255.	250.	240.	244.	244.
19572	243.	243.	242.	242.	242.	243.	241.	242.	241.	244.	245.	248.	245.	245.	242.	241.
19573	235.	235.	234.	241.	242.	242.	238.	235.	238.	240.	241.	237.	237.	238.	239.	237.
19574	258.	254.	258.	258.	256.	258.	258.	259.	252.	247.	253.	254.	250.	242.	249.	249.
19575	247.	254.	255.	256.	256.	254.	255.	260.	261.	257.	255.	256.	256.	253.	248.	244.
19576	254.	250.	253.	250.	252.	253.	259.	260.	259.	253.	261.	257.	257.	253.	266.	260.
19577	241.	243.	242.	239.	241.	241.	238.	234.	232.	232.	228.	227.	222.	224.	226.	225.
19578	256.	257.	261.	262.	261.	259.	262.	262.	260.	259.	264.	264.	258.	249.	255.	257.
19579	212.	219.	215.	213.	221.	220.	220.	220.	218.	213.	215.	214.	213.	214.	211.	210.
19580	239.	238.	238.	245.	244.	243.	239.	247.	243.	245.	241.	247.	244.	242.	237.	242.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 14 (PAGE 10): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP V				3.2 MG/KG/DAY					
DAY	33	34	35	36	37	38	39	40	41	42a
19565	233.	234.	233.	233.	232.	233.	227.	231.	232.	225.
19566	246.	248.	236.	242.	249.	243.	236.	236.	244.	244.
19567	224.	224.	221.	222.	223.	226.	224.	222.	225.	223.
19568	264.	263.	267.	266.	267.	267.	265.	269.	268.	268.
19569	246.	245.	240.	244.	241.	241.	238.	239.	238.	234.
19570	252.	250.	247.	250.	249.	250.	247.	245.	246.	249.
19571	244.	243.	252.	244.	244.	239.	243.	242.	241.	241.
19572	238.	235.	236.	239.	237.	234.	236.	243.	240.	245.
19573	238.	241.	243.	243.	238.	240.	237.	240.	236.	235.
19574	248.	245.	249.	251.	251.	248.	247.	250.	251.	240.
19575	249.	250.	245.	241.	246.	243.	245.	246.	243.	233.
19576	257.	257.	265.	268.	261.	261.	262.	264.	261.	255.
19577	227.	225.	227.	226.	227.	226.	224.	224.	224.	221.
19578	256.	252.	254.	257.	258.	251.	253.	254.	251.	249.
19579	211.	209.	208.	210.	214.	217.	214.	216.	212.	211.
19580	244.	242.	239.	240.	237.	235.	234.	238.	237.	231.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 15 (PAGE 1): MATERNAL BODY WEIGHTS - PRESUMED GESTATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP I 0 (VEHICLE) MG/KG/DAY

RAT #	PREGNANCY STATUS	DAY 0	1	2	3	4	5	6	7	8	9	10	11	12
19501	P	257.	263.	269.	270.	274.	278.	280.	277.	285.	289.	295.	302.	306.
19502	NP	276.	288.	294.	297.	300.	304.	303.	307.	306.	308.	316.	316.	316.
19503	P	NO CONFIRMED MATING DATE; DELIVERED ON DAY 66 OF STUDY												
19504	P	295.	306.	308.	308.	311.	313.	317.	316.	318.	320.	323.	329.	343.
19505	P	281.	294.	301.	301.	302.	308.	309.	306.	309.	316.	323.	332.	328.
19506	P	266.	273.	279.	284.	287.	295.	297.	294.	301.	304.	313.	312.	318.
19507	P	272.	284.	286.	290.	291.	298.	296.	289.	287.	296.	305.	310.	310.
19508	P	291.	297.	306.	313.	314.	313.	315.	319.	318.	325.	333.	339.	344.
19509	P	269.	273.	273.	284.	287.	293.	299.	299.	293.	302.	306.	311.	317.
19510	P	259.	267.	278.	279.	290.	291.	301.	302.	302.	311.	311.	325.	322.
19511	P	291.	299.	305.	307.	311.	311.	316.	322.	310.	321.	324.	329.	334.
19512	P	306.	314.	312.	316.	323.	318.	324.	318.	322.	324.	326.	330.	333.
19513	P	330.	329.	334.	340.	350.	349.	355.	355.	353.	353.	360.	367.	369.
19514	NP	290.	298.	302.	301.	311.	313.	316.	321.	321.	325.	326.	330.	337.
19515	P	258.	267.	274.	274.	278.	283.	285.	286.	287.	295.	295.	302.	308.
19516	P	282.	288.	298.	306.	302.	309.	311.	285.	311.	315.	323.	328.	334.

RAT #	DAY 13	14	15	16	17	18	19	20	21	GRAVID UTERINE WEIGHTS
19501	311.	310.	315.							16.56
19502	318.	314.	310.							-----
19503	NO CONFIRMED MATING DATE; DELIVERED ON DAY 66 OF STUDY									-----
19504	342.	347.	348.	357.	368.	385.	396.	418.	430.	79.93
19505	333.	337.	343.	356.	374.	390.	415.	433.	452.	118.23
19506	325.	326.	335.	340.	360.	376.	397.	412.	431.	99.38
19507	312.	316.	304.	323.	335.	350.	363.	371.	394.	93.66
19508	350.	349.	364.							21.37
19509	322.	324.	328.	333.	345.	363.	380.	399.	377.	86.56
19510	330.	338.	339.							15.76
19511	338.	345.	351.							27.22
19512	336.	341.	344.	354.	376.	388.	406.	416.	431.	96.29
19513	374.	380.	386.							21.00
19514	335.	337.	342.							-----
19515	309.	313.	316.							17.04
19516	334.	347.	350.	357.	375.	390.	412.	422.	446.	99.80

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

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TABLE 15 (PAGE 2): MATERNAL BODY WEIGHTS - PRESUMED GESTATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP II 0.1 MG/KG/DAY

RAT #	PREGNANCY STATUS	DAY 0	1	2	3	4	5	6	7	8	9	10	11	12
19517	NP	277.	276.	268.	270.	272.	272.	271.	272.	272.	273.	272.	274.	281.
19518	P	308.	316.	319.	320.	322.	329.	333.	338.	334.	334.	339.	346.	347.
19519	P	276.	280.	288.	287.	296.	296.	302.	304.	307.	316.	320.	328.	332.
19520		247.	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED											
19521	P	292.	299.	308.	309.	303.	306.	315.	306.	313.	321.	320.	321.	328.
19522	P	292.	295.	305.	305.	308.	318.	320.	320.	319.	328.	328.	332.	338.
19523	P	288.	294.	304.	310.	309.	312.	315.	330.	317.	318.	332.	334.	336.
19524	P	296.	304.	311.	319.	318.	327.	327.	323.	331.	336.	342.	346.	348.
19525	P	259.	261.	271.	272.	274.	277.	278.	281.	281.	285.	283.	287.	291.
19526	P	278.	280.	288.	287.	291.	291.	294.	301.	303.	306.	308.	313.	317.
19527	P	281.	293.	294.	296.	300.	302.	310.	310.	303.	310.	340.	318.	326.
19528	P	258.	275.	285.	290.	300.	300.	300.	308.	315.	317.	317.	330.	327.
19529	P	258.	265.	273.	268.	280.	282.	282.	284.	284.	289.	292.	302.	298.
19530	P	271.	285.	293.	287.	296.	297.	312.	307.	309.	313.	317.	323.	329.
19531	P	270.	270.	281.	280.	290.	292.	294.	296.	296.	298.	308.	312.	323.
19532	P	281.	293.	298.	297.	296.	299.	306.	303.	309.	316.	312.	317.	328.

RAT #	DAY 13	14	15	16	17	18	19	20	21	GRAVID UTERINE WEIGHTS
19517 NP	279.	273.	281.							-----
19518 P	354.	357.	359.	370.	385.	392.	412.	424.	448.	71.83
19519 P	333.	338.	339.	361.	379.	397.	414.	429.	433.	115.99
19520	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED									-----
19521 P	336.	335.	339.	353.	370.	389.	402.	413.	429.	101.43
19522 P	339.	346.	355.							18.40
19523 P	339.	346.	325.	353.	368.	378.	390.	405.	411.	88.34
19524 P	351.	361.	367.	373.	393.	411.	430.	450.	470.	117.23
19525 P	292.	297.	303.							8.80
19526 P	318.	322.	324.							15.42
19527 P	328.	335.	342.							25.35
19528 P	330.	337.	329.	318.	329.	343.	355.	370.	378.	42.75
19529 P	309.	308.	320.	327.	344.	360.	380.	400.	420.	106.57
19530 P	333.	335.	314.							16.30
19531 P	328.	332.	333.							21.41
19532 P	332.	332.	347.							16.54

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

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TABLE 15 (PAGE 3): MATERNAL BODY WEIGHTS - PRESUMED GESTATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP III 0.4 MG/KG/DAY

RAT #	PREGNANCY STATUS	DAY 0	1	2	3	4	5	6	7	8	9	10	11	12
19533	P	267.	270.	273.	273.	275.	274.	278.	276.	281.	278.	282.	289.	288.
19534	P	323.	325.	333.	329.	333.	336.	342.	336.	342.	345.	341.	349.	356.
19535	P	253.	244.	260.	266.	267.	270.	274.	268.	278.	281.	281.	285.	293.
19536	P	274.	275.	282.	285.	286.	289.	289.	290.	293.	295.	296.	304.	310.
19537	P	257.	257.	266.	265.	268.	269.	272.	257.	264.	266.	267.	275.	279.
19538	P	260.	267.	268.	274.	272.	278.	279.	269.	279.	280.	285.	289.	297.
19539	P	262.	261.	271.	277.	280.	284.	286.	279.	292.	298.	296.	303.	306.
19540	P	285.	291.	301.	302.	310.	310.	315.	303.	308.	319.	320.	320.	330.
19541		234.	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED											
19542	P	284.	292.	294.	294.	297.	302.	306.	304.	303.	304.	310.	317.	324.
19543	P	304.	316.	318.	319.	323.	328.	327.	331.	331.	333.	338.	328.	349.
19544	P	290.	304.	314.	310.	308.	306.	313.	315.	327.	313.	320.	328.	330.
19545	NP	MATING NOT CONFIRMED												
19546	P	296.	286.	300.	307.	310.	312.	317.	304.	298.	316.	326.	331.	333.
19547	P	272.	285.	288.	292.	292.	296.	290.	289.	315.	294.	296.	302.	314.
19548	NP	270.	276.	279.	280.	284.	283.	283.	267.	284.	288.	288.	287.	292.

RAT #		DAY 13	14	15	16	17	18	19	20	21	GRAVID UTERINE WEIGHTS
19533	P	294.	298.	298.	306.	323.	338.	352.	371.	387.	85.28
19534	P	363.	367.	334.	378.	380.	400.	405.	438.	445.	94.87
19535	P	303.	305.	312.							9.30
19536	P	315.	320.	317.	338.	353.	371.	397.	418.	429.	123.08
19537	P	281.	284.	285.							6.02
19538	P	301.	302.	291.							18.47
19539	P	310.	321.	319.							19.11
19540	P	331.	340.	335.							20.45
19541		MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED									-----
19542	P	343.	334.	339.							19.46
19543	P	353.	361.	364.	375.	396.	419.	430.	447.	458.	116.27
19544	P	329.	333.	345.							16.14
19545	NP	MATING NOT CONFIRMED									-----
19546	P	334.	339.	333.	350.	353.	371.	375.	377.	396.	36.94
19547	P	314.	323.	333.							21.57
19548	NP	296.	291.	286.	284.	284.	284.	285.	291.	294.	-----

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

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TABLE 15 (PAGE 4): MATERNAL BODY WEIGHTS - PRESUMED GESTATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP IV 1.6 MG/KG/DAY

RAT #	PREGNANCY STATUS	DAY 0	1	2	3	4	5	6	7	8	9	10	11	12
19549	NP	MATING NOT CONFIRMED												
19550	P	276.	276.	279.	279.	278.	284.	290.	266.	281.	291.	294.	303.	312.
19551	NP	MATING NOT CONFIRMED												
19552	NP	MATING NOT CONFIRMED												
19553	P	245.	245.	252.	254.	256.	253.	255.	256.	259.	261.	269.	275.	277.
19554	P	282.	275.	282.	278.	280.	282.	286.	282.	286.	288.	296.	299.	303.
19555	NP	292.	293.	300.	299.	301.	300.	295.	296.	287.	293.	287.	294.	296.
19556	NP	267.	268.	261.	264.	266.	264.	258.	262.	263.	261.	259.	267.	266.
19557		MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION)												
19558	P	286.	287.	302.	305.	303.	308.	299.	297.	309.	302.	306.	317.	324.
19559	NP	260.	260.	264.	267.	265.	269.	268.	268.	271.	273.	276.	279.	273.
19560	P	274.	278.	283.	286.	284.	291.	288.	286.	285.	291.	290.	295.	296.
19561a		254.	260.	262.	259.	264.	265.	262.	268.	262.	266.	268.	271.	265.
19562	P	272.	277.	282.	283.	283.	288.	292.	285.	287.	294.	300.	306.	313.
19563	P	256.	263.	273.	267.	270.	266.	267.	266.	265.	264.	268.	276.	279.
19564	P	258.	257.	264.	266.	268.	268.	273.	271.	274.	276.	279.	283.	288.

RAT #	DAY 13	14	15	16	17	18	19	20	21	GRAVID UTERINE WEIGHTS
19549	MATING NOT CONFIRMED									-----
19550	311.	314.	322.							17.57
19551	MATING NOT CONFIRMED									-----
19552	MATING NOT CONFIRMED									-----
19553	286.	287.	292.							18.42
19554	307.	318.	323.							17.90
19555	298.	296.	299.							-----
19556	268.	263.	261.	268.	270.	265.	272.	276.	270.	-----
19557	MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION)									-----
19558	324.	337.	332.							19.53
19559	267.	259.	250.	252.	254.	252.	258.	258.	257.	-----
19560	309.	308.	319.	328.	349.	363.	377.	389.	410.	86.60
19561a	260.	262.	263.							-----
19562	316.	326.	328.	350.	366.	392.	412.	438.	443.	138.89
19563	278.	285.	295.							16.06
19564	287.	292.	295.							16.16

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Necropsy and Caesarean-section observations were not recorded for rat 19561.

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TABLE 15 (PAGE 5): MATERNAL BODY WEIGHTS - PRESUMED GESTATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP V 3.2 MG/KG/DAY

RAT #	PREGNANCY STATUS	DAY 0	1	2	3	4	5	6	7	8	9	10	11	12
19565	P	234.	224.	227.	228.	229.	230.	234.	219.	234.	240.	250.	251.	257.
19566	P	244.	252.	249.	251.	246.	244.	244.	245.	241.	249.	266.	270.	271.
19567	P	228.	226.	228.	230.	231.	234.	237.	236.	245.	250.	254.	256.	261.
19568	P	259.	263.	264.	266.	266.	270.	270.	264.	270.	275.	276.	284.	287.
19569	P	249.	247.	248.	247.	244.	252.	252.	248.	245.	249.	262.	256.	265.
19570	P	257.	257.	258.	260.	253.	266.	265.	260.	265.	272.	275.	281.	282.
19571	P	245.	247.	249.	253.	256.	255.	259.	245.	245.	259.	268.	275.	278.
19572	P	250.	255.	252.	255.	253.	257.	259.	259.	262.	261.	264.	277.	275.
19573	P	229.	225.	223.	227.	238.	244.	247.	250.	243.	248.	256.	260.	266.
19574	P	252.	253.	256.	261.	261.	261.	266.	245.	254.	262.	275.	275.	279.
19575	P	243.	239.	239.	228.	239.	242.	250.	256.	250.	252.	269.	268.	277.
19576	NP	MATING NOT CONFIRMED												
19577	P	230.	229.	230.	227.	219.	208.	208.	219.	217.	221.	230.	236.	240.
19578	NP	MATING NOT CONFIRMED												
19579	P	219.	216.	213.	211.	213.	227.	223.	217.	222.	229.	238.	236.	236.
19580	P	237.	235.	242.	235.	240.	244.	244.	236.	240.	245.	251.	266.	260.

RAT #	DAY 13	14	15	16	17	18	19	20	21	GRAVID UTERINE WEIGHTS
19565	259.	264.	261.							7.98
19566	278.	286.	285.							15.83
19567	269.	278.	266.							17.59
19568	288.	291.	290.	296.	312.	315.	328.	341.	351.	41.84
19569	264.	266.	278.	287.	306.	319.	334.	346.	349.	86.30
19570	294.	302.	291.							20.43
19571	288.	293.	297.	304.	332.	346.	357.	369.	385.	-----
19572	282.	290.	276.	300.	317.	330.	349.	360.	376.	78.32
19573	268.	273.	275.							17.44
19574	285.	288.	291.							17.60
19575	294.	292.	299.							19.43
19576	MATING NOT CONFIRMED									-----
19577	247.	245.	248.	258.	264.	274.	280.	286.	295.	31.64
19578	MATING NOT CONFIRMED									-----
19579	240.	248.	250.	258.	269.	288.	299.	304.	320.	-----
19580	266.	270.	275.							20.54

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

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TABLE 16 (PAGE 1): FEED CONSUMPTION VALUES - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP I			0 (VEHICLE) MG/KG/DAY			
DAYS	1- 8	8- 15	15- 22	22- 29	29- 36	36- 41	41- 42a
19501	131.	114.	129.	b	124.	92.	18.
19502	131.	123.	128.	119.	107.	81.	19.
19503	138.	145.	150.	128.	141.	100.	23.
19504	144.	147.	152.	140.	141.	95.	20.
19505	144.	b	140.	b	143.	90.	22.
19506	147.	b	147.	133.	122.	b	24.
19507	141.	b	b	146.	148.	97.	19.
19508	124.	131.	144.	129.	130.	b	21.
19509	123.	128.	127.	121.	120.	b	23.
19510	161.	b	b	b	132.	b	18.
19511	149.	b	150.	150.	140.	99.	22.
19512	126.	124.	131.	133.	120.	93.	16.
19513	b	149.	167.	b	158.	114.	b
19514	156.	144.	140.	141.	131.	92.	b
19515	120.	120.	138.	107.	106.	88.	c
19516	130.	122.	123.	120.	135.	87.	14.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.
b. Spilled feed precluded the calculation of this value.
c. Value was incorrectly recorded and was excluded from group averages and statistical analyses.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 16 (PAGE 2): FEED CONSUMPTION VALUES - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP II			0.1 MG/KG/DAY			
DAYS	1- 8	8- 15	15- 22	22- 29	29- 36	36- 41	41- 42a
19517	127.	116.	119.	126.	109.	87.	11.
19518	143.	139.	137.	135.	129.	98.	21.
19519	128.	128.	130.	128.	125.	83.	16.
19520	122.	115.	125.	132.	132.	87.	15.
19521	148.	143.	149.	152.	143.	115.	16.
19522	138.	126.	125.	b	124.	87.	19.
19523	120.	b	150.	119.	120.	83.	18.
19524	140.	150.	149.	153.	127.	99.	18.
19525	121.	119.	123.	108.	114.	73.	17.
19526	140.	148.	146.	148.	151.	97.	18.
19527	145.	138.	144.	124.	121.	82.	23.
19528	137.	124.	128.	126.	111.	84.	16.
19529	146.	131.	127.	117.	112.	88.	20.
19530	129.	135.	145.	140.	128.	94.	19.
19531	154.	142.	153.	144.	143.	102.	20.
19532	133.	121.	133.	136.	135.	85.	14.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.
b. Spilled feed precluded the calculation of this value.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 16 (PAGE 3): FEED CONSUMPTION VALUES - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP III			0.4 MG/KG/DAY			
DAYS	1- 8	8- 15	15- 22	22- 29	29- 36	36- 41	41- 42a
19533	125.	108.	122.	113.	126.	88.	17.
19534	151.	131.	152.	141.	129.	94.	23.
19535	115.	103.	122.	117.	118.	84.	15.
19536	124.	123.	125.	122.	121.	79.	20.
19537	130.	118.	118.	109.	122.	80.	20.
19538	129.	115.	118.	117.	115.	87.	17.
19539	117.	105.	114.	110.	115.	79.	15.
19540	122.	122.	132.	133.	133.	87.	18.
19541	112.	112.	117.	112.	127.	85.	b
19542	144.	135.	132.	c	123.	87.	c
19543	149.	138.	c	150.	126.	101.	c
19544	138.	135.	140.	133.	130.	97.	24.
19545	130.	123.	128.	c	124.	94.	19.
19546	146.	c	168.	162.	158.	c	21.
19547	127.	124.	126.	134.	139.	96.	21.
19548	146.	131.	134.	123.	118.	78.	16.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.
b. Value was incorrectly recorded and was excluded from group averages and statistical analyses.
c. Spilled feed precluded the calculation of this value.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 16 (PAGE 4): FEED CONSUMPTION VALUES - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP IV			1.6 MG/KG/DAY			
DAYS	1- 8	8- 15	15- 22	22- 29	29- 36	36- 41	41- 42a
19549	127.	121.	b	118.	124.	92.	18.
19550	146.	b	123.	107.	108.	75.	15.
19551	141.	142.	137.	126.	125.	74.	16.
19552	121.	103.	108.	105.	104.	82.	17.
19553	131.	145.	116.	b	122.	b	17.
19554	141.	147.	b	b	139.	96.	18.
19555	b	123.	141.	138.	122.	89.	13.
19556	127.	130.	135.	125.	111.	77.	15.
19557	124.	124.	124.	111.	108.	72.	c
19558	144.	143.	140.	140.	140.	98.	b
19559	137.	122.	b	b	115.	92.	b
19560	132.	122.	115.	118.	121.	85.	12.
19561	109.	118.	119.	116.	111.	87.	10.
19562	140.	119.	129.	123.	120.	84.	16.
19563	143.	138.	137.	134.	142.	105.	21.
19564	120.	111.	109.	105.	105.	77.	9.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.
b. Spilled feed precluded the calculation of this value.
c. Value was incorrectly recorded and was excluded from group averages and statistical analyses.

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TABLE 16 (PAGE 5): FEED CONSUMPTION VALUES - PRECOHABITATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP V			3.2 MG/KG/DAY			
DAYS	1- 8	8- 15	15- 22	22- 29	29- 36	36- 41	41- 42a
19565	118.	109.	102.	104.	100.	68.	11.
19566	143.	119.	124.	106.	103.	78.	17.
19567	125.	101.	105.	101.	97.	72.	14.
19568	132.	123.	125.	117.	114.	81.	18.
19569	141.	117.	114.	100.	113.	67.	10.
19570	139.	128.	144.	120.	118.	84.	17.
19571	140.	122.	115.	112.	95.	73.	15.
19572	117.	118.	111.	112.	93.	73.	19.
19573	132.	113.	110.	98.	117.	73.	b
19574	157.	b	b	117.	113.	b	b
19575	144.	125.	114.	115.	97.	77.	c
19576	141.	121.	116.	122.	126.	82.	9.
19577	126.	128.	103.	76.	90.	68.	10.
19578	135.	123.	111.	116.	103.	77.	15.
19579	128.	90.	b	91.	b	80.	13.
19580	127.	103.	111.	112.	106.	74.	13.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.
b. Spilled feed precluded the calculation of this value.
c. Value was incorrectly recorded and was excluded from group averages and statistical analyses.

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TABLE 17 (PAGE 1): MATERNAL FEED CONSUMPTION VALUES - PRESUMED GESTATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP I												
	0 (VEHICLE) MG/KG/DAY												
PREGNANCY STATUS	DAYS 0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13
19501 P	17.	20.	22.	22.	24.	23.	22.	24.	22.	23.	23.	18.	24.
19502 NP	21.	22.	22.	23.	22.	23.	23.	21.	19.	24.	22.	21.	24.
19503 P	NO CONFIRMED MATING DATE; DELIVERED ON DAY 66 OF STUDY												
19504 P	23.	24.	22.	22.	27.	21.	24.	25.	24.	24.	28.	28.	23.
19505 P	22.	21.	24.	23.	24.	25.	22.	22.	23.	26.	25.	26.	25.
19506 P	24.	29.	28.	23.	25.	26.	23.	23.	27.	26.	18.	28.	24.
19507 P	27.	32.	27.	21.	28.	25.	20.	17.	25.	24.	24.	24.	22.
19508 P	21.	23.	26.	24.	23.	24.	24.	21.	25.	25.	25.	29.	26.
19509 P	20.	22.	23.	23.	24.	27.	22.	21.	25.	25.	25.	26.	23.
19510 P	16.	25.	23.	26.	45.	30.	27.	23.	27.	29.	28.	26.	29.
19511 P	18.	23.	23.	24.	25.	23.	26.	22.	22.	22.	21.	22.	24.
19512 P	24.	21.	23.	24.	21.	24.	19.	21.	21.	22.	19.	23.	22.
19513 P	22.	21.	25.	29.	27.	28.	29.	24.	25.	28.	26.	26.	29.
19514 NP	21.	13.	21.	24.	24.	26.	27.	24.	28.	28.	26.	27.	29.
19515 P	22.	21.	22.	22.	23.	23.	24.	22.	24.	23.	23.	27.	21.
19516 P	20.	26.	22.	24.	24.	24.	17.	24.	20.	23.	25.	28.	26.

	DAYS 13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20	20 - 21
19501 P	23.	18.						
19502 NP	19.	16.						
19503 P	NO CONFIRMED MATING DATE; DELIVERED ON DAY 66 OF STUDY							
19504 P	25.	20.	25.	28.	27.	24.	33.	26.
19505 P	26.	23.	23.	29.	28.	28.	27.	24.
19506 P	21.	25.	22.	25.	28.	25.	22.	23.
19507 P	21.	17.	22.	27.	28.	27.	16.	23.
19508 P	23.	25.						
19509 P	20.	25.	18.	20.	25.	23.	24.	11.
19510 P	25.	18.						
19511 P	21.	17.						
19512 P	20.	17.	28.	24.	24.	23.	25.	17.
19513 P	26.	23.						
19514 NP	26.	22.						
19515 P	24.	20.						
19516 P	27.	23.	24.	31.	26.	30.	26.	25.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

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TABLE 17 (PAGE 2): MATERNAL FEED CONSUMPTION VALUES - PRESUMED GESTATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP II			0.1 MG/KG/DAY								
PREGNANCY STATUS DAYS	0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13
19517 NP	21.	12.	16.	19.	18.	15.	18.	16.	16.	14.	17.	21.	18.
19518 P	26.	24.	26.	23.	24.	28.	25.	25.	25.	23.	26.	27.	25.
19519 P	19.	23.	15.	23.	21.	24.	27.	20.	25.	24.	26.	21.	26.
19520	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED												
19521 P	25.	23.	25.	19.	21.	22.	15.	24.	24.	25.	22.	23.	25.
19522 P	17.	23.	20.	22.	23.	23.	24.	19.	25.	22.	23.	24.	25.
19523 P	22.	21.	25.	23.	24.	24.	-21.	22.	25.	25.	24.	26.	26.
19524 P	23.	24.	29.	27.	29.	28.	25.	26.	27.	32.	29.	26.	27.
19525 P	16.	21.	19.	21.	19.	19.	22.	20.	19.	18.	20.	21.	23.
19526 P	20.	25.	23.	26.	23.	23.	28.	22.	24.	25.	23.	24.	26.
19527 P	20.	19.	20.	21.	18.	24.	26.	18.	22.	19.	19.	27.	20.
19528 P	25.	24.	28.	28.	26.	26.	30.	26.	29.	24.	30.	24.	30.
19529 P	21.	23.	20.	26.	22.	21.	24.	18.	24.	21.	22.	20.	26.
19530 P	22.	27.	23.	29.	25.	30.	24.	26.	28.	27.	25.	24.	28.
19531 P	16.	21.	24.	28.	24.	24.	25.	22.	28.	26.	26.	28.	29.
19532 P	20.	23.	20.	23.	23.	25.	22.	23.	27.	24.	22.	25.	28.

	DAYS 13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20	20 - 21
19517 NP	11.	a						
19518 P	22.	a	27.	25.	32.	28.	28.	28.
19519 P	21.	22.	26.	28.	25.	29.	22.	12.
19520	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED							
19521 P	20.	17.	28.	29.	25.	26.	26.	18.
19522 P	21.	22.						
19523 P	26.	18.	21.	23.	26.	28.	24.	20.
19524 P	24.	22.	27.	26.	28.	30.	28.	26.
19525 P	22.	18.						
19526 P	25.	18.						
19527 P	19.	a						
19528 P	21.	19.	4.	16.	16.	28.	26.	23.
19529 P	20.	21.	27.	26.	25.	31.	24.	23.
19530 P	23.	15.						
19531 P	22.	17.						
19532 P	24.	21.						

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.

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TABLE 17 (PAGE 3): MATERNAL FEED CONSUMPTION VALUES - PRESUMED GESTATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP III			0.4 MG/KG/DAY									
PREGNANCY STATUS DAYS	0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13
19533 P	19.	17.	17.	20.	19.	19.	19.	19.	18.	18.	21.	18.	22.
19534 P	24.	27.	23.	24.	22.	26.	a	21.	23.	25.	24.	25.	29.
19535 P	8.	17.	17.	21.	18.	20.	a	20.	21.	22.	20.	21.	27.
19536 P	16.	21.	21.	21.	21.	17.	21.	22.	19.	17.	25.	21.	22.
19537 P	16.	16.	19.	21.	22.	20.	a	18.	21.	20.	19.	21.	23.
19538 P	19.	18.	19.	18.	17.	22.	16.	18.	20.	19.	18.	24.	19.
19539 P	18.	17.	20.	22.	20.	22.	a	25.	22.	20.	22.	22.	23.
19540 P	17.	24.	20.	24.	23.	25.	a	21.	27.	24.	22.	22.	28.
19541	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED												
19542 P	25.	20.	22.	21.	24.	24.	20.	21.	20.	23.	24.	26.	26.
19543 P	27.	23.	25.	26.	23.	26.	24.	24.	25.	26.	25.	26.	28.
19544 P	24.	28.	19.	25.	24.	22.	a	24.	24.	30.	21.	27.	28.
19545 NP	MATING NOT CONFIRMED												
19546 P	12.	22.	24.	27.	26.	30.	a	25.	29.	26.	25.	24.	32.
19547 P	21.	23.	23.	25.	21.	23.	a	21.	22.	23.	23.	23.	27.
19548 NP	16.	21.	20.	21.	20.	17.	a	21.	23.	22.	23.	20.	24.

	DAYS 13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20	20 - 21
19533 P	19.	16.	22.	25.	25.	23.	22.	19.
19534 P	26.	11.	24.	17.	17.	25.	30.	23.
19535 P	22.	19.						
19536 P	21.	10.	21.	20.	25.	26.	28.	24.
19537 P	20.	17.						
19538 P	17.	9.						
19539 P	22.	18.						
19540 P	22.	14.						
19541	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED							
19542 P	22.	b						
19543 P	23.	19.	39.	27.	28.	23.	28.	20.
19544 P	24.	21.						
19545 NP	MATING NOT CONFIRMED							
19546 P	26.	20.	31.	21.	31.	34.	27.	21.
19547 P	22.	18.						
19548 NP	20.	19.	18.	18.	20.	18.	20.	19.

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Value not recorded.
b. Spilled feed precluded the calculation of this value.

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TABLE 17 (PAGE 4): MATERNAL FEED CONSUMPTION VALUES - PRESUMED GESTATION - INDIVIDUAL DATA

RAT #	DOSAGE GROUP IV			1.6 MG/KG/DAY									
PREGNANCY STATUS DAYS	0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13
19549 NP	MATING NOT CONFIRMED												
19550 P	14.	16.	16.	16.	18.	20.	a	17.	23.	22.	23.	25.	21.
19551 NP	MATING NOT CONFIRMED												
19552 NP	MATING NOT CONFIRMED												
19553 P	15.	16.	20.	21.	18.	17.	a	19.	22.	21.	23.	24.	28.
19554 P	b	17.	15.	19.	23.	22.	a	23.	22.	21.	22.	19.	26.
19555 NP	19.	20.	18.	24.	18.	16.	a	17.	19.	15.	20.	20.	23.
19556 NP	19.	11.	18.	16.	15.	11.	17.	18.	18.	15.	19.	22.	16.
19557	MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION)												
19558 P	14.	22.	22.	22.	20.	18.	a	23.	25.	24.	25.	23.	27.
19559 NP	15.	18.	18.	19.	20.	21.	21.	20.	20.	21.	23.	20.	16.
19560 P	24.	23.	21.	22.	23.	18.	16.	21.	17.	22.	21.	20.	26.
19561c	18.	20.	18.	17.	20.	18.	a	19.	20.	20.	21.	15.	13.
19562 P	18.	21.	19.	20.	21.	21.	17.	20.	23.	23.	25.	25.	23.
19563 P	19.	23.	21.	21.	19.	18.	a	18.	22.	22.	22.	23.	24.
19564 P	13.	18.	16.	20.	18.	20.	a	17.	20.	20.	20.	17.	22.

	DAYS 13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20	20 - 21
19549 NP	MATING NOT CONFIRMED							
19550 P	20.	16.						
19551 NP	MATING NOT CONFIRMED							
19552 NP	MATING NOT CONFIRMED							
19553 P	20.	18.						
19554 P	21.	18.						
19555 NP	17.	17.						
19556 NP	14.	b	21.	20.	14.	19.	18.	15.
19557	MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION)							
19558 P	23.	15.						
19559 NP	8.	b	13.	14.	14.	17.	20.	17.
19560 P	17.	24.	28.	26.	27.	27.	29.	22.
19561c	18.	13.						
19562 P	25.	21.	22.	33.	25.	28.	26.	21.
19563 P	22.	18.						
19564 P	19.	15.						

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Value not recorded.
b. Spilled feed precluded the calculation of this value.
c. Necropsy and Caesarean-section observations were not recorded for rat 19561.

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TABLE 17 (PAGE 5): MATERNAL FEED CONSUMPTION VALUES - PRESUMED GESTATION - INDIVIDUAL DATA

RAT # DOSAGE GROUP V 3.2 MG/KG/DAY

PREGNANCY STATUS	DAYS 0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13
19565 P	6.	9.	12.	10.	15.	14.	a	26.	21.	19.	21.	20.	23.
19566 P	19.	17.	17.	13.	10.	13.	14.	14.	20.	22.	21.	27.	27.
19567 P	10.	15.	17.	15.	18.	21.	20.	19.	22.	22.	21.	24.	24.
19568 P	13.	16.	17.	17.	17.	17.	a	19.	20.	19.	21.	19.	24.
19569 P	16.	14.	15.	13.	19.	17.	15.	10.	14.	18.	21.	19.	20.
19570 P	16.	15.	15.	14.	22.	22.	23.	14.	21.	19.	17.	22.	24.
19571 P	18.	15.	19.	22.	19.	18.	11.	16.	21.	22.	21.	24.	22.
19572 P	19.	18.	18.	13.	18.	17.	33.	14.	20.	18.	19.	24.	20.
19573 P	4.	8.	b	19.	22.	20.	a	15.	20.	19.	21.	20.	24.
19574 P	15.	18.	21.	23.	18.	21.	a	18.	20.	23.	24.	22.	27.
19575 P	7.	12.	b	b	19.	20.	a	13.	20.	23.	22.	22.	28.
19576 NP	MATING NOT CONFIRMED												
19577 P	13.	14.	11.	6.	2.	6.	a	b	18.	20.	23.	21.	26.
19578 NP	MATING NOT CONFIRMED												
19579 P	9.	13.	6.	30.	27.	16.	10.	17.	16.	23.	19.	16.	18.
19580 P	9.	16.	15.	13.	18.	15.	a	15.	17.	17.	21.	20.	21.

DAYS	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20	20 - 21
19565 P	18.	14.						
19566 P	20.	b						
19567 P	22.	b						
19568 P	21.	17.	20.	23.	25.	26.	24.	23.
19569 P	14.	17.	24.	22.	24.	17.	21.	11.
19570 P	21.	b						
19571 P	21.	19.	25.	27.	25.	29.	24.	23.
19572 P	18.	14.	25.	25.	31.	26.	23.	30.
19573 P	22.	16.						
19574 P	20.	19.						
19575 P	25.	20.						
19576 NP	MATING NOT CONFIRMED							
19577 P	18.	20.	23.	17.	22.	24.	23.	18.
19578 NP	MATING NOT CONFIRMED							
19579 P	17.	15.	19.	23.	24.	18.	15.	19.
19580 P	18.	15.						

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Value not recorded.
b. Spilled feed precluded the calculation of this value.

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TABLE 18 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

RATS CAESAREAN-SECTION ON DAY 15 OF PRESUMED GESTATION

RAT #	VIABLE EMBRYOS RIGHT HORN	VIABLE EMBRYOS LEFT HORN	VIABLE EMBRYOS TOTAL	NONVIABLE EMBRYOS RIGHT HORN	NONVIABLE EMBRYOS LEFT HORN	NONVIABLE EMBRYOS TOTAL	IMPLANTATION SITES RIGHT HORN	IMPLANTATION SITES LEFT HORN	IMPLANTATION SITES TOTAL	CORPORA LUTEA RIGHT OVARY	CORPORA LUTEA LEFT OVARY	CORPORA LUTEA TOTAL
DOSAGE GROUP I			0 (VEHICLE) MG/KG/DAY									
19501	5	9	14	1	0	1	6	9	15	9	10	19
19502	NOT PREGNANT											
19508	9	7	16	0	0	0	9	7	16	9	7	16
19510	5	6	11	1	1	2	6	7	13	6	7	13
19511	8	11	19	0	0	0	8	11	19	11	12	23
19513	10	6	16	0	0	0	10	6	16	10	6	16
19514	NOT PREGNANT											
19515	7	6	13	2	1	3	9	7	16	10	7	17
DOSAGE GROUP II			0.1 MG/KG/DAY									
19517	NOT PREGNANT											
19522	8	7	15	0	0	0	8	7	15	9	8	17
19525	4	2	6	0	1	1	4	3	7	7	9	16
19526	5	5	10	0	0	0	5	5	10	10	9	19
19527	8	9	17	0	1	1	8	10	18	10	10	20
19530	4	7	11	4	0	4	8	7	15	9	9	18
19531	8	7	15	1	0	1	9	7	16	9	11	20
19532	8	4	12	1	0	1	9	4	13	10	6	16
DOSAGE GROUP III			0.4 MG/KG/DAY									
19535	4	3	7	1	1	2	5	4	9	8	7	15
19537	0	3	3	1	2	3	1	5	6	4	8	12
19538	8	4	12	0	0	0	8	4	12	9	6	15
19539	7	6	13	2	0	2	9	6	15	9	9	18
19540	9	7	16	0	0	0	9	7	16	11	7	18
19542	8	8	16	0	0	0	8	8	16	9	10	19
19544	6	5	11	2	1	3	8	6	14	12	10	22
19547	5	11	16	0	0	0	5	11	16	6	12	18

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 18 (PAGE 2): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

RATS CAESAREAN-SECTION ON DAY 15 OF PRESUMED GESTATION

RAT #	VIABLE EMBRYOS RIGHT HORN	VIABLE EMBRYOS LEFT HORN	VIABLE EMBRYOS TOTAL	NONVIABLE EMBRYOS RIGHT HORN	NONVIABLE EMBRYOS LEFT HORN	NONVIABLE EMBRYOS TOTAL	IMPLANTATION SITES RIGHT HORN	IMPLANTATION SITES LEFT HORN	IMPLANTATION SITES TOTAL	CORPORA LUTEA RIGHT OVARY	CORPORA LUTEA LEFT OVARY	CORPORA LUTEA TOTAL
DOSAGE GROUP IV 1.6 MG/KG/DAY												
19550	9	5	14	0	0	0	9	5	14	9	10	19
19553	6	7	13	1	0	1	7	7	14	10	10	20
19554	9	5	14	0	1	1	9	6	15	10	7	17
19555	NOT PREGNANT											
19558	7	6	13	3	0	3	10	6	16	10	8	18
19561	NECROPSY AND CAESAREAN-SECTION OBSERVATIONS WERE NOT RECORDED											
19563	5	7	12	1	1	2	6	8	14	8	11	19
19564	7	4	11	0	1	1	7	5	12	13	13	26
DOSAGE GROUP V 3.2 MG/KG/DAY												
19565	2	4	6	0	0	0	2	4	6	6	7	13
19566	7	5	12	1	0	1	8	5	13	9	8	17
19567	8	5	13	0	0	0	8	5	13	9	8	17
19570	6	9	15	1	1	2	7	10	17	7	10	17
19573	5	9	14	0	1	1	5	10	15	5	10	15
19574	7	6	13	0	0	0	7	6	13	10	10	20
19575	7	6	13	0	4	4	7	10	17	8	11	19
19580	7	8	15	0	0	0	7	8	15	11	10	21

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 18 (PAGE 3): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF PRESUMED GESTATION

	SEX		VIABLE FETUSES			DEAD FETUSES			EARLY RESORPTIONS			LATE RESORPTIONS			IMPLANTATION SITES			CORPORA LUTEA		
RAT #	M	F	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
DOSAGE GROUP I					0 (VEHICLE) MG/KG/DAY															
19503	NO CONFIRMED MATING DATE; DELIVERED ON DAY 66 OF STUDY																			
19504	8	4	5	7	12	0	0	0	2	3	5	0	0	0	7	10	17	10	13	23
19505	8	9	4	13	17	0	0	0	0	2	2	0	0	0	4	15	19	4	16	20
19506	4	9	5	8	13	0	0	0	1	0	1	0	0	0	6	8	14	7	8	15
19507	7	7	9	5	14	0	0	0	0	1	1	0	0	0	9	6	15	13	12	25
19509	5	7	6	6	12	0	0	0	1	0	1	0	0	0	7	6	13	7	8	15
19512	7	7	4	10	14	0	0	0	0	0	0	0	0	0	4	10	14	4	11	15
19516	5	9	6	8	14	0	0	0	0	0	0	0	0	0	6	8	14	8	9	17
DOSAGE GROUP II					0.1 MG/KG/DAY															
19518	6	5	6	5	11	0	0	0	2	0	2	0	0	0	8	5	13	8	7	15
19519	9	7	9	7	16	0	0	0	0	0	0	0	0	0	9	7	16	10	7	17
19520	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED																			
19521	7	8	9	6	15	0	0	0	0	0	0	0	0	0	9	6	15	9	6	15
19523	3	7	8	2	10	0	0	0	1	1	2	1	1	2	10	4	14	10	4	14
19524	12	4	8	8	16	0	0	0	0	1	1	0	0	0	8	9	17	9	12	21
19528	3	2	1	4	5	0	0	0	1	2	3	0	0	0	2	6	8	6	9	15
19529	6	8	7	7	14	0	0	0	1	0	1	0	0	0	8	7	15	8	8	16
DOSAGE GROUP III					0.4 MG/KG/DAY															
19533	8	4	4	8	12	0	0	0	0	0	0	0	0	0	4	8	12	4	12	16
19534	8	6	7	7	14	0	0	0	0	0	0	0	0	0	7	7	14	13	15	28
19536	5	13	6	12	18	0	0	0	0	0	0	0	0	0	6	12	18	7	12	19
19541	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED																			
19543	11	6	10	7	17	0	0	0	1	1	2	0	0	0	11	8	19	11	10	21
19545	MATING NOT CONFIRMED																			
19546	4	1	4	1	5	0	0	0	1	3	4	0	0	0	5	4	9	14	11	25
19548	NOT PREGNANT																			

M = MALE F = FEMALE
PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 18 (PAGE 4): CAESAREAN-SECTIONING OBSERVATIONS - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF PRESUMED GESTATION

			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	
RAT #	M	F	HORN	TOTAL	HORN	TOTAL	HORN	TOTAL	HORN	TOTAL	HORN	TOTAL	OVARY	TOTAL
DOSAGE GROUP IV 1.6 MG/KG/DAY														
19549	MATING NOT CONFIRMED													
19551	MATING NOT CONFIRMED													
19552	MATING NOT CONFIRMED													
19556	NOT PREGNANT													
19557	MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION)													
19559	NOT PREGNANT													
19560	5	7	9	3	12	0	0	0	0	0	0	0	0	0	9	3	12	12	7	19
19562	10	10	8	12	20	0	0	0	0	2	2	0	0	0	8	14	22	8	14	22
DOSAGE GROUP V 3.2 MG/KG/DAY														
19568	6	0	6	0	6	0	0	0	0	3	3	0	0	0	6	3	9	8	11	19
19569	6	7	9	4	13	0	0	0	0	0	0	0	0	0	9	4	13	10	6	16
19571	DELIVERED ON DAY 21 OF GESTATION													
19572	6	6	7	5	12	0	0	0	1	2	3	0	0	0	8	7	15	8	9	17
19576	MATING NOT CONFIRMED													
19577	1	3	4	0	4	0	0	0	1	0	1	0	0	0	5	0	5	8	5	13
19578	MATING NOT CONFIRMED													
19579	DELIVERED ON DAY 21 OF GESTATION													

M = MALE F = FEMALE
PLACENTAE APPEARED NORMAL UNLESS NOTED OTHERWISE.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 19 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED EMBRYOS OR FETUSES) - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

RAT #	NUMBER OF LIVE EMBRYOS TOTAL	CONCEPTUSES N	NONVIABLE N	NONVIABLE %
DOSAGE GROUP I	0 (VEHICLE) MG/KG/DAY			
19501	14	15	1	6.7
19502	NOT PREGNANT			
19508	16	16	0	0.0
19510	11	13	2	15.4
19511	19	19	0	0.0
19513	16	16	0	0.0
19514	NOT PREGNANT			
19515	13	16	3	18.8
DOSAGE GROUP II	0.1 MG/KG/DAY			
19517	NOT PREGNANT			
19522	15	15	0	0.0
19525	6	7	1	14.3
19526	10	10	0	0.0
19527	17	18	1	5.6
19530	11	15	4	26.7
19531	15	16	1	6.2
19532	12	13	1	7.7
DOSAGE GROUP III	0.4 MG/KG/DAY			
19535	7	9	2	22.2
19537	3	6	3	50.0
19538	12	12	0	0.0
19539	13	15	2	13.3
19540	16	16	0	0.0
19542	16	16	0	0.0
19544	11	14	3	21.4
19547	16	16	0	0.0

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 19 (PAGE 2): LITTER OBSERVATIONS (CAESAREAN-DELIVERED EMBRYOS OR FETUSES) - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 15 OF GESTATION

RAT #	NUMBER OF LIVE EMBRYOS TOTAL	CONCEPTUSES N	NONVIABLE N	NONVIABLE %
DOSAGE GROUP IV	1.6 MG/KG/DAY			
19550	14	14	0	0.0
19553	13	14	1	7.1
19554	14	15	1	6.7
19555	NOT PREGNANT			
19558	13	16	3	18.8
19561	NECROPSY AND CAESAREAN-SECTION OBSERVATIONS WERE NOT RECORDED			
19563	12	14	2	14.3
19564	11	12	1	8.3
DOSAGE GROUP V	3.2 MG/KG/DAY			
19565	6	6	0	0.0
19566	12	13	1	7.7
19567	13	13	0	0.0
19570	15	17	2	11.8
19573	14	15	1	6.7
19574	13	13	0	0.0
19575	13	17	4	23.5
19580	15	15	0	0.0

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 19 (PAGE 3): LITTER OBSERVATIONS (CAESAREAN-DELIVERED EMBRYOS OR FETUSES) - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			---- CONCEPTUSES ------		
								RESORBED	
RAT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP I			0 (VEHICLE) MG/KG/DAY						
19503	NO CONFIRMED DATE OF MATING; DELIVERED ON DAY 66 OF STUDY								
19504	8	4	12	4.75	4.44	4.64	17	5	29.4
19505	8	9	17	5.13	5.10	5.12	19	2	10.5
19506	4	9	13	5.66	5.48	5.54	14	1	7.1
19507	7	7	14	5.47	5.21	5.34	15	1	6.7
19509	5	7	12	5.34	5.14	5.22	13	1	7.7
19512	7	7	14	5.14	4.87	5.01	14	0	0.0
19516	5	9	14	5.17	4.87	4.98	14	0	0.0
DOSAGE GROUP II			0.1 MG/KG/DAY						
19518	6	5	11	4.80	4.52	4.67	13	2	15.4
19519	9	7	16	5.29	5.16	5.23	16	0	0.0
19520	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED								
19521	7	8	15	5.12	4.79	4.94	15	0	0.0
19523	3	7	10	5.47	5.28	5.34	14	4	28.6
19524	12	4	16	5.51	5.11	5.41	17	1	5.9
19528	3	2	5	6.45	5.06	5.89	8	3	37.5
19529	6	8	14	5.87	5.54	5.68	15	1	6.7
DOSAGE GROUP III			0.4 MG/KG/DAY						
19533	8	4	12	5.30	4.89	5.16	12	0	0.0
19534	8	6	14	5.00	4.82	4.92	14	0	0.0
19536	5	13	18	5.31	5.05	5.12	18	0	0.0
19541	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED								
19543	11	6	17	5.18	4.72	5.02	19	2	10.5
19545	MATING NOT CONFIRMED								
19546	4	1	5	5.18	4.70	5.09	9	4	44.4
19548	NOT PREGNANT								

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 19 (PAGE 4): LITTER OBSERVATIONS (CAESAREAN-DELIVERED EMBRYOS OR FETUSES) - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

	NUMBER OF LIVE FETUSES			AVERAGE FETAL BODY WEIGHT (G)			---- CONCEPTUSES ------		
								RESORBED	
RAT #	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL a	N	N	%
DOSAGE GROUP IV			1.6 MG/KG/DAY						
19549	MATING NOT CONFIRMED								
19551	MATING NOT CONFIRMED								
19552	MATING NOT CONFIRMED								
19556	NOT PREGNANT								
19557	MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION)								
19559	NOT PREGNANT								
19560	5	7	12	5.26	5.06	5.14	12	0	0.0
19562	10	10	20	4.97	4.84	4.91	22	2	9.1
DOSAGE GROUP V			3.2 MG/KG/DAY						
19568	6	0	6	4.50	----	4.50	9	3	33.3
19569	6	7	13	4.84	4.50	4.66	13	0	0.0
19571	DELIVERED ON DAY 21 OF GESTATION								
19572	6	6	12	4.78	4.57	4.68	15	3	20.0
19576	MATING NOT CONFIRMED								
19577	1	3	4	5.18	5.00	5.04	5	1	20.0
19578	MATING NOT CONFIRMED								
19579	DELIVERED ON DAY 21 OF GESTATION								

a. TOTAL = SUM OF FETAL WEIGHTS/NUMBER OF LIVE FETUSES.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 20 (PAGE 1): EMBRYONAL VITAL STATUS OR FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 15 OF PRESUMED GESTATION

EMBRYO #		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DOSAGE GROUP I							0 (VEHICLE) MG/KG/DAY																	
RAT #	CLs																							
19501	9/10	E	A	A	A	A	A /	A	A	A	A	A	A	A	A	A								
19502		NOT PREGNANT																						
19508	9/ 7	A	A	A	A	A	A	A	A	A /	A	A	A	A	A	A	A							
19510	6/ 7	E	A	A	A	A	A /	A	A	A	A	A	E	A										
19511	11/12	A	A	A	A	A	A	A	A /	A	A	A	A	A	A	A	A	A	A	A				
19513	10/ 6	A	A	A	A	A	A	A	A	A	A /	A	A	A	A	A	A							
19514		NOT PREGNANT																						
19515	10/ 7	E	A	A	E	A	A	A	A	A /	A	A	E	A	A	A	A							
DOSAGE GROUP II							0.1 MG/KG/DAY																	
19517		NOT PREGNANT																						
19522	9/ 8	A	A	A	A	A	A	A	A /	A	A	A	A	A	A	A								
19525	7/ 9	A	A	A	A /	E	A	A																
19526	10/ 9	A	A	A	A	A /	A	A	A	A	A													
19527	10/10	A	A	A	A	A	A	A	A /	A	A	E	A	A	A	A	A	A	A					
19530	9/ 9	E	A	E	A	A	E	A	E /	A	A	A	A	A	A	A								
19531	9/11	A	A	A	A	A	A	A	A	E /	A	A	A	A	A	A	A							
19532	10/ 6	A	E	A	A	A	A	A	A	A /	A	A	A	A										

A = VIABLE EMBRYO E = NONVIABLE EMBRYO "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 20 (PAGE 2): EMBRYONAL VITAL STATUS OR FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 15 OF PRESUMED GESTATION

EMBRYO #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DOSAGE GROUP III 0.4 MG/KG/DAY																							
RAT # CLs																							
19535 8/ 7	A	E	A	A	A /	E	A	A	A														
19537 4/ 8	E /	E	A	A	A	E																	
19538 9/ 6	A	A	A	A	A	A	A	A /	A	A	A	A											
19539 9/ 9	A	A	A	A	E	E	A	A	A /	A	A	A	A	A	A								
19540 11/ 7	A	A	A	A	A	A	A	A	A /	A	A	A	A	A	A	A							
19542 9/10	A	A	A	A	A	A	A	A /	A	A	A	A	A	A	A	A							
19544 12/10	E	A	A	A	A	A	A	E /	A	A	A	A	E	A									
19547 6/12	A	A	A	A	A /	A	A	A	A	A	A	A	A	A	A	A							
DOSAGE GROUP IV 1.6 MG/KG/DAY																							
19550 9/10	A	A	A	A	A	A	A	A	A /	A	A	A	A	A									
19553 10/10	A	A	A	A	E	A	A /	A	A	A	A	A	A	A									
19554 10/ 7	A	A	A	A	A	A	A	A	A /	A	A	A	E	A	A								
19555	NOT PREGNANT																						
19558 10/ 8	E	E	E	A	A	A	A	A	A	A /	A	A	A	A	A	A							
19561a																							
19563 8/11	A	E	A	A	A	A /	A	A	E	A	A	A	A	A									
19564 13/13	A	A	A	A	A	A	A /	A	E	A	A	A											

A = VIABLE EMBRYO E = NONVIABLE EMBRYO "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY
a. Necropsy and Caesarean-section observations were not recorded for rat 19561.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 20 (PAGE 3): EMBRYONAL VITAL STATUS OR FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 15 OF PRESUMED GESTATION

EMBRYO #		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DOSAGE GROUP V					3.2 MG/KG/DAY																			
RAT #	CLs																							
19565	6/ 7	A	A /	A	A	A	A																	
19566	9/ 8	E	A	A	A	A	A	A	A /	A	A	A	A	A										
19567	9/ 8	A	A	A	A	A	A	A	A /	A	A	A	A	A										
19570	7/10	A	A	A	A	A	A	E /	E	A	A	A	A	A	A	A	A	A						
19573	5/10	A	A	A	A	A /	A	A	E	A	A	A	A	A	A	A								
19574	10/10	A	A	A	A	A	A	A /	A	A	A	A	A	A										
19575	8/11	A	A	A	A	A	A	A /	A	A	E	E	A	A	E	E	A	A						
19580	11/10	A	A	A	A	A	A	A /	A	A	A	A	A	A	A	A								

A = VIABLE EMBRYO E = NONVIABLE EMBRYO "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 20 (PAGE 4): EMBRYONAL VITAL STATUS OR FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF PRESUMED GESTATION

RAT #	CLs	FETUS # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DOSAGE GROUP I 0 (VEHICLE) MG/KG/DAY																								
19503		NO CONFIRMED MATING DATE; DELIVERED ON DAY 66 OF STUDY																						
19504	10/13	FA	E	MA	E	MA	MA	MA /	E	MA	E	MA	FA	MA	E	FA	MA	FA						
		4.29		4.79		4.76	4.62	4.71		5.10		4.01	4.84	5.21		3.80	4.77	4.83						
19505	4/16	FA	FA	MA	FA / MA	FA	MA	E	FA	MA	MA	FA	MA	MA	MA	E	FA	FA	FA					
		5.04	5.46	5.37	5.41	5.47	4.69	4.15		5.13	5.21	4.81	5.12	5.31	5.36	5.38	4.78	5.34	4.93					
19506	7/ 8	E	FA	MA	FA	MA	MA / FA	FA	FA	MA	FA	FA	FA	FA										
			5.53	5.93	5.66	5.31	5.43	5.40	5.68	5.64	5.95	4.67	5.87	5.22	5.70									
19507	13/12	MA	FA	MA	MA	MA	MA	FA	FA	FA /	E	FA	MA	MA	FA	FA								
		5.48	5.42	5.34	5.53	5.47	5.64	5.06	5.06	5.28		5.32	5.32	5.49	5.18	5.13								
19509	7/ 8	FA	FA	FA	FA	MA	FA	E / MA	MA	FA	FA	MA	MA											
		5.03	5.31	4.90	5.51	5.12	5.14		5.44	5.31	5.12	4.98	5.27	5.54										
19512	4/11	MA	FA	FA	FA / MA	FA	MA	MA	MA	MA	FA	FA	FA	MA										
		5.45	4.87	4.76	5.07	4.78	4.54	4.82	5.29	5.21	4.98	5.30	5.00	4.58	5.47									
19516	8/ 9	MA	FA	FA	FA	FA	FA / MA	MA	MA	FA	FA	FA	MA	FA										
		5.34	4.85	4.87	5.03	5.46	4.92	4.84	5.38	5.06	3.84	5.13	4.91	5.23	4.85									
DOSAGE GROUP II 0.1 MG/KG/DAY																								
19518	8/ 7	FA	E	MA	FA	FA	E	FA	FA / MA	MA	MA	MA	MA											
		4.21		4.70	4.78	4.52		4.53	4.56	4.76	4.70	4.85	4.72	5.07										
19519	10/ 7	FA	MA	FA	FA	MA	FA	FA	MA	MA / FA		MA	MA	MA	FA	MA	MA							
		4.99	5.31	5.48	5.14	5.63	5.06	4.63	5.31	5.83	5.59	5.16	4.98	4.88	5.22	5.00	5.51							
19520		MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED																						
19521	9/ 6	FA	FA	FA	MA	FA	FA	MA	MA	MA / FA		FA	MA	FA	MA	MA								
		4.89	4.74	4.62	4.66	4.90	4.81	4.92	4.92	5.28	4.49	4.86	5.20	4.98	5.45	5.42								
19523	10/ 4	E	FA	L	FA	FA	FA	FA	FA	MA	MA / MA		E	FA	L									
			4.80		5.52	5.42	5.23	5.57	4.81	5.58	5.32	5.52		5.62										
19524	9/12	FA	MA	MA	MA	MA	FA	MA	MA / MA		MA	E	MA	MA	MA	FA	MA	FA						
		5.16	5.37	5.51	5.64	5.54	5.11	5.70	5.55	5.00	5.49		5.73	5.32	5.56	5.16	5.72	5.00						
19528	6/ 9	E	MA / MA		E	E	FA	MA	FA															
			6.86	6.59			4.83	5.89	5.30															
19529	8/ 8	FA	MA	FA	MA	E	FA	FA	MA / FA		MA	FA	MA	FA	MA	FA								
		5.13	5.87	5.63	5.97		5.54	5.64	5.19	5.68	6.11	5.27	6.25	5.71	5.81	5.74								

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).

Caesarean-Sectioning Observations - Day 21 Presumed Gestation:

All remaining female rats in each dosage group will be Caesarean-sectioned on day 21 of presumed gestation. The gravid uterus will be excised and weighed. Individual placental weights will be recorded. The fetuses will be removed from the uterus, weighed and placed in individual containers. The female rats (pregnant dams) 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 defined as an early resorption.)

Fetal Observations:

Caps and labeled tubes will be weighed (combined weight, to the nearest .001 gram) before and after retention of pooled fetal samples for subsequent use in pharmacokinetic analyses. These weights will be documented in the raw data, and copies of these weights will be included with the packing list prior to shipment.

Placental Samples and Amniotic Fluid:

Samples of the amniotic fluid (when possible) and the placenta of each fetus will be collected, individually pooled (per litter), frozen and stored (-70°C or below) until shipment to the Sponsor for analysis.

Gross External Alterations, Sex, Body Weights and Identification:

Fetuses will be examined for sex and for gross external alterations. Late resorptions and dead fetuses will be examined for gross external alterations to the extent possible. The individual body weight of each fetus will be recorded. Only body weights of live fetuses will be used to determine litter fetal body weight averages. Representative photographs of fetal gross external alterations will be taken.

Blood Samples:

Blood samples will be collected from each pup via decapitation, pooled (per litter) and transferred into serum separator tubes. The samples will be spun in a refrigerated centrifuge. The serum will be transferred into polypropylene tubes labeled with the study number, animal identification, date of collection, study day and collection timepoint. All samples will be immediately frozen on dry ice and maintained frozen (-70°C or below) until shipment to the Sponsor for analysis.

Liver Samples:

The liver of each fetus will be collected, pooled (per litter), frozen and stored (-70°C or below) until shipment to the Sponsor for analysis.

Fetal Carcasses:

The remaining carcass (and head) of each fetus will be collected, frozen and stored (-70°C or below) until shipment to the Sponsor for analysis.

Shipping Instructions:

All samples will be maintained frozen (-70°C or below) until shipment for analysis. Samples will be shipped frozen on dry ice via overnight mail. A packing list will be included with the samples and sent to Kris J. Hansen, Ph.D., at the previously cited address. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

METHOD OF SACRIFICE:

Rats will be sacrificed by carbon dioxide asphyxiation. Live fetuses will be sacrificed via decapitation.

NECROPSY:

Gross lesions will be retained in neutral buffered 10% formalin for possible future evaluation (a table of random units will be used to select one control group rat from which all tissues examined at necropsy will be retained, in order to provide control tissues for any possible histopathological evaluations of gross lesions). Unless specifically cited below, all other tissues will be discarded.

Scheduled Sacrifice:

On either day 15 of presumed gestation or day 21 of presumed gestation, following the final collection interval for urine and fecal samples and blood sample collection, female rats will be sacrificed, and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. The number and distribution of implantation sites will be recorded. A liver section (right lateral lobe) and the milk-secreting glands from the axillary, thoracic, abdominal and inguinal regions of each dam (left side only) will be collected, frozen and stored (-70°C or below) until shipment to the Sponsor for analysis. Uteri of apparently nonpregnant rats will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5].

Rats Found Dead or Moribund:

Rats 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. The rats will be examined for gross lesions. A liver section (right lateral lobe) and the milk-secreting glands from the axillary, thoracic, abdominal and inguinal regions of each dam (left side only) will be collected, frozen and stored (-70°C or below) until shipment to the Sponsor for analysis. Pregnancy status and uterine contents of female rats will be recorded. Aborted fetuses and/or delivered pups will be examined to the extent possible, using the same methods described for term fetuses. Uteri of apparently nonpregnant rats will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5].

Shipping Instructions:

All samples will be maintained frozen (-70°C or below) until shipment for analysis. Samples will be shipped frozen on dry ice via overnight mail. A packing list will be included with the samples and sent to Kris J. Hansen, Ph.D., at the previously cited address. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

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.
Mating History.
Treatment (if prescribed by Staff Veterinarian).
General Comments.
Clinical Observations and/or General Appearance.
Tissue and Sample Collection, Processing and Shipment.
Cap and Labeled Tube Weights.
Body Weights.
Feed Consumption Values.
Caesarean-Sectioning and Fetal Observations.
Gross Necropsy Observations.
Organ Weights.
Photographs (if required).
Study Maintenance (room and environmental records).
Feed, Water and Bedding 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.
Director of Study Management: Valerie A. Sharper, M.S.
Manager of Animal Operations and Member, Institutional Animal Care and Use Committee: Dena C. Lebo, V.M.D.
Director of Operations and Compliance: Barbara J. Patterson, B.A.
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. and Voytek, P.E. (1982). *In Vivo Reproductive and Mutagenicity Tests*. Environmental Protection Agency, Washington, D.C. National Technical Information Service, U.S. Department of Commerce, Springfield, VA 22161.

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. Lang, P.L. (1988). *Embryo and Fetal Developmental Toxicity (Teratology) Control Data in the Charles River Crl:CD®BR Rat*. Charles River Laboratories, Inc., Wilmington, MA 01887-0630. (Data base provided by Argus Research Laboratories, Inc.)

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

09-NOV-98

Alan M. Hoberman, Ph.D., DABT
Director of Research

Date

09-NOV-98

Raymond G. York, Ph.D., DABT
Associate Director of Research
Study Director

Date

09-NOV-98

Barbara J. Patterson, B.A.
Chairperson, Institutional Animal Care and Use Committee

Date

FOR THE SPONSOR

10 Nov 98

Marvin T. Case, D.V.M., Ph.D.
Study Monitor

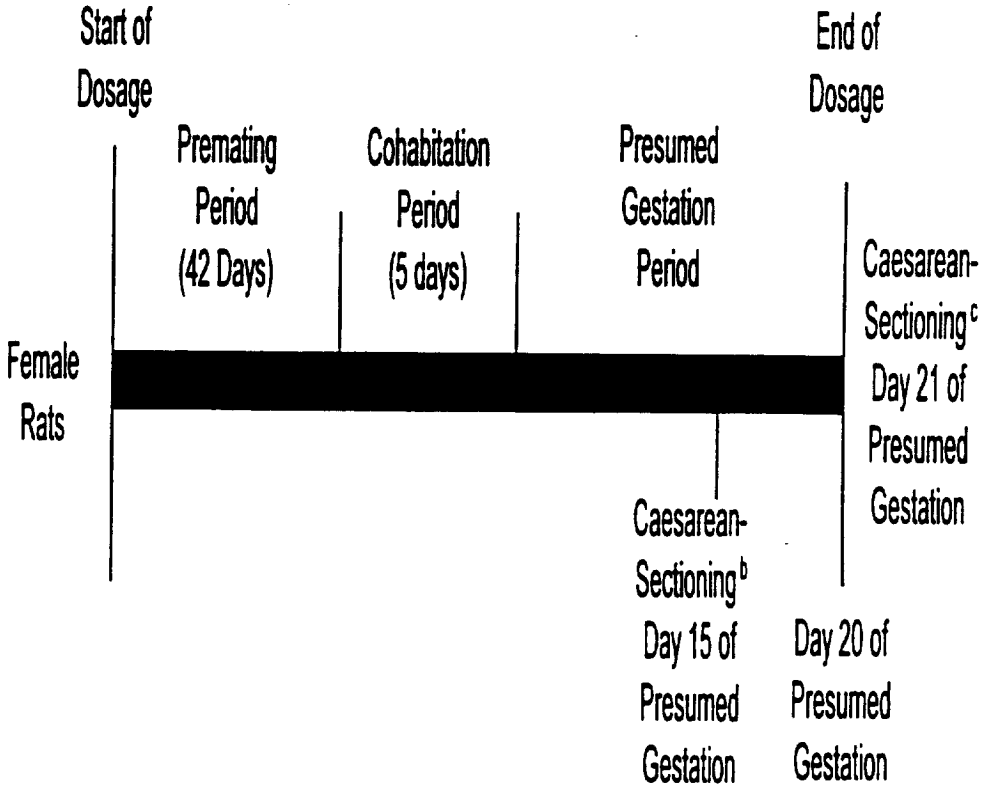
Date

ATTACHMENT 1

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE

STUDY SCHEMATIC

PHARMACOKINETIC STUDY[a]



Dosage Period.

a. For additional details see "Tests, Analyses and Measurements" section of the protocol.

b. Evaluation of corpora lutea, implantation sites and viable and nonviable embryos.

c. Fetal evaluations (all fetuses - external examinations).

SCHEDULE[a]

10 NOV 98	Animals Arrive - Acclimation Begins.
16 NOV 98 - 21 JAN 99	Dosage Period - Female Rats (42 days prior to cohabitation until day 14 or 20 of presumed gestation).
27 DEC 98 PM - 01 JAN 99 AM	Cohabitation Period.
28 DEC 98 - 01 JAN 99	Day 0 of Presumed Gestation.
12 JAN 99 - 16 JAN 99	Caesarean-Sectioning Period (Day 15 of presumed gestation).
18 JAN 99 - 22 JAN 99	Caesarean-Sectioning Period (Day 21 of presumed gestation).
26 MAY 99	Draft Final Report.

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a. The study initiation date is the day 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)

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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.

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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. 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 rats on Argus Study 418-013.

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 0.64 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 0.32 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 0.08 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.

ATTACHMENT 3

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: _____

Approved by: _____ Date: 09-NOV-98

Clarification: X No ___ Yes (See attached clarification form.)

Initials/Date : MC 2-2-99

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

PROTOCOL 418-013

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

SPONSOR'S STUDY NUMBER: T-6295.12

Amendment 1 – 6 January 1999

1. Caesarean-Sectioning Observations - Day 21 Presumed Gestation (pages 12 and 13 of the protocol) and Necropsy (page 14 of the protocol):

At Caesarean-sectioning on day 21 of presumed gestation, lung and liver samples will be collected from three fetuses per litter from five litters per dosage group. These collections will occur after weighing and examination for sex and gross external alterations.

One half of the lungs and one lateral lobe of the liver will be individually retained in neutral buffered 10% formalin in scintillation vials. Prior to fixation, several (minimum of two) sections (approximately 1 mm thick) of this half of the lungs and this lobe of the liver will be removed using a scalpel and will be retained in McDowell-Trump's Fixative (stored under refrigeration in scintillation vials) for future evaluation. The other half of the lungs and the other lateral lobe of the liver will be flash frozen in liquid nitrogen and stored in heat-sealable pouches on dry ice. The procedure for collecting and flash freezing these tissues will be available in the raw data. The liver of each of the remaining fetuses, plus any remaining liver from the three selected fetuses, will be collected, pooled (per litter), frozen and stored (-70°C or below) until shipment to the Sponsor for analysis as described on page 13 of the protocol.

The lung and liver sections in McDowell-Trump's Fixative and the lung and liver samples in neutral buffered formalin will be shipped (on cold packs and ambient conditions, respectively), for possible future evaluation by electron microscopy or light microscopy, respectively, to:

Jeanne deWard
Pathology Associates International
4915 D Prospectus Drive
Durham, North Carolina 27713
Telephone: (919) 544-5257
Telefax: (919) 544-3218

The frozen samples will be shipped (on dry ice) to Kris J. Hansen, Ph.D., at the address cited in the protocol for possible future biochemical evaluation.

All recipients will be notified in advance of sample shipment.

Reason for Change:

The Sponsor has requested that these additional samples be retained for continued evaluation of the reasons for reduced pup survival observed in previous studies.

6-JAN-99	06-JAN-99
Alan M. Hoberman, Ph.D., DABT Director of Research	Raymond G. York, Ph.D., DABT Associate Director of Research Study Director
Date	Date
6 Jan 99	06 Jan 99
Dena C. Lebo, V.M.D. Chairperson, Institutional Animal Care and Use Committee	Marvin T. Case, D.V.M., Ph.D. Study Monitor
Date	Date

APPENDIX D

DEVIATIONS FROM THE PROTOCOL AND THE STANDARD OPERATING PROCEDURES OF THE TESTING FACILITY

DEVIATION FROM THE PROTOCOL AND THE STANDARD OPERATING PROCEDURES OF THE TESTING FACILITY

1. Eighty virgin female rats were randomly assigned to the study and received the test article for forty-two days prior to cohabitation, rather than 120 virgin females assigned to study, dosed, mated and then assigned as the population for the study.

   Female rats with confirmed date of mating were randomly selected for gestation day 15 (DG 15) Caesarean-sectioning. All remaining rats (with and without confirmed dates of mating) were assigned to DG 21 Caesarean-sectioning. Rats with no confirmed date of mating were Caesarean-sectioned on their estimated DG 21.

   These deviations did not adversely affect the outcome or interpreation of the study because sufficient rats were available to collect the pharmacokinetic data.

   All deviations are documented in the raw data.

Raymond G. York 21-JUN 99

Raymond G. York, Ph.D., DABT Date
Associate Director of Research
and Study Director

APPENDIX E

TEMPERATURE AND RELATIVE HUMIDITY REPORTS

ARGUS

Temperature and Relative Humidity Report
Location: Room 04

Protocol Number: 418-013

Range of Dates: 10-Nov-1998 15:00 to 23-Nov-1998 15:49

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: Rat				
Total Number of Days:	14		14	
Total Number of Hours:	312.75		312.75	
Total Number of Data Points:	312		312	
Mean (± SD):	69.1	(± 2.5)	58.5	(± 1.7)
Maximum:	76.4		64.4	
Median:	68.3		58.6	
Minimum:	66.8		53.6	
Number of Points in Range (%):	312	(100.0)	312	(100.0)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 03-Feb-1999 at 13:44

COMMENTS: _____

REVIEWED BY: Joseph Leach **DATE:** 2/3/99

ARGUS

Temperature and Relative Humidity Report
Location: Room 27

Protocol Number: 418-013

Range of Dates: 23-Nov-1998 15:49 to 22-Jan-1999 16:00

	Temperature 64°F to 79°F		Relative Humidity 30% to 70%	
Target Range:				
Species: Rat				
Total Number of Days:	61		61	
Total Number of Hours:	1439.74		1439.74	
Total Number of Data Points:	1442		1442	
Mean (± SD):	70.7	(± 0.7)	54.1	(± 8.2)
Maximum:	72.6		69.8	
Median:	70.8		56.1	
Minimum:	69.0		14.9	
Number of Points in Range (%):	1442	(100.0)	1418	(98.3)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	24	(1.7)

Report Generated: 03-Feb-1999 at 13:48

COMMENTS: _____

REVIEWED BY: _____ **DATE:** 2/3/99

ARGUS

Relative Humidity Deviations Report
Location: Room 27

Protocol Number: 418-013

Range of Dates: 23-Nov-1998 15:49 to 22-Jan-1999 16:00

Humidity Target Range: 30% to 70%
Species: Rat

Date	Time	R.H.
04-Jan-1999	16:00	29.1 L
05-Jan-1999	23:00	26.1 L
06-Jan-1999	00:00	23.7 L
06-Jan-1999	01:00	23.3 L
06-Jan-1999	02:00	20.1 L
06-Jan-1999	03:00	18.4 L
06-Jan-1999	04:00	17.6 L
06-Jan-1999	05:00	16.9 L
06-Jan-1999	06:00	14.9 L
06-Jan-1999	07:00	27.0 L
11-Jan-1999	11:00	27.9 L
11-Jan-1999	12:00	29.1 L
11-Jan-1999	13:00	26.1 L
11-Jan-1999	14:00	28.6 L
11-Jan-1999	15:00	26.9 L
11-Jan-1999	16:00	28.7 L
14-Jan-1999	00:00	29.6 L
14-Jan-1999	02:00	28.6 L
14-Jan-1999	03:00	27.8 L
14-Jan-1999	04:00	27.5 L
14-Jan-1999	05:00	26.3 L
14-Jan-1999	06:00	27.0 L
14-Jan-1999	07:00	26.3 L
14-Jan-1999	08:00	25.8 L

H = Value out of range - High L = Value out of range - Low
R.H. = Relative Humidity (%)

Report Generated: 03-Feb-1999 at 13:57

✓ These deviations did not adversely affect the outcome or interpretation of the study.

_____ The following deviation(s) impacted on the outcome of the study as described:

Study Director: [signature] Date: 17-Feb-99

APPENDIX F

HISTORICAL CONTROL DATA

SUMMARY OF REPRODUCTIVE INDICES
CD RAT

PERIOD	JUNE 1996 - JUNE 1998	
NUMBER OF STUDIES		105
NUMBER OF RATS:		
	TESTED	2233
	PREGNANT	2065
	FOUND DEAD	4
	ABORTED	0
	DELIVERED	0
NUMBER OF RATS PREGNANT AT CAESAREAN-SECTIONING		2052
NUMBER OF RATS WITH SINGLE CONCEPTUS LITTER:		
	LIVE	6
	RESORBED	1
	ABORTED	0

	MEAN or %	RANGE/STUDY MEAN or %
% PREGNANT	92.8	(64.0-100)
AVERAGE # CORPORA LUTEA	16.9	(14.0-21.0)
AVERAGE # IMPLANTATIONS	15.3	(12.9-18.0)
AVERAGE LITTER SIZE		
AVERAGE # LIVE FETUSES	14.5	(11.8-16.9)
AVERAGE # DEAD FETUSES	0.1	(0-1.4)
AVERAGE # RESORPTIONS	0.7	(0-2.0)
AVERAGE # EARLY RESORPTIONS	0.7	(0-1.9)
AVERAGE # LATE RESORPTIONS	0.0	(0-0.1)

SUMMARY OF REPRODUCTIVE INDICES
CD RAT

	MEAN or %	RANGE/STUDY MEAN or %
AVERAGE % DAMS WITH ANY RESORPTIONS	46.1	(0-87.5)
AVERAGE % DAMS WITH ALL CONCEPTUSES RESORBED	0.1	(0-4.5)
AVERAGE % DAMS WITH ONE OR MORE LIVE FETUSES	99.8	(95.4-100)
AVERAGE SEX RATIO, (% MALES/LITTER)	50.2	(42.8-57.0)
AVERAGE FETAL BODY WEIGHT (G)	3.48	(3.10-3.78)
AVERAGE FOR MALES (G)	3.58	(3.17-3.90)
AVERAGE FOR FEMALES (G)	3.39	(2.98-3.67)
AVERAGE % DEAD OR RESORBED CONCEPTUSES/LITTER	4.7	(0-12.4)

SUMMARY OF MATERNAL NECROPSY OBSERVATIONS
CD RAT

PERIOD	JUNE 1996 - JUNE 1998
# STUDIES	124
# RATS TESTED	2668
# RATS PREGNANT	2480*
# RATS DIED	8**
# RATS ABORTED	0
# RATS DELIVERED	431
# RATS WITH 100% RESORPTION	3

EXTERNAL OBSERVATIONS	N	MEAN %	RANGE N	/STUDY %
Red substance around nose and/or mouth	2	0.07	0-1	(0-12.5)
White substance present in anterior chamber of eyes	1	0.04	0-1	(0-4.2)
Red perivaginal substance	1	0.04	0-1	(0-4.0)
GROSS LESIONS				
ESOPHAGUS				
Torn(attributed to an intubation accident)	1	0.04	0-1	(0-4.0)
LUNGS				
Dark red (attributed to an intubation accident)	1	0.04	0-1	(0-4.0)
THORACIC CAVITY				
Filled with light red or cloudy red fluid	2	0.07	0-1	(0-4.0)
THYMUS				
Large	1	0.04	0-1	(0-4.0)
AXILLA				
Mass present	1	0.04	0-1	(0-4.0)
Lymph nodes enlarged on left side	1	0.04	0-1	(0-4.2)

* Pregnancy status for one dam could not be determined
** One was a moribund sacrifice and one was attributed to an intubation accident

SUMMARY OF MATERNAL NECROPSY OBSERVATIONS
CD RAT

GROSS LESIONS		MEAN	RANGE /STUDY	
	N	%	N	%
LIVER				
Adhesion between left lateral lobe and left lateral abdominal wall	1	0.04	0-1	(0-4.0)
Right lateral and median lobes protruded through diaphragm	1	0.04	0-1	(0-4.0)
STOMACH/INTESTINE				
Stomach and intestines distended with gas	1	0.04	0-1	(0-4.0)
Cecum contained a black substance	1	0.04	0-1	(0-4.0)
Stomach contained a dark red, granular substance	1	0.04	0-1	(0-4.2)
BACK				
Spine, protrusion of bone on dorsal thoracic portion	1	0.04	0-1	(0-4.0)
KIDNEY(S)				
Pelvis, slight/moderate dilation with or without fluid	16	0.60	0-2	(0-12.5)
Pelvis, marked/extreme dilation	7	0.26	0-1	(0-4.0)
Mottled	1	0.04	0-1	(0-4.0)
Large	2	0.07	0-1	(0-4.0)
Small	1	0.04	0-1	(0-4.0)
Cortex pitted	1	0.04	0-1	(0-3.4)
Left, pelvis contained yellow fluid	1	0.04	0-1	(0-4.0)
Bilateral, pelvis contained numerous calculi	1	0.04	0-1	(0-4.0)
Right, indentation	1	0.04	0-1	(0-4.0)
Pale	1	0.04	0-1	(0-12.5)
Left, small; right, enlarged cortex with numerous pinpoint yellow areas and viscous yellow fluid in pelvis	1	0.04	0-1	(0-4.0)
ADRENAL GLAND(S)				
Large	1	0.04	0-1	(0-4.0)

SUMMARY OF MATERNAL NECROPSY OBSERVATIONS
CD RAT

GROSS LESIONS		N	MEAN %	RANGE /STUDY N	%
ABDOMINAL CAVITY					
	Filled with light red fluid	1	0.04	0-1	(0-4.0)
SPLEEN					
	Two white areas on serosal surface	1	0.04	0-1	(0-4.0)
	Large	2	0.07	0-2	(0-8.0)
BLADDER					
	Wall thick and contained one calculus	1	0.04	0-1	(0-4.0)
URETERS					
	Distended with clear fluid	2	0.07	0-1	(0-4.0)
VAGINA/CERVIX					
	Cervix contained a dark red, gelatinous substance	1	0.04	0-1	(0-4.0)
	Cervix contained green, viscous fluid	1	0.04	0-1	(0-4.0)
	Vagina contained brown, viscous fluid	1	0.04	0-1	(0-2.1)
UTERUS					
	Contained red-brown fluid	1	0.04	0-1	(0-4.0)
	Right horn, absent	1	0.04	0-1	(0-4.0)
	Right horn, threadlike	1	0.04	0-1	(0-2.1)
	Left horn, lumen absent on cervical end; ovarian end, distended with clear fluid	1	0.04	0-1	(0-4.0)
	Right horn, clear masses containing gelatinous substance present	1	0.04	0-1	(0-4.0)
	Portion of left uterine horn prolapsed	1	0.04	0-1	(0-4.0)
OVARIES					
	Right contained a red, fluid-filled bursal cyst	1	0.04	0-1	(0-4.0)

SUMMARY OF FETAL GROSS EXTERNAL ALTERATIONS
CD RAT

PERIOD JUNE 1996 - JUNE 1998

# STUDIES INCLUDED	84
# LITTERS EXAMINED	1652
# LIVE FETUSES EXAMINED	23689

ALTERATION			N	%	RANGE N	/STUDY %
HEAD						
	Exencephaly	L	4	0.24	0-1	(0-4.2)
		F	4	0.02	0-1	(0-0.3)
	Hematoma	L	1	0.06	0-1	(0-2.2)
		F	1	0.00	0-1	(0-0.2)
	Microcephaly	L	1	0.06	0-1	(0-4.0)
		F	1	0.00	0-1	(0-0.3)
EYE(S)						
	Eye bulges depressed	L	9	0.54	0-2	(0-12.5)
		F	9	0.04	0-2	(0-0.9)
	Eyelids open	L	2	0.12	0-1	(0-4.2)
		F	2	0.01	0-1	(0-0.3)
	Microphthalmia	L	1	0.06	0-1	(0-4.0)
		F	1	0.00	0-1	(0-0.3)
EARS						
	Low set	L	1	0.06	0-1	(0-12.5)
		F	1	0.00	0-1	(0-0.9)
SNOUT						
	Short	L	2	0.12	0-1	(0-4.2)
		F	2	0.01	0-1	(0-0.3)
TONGUE						
	Absent	L	1	0.06	0-1	(0-4.0)
		F	1	0.00	0-1	(0-0.3)
	Protrudes	L	1	0.06	0-1	(0-4.2)
		F	1	0.00	0-1	(0-0.3)
	Fused to lower margin of oral opening	L	1	0.06	0-1	(0-4.8)
		F	1	0.00	0-1	(0-0.4)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL GROSS EXTERNAL ALTERATIONS
CD RAT

ALTERATION			N	%	RANGE N	/STUDY %
JAWS						
	Micrognathia	L	2	0.12	0-1	(0-4.5)
		F	2	0.01	0-1	(0-0.3)
	Agnathia	L	1	0.06	0-1	(0-12.5)
		F	1	0.00	0-1	(0-0.9)
	Cleft	L	1	0.06	0-1	(0-4.8)
		F	1	0.00	0-1	(0-0.4)
BODY						
	Edema	L	2	0.12	0-1	(0-4.3)
		F	2	0.01	0-1	(0-0.3)
	Umbilical hernia	L	5	0.30	0-1	(0-4.3)
		F	5	0.02	0-1	(0-0.3)
	Spina bifida	L	1	0.06	0-1	(0-4.2)
		F	1	0.00	0-1	(0-0.3)
	Hematoma	L	2	0.12	0-1	(0-4.0)
		F	2	0.01	0-1	(0-0.3)
	Conjoined twin	L	1	0.06	0-1	(0-4.0)
		F	1	0.00	0-1	(0-0.3)
HINDLIMBS						
	Third limb present	L	1	0.06	0-1	(0-14.3)
		F	1	0.00	0-1	(0-0.9)
ANUS						
	No opening present	L	1	0.06	0-1	(0-2.6)
		F	1	0.00	0-1	(0-0.2)
TAIL						
	Threadlike	L	5	0.30	0-1	(0-12.5)
		F	5	0.02	0-1	(0-0.9)
	Agenesis	L	2	0.12	0-1	(0-4.5)
		F	2	0.01	0-1	(0-0.3)
	Short	L	1	0.06	0-1	(0-2.4)
		F	1	0.00	0-1	(0-0.2)
	Constricted	L	1	0.06	0-1	(0-2.4)
		F	1	0.00	0-1	(0-0.2)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SOFT TISSUE ALTERATIONS
CD RAT

PERIOD	JUNE 1996 - JUNE 1998
# STUDIES INCLUDED	36
# LITTERS EXAMINED	830
# FETUSES EXAMINED	5753

	ALTERATION		N	%	RANGE/STUDY N	RANGE/STUDY %
BRAIN						
	Lateral ventricles, moderate dilation	L	1	0.12	0-1	(0-4.3)
		F	1	0.02	0-1	(0-0.6)
	Lateral ventricles, marked dilation	L	2	0.24	0-1	(0-4.3)
		F	2	0.03	0-1	(0-0.6)
	Third ventricle, marked dilation	L	1	0.12	0-1	(0-4.2)
		F	1	0.02	0-1	(0-0.6)
	Lateral and third ventricles, irregularly shaped	L	1	0.12	0-1	(0-4.2)
		F	1	0.02	0-1	(0-0.6)
	Irregularly shaped	L	1	0.12	0-1	(0-4.2)
		F	1	0.02	0-1	(0-0.6)
EYES						
	Microphthalmia	L	3	0.36	0-1	(0-4.3)
		F	3	0.05	0-1	(0-0.7)
TONGUE						
	Absent	L	1	0.12	0-1	(0-4.0)
		F	1	0.02	0-1	(0-0.6)
JAW						
	Micrognathia	L	1	0.12	0-1	(0-4.0)
		F	1	0.02	0-1	(0-0.6)
HEART						
	Septal defect	L	1	0.12	0-1	(0-4.0)
		F	1	0.02	0-1	(0-0.6)
VESSELS						
	Innominate, absent	L	6	0.72	0-2	(0-8.3)
		F	6	0.10	0-2	(0-1.2)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SOFT TISSUE ALTERATIONS
CD RAT

ALTERATION		N	%	RANGE/STUDY N	RANGE/STUDY %
VESSELS (CONT.)					
Innominate, arises on left	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
Umbilical artery, descends to left of urinary bladder	L	15	1.81	0-2	(0-8.7)
	F	16	0.28	0-2	(0-1.4)
Situs inversus	L	1	0.12	0-1	(0-4.2)
	F	1	0.02	0-1	(0-0.6)
Aorta, descends to right	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
Pulmonary artery, descends to right behind aorta	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
LUNGS					
Right apical, cardiac and diaphragmatic lobes appear as one	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
Intermediate lobe, absent	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
BODY					
Edema	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
ABDOMINAL CAVITY					
Situs inversus of liver, intestines, stomach, spleen, pancreas and kidneys	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
KIDNEY(S)					
Pelvis, slight dilation	L	2	0.24	0-2	(0-8.7)
	F	2	0.03	0-2	(0-1.3)
Pelvis, moderate dilation	L	1	0.12	0-1	(0-4.3)
	F	1	0.02	0-1	(0-0.6)
ADRENAL(S)					
Dark red	L	2	0.24	0-1	(0-4.3)
	F	2	0.03	0-1	(0-0.6)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SKELETAL ALTERATIONS
CD RAT

PERIOD	JUNE 1996 - JUNE 1998
# STUDIES INCLUDED	36
# LITTERS EXAMINED	816
# FETUSES EXAMINED	6151

ALTERATION		N	%	RANGE/STUDY N	RANGE/STUDY %
SKULL					
Frontal(s): incompletely or not ossified	L	2	0.24	0-1	(0-4.2)
	F	2	0.03	0-1	(0-0.6)
Parietal(s): incompletely or not ossified	L	2	0.24	0-1	(0-4.2)
	F	2	0.03	0-1	(0-0.6)
Nasal(s): short	L	3	0.37	0-1	(0-4.2)
	F	3	0.05	0-1	(0-0.6)
Sphenoid: incompletely ossified	L	1	0.12	0-1	(0-4.2)
	F	1	0.02	0-1	(0-0.6)
Maxillae and Premaxillae: short	L	3	0.37	0-1	(0-4.2)
	F	3	0.05	0-1	(0-0.6)
Skull: incompletely or not ossified	L	1	0.12	0-1	(0-4.2)
	F	2	0.03	0-2	(0-1.2)
VERTEBRAE					
Thoracic: Centrum, bifid	L	67	8.21	0-5	(0-20.8)
	F	74	1.20	0-6	(0-3.4)
: Centra, unilateral ossification	L	6	0.74	0-2	(0-8.0)
	F	6	0.10	0-2	(0-1.0)
: Centrum, incompletely or not ossified	L	3	0.37	0-2	(0-8.3)
	F	4	0.06	0-3	(0-1.8)
: Centra, fused	L	1	0.12	0-1	(0-4.2)
	F	1	0.02	0-1	(0-0.5)
: Arch, small	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
Lumbar: Centrum, bifid	L	1	0.12	0-1	(0-4.3)
	F	1	0.02	0-1	(0-0.6)
: Centrum, incompletely or not ossified	L	2	0.24	0-2	(0-8.3)
	F	3	0.05	0-3	(0-1.8)
: Arches, incompletely or not ossified	L	12	1.47	0-3	(0-12.0)
	F	17	0.28	0-4	(0-2.1)
: Centra, unilateral ossification	L	2	0.24	0-1	(0-4.2)
	F	2	0.03	0-1	(0-0.6)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL SKELETAL ALTERATIONS
CD RAT

ALTERATION		N	%	RANGE/STUDY N	%
RIBS					
Cervical Rib(s) present	L	23	2.82	0-4	(0-17.4)
	F	26	0.42	0-5	(0-2.9)
One or more, wavy	L	32	3.92	0-4	(0-33.3)
	F	52	0.84	0-8	(0-5.1)
One or more, incompletely ossified (hypoplastic), or not ossified	L	22	2.70	0-2	(0-9.1)
	F	36	0.58	0-5	(0-2.6)
Extra ribs, 14th, unilateral	L	4	0.49	0-4	(0-16.0)
	F	4	0.06	0-4	(0-2.2)
Extra ribs, 14th, bilateral	L	1	0.12	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)
STERNEBRAE					
One or more incompletely ossified or not ossified	L	109	13.36	0-9	(0-36.0)
	F	150	2.44	0-12	(0-6.7)
Fused	L	1	0.12	0-1	(0-4.2)
	F	1	0.02	0-1	(0-0.6)
Asymmetric	L	1	0.12	0-1	(0-4.2)
	F	1	0.02	0-1	(0-0.5)
SCAPULAE					
Bent	L	1	0.12	0-1	(0-4.2)
	F	1	0.02	0-1	(0-0.5)
PELVIS					
Pubis(es) and/or Ischium(a): incompletely or not ossified	L	116	14.22	0-7	(0-30.4)
	F	180	2.93	0-16	(0-9.5)
Pubis(es): incompletely ossified	L	96	11.76	0-7	(0-30.4)
	F	147	2.39	0-16	(0-9.5)
Pubis(es): not ossified	L	3	0.37	0-1	(0-4.2)
	F	3	0.05	0-1	(0-0.6)
Ischium(a): incompletely or not ossified	L	39	4.78	0-4	(0-16.7)
	F	58	0.94	0-9	(0-4.7)
FORELIMB(S)					
Radius and Ulna: Bent	L	1	0.12	0-1	(0-4.2)
	F	1	0.02	0-1	(0-0.6)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

SUMMARY OF FETAL OSSIFICATION SITES
SKELETAL AVERAGES
CD RAT
(CAESAREAN-SECTIONED DAY 20 GESTATION)

PERIOD:	JUNE 1996 - JUNE 1998
# STUDIES INCLUDED	35
# LITTERS EXAMINED	793
# FETUSES EXAMINED	5961

SKELETAL AVERAGES	FETUS/LITTER MEAN	RANGE/STUDY
HYOID	0.86	(0.69-0.98)
VERTEBRAE		
CERVICAL	7.00	–
THORACIC	13.04	(13.00-13.15)
LUMBAR	5.95	(5.85-5.99)
SACRAL	3.00	(2.96-3.01)
CAUDAL	4.82	(4.35-5.16)
RIBS (pairs)	13.03	(13.00-13.09)
STERNUM		
MANUBRIUM	1.00	(0.98-1.01)
STERNAL CENTERS	3.61	(3.26-3.75)
XIPHOID	0.99	(0.94-1.00)
FOREPAWS (Calculated as average per limb)		
CARPALS	0.00	–
METACARPALS	3.54	(3.33-3.74)
DIGITS	5.00	–
PHALANGES	5.04	(4.90-5.27)
HINDPAWS (Calculated as average per limb)		
TARSALS	0.00	–
METATARSALS	3.99	(3.93-4.03)
DIGITS	5.00	–
PHALANGES	4.98	(4.82-5.08)

FETAL OSSIFICATION SITES
SKELETAL AVERAGES
CD RAT
(CAESAREAN-SECTIONED DAY 21 GESTATION)

STUDY #	580
# LITTERS EXAMINED	23
# FETUSES EXAMINED	190
SKELETAL AVERAGES	
HYOID	0.96
VERTEBRAE	
CERVICAL	7.00
THORACIC	13.06
LUMBAR	5.94
SACRAL	3.00
CAUDAL	7.23
RIBS (pairs)	13.05
STERNUM	
MANUBRIUM	1.00
STERNAL CENTERS	3.97
XIPHOID	1.00
FOREPAWS (Calculated as average per limb)	
CARPALS	0.00
METACARPALS	3.99
DIGITS	5.00
PHALANGES	7.53
HINDPAWS (Calculated as average per limb)	
TARSALS	0.02
METATARSALS	4.62
DIGITS	5.00
PHALANGES	5.78

APPENDIX G

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 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS
SPONSOR'S STUDY NUMBER: T-6295.12

STATEMENT OF THE STUDY DIRECTOR

This final report accurately reflects the raw data obtained during the performance of the study. No 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.

Raymond G. York 24-Jun-99

Raymond G. York, Ph.D., DABT Date
Associate Director of Research
and Study Director

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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 H

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 418-013: Oral (Gavage) Pharmacokinetic Study of PFOS in Rats
Sponsor's Study Number: T-6295.12

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 18 OCT 98.

Critical phases of this study were inspected six 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 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 25 APR 99 and 26 MAY 99, and for revisions requested by the Sponsor 17 JUN and 18 JUN 99 and for finalization on 24 JUN 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.

Nancy J. Gongliewski 24 Jun 99 — Gina M. Bach 24 June 99

Nancy J. Gongliewski — Date — for Lisa A. Zaborowski, B.S. — Date

Quality Assurance Manager

Senior Quality Assurance Associate and Principal Auditor

TABLE 1

CRITICAL PHASES INSPECTED

Test Article Preparation

Date of inspection: 19 NOV 98

Date results reported to the Study Director and Management: 20 NOV 98

Test Article Administration - Gavage

Date of inspection: 19 NOV 98

Date results reported to the Study Director and Management: 20 NOV 98

Cohabitation

Date of Inspection: 28 DEC 98

Date results reported to the Study Director and Management: 31 DEC 98

Blood Collection

Date of inspection: 04 JAN 99

Date results reported to the Study Director and Management: 04 JAN 99

Urine and Fecal Collection

Date of inspection: 04 JAN 99

Date results reported to the Study Director and Management: 04 JAN 99

Caesarean-Sectioning

Date of inspection: 13 JAN 99

Date results reported to the Study Director and Management: 19 JAN 99

TABLE 2

RAW DATA AUDIT(S)

The following study information and raw data were audited from 18 MAR 99 to 25 MAR 99:

Protocol.
Protocol amendment.
List of personnel and computer operator codes.
Error codes and codes for clinical sign observations.
Animal receipt, randomization, physical examination and acclimation.
In-life transaction record.
Feed consumption.
Cohabitation.
Caesarean-sectioning.
Maternal gross observations.
Fetal gross observations.
Necropsy.
Gravid uterine weights.
Placental weights.
Tissue packing lists.
Male breeder colony records.
General comments.
Study maintenance records.
Temperature and relative humidity reports.
Feed and water analyses.
Edit requests.
Randomizations.
Dosage volumes.
Deviations.
Data review pages.
Blood and liver collection data and packing lists.
Maternal packing lists for liver/glands.
Fetal liver/lung tissue packing lists.
Pooled liver, placenta, liver and lung packing lists.
Fecal/urine collection data and packing lists.
Embryo/placenta collection and packing lists.
Fetal carcass packing lists.
Amniotic fluid collection and packing lists.

The results of this audit were reported to the Study Director and Management on 26 MAR 99.

The following study information and raw data were audited on 25 MAR 99:

Vehicle/Control article receipt, preparation and use.
Test article/substance receipt, preparation and use.
Test article/substance packing lists.
Test article/substance analysis.

The results of this audit were reported to the Study Director and Management on 29 MAR 99.

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 20 (PAGE 5): EMBRYONAL VITAL STATUS OR FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF PRESUMED GESTATION

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DOSAGE GROUP III							0.4 MG/KG/DAY																
RAT # CLs																							
19533 4/12	MA	MA	FA	MA / FA	FA	MA	MA	MA	MA	MA	FA												
	5.19	5.23	4.92	5.42	4.80	5.01	5.13	5.09	5.57	5.40	5.35	4.82											
19534 13/15	FA	MA	MA	MA	MA	MA	FA / FA	FA	MA	MA	FA	FA	MA										
	4.08	4.80	4.80	4.79	5.20	5.06	5.24	4.78	5.00	5.12	4.87	4.60	5.22	5.32									
19536 7/12	MA	FA	FA	FA	MA	FA / FA	FA	FA	FA	MA	FA	FA	FA	MA	FA	FA	MA						
	5.57	5.15	5.13	4.97	5.89	5.36	4.88	5.22	5.08	4.93	5.16	4.82	4.74	5.23	4.46	5.12	4.97	5.46					
19541	MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED																						
19543 11/10	E	FA	MA	FA	MA	MA	FA	MA	MA	FA	MA / FA	E	MA	MA	MA	MA	MA	FA					
		4.83	5.07	4.60	5.10	5.26	4.66	5.08	5.19	4.47	5.51	4.60	4.74	5.41	5.48	5.38	4.71	5.17					
19545	MATING NOT CONFIRMED																						
19546 14/11	MA	MA	FA	E	MA / MA	E	E	E															
	5.04	5.56	4.70		5.30	4.84																	
19548	NOT PREGNANT																						
DOSAGE GROUP IV							1.6 MG/KG/DAY																
19549	MATING NOT CONFIRMED																						
19551	MATING NOT CONFIRMED																						
19552	MATING NOT CONFIRMED																						
19556	NOT PREGNANT																						
19557	MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION)																						
19559	NOT PREGNANT																						
19560 12/ 7	FA	FA	MA	MA	MA	FA	FA	FA	MA / MA	FA	FA												
	5.17	5.13	5.20	5.43	5.44	5.12	5.27	4.70	5.13	5.08	4.85	5.15											
19562 8/14	MA	MA	FA	FA	FA	FA	MA	FA / FA	FA	E	MA	E	MA	FA	MA	FA	MA	FA	MA	MA	MA		
	5.50	5.70	4.75	5.27	5.28	4.61	4.84	4.78	4.97	4.74	4.79		4.95	4.93	4.94	4.37	4.75	4.73	5.04	4.77	4.43		

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).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 20 (PAGE 6): EMBRYONAL VITAL STATUS OR FETAL SEX, VITAL STATUS AND BODY WEIGHT - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF PRESUMED GESTATION

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DOSAGE GROUP V						3.2 MG/KG/DAY																	
RAT # CLs																							
19568 8/11	MA	MA	MA	MA	MA	MA /	E	E	E														
	4.12	3.73	4.16	5.19	4.98	4.84																	
19569 10/ 6	FA	MA	FA	MA	FA	FA	MA	MA	FA /	MA	FA	FA	MA										
	4.33	4.85	3.43	5.12	4.78	4.33	4.81	4.86	4.66	5.12	5.34	4.61	4.30										
19571	DELIVERED ON DAY 21 OF GESTATION																						
19572 8/ 9	FA	MA	FA	MA	MA	E	MA	FA /	FA	E	FA	E	FA	MA	MA								
	4.51	4.56	4.55	5.02	4.96		4.31	4.76	4.39		4.67		4.55	4.67	5.15								
19576	MATING NOT CONFIRMED																						
19577 8/ 5	FA	E	FA	MA	FA /																		
	4.98		5.15	5.18	4.87																		
19578	MATING NOT CONFIRMED																						
19579	DELIVERED ON DAY 21 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).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 21 (PAGE 1): PLACENTAL WEIGHTS - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

RAT #	MEAN	FETUS # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
		DOSAGE GROUP I							0 (VEHICLE) MG/KG/DAY															
19503		NO CONFIRMED MATING DATE; DELIVERED ON DAY 66 OF STUDY																						
19504		FA	E	MA	E	MA	MA	MA /	E	MA	E	MA	FA	MA	E	FA	MA	FA						
	0.58	0.52		0.47		0.59	0.59	0.64		0.45		0.61	0.60	0.69		0.65	0.64	0.50						
19505		FA	FA	MA	FA /	MA	FA	MA	E	FA	MA	MA	FA	MA	MA	MA	E	FA	FA	FA				
	0.44	0.53	0.48	0.52	0.47	0.48	0.43	0.40		0.37	0.47	0.38	0.41	0.41	0.48	0.41		0.38	0.46	0.43				
19506		E	FA	MA	FA	MA	MA /	FA	FA	FA	MA	FA	FA	FA	FA									
	0.54		0.63	0.48	0.63	0.41	0.61	0.55	0.53	0.52	0.65	0.46	0.53	0.45	0.51									
19507		MA	FA	MA	MA	MA	MA	FA	FA	FA /	E	FA	MA	MA	FA	FA								
	0.41	0.41	0.44	0.38	0.39	0.38	0.44	0.32	0.35	0.38		0.43	0.37	0.44	0.44	0.52								
19509		FA	FA	FA	FA	MA	FA	E /	MA	MA	FA	FA	MA	MA										
	0.53	0.46	0.53	0.56	0.53	0.55	0.45		0.57	0.54	0.57	0.54	0.55	0.47										
19512		MA	FA	FA	FA /	MA	FA	MA	MA	MA	MA	FA	FA	FA	MA									
	0.49	0.56	0.54	0.54	0.54	0.53	0.36	0.46	0.47	0.51	0.40	0.48	0.51	0.47	0.53									
19516		MA	FA	FA	FA	FA	FA /	MA	MA	MA	FA	FA	FA	MA	FA									
	0.51	0.60	0.46	0.49	0.63	0.63	0.47	0.44	0.48	0.57	0.33	0.61	0.42	0.59	0.42									
		DOSAGE GROUP II							0.1 MG/KG/DAY															
19518		FA	E	MA	FA	FA	E	FA	FA /	MA	MA	MA	MA	MA										
	0.51	0.49		0.53	0.55	0.54		0.53	0.42	0.66	0.44	0.41	0.46	0.54										
19519		FA	MA	FA	FA	MA	FA	FA	MA	MA /	FA	MA	MA	MA	FA	MA	MA							
	0.45	0.43	0.48	0.47	0.47	0.47	0.42	0.38	0.46	0.43	0.44	0.50	0.45	0.55	0.38	0.45	0.49							
19520		MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED																						
19521		FA	FA	FA	MA	FA	FA	MA	MA	MA /	FA	FA	MA	FA	MA	MA								
	0.45	0.44	0.46	0.40	0.44	0.49	0.49	0.48	0.42	0.46	0.37	0.47	0.41	0.48	0.42	0.50								
19523		E	FA	L	FA	FA	FA	FA	FA	MA	MA /	MA	E	FA	L									
	0.53		0.49		0.55	0.56	0.44	0.55	0.55	0.56	0.49	0.52		0.59										
19524		FA	MA	MA	MA	MA	FA	MA	MA /	MA	MA	E	MA	MA	MA	FA	MA	FA						
	0.53	0.60	0.52	0.58	0.67	0.47	0.55	0.52	0.58	0.46	0.46		0.67	0.54	0.53	0.40	0.49	0.42						
19528		E	MA /	MA	E	E	FA	MA	FA															
	0.66		1.08	0.53			0.63	0.52	0.54															
19529		FA	MA	FA	MA	E	FA	FA	MA /	FA	MA	FA	MA	FA	MA	FA								
	0.62	0.93	0.77	0.54	0.63		0.56	0.67	0.69	0.49	0.51	0.50	0.59	0.68	0.53	0.58								

M = MALE F = FEMALE A = ALIVE E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY PLACENTAL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 21 (PAGE 2) : PLACENTAL WEIGHTS - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

RAT #	MEAN	FETUS # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DOSAGE GROUP III								0.4 MG/KG/DAY																
19533		MA	MA	FA	MA / FA		FA	MA	MA	MA	MA	MA	FA											
	0.45	0.52	0.38	0.34	0.44	0.42	0.46	0.40	0.43	0.47	0.58	0.50	0.47											
19534		FA	MA	MA	MA	MA	MA	FA / FA		FA	MA	MA	FA	FA	MA									
	0.57	0.49	0.58	0.50	0.51	0.60	0.61	0.62	0.50	0.61	0.53	0.65	0.61	0.53	0.64									
19536		MA	FA	FA	FA	MA	FA / FA		FA	FA	FA	MA	FA	FA	FA	MA	FA	FA	MA					
	0.46	0.45	0.48	0.50	0.54	0.53	0.40	0.38	0.54	0.42	0.39	0.49	0.47	0.45	0.47	0.33	0.48	0.46	0.47					
19541		MORIBUND SACRIFICED ON DAY 0 OF PRESUMED GESTATION; PREGNANCY STATUS COULD NOT BE DETERMINED																						
19543		E	FA	MA	FA	MA	MA	FA	MA	MA	FA	MA / FA		E	MA	MA	MA	MA	MA	FA				
	0.41		0.50	0.39	0.44	0.37	0.49	0.38	0.42	0.43	0.41	0.42	0.28		0.38	0.43	0.43	0.40	0.39	0.42				
19545		MATING NOT CONFIRMED																						
19546		MA	MA	FA	E	MA / MA		E	E	E														
	0.51	0.44	0.47	0.36		0.49	0.79																	
19548		NOT PREGNANT																						
DOSAGE GROUP IV								1.6 MG/KG/DAY																
19549		MATING NOT CONFIRMED																						
19551		MATING NOT CONFIRMED																						
19552		MATING NOT CONFIRMED																						
19556		NOT PREGNANT																						
19557		MORIBUND SACRIFICED ON DAY 43 OF STUDY (DAY 2 OF COHABITATION)																						
19559		NOT PREGNANT																						
19560		FA	FA	MA	MA	MA	FA	FA	FA	MA / MA		FA	FA											
	0.54	0.48	0.54	0.59	0.67	0.74	0.54	0.50	0.53	0.39	0.56	0.44	0.50											
19562		MA	MA	FA	FA	FA	FA	MA	FA / FA		FA	E	MA	E	MA	FA	MA	FA	MA	FA	MA	MA	MA	
	0.44	0.46	0.54	0.43	0.43	0.41	0.45	0.49	0.54	0.53	0.38		0.57		0.39	0.34	0.39	0.43	0.45	0.41	0.49	0.48	0.32	

M = MALE F = FEMALE A = ALIVE E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY PLACENTAL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-013: ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.12)

TABLE 21 (PAGE 3) : PLACENTAL WEIGHTS - INDIVIDUAL DATA

RATS CAESAREAN-SECTIONED ON DAY 21 OF GESTATION

FETUS #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RAT # MEAN	DOSAGE GROUP V								3.2 MG/KG/DAY														
19568	MA	MA	MA	MA	MA	MA /	E	E	E														
0.62	0.71	0.54	0.76	0.59	0.59	0.54																	
19569	FA	MA	FA	MA	FA	FA	MA	MA	FA /	MA	FA	FA	MA										
0.50	0.54	0.45	0.47	0.55	0.51	0.44	0.48	0.50	0.58	0.42	0.56	0.53	0.48										
19571	DELIVERED ON DAY 21 OF GESTATION																						
19572	FA	MA	FA	MA	MA	E	MA	FA /	FA	E	FA	E	FA	MA	MA								
0.42	0.38	0.38	0.39	0.58	0.56		0.39	0.42	0.38		0.51		0.36	0.42	0.30								
19576	MATING NOT CONFIRMED																						
19577	FA	E	FA	MA	FA /																		
0.67	0.69		0.68	0.68	0.63																		
19578	MATING NOT CONFIRMED																						
19579	DELIVERED ON DAY 21 OF GESTATION																						

M = MALE F = FEMALE A = ALIVE E = EARLY RESORPTION L = LATE RESORPTION "/" DENOTES POSITION OF CERVIX
CLs = CORPORA LUTEA/OVARY PLACENTAL WEIGHTS WERE RECORDED IN GRAMS (G).

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 418-013

SPONSOR'S STUDY NUMBER: T-6295.12

STUDY TITLE: Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

PURPOSE: The purpose of this study is to evaluate the pharmacokinetics of PFOS in Fo generation dams and F1 generation fetuses following PFOS treatment of Crl:CD®BR VAF/Plus® female rats during premating and gestation.

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 Corporate Toxicology
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 (Synonym: FC-95).
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 (ATTACHMENT 2).

Storage:

Bulk Test Article:	Room temperature.
Vehicle Components:	Room temperature.
Prepared Vehicle:	Room temperature.
Prepared Formulations:	Room temperature.

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. Data verifying the stability of the test article in the vehicle for 48 hours under the conditions of administration are on file with the Sponsor.

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 frozen (-70°C or below) 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 Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat was selected as the Test System because: 1) this strain of rat was used in the reproductive and developmental toxicity studies; 2) historical data and experience exist at the Testing Facility[1-3]; and 3) the test article is pharmacologically active in the species and strain.

Number:

Initial population acclimated:	120 virgin female rats.
Population selected for study:	80 mated female rats (16 per dosage group).

Body Weight and Age:

Female rats will be ordered to have body weights of 200 g to 225 g each at receipt, at which time they will be expected to be at least 60 days of age. Actual body weights will be recorded the day after receipt and will be documented in the raw data. The weight range will be included in the final report.

Sex:

Female rats will be given the test article. Male rats of the same source and strain will be used only as breeders and are not considered part of the Test System.

Source:

Charles River Laboratories, Inc.

The rats will be shipped in filtered cartons by air freight and/or truck from Charles River Laboratories, Inc., to the Testing Facility.

Identification:

Rats are permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20101). Male rats are given unique permanent identification numbers upon assignment to the Testing Facility's breeder male rat population. Female rats are assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study.

ANIMAL HUSBANDRY:

All cage sizes and housing conditions are in compliance with the *Guide for the Care and Use of Laboratory Animals*[4].

Housing:

Female rats will be individually housed in stainless steel, wire-bottomed cages, except during the cohabitation period and during collection intervals for urine and fecal samples. During cohabitation, each pair of rats will be housed in the male rat's cage. During collection intervals for urine and fecal samples, the female rats will be housed individually in metabolism cages. No nesting materials will be supplied because the female rats 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 (Airo Clean® room). Room temperature will be maintained at 64°F (18°C) to 79°F (26°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:

Rats will be given Certified Rodent Diet® #5002 (PMI Nutrition International) available *ad libitum* from individual feeders.

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 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.

RANDOMIZATION AND COHABITATION:

Upon arrival, male and female rats will be assigned to individual housing on the basis of computer-generated random units. After acclimation, virgin female rats will be selected for study on the basis of physical appearance and body weights recorded during acclimation. The female rats will be assigned to dosage groups based on computer-generated (weight-ordered) randomization procedures.

Within each dosage group, consecutive order will be used to assign female rats to cohabitation with breeder male rats, one male rat per female rat. The cohabitation period will consist of a maximum of five days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* will be considered to be at day 0 of presumed gestation and assigned to individual housing.

A table of random units or a computer-generated randomization procedure will be used to select eight female rats per dosage group for Caesarean-section examinations on day 15 of presumed gestation. The remaining female rats in each dosage group will be examined on day 21 of presumed gestation.

ADMINISTRATION:

Route and Reason for Choice:

The oral (gavage) route was selected for use because: 1) this was the route of administration in the developmental and reproductive toxicology studies; and 2) it is one of the possible routes of human exposure.

Method and Frequency:

Female rats will be given the test article or vehicle once daily beginning 42 days prior to cohabitation through either day 14 of presumed gestation or day 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 previous study conducted with the test article (Argus Research Laboratories, Inc., Protocol 418-008).

Dosage Levels, Concentrations and Volumes:

Dosage Group	Number of Female Rats	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Argus Batch Number
I	16	0 (Vehicle)	0	5	B-418-013-A(Day.Month.Year)
II	16	0.1	0.02	5	B-418-013-B(Day.Month.Year)
III	16	0.4	0.08	5	B-418-013-C(Day.Month.Year)
IV	16	1.6	0.32	5	B-418-013-D(Day.Month.Year)
V	16	3.2	0.64	5	B-418-013-E(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:

Acclimation Period:	At least once.
Dosage Period:	Twice daily. Prior to administration and once approximately one hour postdosage.
Postdosage Period:	Once prior to sacrifice.

Clinical observations may be recorded more frequently than cited above, if deemed appropriate by the Study Director and/or Study Monitor.

Body Weights:

Acclimation Period:	At least once.
Dosage Period:	Daily.
Sacrifice:	Terminal weight.

Feed Consumption Values (recorded and tabulated):

Acclimation Period:	At least once.
Dosage Period:	Weekly to cohabitation and daily during presumed gestation.

Feed consumption values may be recorded more frequently if it is necessary to replenish the feed. These intervals will not be tabulated.

Mating Performance:

Mating will be evaluated daily during the cohabitation period and confirmed by observation of spermatozoa in a smear of the vaginal contents and/or a copulatory plug observed *in situ*.

Pharmacokinetic Sample Collection:

Urine and Fecal Samples:

Female rats will be housed individually in metabolism cages for collection of urine and fecal samples for the following intervals: one day prior to initiation of cohabitation to the following morning and days 6 to 7, 14 to 15 and 20 to 21 of presumed gestation. Following each 24-hour collection interval, samples will be collected into centrifuge tubes, placed on dry ice and stored frozen (-70°C or below) until shipment for analysis.

In the event that a dam begins to deliver before completion of urine and fecal sample collection (day 20 to day 21 of presumed gestation), the dam will be removed from the metabolism cage and placed in a nesting box with sufficient bedding until sacrifice.

Blood Samples:

Blood samples will be collected from each of the female rats following removal from metabolism caging (prior to administration) on each of the following days: on the day cohabitation is initiated (prior to cohabitation) and on days 7 and 15 of presumed gestation, as well as day 21 of presumed gestation. The time of blood collection will be recorded in the raw data.

On all days of collection except days 15 and 21 of presumed gestation (dams at their terminal collection interval), blood samples (approximately 1 mL each) will be collected from the orbital sinus. If necessary, whole blood may be collected from an alternate site; if so, the alternate site will be documented in the raw data. On day 21 of presumed gestation, blood samples (approximately 4 mL each) will be collected via the inferior vena cava. If blood collection on day 15 of presumed gestation will be a final bleed for the respective dam (i.e., the dam will be Caesarean-sectioned that day), the sample will be approximately 4 mL and will be collected via the inferior vena cava.

Blood will be collected and transferred into serum separator tubes. The samples will be spun in a refrigerated centrifuge. The serum will be transferred into polypropylene tubes labeled with the study number, animal identification, date of collection, study day and collection timepoint. All samples will be immediately frozen on dry ice and maintained frozen (-70°C or below) until shipment to the Sponsor for analysis.

Shipping Instructions:

All samples will be maintained frozen (-70°C or below) until shipment for analysis. Samples will be shipped frozen on dry ice via overnight mail. A packing list will be included with the samples and sent to Kris J. Hansen, Ph.D., at the previously cited address. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

Caesarean-Sectioning Observations - Day 15 Presumed Gestation:

Eight randomly selected female rats per dosage group will be Caesarean-sectioned on day 15 of presumed gestation. The gravid uterus will be excised and weighed. Placentae that appear abnormal (size, color or shape) will be noted in the raw data. The female rats (pregnant dams) will be examined for number and distribution of:

Corpora Lutea.

Implantation Sites.

Viable and Nonviable Embryos.
(A viable embryo is oval or crescent shaped, pink, firm and enclosed in an amniotic sac filled with clear fluid. A nonviable embryo is amorphous, small, pale pink to tan or deep red to black, soft and enclosed in an amniotic sac filled with clear, cloudy or opaque fluid.)

Sample Collection:

Caps and labeled tubes will be weighed (combined weight, to the nearest .001 gram) before and after retention of pooled embryonic samples for subsequent use in pharmacokinetic analyses. These weights will be documented in the raw data, and copies of these weights will be included with the packing list prior to shipment.

Samples of the amniotic fluid of each viable embryo will be collected, pooled (per litter), frozen and stored (-70°C or below) until shipment to the Sponsor for analysis.

Each viable embryo will be removed from the uterus with the attached placenta, pooled (per litter), frozen and stored (-70°C or below) until shipment to the Sponsor.

Shipping Instructions:

All samples will be maintained frozen (-70°C or below) until shipment for analysis. Samples will be shipped frozen on dry ice via overnight mail. A packing list will be included with the samples and sent to Kris J. Hansen, Ph.D., at the previously cited address. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

3M

Study Title

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

PROTOCOL

Author
Lisa Clemen

Date:
June 8, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
FACT-TOX-110
U2849

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Date 4/19/2000

Study Identification

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

Test Material	Perfluorooctane sulfonic acid potassium salt (T-6295)
Sponsor	3M Toxicology Services - Medical Department 3M Center, Building 220-2E-02 St. Paul, MN 55144-1000
Sponsor Representative	Marvin T. Case, D.V.M., Ph.D. 3M Toxicology Services Telephone: 651-733-5180 Facsimile: 651-733-1773
Study Director	Kristen J. Hansen, Ph.D. 3M Environmental Technology and Safety Services Building 2-3E-09 651-778-6018
Study Location(s)	
In vivo Testing Facility	Argus Research Laboratories, Inc. 905 Sheehy Drive, Building A Horsham, PA 19044
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106

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Sub-Contract Laboratory	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850
	Battelle Memorial Institute 505 King Avenue Columbus, Ohio 43201-2693

Proposed Study Timetable

Study Initiation Date	June 8, 1999
Study Completion Date	August 8, 2000

1. STUDY

Oral (gavage) pharmacokinetic study of potassium perfluorooctane sulfonic acid (PFOS) in rats.

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rats. The in-life portion of this study was conducted at Argus Research Laboratories, study #418-013. All serum samples will be extracted and analyzed at Advanced Bioanalytical Services, Inc. and all liver samples extracted and analyzed at Battelle Memorial Institute. Additional analyses may be performed at the 3M Environmental Laboratory as methods are developed and validated. If additional analyses are performed an amendment to this protocol will be written.

3. REGULATORY COMPLIANCE

This study will be conducted in accordance with the United States Food and Drug Administration, Good Laboratory Practices Standards, Final Rule 21 CFR 58, with the exception that analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor.

4. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will review the protocol and audit study conduct, data, and final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures.

The QA Unit at the sub-contract laboratory will audit their study conduct, data, and results report prior to submitting to the 3M Environmental Laboratory.

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5. TEST MATERIAL

5.1 Refer to Argus Research Laboratory protocol for study #418-013.

6. CONTROL MATRICES

6.1 Identification Rat liver and serum and/or rabbit liver and serum, traceability numbers will be recorded in the raw data and included in the final report

6.2 Source Argus Research and/or Sigma Chemical

6.3 Physical Description Rat liver and serum and/or rabbit liver and serum

6.4 Purity and Stability Not applicable

6.5 Storage Conditions Frozen at -20 °C ± 10 °C or –50 °C ± 10 °C

6.6 Reserve Matrix A portion of the control matrix will be retained in the 3M archives for as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

6.7 Disposition Matrices will be retained at the 3M Environmental Laboratory per GLP regulation. Certain matrices (feces, urine, and blood) may be disposed after QAU verification.

6.8 Safety Precautions Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

7. REFERENCE MATERIAL

7.1 Identification Potassium perfluorooctanesulfonate (PFOS), lot #s 171, 215, or 217 (equivalent lots)

7.2 Source 3M Specialty Chemicals

7.3 Physical Description White powder

7.4 Purity and Stability Purity of PFOS is 99% or greater. Stability has not been determined.

7.5 Storage Conditions Room temperature

7.6 Reserve Material A reserve sample from each batch of PFOS used in this study will be retained as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

7.7 Disposition Unused reference material will be retained for use by the 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation.

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7.8 Safety Precautions Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

8. TEST SYSTEM

Rats were used as the test system and were maintained and dosed as described in the Argus Research protocol #418-013. The female rats will be given the test material or control once daily beginning 42 days prior to cohabitation and continue through day 14 or 20 of presumed gestation. See table 1 for more dosage information.

Table 1
Dosage Levels, Concentration, and Volumes

Dosage Group	Number of female rats	Dosage mg/kg/day	Concentration mg/kg/day	Dosage volume mL/kg
1	16	0	0	5
2	16	0.1	0.02	5
3	16	0.4	0.08	5
4	16	1.6	0.32	5
5	16	3.2	0.64	5

9. SPECIMEN AND SAMPLE RECEIPT

The 3M Environmental Laboratory will receive homogeneity samples for dose analysis and specimens of the following body tissues and fluids from the indicated points in the study. See table 2 for specimen information. All specimens will be packed on dry ice for shipping.

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Fetus animals	Dam-Predose, Days 7, 15, and 21 Fetus-At termination of the study	320 Dam and 320 Fetus (pooled)
Urine and feces – Dam animals	Predose, Days 7, 15, and 21	320 urine and 320 feces
Liver – Dam and Fetus animals	Dam and Fetus-At termination of the study	80 Dam and 80 Fetus (pooled)
Milk Secreting Glands – Dam animals	At the termination of the study	80
Amniotic Fluid – Dam animals	Day 15 and Day 21	80

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Table 2 Cont. Specimen Information		
Body tissue/fluid	**Collected**	**Expected # of specimens**
Embryo's with Placentae – Dam animals	Day 15 and Day 21	80
Carcass – Fetus animal	At the termination of the study	160
Placentae – Fetus animal	Day 15 and Day 21	80
Liver/lung – Fetus animal	At the termination of the study	80

Total number of expected specimens: 2000
Total number of test animals: 64
Total number of control animals: 16

Specimens sent to 3M Environmental Laboratories will be received and tracked according to applicable Standard Operating Procedures.

10. PREPARATORY METHODS

10.1 FACT-M-1.1, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.2 ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.3 If preparatory methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

10.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

11. ANALYTICAL METHODS

11.1 FACT-M-2.1, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

11.2 ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry

11.3 If analytical methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

11.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

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12. DATA QUALITY OBJECTIVES

The number of spikes/duplicates, use of surrogates, and information on other data quality indicators are included in the analytical methods. In addition, the following criteria will be met:

12.1 Linearity $r^2 \geq 0.98$

12.2 Limits of detection / quantitation

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

12.2.2 Limit of Quantitation (LOQ) – Equal to the lowest acceptable standard in the calibration curve

12.3 Duplicate acceptable precision < 30% for the method

12.4 Spike acceptable recoveries 70% - 130%

12.5 Use of confirmatory methods Indeterminate samples will be re-analyzed using a confirmatory method. If a confirmatory method is used, an amendment to this protocol will be written.

12.6 Demonstration of specificity Chromatographic retention time, mass spectral daughter ion characterization.

13. SUB-CONTRACTED ANALYSIS

13.1 All analyses as detailed in this protocol will be performed at 3M Environmental Laboratories, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106, at Advanced Bioanalytical Services, Inc., 15 Catherwood Road, Ithaca, NY 14850, or at Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693.

13.2 An amendment to this protocol will be written if analyses are performed at laboratories other than 3M Environmental Laboratories, Advanced Bioanalytical Services, Inc., or Battelle Memorial Institute.

14. STATISTICAL ANALYSIS

Averages and standard deviations will be calculated. The statistical methods that will be used are described below:

14.1 Data transformations and analysis Data will be reported as the concentration (weight/weight or weight/vol) of PFOS or metabolite per tissue or fluid.

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14.2 ***Statistical analysis*** Statistics used may include regression analysis of concentrations over time, and standard deviations calculated for the concentrations within each dose group. If necessary, simple statistical tests, such as Student's t test, may be applied to evaluate statistical difference.

15. REPORT

A report containing all the results of the study will be prepared by the 3M Environmental Laboratory. If analyses are sub-contracted to other laboratories, each laboratory will prepare a report and submit it to the 3M Environmental Laboratory for inclusion in the 3M Environmental Laboratory report. Each report will include, but not be limited to, the following, when applicable:

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

15.3 A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards

15.4 Objectives and procedures as stated in the approved protocol, including any changes in the original protocol

15.5 The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor

15.6 Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor

15.7 A description of the methods used to conduct the test(s)

15.8 A description of the test system

15.9 A description of any circumstances that may have affected the quality or the integrity of the data

15.10 The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study

15.11 A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses

15.12 Statistical methods used to evaluate the data, if applicable

15.13 The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable

15.14 The location where raw data and the final report are to be stored

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Date

15.15 A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management

If it is necessary to make corrections or additions to a report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

16. Location of Raw Data, Records, and Final Report

Original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

16.1 The following raw data and records will be retained in the study folder in the study/project archives according to 3M Environmental Laboratory Standard Operating Procedures:

- **16.1.1** Approved protocol and amendments
- **16.1.2** Study correspondence
- **16.1.3** Shipping records
- **16.1.4** Raw data
- **16.1.5** Approved final report (original signed copy)
- **16.1.6** Electronic copies of data

16.2 The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory Standard Operating Procedures:

- **16.2.1** Training records
- **16.2.2** Calibration records
- **16.2.3** Instrument maintenance logs
- **16.2.4** Standard Operating Procedures, Equipment Procedures, and Methods

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Date 4/19

17. SPECIMEN RETENTION

Specimens will be maintained in the 3M Environmental Laboratory specimen archives for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable), and as established by 3M Environmental Laboratory Standard Operating Procedures.

18. PROTOCOL AMENDMENTS AND DEVIATIONS

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and filed with the raw data.

19. ATTACHMENTS

19.1 Attachment A Preparatory and analytical methods

20. SIGNATURES

Marvin T Case — 9 June 1999

Marvin T. Case, D.V.M., Ph.D., Sponsor Representative — Date

Kristen Hansen — 6/16/99

Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director — Date

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Study Title
Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:
August 12, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS FACT-TOX110
LIRN U2849

Exact Copy of Original
Initial 4/19
Date

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:*** Section 2.0 states this study is designed to determine potassium perfluorooctane sulfonic acid (PFOS) in specimens of liver and serum of rats.

AMEND TO READ: This study is designed to determine PFOS in specimens of rat liver, serum, and urine. All Day -1 and Gestation Day 21 rat urine specimens will be extracted and analyzed by the 3M Environmental Laboratory.

REASON: The urine extraction and analytical methods were not validated and approved prior to protocol approval.

2. ***PROTOCOL READS:*** Section 6.0 lists rat or rabbit liver and serum.

AMEND TO READ: Rat or rabbit urine from 3M Toxicology with a physical description of rat or rabbit urine.

REASON: The rat urine matrix was added after the protocol was approved.

3. ***PROTOCOL READS:*** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
FACT-M-2.1 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

AMEND TO READ: The extraction and analytical methods to follow at the 3M Environmental Laboratory are:

ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-96.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"
ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-97.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"

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REASON: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to ETS-8-6.0 and ETS-8-7.0.
Methods ETS-8-96.0 and ETS-8-97.0 were not validated and approved until after the protocol was approved.

4. ***PROTOCOL READS:*** Section 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.

AMEND TO READ: The extraction and analytical methods to follow at Advanced Bioanalytical Services will be attached to the protocol.

The extraction and analytical method to follow at Battelle Memorial Institute is:

"Method for Analysis of Perfluorooctane Sulfonate (PFOS) in Rat ~~Sera~~ Liver by LC/MS/MS, Version ~~1~~ 1.0"

REASON: The analytical methods at the sub-contract laboratories were not included in the original protocol.

5. ***PROTOCOL READS:*** Section 12.2.1 b) Liver method detection limit is 15 ppb.

AMEND TO READ: 12.2.1 b) Liver method detection limit is 8.50 ppb (ng/g).
12.2.1 c) Urine method detection limit is 1.5 ppb (ng/g).

REASON: The validation supporting methods ETS-8-6.0 and ETS-8-7.0 included a lower method detection limit for PFOS in liver. A validation in urine was performed, after protocol approval, to support this method detection limit.

RE UAC 9/27/99

kjh 9/27/99

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Date 4/15

Amendment Approval

Marvin T Case — 17 August 1999

Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kris H — 8/18/99

Kris J. Hansen, Ph.D., Study Director — Date

Exact Copy of Original
Initial — Date 4/19

Study Title
Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 28, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS FACT-TOX110
LIRN U2849

Exact Copy of Original
Initial
4/17
Date

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
   Section 2 states that all liver samples will be extracted and analyzed at Battelle Memorial Institute.

   AMEND TO READ:
   All dam liver specimens (female mother) and 27 fetus (pooled) liver specimens will be analyzed at Battelle Memorial Institute.

   REASON:
   The fetal liver/lung is not a target matrix and will not be extracted or analyzed.

2. ***PROTOCOL READS:***
   Section 9 states that 80 dam liver specimens (female mother) and 80 fetus (pooled) liver specimens will be sent to the Environmental Laboratory.

   AMEND TO READ:
   Seventy-three dam liver specimens (female mother), 27 fetus (pooled) liver specimens, and 81 fetal liver/lung specimens (27 liver/lung specimens sampled in triplicate) were sent to the Environmental Laboratory.

   REASON:
   The number of animals changed during the course of the study.

3. ***PROTOCOL READS:***
   Section 12.2.1 a) serum method detection limit is 1.75 ppb b) Liver method detection limit is 15 ppb.

   AMEND TO READ:
   The method detection limits for all compounds and matrices will be taken from the methods used for extraction and analysis.

   REASON:
   The method detection limits listed are specific to the 3M Environmental Laboratory. Statement was added to allow for sub-contracted analyses and/or revised methods.

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Date 4/19

4. ***PROTOCOL READS:***
Section 16 states that the original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, electronic copies of data, training records, calibration records, instrument maintenance logs and standard operating procedures, equipment procedures, and methods will be retained in the archives of the 3M Environmental Laboratory.

AMEND TO READ:
Section 16 states that the original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis.

REASON:
Clarification of the disposition of archived records if analyses are performed at a sub-contract laboratory.

5. ***PROTOCOL READS:***
Section 17 states that specimens will be maintained in the 3M Environmental Laboratory specimen archives.

AMEND TO READ:
Specimens will be maintained in the 3M Environmental Laboratory specimen archives. All specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. The specimens will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.

REASON:
Clarification of the disposition of documentation when shipping specimens for analyses performed by a sub-contract laboratory.

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Amendment Approval

Marvin T Case — 4 Oct 1999
Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kristen Hansen — 5 Oct 1999
Kristen J. Hansen, Ph.D., Study Director — Date

Exact Copy of Original
Initial — Date 4/19

Study Title

Analytical Laboratory Report on the Determination of Perfluorooctanesulfonate (PFOS) Presence and Concentration in Serum, Liver, and Urine from the Gavage Study of T-6295.12

PROTOCOL AMENDMENT NO. 3

Amendment Date:

20 January 2000

Performing Laboratories

Urine Analyses
3M Environmental Technology and Safety Services
Fluorine Analytical Chemistry Team
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Liver Analyses
Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

Serum Analyses
Advanced Bioanalytical Services, Inc.
15 Catherwood Road
Ithaca, NY 14850

Laboratory Project Identification

ET&SS LRN-U2849
FACT TOX-110
Argus Study: 418-013
3M Medical Department Study: T-6295.12

Exact Copy of Original
Initial
Date 4/17

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
   The study director for the present study was identified in the protocol as Kristen J. Hansen, Ph.D.
   AMEND TO READ:
   The role of study director for the present study was reassigned to Marvin T. Case, D.V.M., Ph.D., as of 20 January 2000. The previous study director, Kristen J. Hansen, has been reassigned to the role of Principle Analytical Investigator.
   REASON:
   The role of study director was reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements (refer to 21 CFR Part 58).

2. ***PROTOCOL READS:***
   The sponsor for the present study was identified as Marvin T. Case, D.V.M., Ph.D.
   AMEND TO READ:
   The role of sponsor for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000.
   REASON:
   To ensure that the study director does not also carry the duties of study sponsor, the sponsor role was reassigned. In this manner, personnel responsibilities and workload are more evenly balanced.

Exact Copy of Original
Initial
Date 4/19

Amendment Approval

John L. Butenhoff — February 10, 2000

John L. Butenhoff, Ph.D., Sponsor Representative — Date

Kristen Hansen — 11-Feb-2000

Kristen J. Hansen, Ph.D., Outgoing Study Director — Date

Marvin T Case — 10 February 2000

Marvin T. Case, D.V.M., Ph.D., Incoming Study Director — Date

Exact Copy of Original
Initial — Date 4/9

Study Title

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 4

Amendment Date:

20 April 2000

Performing Laboratories

URINE ANALYSES	FECES ANALYSES
3M Environmental Technology and Safety Services Fluorine Analytical Chemistry Team Building 2-3E-09, 935 Bush Avenue St. Paul, MN 55106	**Centre Analytical Laboratories, Inc.** 3048 Research Drive State College, PA 16801
LIVER ANALYSES	**SERUM ANALYSES**
Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693	**Advanced Bioanalytical Services, Inc.** 15 Catherwood Road Ithaca, NY 14850

Laboratory Project Identification

ET&SS LRN-U2849

FACT TOX-110

Argus Study: 418-013

3M Medical Department Study: T-6295.12

Exact Copy of Original
Initial
Date 4/19/2000

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
   The amended section 2.0 text states that this study is designed to determine PFOS in specimens of rat liver, serum, and urine.
   AMEND TO READ:
   This study is designed to determine PFOS in specimens of rat liver, serum, feces, and urine.
   REASON:
   The analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the protocol. Feces extraction and analytical methods were not validated and approved prior to protocol approval.

2. ***PROTOCOL READS:***
   The amended section 6.0 lists rat or rabbit liver, serum, and urine.
   AMEND TO READ:
   Add: rat or rabbit feces with a physical description of rat or rabbit feces.
   REASON:
   Analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

3. ***PROTOCOL READS:***
   Section 13.1 lists all of the laboratories that will be conducting analyses for this study.
   AMEND TO READ:
   Add: Centre Analytical Laboratories, Inc., 3048 Research Drive, State College, PA 16801
   REASON:
   Feces analyses were added to the scope of this study. The sub-contract laboratory performing analyses was not in the original protocol.

4. ***PROTOCOL READS:***
   Sections 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.
   AMEND TO READ:
   The feces extraction and analytical method used by **Centre Analytical Laboratories** will be; 00M–023–003 (Revision 2), "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS."
   REASON:
   The sub-contract laboratory performing feces analyses was added to the scope of this study; this method was not validated and approved prior to protocol approval.

Exact Copy of Original
Initial
Date

Amendment Approval

John L. Butenhoff — April 19, 2000

John L. Butenhoff, Ph.D., Sponsor Representative — Date

Marvin Case — 19 April 2000

Marvin T. Case, D.V.M., Ph.D., Study Director — Date

Exact Copy of Original
Initial — Date

Perfluorooctanesulfonate:

PK During Pregnancy in Recovery + Analytical Protocol

FINAL REPORT

PROTOCOL 418-015

ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: T-6295.14

FINAL REPORT DATE: 23 JULY 1999

PROTOCOL 418-015

ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: T-6295.14

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TITLE: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

ARGUS RESEARCH LABORATORIES, INC.
PROTOCOL NUMBER: 418-015
SPONSOR'S STUDY NUMBER: T-6295.14

I. SUMMARY AND CONCLUSION

A. Methods[a]

Twenty-four presumed pregnant Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats were assigned to three dosage groups (Groups I through III), eight rats per dosage group. The rats were administered the test article, PFOS (FC-95), or the vehicle, 0.5% Tween® 80 in reverse osmosis membrane processed deionized water (R.O. deionized water), orally (via gavage), once daily beginning 43 days prior to cohabitation until confirmed evidenced of mating. Dosages of 0 (Vehicle), 0.1 and 1.6 mg/kg/day were administered at a dosage volume of 5 mL/kg.

A.1. Fo Generation Rats:

The female rats were observed for viability at least twice each day of the study. The rats were examined for clinical observations of effects of the test article, abortions, premature deliveries and deaths before and approximately one hour after dosage. Rats were observed for clinical observations and general appearance once daily during all other periods of study. Body weights were recorded daily during the dosage and postdosage periods and at sacrifice. Feed consumption values were recorded weekly prior to cohabitation, daily during gestation and on days 1, 4, 7, 10 and 14 of lactation (DLs 1, 4, 7, 10 and 14).

The female rats were evaluated for duration of gestation, litter sizes and pup viability at birth. Pups that either appeared stillborn or that died before initial examination of the litters for viability were examined for vital status at birth. Maternal behavior of the dams was evaluated daily when the pups were examined during the 21-day postpartum period. Observed maternal behavior was recorded on DLs 1, 4, 7, 10, 14 and 21.

---

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).

Urine and fecal sample were collected from female rats for the following intervals: one day prior to initiation of cohabitation to the following morning, days 6 to 7, 14 to 15 and 20 to 21 of presumed gestation (DGs 6 to 7, 14 to 15 and 20 to 21), and DLs 21 to 22. Following each 24-hour collection interval, samples were shipped to the Sponsor for analysis. Blood samples were collected from each of the maternal rats on the day cohabitation was initiated (prior to cohabitation), DGs 7, 15 and 21, and DLs 14 and 22. Serum samples were shipped to the Sponsor for analysis.

All surviving rats assigned to the study were sacrificed on DL 22 following the final collection interval for urine and fecal samples and blood sample collection and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Tissues with gross lesions were retained. The number and distribution of implantation sites was recorded. A liver section from each dam was collected and shipped to the Sponsor for analysis.

A.2. <u>F1 Generation Litters</u>:

Day 1 of lactation (postpartum) was defined as the day of birth and was also the first day on which all pups in a litter were individually weighed. The litters were observed for viability at least twice each day during the postpartum period. Litters were observed for clinical observations and general appearance once daily during the postpartum period. The pups in each litter were counted once daily. Body weights were recorded on DLs 1 (birth), 4, 7, 14 and 21.

Pups found dead were examined for gross lesions and for the cause of death. For all pups found dead on DLs 2 to 4, all lungs were preserved.

On DL 4, litters were culled to five male pups and five female pups per litter, where possible. The lungs and the livers were collected from the first ten pups culled determined to be at DL 4 from each dosage group (irrespective of litter). The lungs and the livers were individually retained. Remaining culled pups were sacrificed and discarded without evaluation.

All remaining pups on study were sacrificed and examined for gross lesions on DL 21. Gross lesions were retained. The liver from each pup was collected, pooled (per litter) and shipped to the Sponsor for analysis. Blood samples were collected and pooled (per litter). Serum samples were shipped to the Sponsor for analysis.

B. Results

No deaths or premature deliveries were attributed to PFOS treatment. One vehicle control group rat was injured and died after orbital sinus bleeding on DG 15. All other rats survived to scheduled sacrifice. All adverse clinical observations during the precohabitation, gestation and lactation periods were considered unrelated to the test article. A red substance was found in the thoracic cavity of the rat that died. All other rats appeared normal at necropsy.

Rats administered the 1.6 mg/kg/day dosage of the test article had reduced body weight gains or body weight losses in each week of the precohabitation period. Dams in the 1.6 mg/kg/day dosage group had reduced body weight gains during the first week of the gestation period (DGs 0 to 7). Body weight gains were then increased as compared to the control group value on DGs 7 to 10, 13 to 15 and 15 to 18. Reflecting this rebound effect, body weight gains were increased for the entire gestation period (DGs 0 to 20) in the 1.6 mg/kg/day dosage group, as compared to the control group value. Body weight gains for dams administered the 1.6 mg/kg/day dosage of the test article during the precohabitation period generally continued to be increased during the lactation period.

Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced in the 1.6 mg/kg/day dosage group in each week of the precohabitation period. Dams in the 1.6 mg/kg/day dosage group had reduced absolute and relative feed consumption values during the first week of the gestation period (DGs 0 to 7). Feed consumption values were then generally comparable to control group values for the remainder of the gestation period. Absolute and relative feed consumption values in this dosage group were slightly reduced, as compared to control group values, for the entire gestation period (DGs 0 to 20). Absolute and relative feed consumption values during the lactation period were reduced in the groups administered 0.1 or 1.6 mg/kg/day of the test article during the precohabitation period, however the reductions were not strictly dosage-dependent.

Administration of the test article at dosages as high as 1.6 mg/kg/day did not adversely affect any parameter evaluated at natural delivery or during the 22-day lactation period. No clinical or necropsy observations in the F1 generation pups were attributable to dosages of the test article as high as 1.6 mg/kg/day.

C. Conclusion

The purpose of the study, as stated in the protocol, was to evaluate the pharmacokinetics of PFOS in Fo generation pregnant female rats following PFOS treatment during the pre-cohabitation period. This report includes study data from the in-life portion which was conducted at Argus Research laboratories, Inc. The pharmacokinetic samples that were collected during the in-life portion of the study were sent to the Sponsor for analysis and will be reported separately. It is the responsibility of the Sponsor, 3M Corporate Toxicology, to combine the in-life results with the analytical results into a final pharmacokinetic report.

23 JUL 98

Mildred S. Christian, Ph.D., Fellow, ATS Date
Executive Director of Research

23 JUL 98

Alan M. Hoberman, Ph.D., DABT Date
Director of Research

23 JUL 98

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 Toxicololgy, 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:

418-015

A.4. Sponsor's Study Number:

T-6295.14

A.5. Purpose of the Study:

The purpose of this study was to evaluate the pharmacokinetics of PFOS in Fo generation and F1 generation rats during gestation and lactation following cessation of PFOS treatment of Crl:CD®BR VAF/Plus® female rats at confirmed mating.

A.6. Study Design:

The requirements of the U.S. Food and Drug Administration (FDA)[1] were used as the basis of study design.

A.7. Regulatory Compliance:

The study was conducted in compliance with 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 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)
Paul E. Ciotti (Team Leader)
Todd J. Killino, B.S. (Laboratory Technician)

A.13. Report Preparation:

Raymond G. York, Ph.D., DABT
Jo Ann Frazee, M.S. (Study Coordinator)
Denise P. Gasiorowski (Data Management Specialist)
Karen G. Parker, A.A. (Report Administrator)

A.14. Report Review:

Mildred S. Christian, Ph.D., Fellow, ATS (Executive Director of Research)
Alan M. Hoberman, Ph.D, DABT (Director of Research)

A.15. Date Protocol Signed:

16 November 1998

A.16. Dates of Technical Performance:

Rat Arrival Date	17 NOV 98
Dosage Period [43 days prior to the first day of cohabitation[a] until confirmed mating (DG 0)]	23 NOV 98 - 08 JAN 99
Cohabitation Period	04 JAN 99 PM - 09 JAN 99 AM
DG[b] 0	05 JAN 99 - 08 JAN 99
Delivery Period (DL[c] 1)	26 JAN 99 - 30 JAN 99
Sacrifice of Fo generation rats not selected for continued evaluation	15 JAN 99
DL 4 Culling (pups not selected for continued observation)	29 JAN 99 - 02 FEB 99
DG 25 Sacrifice (dam with no confirmed date of mating)	02 FEB 99
DL 21 Scheduled Sacrifice (F1 generation pups)	15 FEB 99 - 19 FEB 99
DL 22 Scheduled Sacrifice (Fo generation dams)	16 FEB 99 - 20 FEB 99

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 final report, after which time the Sponsor will decide their final disposition. All unused prepared formulations were discarded at the Testing Facility. All remaining bulk test article was returned to the Study Monitor upon completion of all work with the test article.

B. Test Article Information:

B.1. Description:

PFOS (FC-95) - off-white powder

B.2. Lot/Batch Number:

217 (Expiration date: May 2000)

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a. See APPENDIX D (DEVIATIONS FROM THE PROTOCOL AND THE STANDARD OPERATING PROCEDURES OF THE TESTING FACILITY), item 1.

b. DG is used as an abbreviation for day of (presumed) gestation.

c. DL is used as an abbreviation for day of lactation or day postpartum.

B.3. Date Received and Storage Conditions:

The test article was received on 21 October 1998, and stored at room temperature.

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 Activity:

Information regarding the identity, composition, strength and purity of the test article is on file with the Sponsor.

C. Vehicle Information:

C.1. Description:

0.5% Tween® 80 in Reverse Osmosis Membrane Processed Deionized Water (R.O. Deionized Water).
Tween® 80 - a clear or yellow viscous liquid

C.2. Lot Numbers:

Tween® 80 - M29477 and M03H05

C.3. Dates Received, Source and Storage Conditions:

Tween® 80 was received from J.T. Baker, Phillipsburg, New Jersey, on 17 September 1998 (lot M29477) and 3 December 1998 (lot M03H05), and stored at room temperature. The 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 and Storage Conditions:

Suspensions of PFOS (PC-95) were prepared daily at concentrations of 0, 0.02, and 0.32 mg/mL. Prepared formulations were stored at room temperature.

D.1. Sample Information:

Sample Type	Size	Date Retained	Storage Conditions	Shipped To	Date Shipped
Concentration (all levels)	2 mL[a]	22 NOV 98 07 JAN 99	Frozen	Sponsor	23 NOV 98 12 JAN 99
Bulk Test Article Reserve	1 g	19 NOV 98	Room temperature	Testing Facility Archives	27 JAN 99
Vehicle Components Reserve Tween® 80 Lot M29477 Lot M03H05 R.O. Water	 5 mL 5 mL 5 mL	 19 NOV 98 10 DEC 98 19 NOV 98	Room temperature	Testing Facility Archives	 27 JAN 99 27 JAN 99 27 JAN 99

a. Duplicate samples were taken from the first and last preparation. One sample of each set was shipped for analysis; the remaining samples were retained as backups. Backup samples were stored frozen (-70°C or below) and will be discarded at the Testing Facility upon the request of the Sponsor.

D.2. Analytical Results:

Information on the stability and homogeneity of the prepared formulations and of the stability of the bulk test article are on file with the Sponsor. Data verifying the stability of the test article in the vehicle for 48 hours under the conditions of administration are on file with the Sponsor. Records were maintained to document how the test article formulations were prepared.

Results of the concentration analyses were not available at the time of the writing of this report.

E. Test System:

E.1. Species:

Rat

E.2. Strain:

Crl:CD®BR VAF/Plus® (Sprague-Dawley)

E.3. Supplier (Source):

Charles River Laboratories, Inc., Raleigh, North Carolina

E.4. Sex:

Female (Note: Male rats were used only for the purposes of breeding and are not considered part of the Test System.)

E.5. Rationale for Test System:

The Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat was selected as the Test System because: 1) this strain of rat was used in the reproductive and developmental toxicity studies; 2) historical data and experience exist at the Testing Facility[5-7]; and 3) the test article is pharmacologically active in the species and strain.

E.6. Test System Data:

Number of Rats	37
Approximate Date of Birth	14 SEP 98
Approximate Age at Arrival	65 days
Weight (g) on the Day After Arrival	181 - 222
Weight (g) at Study Assignment	192 - 231

E.7. Breeder Male Rat Data:

Number of Rats	112
Approximate Date of Birth	13 JAN 98
Approximate Age at Arrival	78 days
Weight (g) on the Day After Arrival	300 - 356
Weight (g) at Cohabitation	515 - 893

E.8. Method of Randomization:

Upon arrival, rats were assigned to individual housing on the basis of computer-generated random units. After acclimation, 36 virgin female rats were placed into groups on the basis of physical appearance and body weights recorded during acclimation. Female rats were assigned to three dosage groups (Groups I through III), 12 rats per dosage group, using a computer-generated (weight-ordered) randomization procedure. After these 36 rats were cohabitated,

eight mated female rats (those with confirmed evidence of mating) were selected for the study from each dosage group.

On DL 4, a table of random units was used to cull the litters to five male pups and five female pups per litter, where possible.

E.9. System of Identification:

E.9.a. Fo Generation Rats:

Male rats were given unique permanent identification numbers upon assignment to the Testing Facility's breeder male rat population. Female rats were assigned temporary numbers at receipt and given unique permanent identification numbers before cohabitation. Each rat was individually identified with a Monel® self-piercing ear tag (Gey Band and Tag Co., Inc., No. MSPT 20101).

E.9.b. F1 Generation Pups:

Pups were not individually identified during lactation; all parameters were evaluated in terms of the litter.

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. Room temperature and humidity were monitored constantly throughout the study. Room temperature was targeted at 64°F to 79°F (18°C to 26°C); relative humidity was targeted at 30% to 70%. See APPENDIX E (TEMPERATURE AND RELATIVE HUMIDITY REPORTS).

F.3. Housing:

All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals*[8]. Fo generation rats were individually housed in stainless steel, wire-bottomed cages, except during the cohabitation and postpartum periods. During cohabitation, each pair of rats was housed in the male rat's cage. Beginning no later than DG 20, Fo generation female rats

were individually housed in nesting boxes, except during collection intervals for urine and fecal samples. During these collection intervals, the female rats were housed individually in metabolism cages. Each dam and delivered litter were housed in a common nesting box during the postpartum period.

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:

Rats were given *ad libitum* access to Certified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, Missouri) in individual feeders.

F.7. Feed Analysis:

Analyses were routinely performed by the feed supplier. No contaminants at levels exceeding the maximum concentration 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 potential contaminants likely to have been present in the feed that would have interfered 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 rats *ad libitum* from an automatic watering access system and/or individual water bottles. 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 nor the Sponsor was aware of any potential contaminants likely to have been present in the water that would have interfered with the results of this study.

F.10. Bedding:

Bed-o'cobs® was used as the nesting material (The Andersons' Industrial Products Groups, Maumee, Ohio).

F.11. Bedding Analysis:

Bedding was changed as often as necessary to keep the animals dry and clean. Analyses for possible contamination were conducted annually and documented in the raw data.

Neither the Study Director nor the Sponsor was aware of any agent present in the bedding that was known to interfere with the results of this study.

G. Methods:

G.1. Dosage Administration:

Dosage Group	Dosage (mg/kg/day)[a]	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Female Rats	Assigned Rat Numbers
I	0 (Vehicle)	0	5	8	13726 - 13728, 13731, 13734 – 13737
II	0.1	0.02	5	8	13739 - 13741, 13744 – 13746, 13748 – 13749
III	1.6	0.32	5	8	13751 - 13756, 13758 – 13859

a. The test article was considered 100% pure for the purpose of dosage calculations.

G.2. Rationale for Dosage Selection:

Dosages were selected on the basis of a previous study conducted with the test article (Argus Research Laboratories, Inc., Protocol 418-008). In this study the Fo generation maternal and paternal no-observable-effect-level (NOEL) of PFOS was 0.1 mg/kg/day (0.4 mg/kg/day and higher dosages caused reductions in body weight gain and reduced feed consumption values).

The Fo generation reproductive NOEL was greater than 3.2 mg/kg/day; no effects on mating, fertility or estrous cycling occurred. The NOEL for viability and growth in the F1 generation offspring was 0.4 mg/kg/day (1.6 mg/kg/day and higher dosages caused preimplantation loss and reductions in litter size, pup viability, growth and survival).

The F1 generation maternal and paternal NOEL of PFOS was 0.1 mg/kg/day (0.4 mg/kg/day dosage caused reductions in body weight gain and reduced feed consumption values). The F1 generation reproductive NOEL was greater than a dosage of 0.4 mg/kg/day; no effects on mating or fertility occurred. The NOEL for viability and growth in the F2 generation offspring was 0.1 mg/kg/day (0.4 mg/kg/day dosage caused stillbirths and reductions in litter size, pup viability, growth and survival).

G.3. Route of Administration:

Oral (gavage)

G.4. Rationale for Route of Administration:

The oral (gavage) route was selected for use because: 1) this was the route of administration in the developmental and reproductive toxicology studies; and 2) it is one of the possible routes of human exposure.

G.5. Frequency of Administration:

G.5.a. Fo Generation Female Rats:

Appropriate dosages[a] of the test article or vehicle were administered orally (via gavage) once daily to female rats beginning 43[b] days prior to cohabitation until DG 0 (confirmed evidenced of mating, such as observation of spermatozoa in a smear of the vaginal contents or a copulatory plug *in situ*). Female rats were not given the test article or vehicle on DG 0. Dosages were adjusted daily on the basis of the individual body weights recorded before intubation. The rats were intubated once daily at approximately the same time each day.

G.5.b. F1 Generation Pups:

F1 generation pups were not directly given the test article, but may have been possibly exposed to the test article during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period.

G.6. Length of Study:

Approximately 14 weeks

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a. See APPENDIX D, item 2.
b. See APPENDIX D, item 1.

G.7. Method of Study Performance:

G.7.a. Fo Generation Rats:

After acclimation and 43 days of dosage administration, 36 healthy virgin female rats were placed into cohabitation with 36 breeder male rats (one male rat per female rat in the male rat's cage). The cohabitation period consisted of a maximum of five days. Mating was evaluated daily during the cohabitation period. Female rats with spermatozoa observed in a smear of the vaginal contents or a copulatory plug *in situ* were considered to be at DG 0 and returned to individual housing. Twenty-four mated female rats, eight per dosage group, were selected for the study, as previously discussed.

The female rats were observed for viability at least twice each day of the study and for general appearance at least once during acclimation. The rats were also examined for clinical observations of effects of the test article, abortions, premature deliveries and deaths before and approximately one hour after dosage. Rats were observed for clinical observations and general appearance once daily during all other periods of study.

Body weights were recorded once during acclimation. Body weights were recorded daily during the dosage and postdosage periods and at sacrifice. Feed consumption values were recorded once during acclimation, weekly prior to cohabitation, daily during gestation and on DLs 1, 4, 7, 10 and 14. Feed consumption was not tabulated after DL 14, when it was expected that pups would begin to consume maternal feed.

The female rats were evaluated for duration of gestation (DG 0 to the day the first pup was observed), litter sizes (defined as all pups delivered), live litter size (live born pups only) and pup viability at birth. Pups that either appeared stillborn or that died before initial examination of the litters for viability were examined for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn; pups with lungs that floated were considered liveborn and to have died shortly after birth. Maternal behavior of the dams was evaluated daily when the pups were examined during the 21-day postpartum period. Observed maternal behavior was recorded on DLs 1, 4, 7, 10, 14 and 21. Deviations from expected maternal behavior were recorded, if and when present, on all other days during the postpartum period.

G.7.b. F1 Generation Rats:

Day 1 of lactation (postpartum) was defined as the day of birth and was also the first day on which all pups in a litter were individually weighed (pup body weights were recorded after all pups in a litter were delivered and groomed by the dam).

The litters were observed for viability at least twice each day during the postpartum period. Dead pups observed at these times were removed from the nesting box. Litters were observed for clinical observations and general appearance once daily during the postpartum period. The pups in each litter were counted once daily. Body weights were recorded on DLs 1 (birth), 4, 7, 14 and 21.

G.8. Pharmacokinetic Sample Collection:

G.8.a. Fo Generation Rats:

Urine and fecal sample were collected from female rats for the following intervals: one day prior to initiation of cohabitation to the following morning, DGs 6 to 7, 14 to 15 and 20 to 21, and DLs 21 to 22. Following each 24-hour collection interval, samples were collected into centrifuge tubes, placed on dry ice and stored frozen (-70°C or below) and shipped, frozen on dry ice, to the Sponsor for analysis.

Blood samples were collected from each of the maternal rats following removal from metabolism caging (prior to test article or vehicle administration) on each of the following days: on the day cohabitation is initiated (prior to cohabitation), DGs 7, 15 and 21, and DLs 14 and 22.

On all days of collection except DL 22, blood samples (approximately 1 mL each) were collected from the orbital sinus. On DL 22, blood samples (approximately 4 mL each) were collected via the inferior vena cava. Blood was collected and transferred into serum separator tubes. The samples were spun in a refrigerated centrifuge. The serum was transferred into polypropylene tubes labeled with the study number, rat identification, date of collection, study day and collection timepoint. All samples were immediately frozen on dry ice and maintained frozen (-70°C or below), and shipped, frozen on dry ice, to the Sponsor for analysis.

G.8.b. F1 Generation Litters:

On DL 21, blood samples were collected from all remaining pups on study. Blood samples were collected via the inferior vena cava from each pup, pooled (per litter) and transferred into serum separator tubes. The samples were spun in a refrigerated centrifuge. The serum was transferred into polypropylene tubes labeled with the study number, rat identification, date of collection, study day and collection timepoint. All samples were immediately frozen on dry ice and maintained frozen (-70°C or below) and shipped to the Sponsor for analysis.

G.9. Gross Necropsy:

G.9.a. Fo Generation Rats:

Female rats, with or without confirmed evidence of mating, that were cohabited but not assigned to the study, were sacrificed by carbon dioxide asphyxiation and discarded without further evaluation.

All surviving rats assigned to the study were sacrificed by carbon dioxide asphyxiation on DL 22 following the final collection interval for urine and fecal samples and blood sample collection. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Tissues with gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation. Representative photographs of maternal lesions are available in the raw data. The number and distribution of implantation sites was recorded. A liver section (right lateral lobe) from each dam was collected, frozen and stored (-70°C or below) until shipment to the Sponsor for analysis. All other maternal tissues were discarded.

The rat that did not deliver a litter was sacrificed on DG 25 and examined for gross lesions. Uteri were stained with 10% ammonium sulfide to confirm the absence of implantation sites[9]. The rat that was found dead was examined for the cause of death on the day of the death. The rat was examined for gross lesions. A liver section (right lateral lobe) was collected and stored, as described above. Pregnancy status was recorded; fetuses were examined to the extent possible.

G.9.b. F1 Generation Pups:

All pups culled on DL 4 were sacrificed via decapitation. The lungs and the livers were collected from the first ten pups culled determined to be at DL 4 from each dosage group (irrespective of litter) and preserved for possible future histopathological evaluation. The lungs were individually retained in Bouin's solution, and the livers were individually retained in neutral buffered 10% formalin for possible future evaluation. Remaining culled pups were sacrificed and discarded without evaluation.

Pups found dead were examined for gross lesions and for the cause of death. For all pups found dead on DLs 2 to 4, all lungs were preserved in Bouin's solution for possible future evaluation.

All remaining pups on study were sacrificed on DL 21 by carbon dioxide asphyxiation and examined for gross lesions. Gross lesions were preserved in neutral buffered 10% formalin. The liver from each pup was collected, pooled

(per litter), frozen and stored (-70°C or below) and shipped to the Sponsor for analysis.

G.10. Statistical Analyses:

Averages and percentages were calculated. Litter values were used where appropriate.

III. RESULTS

A. Mortality, Clinical and Necropsy Observations (Summaries - Tables 1 and 2; Individual Data - Tables 19 and 20)

A.1. Mortality

No deaths or premature deliveries were attributed to PFOS treatment. One vehicle control group rat was injured and died after orbital sinus bleeding on DG `15. All other rats survived to scheduled sacrifice.

A.2. Clinical Observations

All adverse clinical observations during the precohabitation, gestation and lactation periods were considered unrelated to the test article because the incidences were not dosage-dependent and/or the observation occurred in only one rat in a group. These observations included dental problems (missing, broken and/or misaligned incisors), chromodacryorrhea, abrasion on the forepaw, localized alopecia on the head or limbs, chromorhinorrhea, exophthalmos, hemorrhagic area on the eye, urine-stained abdominal fur, missing eye, corneal opacity, swollen area on chest or around eye, red, dried perioral substance, torn ear and soft or liquid feces. A red perinasal substance, blue paws and gasping were agonal signs for the rat that died.

A.3. Necropsy Observations

A red substance was found in the thoracic cavity of the rat that died. All other rats appeared normal at necropsy.

B. Body Weights and Body Weight Changes (Figure 1; Summaries - Tables 3 through 8; Individual Data - Tables 21 through 23)

B.1. Precohabitation

Rats administered 1.6 mg/kg/day dosage of the test article had reduced body weight gains or body weight losses in each week of the precohabitation period. Reflecting these effects of the test article, body weight gains were 46.1% of the control group value for the entire precohabitation period (DSs 1 to 43) in the 1.6 mg/kg/day dosage group.

Precohabitation body weights and body weight gains were unaffected by the 0.1 mg/kg/day dosage of the test article.

B.2. Gestation

Dams administered 1.6 mg/kg/day dosage of the test article during the precohabitation period (treatment ended on DG 0) had reduced body weight gains during the first week of the gestation period (DGs 0 to 7). Body weight gains were then increased as compared to the control group value on DGs 7 to 10, 13 to 15 and 15 to 18. Reflecting this rebound effect, body weight gains were increased for the entire gestation period (DGs 0 to 20) in the 1.6 mg/kg/day dosage group, as compared to the control group value.

Gestation body weights and body weight gains were unaffected by the 0.1 mg/kg/day dosage of the test article.

B.3. Lactation

Body weight gains for dams administered the 1.6 mg/kg/day dosage of the test article during the precohabitation period generally continued to be increased during the lactation period.

Lactation body weights and body weight gains were unaffected by the 0.1 mg/kg/day dosage of the test article.

C. Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables 9 through 14; Individual Data - Tables 24 through 26)

C.1. Precohabitation

Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced in the 1.6 mg/kg/day dosage group in each week of the precohabitation period. Reflecting these effects of the test article, feed consumption values were reduced for the entire precohabitation period (DSs 1 to 43) in the 1.6 mg/kg/day dosage group.

Precohabitation feed consumption values were unaffected by the 0.1 mg/kg/day dosage of the test article.

C.2. Gestation

Dams administered 1.6 mg/kg/day dosage of the test article during the precohabitation period (treatment ended on DG 0) had reduced absolute and relative feed consumption values during the first week of the gestation period (DGs 0 to 7). Feed consumption values were then generally comparable to control group values for the remainder of the gestation period. Absolute and

relative feed consumption values in this dosage group were slightly reduced, as compared to control group values, for the entire gestation period (DGs 0 to 20).

Gestation feed consumption values were unaffected by the 0.1 mg/kg/day dosage of the test article.

C.3. Lactation

Absolute and relative feed consumption values during the lactation period were reduced in the groups administered 0.1 or 1.6 mg/kg/day of the test article during the precohabitation period, however the reductions were not strictly dosage-dependent.

D. Natural Delivery and Litter Observations (Summaries - Tables 15 and 16; Individual Data - Tables 27 through 30)

Natural delivery observations were based on 7, 8 and 7 pregnant rats in each of the three respective dosage groups.

Administration of the test article at dosages as high as 1.6 mg/kg/day did not adversely affect any parameter evaluated at natural delivery or during the 22-day lactation period (duration of gestation, averages for implantations and live litter sizes, numbers of dams with no liveborn pups or all pups dying during lactation, gestation, viability and lactation indices, surviving pups per litter, litter size at weighing, pup weight per litter and pup sex ratios).

E. Clinical Observations from Birth to Day 21 Postpartum and Necropsy Observations (Summaries - Tables 17 and 18; Individual Data - Tables 31 and 32)

No clinical or necropsy observations in the F1 generation pups were attributable to maternal dosages of the test article as high as 1.6 mg/kg/day because: 1) the incidences were not dosage-dependent; and/or 2) the observation occurred in only one or two pups. The only adverse clinical observation was a black tip of tail in one 0.1 mg/kg/day dosage group pup. No milk in stomach occurred in 0, 1 and 2 pups that were found dead in the three respective dosage groups. All pups appeared normal at necropsy on DL 4 or 21.

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.

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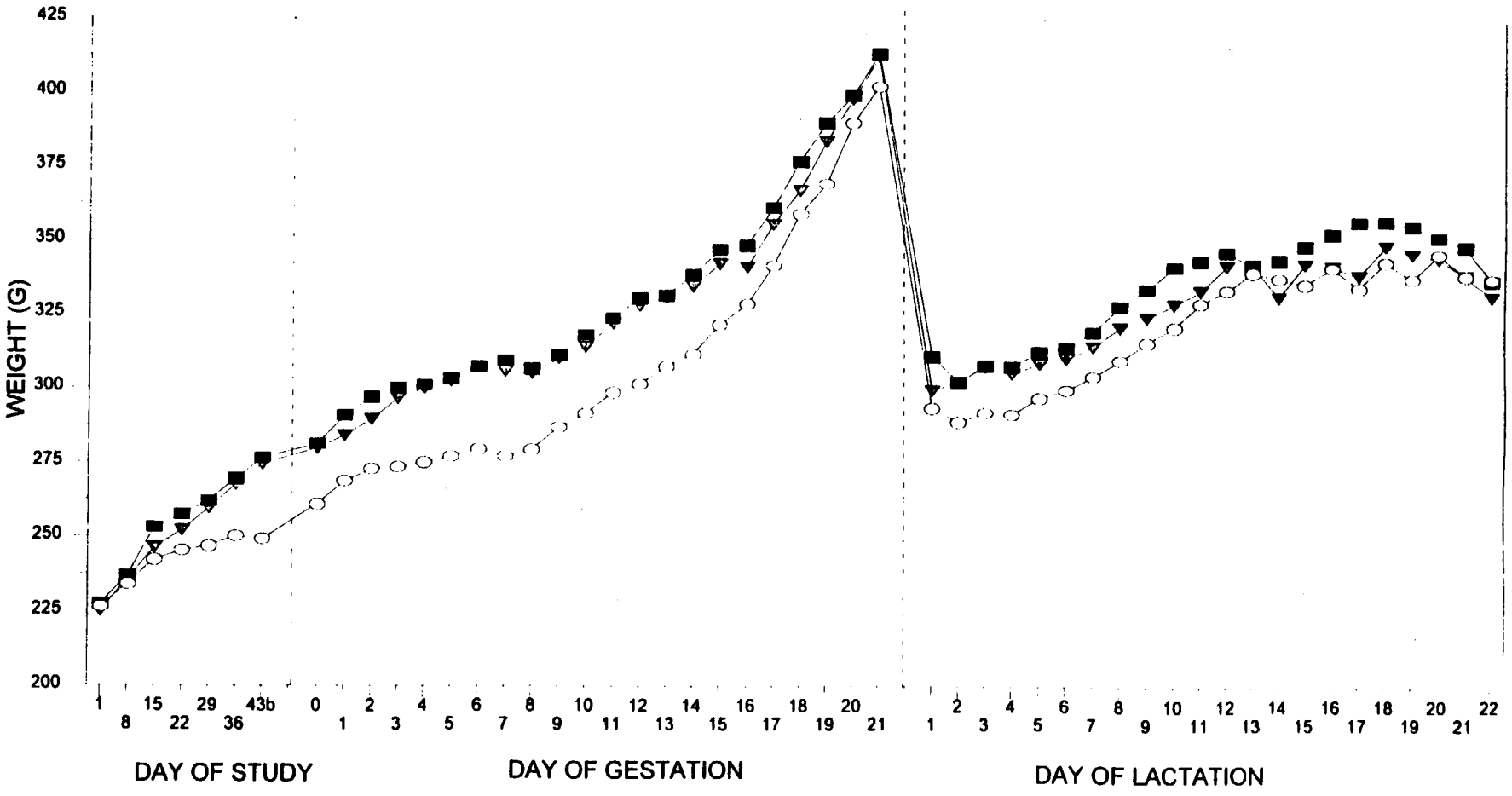
APPENDIX A

REPORT FIGURE

BODY WEIGHTS

Fo GENERATION FEMALE RATS

Figure 1



a. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
b. Last value recorded before cohabitation.

▼ 0 (VEHICLE) MG/KG/DAY ■ 0.1 MG/KG/DAY a ○ 1.6 MG/KG/DAY a

APPENDIX B

REPORT TABLES

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	I 0 (VEHICLE)	II 0.1b	III 1.6b
PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION):			
MAXIMUM POSSIBLE INCIDENCE	516/ 12	516/ 12	516/ 12
MORTALITY	0	0	0
INCISORS: TOTAL	0/ 0	0/ 0	16/ 1
MISALIGNED	0/ 0	0/ 0	16/ 1
MISSING/BROKEN	0/ 0	0/ 0	5/ 1
CHROMODACRYORRHEA	0/ 0	0/ 0	10/ 1
RIGHT FOREPAW: ABRASION	0/ 0	0/ 0	5/ 1
LOCALIZED ALOPECIA: HEAD	0/ 0	2/ 1	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on day 1 of study until day 0 of presumed gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	I 0 (VEHICLE)	II 0.1b	III 1.6b
PRESUMED GESTATION: c			
MAXIMUM POSSIBLE INCIDENCE	190/ 12	192/ 11	201/ 12
ACCIDENTAL DEATH	1d	0	0
LOCALIZED ALOPECIA: LIMBS	0/ 0	5/ 2	11/ 2
CHROMODACRYORRHEA	5/ 1	12/ 3	5/ 1
CHROMORHINORRHEA	1/ 1	9/ 2	3/ 1
INCISORS: MISALIGNED	0/ 0	0/ 0	5/ 1
EXOPHTHALMOS	5/ 1	4/ 1	0/ 0
EYES: HEMORRHAGIC AREA	5/ 1	4/ 1	0/ 0
URINE-STAINED ABDOMINAL FUR	0/ 0	2/ 1	0/ 0
RIGHT EYE: REMOVED	0/ 0	1/ 1	0/ 0
GASPING	1/ 1d	0/ 0	0/ 0
PAWS: BLUE	1/ 1d	0/ 0	0/ 0
RED PERINASAL SUBSTANCE	1/ 1d	0/ 0	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.

a. Dosage occurred on day 1 of study until day 0 of presumed gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Restricted to rats with a confirmed mating date.
d. Dam 13731 had an accidental death on day 15 of gestation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 1 (PAGE 3): CLINICAL OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	I 0 (VEHICLE)	II 0.1b	III 1.6b
LACTATION:			
MAXIMUM POSSIBLE INCIDENCE	154/ 7	176/ 8	154/ 7
MORTALITY	0	0	0
CHROMODACRYORRHEA	0/ 0	10/ 2	1/ 1
CORNEAL OPACITY	0/ 0	0/ 0	7/ 1
CHROMORHINORRHEA	0/ 0	20/ 4	0/ 0
RIGHT EYE: MISSING	0/ 0	22/ 1	0/ 0
CHEST OR AROUND RIGHT EYE: SWOLLEN	1/ 1	7/ 1	0/ 0
RED DRIED PERIORAL SUBSTANCE	0/ 0	1/ 1	0/ 0
RIGHT EAR: TORN	3/ 1	0/ 0	0/ 0
SOFT OR LIQUID FECES	3/ 1	0/ 0	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
RATS EXAMINED c	N	8d	8d	8d
ACCIDENTAL DEATH	N	1e	0	0
APPEARED NORMAL	N	7	8	8
THORACIC CAVITY:				
RED SUBSTANCE	N	1e	0	0

a. Dosage occurred on day 1 of study until day 0 of presumed gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Refer to the individual clinical observations table (Table 19) for external observations confirmed at necropsy.
d. Excludes values for rats that were not selected for continuation on study.
e. Dam 13731 had an accidental death on day 15 of gestation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 3 (PAGE 1): BODY WEIGHTS - PRECOHABITATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
RATS TESTED	N	12	12	12
BODY WEIGHT (G)				
DAY 1	MEAN±S.D	225.0 ± 10.2	227.2 ± 8.9	226.3 ± 12.3
DAY 8	MEAN±S.D	235.4 ± 10.8	236.6 ± 10.5	233.9 ± 13.0
DAY 15	MEAN±S.D	246.2 ± 10.9	253.1 ± 12.0	242.3 ± 14.4
DAY 22	MEAN±S.D	252.2 ± 13.4	257.5 ± 14.4	245.2 ± 15.7
DAY 29	MEAN±S.D	259.6 ± 12.7	262.0 ± 15.5	246.9 ± 14.5
DAY 36	MEAN±S.D	267.6 ± 15.8	269.6 ± 15.8	250.3 ± 18.6
DAY 43c	MEAN±S.D	274.5 ± 17.9	276.4 ± 18.6	249.2 ± 21.5

DAY = DAY OF STUDY

a. Dosage occurred on day 1 of study until day 0 of presumed gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

c. Last value recorded before cohabitation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 4 (PAGE 1): BODY WEIGHT CHANGES - PRECOHABITATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
BODY WEIGHT CHANGE (G)				
DAYS 1 - 8	MEAN±S.D.	+10.4 ± 4.9	+9.4 ± 3.3	+7.6 ± 6.7
DAYS 8 - 15	MEAN±S.D.	+10.8 ± 5.6	+16.5 ± 6.8	+8.4 ± 4.4
DAYS 15 - 22	MEAN±S.D.	+6.1 ± 6.6	+4.4 ± 6.5	+2.9 ± 3.8
DAYS 22 - 29	MEAN±S.D.	+7.3 ± 6.9	+4.5 ± 6.8	+1.7 ± 3.0
DAYS 29 - 36	MEAN±S.D.	+8.0 ± 6.3	+7.6 ± 6.8	+3.4 ± 5.8
DAYS 36 - 43c	MEAN±S.D.	+6.9 ± 6.6	+6.8 ± 6.9	-1.2 ± 5.3
DAYS 1 - 43c	MEAN±S.D.	+49.5 ± 14.5	+49.2 ± 14.0	+22.8 ± 16.3

DAYS = DAYS OF STUDY

a. Dosage occurred on day 1 of study until day 0 of presumed gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

c. Last value recorded before cohabitation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 5 (PAGE 1): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
MATERNAL BODY WEIGHT (G)				
DAY 0	MEAN±S.D.	279.6 ± 20.2	281.1 ± 20.9	261.0 ± 19.7
DAY 1	MEAN±S.D.	284.1 ± 18.0	290.8 ± 18.6	268.7 ± 19.9
DAY 2	MEAN±S.D.	289.5 ± 16.7	297.2 ± 17.5	272.7 ± 21.0
DAY 3	MEAN±S.D.	296.5 ± 19.4	300.2 ± 19.0	273.6 ± 20.2
DAY 4	MEAN±S.D.	299.9 ± 19.7	301.1 ± 19.2	275.1 ± 19.4
DAY 5	MEAN±S.D.	302.6 ± 18.9	303.5 ± 17.6	277.0 ± 16.7
DAY 6	MEAN±S.D.	307.2 ± 21.3	307.6 ± 16.6	279.7 ± 15.0
DAY 7	MEAN±S.D.	305.9 ± 18.0	309.5 ± 15.5	277.1 ± 15.0
DAY 8	MEAN±S.D.	305.1 ± 21.1	306.9 ± 17.7	279.4 ± 16.2
DAY 9	MEAN±S.D.	310.2 ± 20.3	311.5 ± 17.3	287.0 ± 16.1
DAY 10	MEAN±S.D.	314.1 ± 19.2	318.1 ± 19.3	291.6 ± 17.5
DAY 11	MEAN±S.D.	322.0 ± 24.2	323.8 ± 18.9	298.8 ± 21.1
DAY 12	MEAN±S.D.	328.1 ± 20.8	330.6 ± 16.3	301.7 ± 15.9
DAY 13	MEAN±S.D.	330.8 ± 22.9	331.5 ± 18.6	307.6 ± 21.7

DAY = DAY OF GESTATION

a. Dosage occurred on day 1 of study until day 0 of gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

c. Excludes values for rats that were not selected for continuation on study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 5 (PAGE 2): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
MATERNAL BODY WEIGHT (G)				
DAY 14	MEAN±S.D.	334.5 ± 22.6	338.5 ± 17.9	311.7 ± 19.2
DAY 15	MEAN±S.D.	342.2 ± 24.1 [7]d	347.1 ± 20.9	321.8 ± 22.0
DAY 16	MEAN±S.D.	341.1 ± 23.5 [7]d	348.5 ± 18.6	328.6 ± 20.8
DAY 17	MEAN±S.D.	355.6 ± 25.8 [7]d	361.2 ± 17.2	341.6 ± 19.3
DAY 18	MEAN±S.D.	367.1 ± 27.2 [7]d	376.9 ± 21.2	359.3 ± 19.6
DAY 19	MEAN±S.D.	383.7 ± 29.5 [7]d	390.1 ± 20.5	369.4 ± 18.0
DAY 20	MEAN±S.D.	398.0 ± 31.0 [7]d	399.4 ± 21.7	390.0 ± 25.1
DAY 21	MEAN±S.D.	412.4 ± 34.5 [7]d	413.4 ± 31.2	402.3 ± 20.6

DAY = DAY OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values for rats that were not selected for continuation on study.
d. Excludes values for dam 13731, which had an accidental death on day 15 of gestation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 6 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
MATERNAL BODY WEIGHT CHANGE (G)				
DAYS 0 - 7	MEAN±S.D.	+26.2 ± 7.0	+28.4 ± 9.4	+16.1 ± 7.0
DAYS 7 - 10	MEAN±S.D.	+8.2 ± 8.1	+8.6 ± 9.0	+14.4 ± 7.8
DAYS 10 - 13	MEAN±S.D.	+16.6 ± 6.5	+13.4 ± 8.8	+16.0 ± 7.9
DAYS 13 - 15	MEAN±S.D.	+11.5 ± 6.9	+15.6 ± 12.1	+14.3 ± 8.6
DAYS 15 - 18	MEAN±S.D.	+28.7 ± 7.4 [7]d	+29.8 ± 14.1	+37.4 ± 5.3
DAYS 18 - 20	MEAN±S.D.	+30.8 ± 6.1 [7]d	+22.5 ± 8.7	+30.7 ± 6.9
DAYS 0 - 20	MEAN±S.D.	+119.6 ± 16.3 [7]d	+118.2 ± 18.4	+129.0 ± 16.8

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values for rats that were not selected for continuation on study.
d. Excludes values for dam 13731, which had an accidental death on day 15 of gestation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 7 (PAGE 1): MATERNAL BODY WEIGHTS - LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
DELIVERED LITTERS	N	7d	8	7
MATERNAL BODY WEIGHT (G)				
DAY 1	MEAN±S.D.	299.6 ± 20.6	311.1 ± 19.7	293.6 ± 23.8
DAY 2	MEAN±S.D.	302.6 ± 25.8	302.4 ± 23.3	289.0 ± 20.8
DAY 3	MEAN±S.D.	307.7 ± 23.5	308.1 ± 28.4	292.0 ± 15.9
DAY 4	MEAN±S.D.	305.3 ± 22.9	307.8 ± 31.7	291.4 ± 19.6
DAY 5	MEAN±S.D.	308.7 ± 23.5	312.8 ± 18.6	297.1 ± 16.7
DAY 6	MEAN±S.D.	310.4 ± 23.1	314.1 ± 16.5	299.7 ± 15.4
DAY 7	MEAN±S.D.	314.4 ± 17.9	319.2 ± 12.5	304.4 ± 17.6
DAY 8	MEAN±S.D.	321.0 ± 21.2	328.1 ± 14.4	309.8 ± 14.3
DAY 9	MEAN±S.D.	324.3 ± 20.3	333.8 ± 12.7	316.0 ± 13.3
DAY 10	MEAN±S.D.	328.8 ± 19.6	341.4 ± 13.1	321.1 ± 14.6
DAY 11	MEAN±S.D.	333.4 ± 21.4	343.6 ± 13.1	329.1 ± 11.8
DAY 12	MEAN±S.D.	341.8 ± 21.2	346.4 ± 11.5	333.6 ± 12.6

DAY = DAY OF LACTATION

a. Dosage occurred on day 1 of study until day 0 of gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

c. Excludes values for rats that were not selected for continuation on study.

d. Excludes values for dam 13731, which had an accidental death on day 15 of gestation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 7 (PAGE 2): MATERNAL BODY WEIGHTS - LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
DELIVERED LITTERS	N	7d	8	7
MATERNAL BODY WEIGHT (G)				
DAY 13	MEAN±S.D.	342.4 ± 23.5	342.2 ± 17.0	339.8 ± 23.5
DAY 14	MEAN±S.D.	331.4 ± 16.9	344.1 ± 20.7	337.8 ± 16.2
DAY 15	MEAN±S.D.	342.6 ± 19.2	348.9 ± 19.1	335.8 ± 17.8
DAY 16	MEAN±S.D.	341.8 ± 19.4	353.0 ± 15.5	341.4 ± 19.9
DAY 17	MEAN±S.D.	338.8 ± 21.2	356.8 ± 14.0	334.7 ± 23.1
DAY 18	MEAN±S.D.	348.8 ± 19.2	357.1 ± 16.8	343.4 ± 21.7
DAY 19	MEAN±S.D.	346.0 ± 11.1	355.5 ± 13.7	337.7 ± 19.6
DAY 20	MEAN±S.D.	344.8 ± 14.5	351.8 ± 19.4	346.0 ± 17.4
DAY 21	MEAN±S.D.	339.1 ± 14.0	348.8 ± 20.5	339.1 ± 20.9
DAY 22	MEAN±S.D.	331.7 ± 16.7	338.5 ± 22.5	337.8 ± 23.3

DAY = DAY OF LACTATION
a. Dosage occurred on day 1 of study until day 0 of presumed gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values for rats that were not selected for continuation on study.
d. Excludes values for dam 13731, which had an accidental death on day 15 of gestation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 8 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
DELIVERED LITTERS	N	7d	8	7
MATERNAL BODY WEIGHT CHANGE (G)				
DAYS 1 - 4	MEAN±S.D.	+5.7 ± 6.7	-3.4 ± 30.1	-2.1 ± 11.4
DAYS 4 - 7	MEAN±S.D.	+9.1 ± 6.3	+11.5 ± 25.5	+13.0 ± 8.8
DAYS 7 - 10	MEAN±S.D.	+14.4 ± 4.2	+22.1 ± 4.7	+16.7 ± 5.4
DAYS 10 - 14	MEAN±S.D.	+2.6 ± 12.7	+2.8 ± 13.0	+16.7 ± 5.5
DAYS 14 - 22	MEAN±S.D.	+0.3 ± 14.5	-5.6 ± 24.3	+0.0 ± 11.5
DAYS 1 - 22	MEAN±S.D.	+32.1 ± 10.0	+27.4 ± 22.2	+44.3 ± 21.7

DAYS = DAYS OF LACTATION

a. Dosage occurred on day 1 of study until day 0 of gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

c. Excludes values for rats that were not selected for continuation on study.

d. Excludes values for dam 13731, which had an accidental death on day 15 of gestation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 9 (PAGE 1): ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - PRECOHABITATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
FEED CONSUMPTION (G)				
DAYS 1 - 8	MEAN±S.D.	19.6 ± 1.2 [11]c	18.9 ± 1.2 [10]c	18.3 ± 1.7
DAYS 8 - 15	MEAN±S.D.	18.8 ± 1.5 [10]c	20.2 ± 1.9	17.8 ± 1.4
DAYS 15 - 22	MEAN±S.D.	19.6 ± 1.8 [10]c	19.2 ± 1.4 [10]c	17.5 ± 1.3 [11]c
DAYS 22 - 29	MEAN±S.D.	18.4 ± 2.2 [10]c	18.1 ± 2.1 [10]c	15.8 ± 1.5
DAYS 29 - 36	MEAN±S.D.	19.1 ± 2.0 [10]c	19.2 ± 1.6 [9]c	16.6 ± 1.6 [11]c
DAYS 36 - 43d	MEAN±S.D.	20.4 ± 1.9 [11]c	20.3 ± 2.1 [11]c	17.5 ± 2.8
DAYS 1 - 43d	MEAN±S.D.	19.4 ± 1.5 [11]c	19.6 ± 1.8 [11]c	17.4 ± 1.6

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study until day 0 of presumed gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values that were associated with spillage.
d. Last value recorded before cohabitation.

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TABLE 10 (PAGE 1): RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - PRECOHABITATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
RATS TESTED	N	12	12	12
FEED CONSUMPTION (G/KG/DAY)				
DAYS 1 - 8	MEAN±S.D.	85.0 ± 5.2 [11]c	82.3 ± 3.8 [10]c	79.3 ± 4.9
DAYS 8 - 15	MEAN±S.D.	77.4 ± 5.4 [10]c	82.2 ± 5.6	74.8 ± 4.5
DAYS 15 - 22	MEAN±S.D.	78.8 ± 6.7 [10]c	76.1 ± 4.2 [10]c	71.7 ± 3.3 [11]c
DAYS 22 - 29	MEAN±S.D.	71.7 ± 7.8 [10]c	69.1 ± 5.3 [10]c	64.4 ± 5.2
DAYS 29 - 36	MEAN±S.D.	72.2 ± 5.9 [10]c	72.4 ± 4.5 [9]c	66.8 ± 4.6 [11]c
DAYS 36 - 43d	MEAN±S.D.	74.5 ± 4.5 [11]c	73.6 ± 5.8 [11]c	70.0 ± 9.2
DAYS 1 - 43d	MEAN±S.D.	76.7 ± 4.9 [11]c	76.6 ± 4.7 [11]c	71.7 ± 4.7

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study until day 0 of presumed gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values that were associated with spillage.
d. Last value recorded before cohabitation.

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TABLE 11 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
MATERNAL FEED CONSUMPTION (G/DAY)				
DAYS 0 - 7	MEAN±S.D.	25.2 ± 1.2 [7]d	24.2 ± 2.0	21.1 ± 2.4
DAYS 7 - 10	MEAN±S.D.	23.8 ± 2.4	24.1 ± 2.3	22.1 ± 3.0
DAYS 10 - 13	MEAN±S.D.	26.5 ± 2.6	26.4 ± 1.4	22.5 ± 2.8
DAYS 13 - 15	MEAN±S.D.	24.9 ± 3.0	25.5 ± 2.3	26.1 ± 2.7
DAYS 15 - 18	MEAN±S.D.	26.0 ± 1.9 [7]e	27.5 ± 2.8	26.2 ± 1.6
DAYS 18 - 20	MEAN±S.D.	25.4 ± 3.3 [7]e	27.1 ± 2.4	23.8 ± 3.7
DAYS 0 - 20	MEAN±S.D.	25.2 ± 1.3 [7]e	25.4 ± 0.8	23.0 ± 2.0

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values for rats that were not selected for continuation on study.
d. Excludes values that were associated with spillage.
e. Excludes values for rat 13731, which had an accidental death on day 15 of gestation.

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TABLE 12 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
MATERNAL FEED CONSUMPTION (G/KG/DAY)				
DAYS 0 - 7	MEAN±S.D.	84.5 ± 6.6 [7]d	81.1 ± 7.6	77.9 ± 11.9
DAYS 7 - 10	MEAN±S.D.	76.9 ± 5.3	77.3 ± 6.2	78.1 ± 12.3
DAYS 10 - 13	MEAN±S.D.	81.9 ± 5.5	81.0 ± 6.5	75.0 ± 9.0
DAYS 13 - 15	MEAN±S.D.	74.6 ± 10.5	75.2 ± 6.1	83.4 ± 8.8
DAYS 15 - 18	MEAN±S.D.	74.1 ± 4.5 [7]e	77.0 ± 10.3	77.8 ± 5.9
DAYS 18 - 20	MEAN±S.D.	66.3 ± 5.9 [7]e	70.1 ± 8.9	63.7 ± 7.1
DAYS 0 - 20	MEAN±S.D.	78.6 ± 4.3 [7]e	77.8 ± 5.0	76.1 ± 7.8

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values for rats that were not selected for continuation on study.
d. Excludes values that were associated with spillage.
e. Excludes values for rat 13731, which had an accidental death on day 15 of gestation.

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TABLE 13 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
DELIVERED LITTERS	N	7d	8	7
MATERNAL FEED CONSUMPTION (G/DAY)				
DAYS 1 - 4	MEAN±S.D.	31.3 ± 5.1	30.1 ± 18.9	23.2 ± 3.5
DAYS 4 - 7	MEAN±S.D.	47.7 ± 10.7 [6]e	42.1 ± 12.4 [7]e	40.1 ± 5.6
DAYS 7 - 10	MEAN±S.D.	54.4 ± 3.1	54.8 ± 8.6	51.0 ± 6.3
DAYS 10 - 14f	MEAN±S.D.	68.1 ± 2.9 [6]e	61.4 ± 5.2 [7]e	59.5 ± 3.4
DAYS 1 - 14f	MEAN±S.D.	52.9 ± 2.8 [6]e	41.7 ± 8.3 [7]e	44.7 ± 3.9

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values for rats that were not selected for continuation on study.
d. Excludes values for rat 13731, which had an accidental death on day 15 of gestation.
e. Excludes values that were associated with spillage.
f. Because it is presumed that the pups begin to consume maternal feed after day 14 of lactation, maternal feed consumption values were not tabulated on days 14 to 22 of lactation.

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TABLE 14 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
RATS TESTED	N	12	12	12
INCLUDED IN ANALYSES	N	8c	8c	8c
PREGNANT	N	8	8	7
DELIVERED LITTERS	N	7d	8	7
MATERNAL FEED CONSUMPTION (G/KG/DAY)				
DAYS 1 - 4	MEAN±S.D.	103.6 ± 20.3	98.7 ± 65.9	80.1 ± 14.9
DAYS 4 - 7	MEAN±S.D.	152.0 ± 33.1 [6]e	134.8 ± 44.5 [7]e	135.2 ± 23.8
DAYS 7 - 10	MEAN±S.D.	169.3 ± 12.9	166.2 ± 28.4	163.2 ± 21.1
DAYS 10 - 14f	MEAN±S.D.	202.0 ± 17.6 [6]e	178.4 ± 16.5 [7]e	179.3 ± 13.1
DAYS 1 - 14f	MEAN±S.D.	165.3 ± 16.6 [6]e	144.5 ± 28.9 [7]e	144.1 ± 16.6

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Excludes values for rats that were not selected for continuation on study.
d. Excludes values for rat 13731, which had an accidental death on day 15 of gestation.
e. Excludes values that were associated with spillage.
f. Because it is presumed that the pups begin to consume maternal feed after day 14 of lactation, maternal feed consumption values were not tabulated on days 14 to 22 of lactation.

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TABLE 15 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
RATS ASSIGNED TO NATURAL DELIVERY	N	8	8	8
PREGNANT	N	8	8	7
DELIVERED LITTERS	N	7c	8	7
DURATION OF GESTATION d	MEAN±S.D.	22.6 ± 0.5 [5]e	23.0 ± 0.0 [6]e	22.2 ± 0.4 [6]e
IMPLANTATION SITES	N	106	119	108
PER DELIVERED LITTER	MEAN±S.D.	15.1 ± 0.9	14.9 ± 2.0	15.4 ± 2.2
DAMS WITH STILLBORN PUPS	N(%)	1(14.3)	1(12.5)	0(0.0)
DAMS WITH NO LIVEBORN PUPS	N	0	0	0
GESTATION INDEX f	%	100.0	100.0	100.0
	N/N	7/ 7	8/ 8	7/ 7
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N	0	0	0
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N	0	0	0

[ ] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 1 of study until day 0 of gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

c. Excludes values for dam 13731, which had an accidental death on day 15 of gestation.

d. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as day 0) and the time (in days) the first pup was delivered.

e. Excludes values for rats that did not have delivery observations recorded.

f. Number of rats with live offspring/number of pregnant rats.

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TABLE 16 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	7	8	7
PUPS DELIVERED (TOTAL)	N	97	103	97
	MEAN±S.D.	13.8 ± 1.6	12.9 ± 3.0	13.8 ± 1.7
LIVEBORN	MEAN±S.D.	13.7 ± 1.4	12.8 ± 3.0	13.8 ± 1.7
	N(%)	96(99.0)	102(99.0)	97(100.0)
STILLBORN	MEAN±S.D.	0.1 ± 0.4	0.1 ± 0.4	0.0 ± 0.0
	N(%)	1(1.0)	1(1.0)	0(0.0)
CULLED	N	23	26	24
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED				
DAY 1	N/N(%)	0/ 96(0.0)	0/102(0.0)	1/ 97(1.0)
DAYS 2- 4	N/N(%)	3/ 96(3.1)	1/102(1.0)	2/ 96(2.1)
DAYS 5- 7	N/N(%)	0/ 70(0.0)	0/ 75(0.0)	0/ 70(0.0)
DAYS 8-14	N/N(%)	0/ 70(0.0)	0/ 75(0.0)	0/ 70(0.0)
DAYS 15-21	N/N(%)	0/ 70(0.0)	0/ 75(0.0)	0/ 70(0.0)
VIABILITY INDEX c	%	96.9	99.0	96.9
	N/N	93/ 96	101/102	94/ 97
LACTATION INDEX d	%	100.0	100.0	100.0
	N/N	70/ 70	75/ 75	70/ 70

DAY(S) = DAY(S) POSTPARTUM

a. Dosage occurred on day 1 of study until day 0 of gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

c. Number of live pups on day 4 (preculling) postpartum/number of liveborn pups on day 1 postpartum.

d. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 4 (postculling) postpartum.

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TABLE 16 (PAGE 2): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1b	III 1.6b
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	7	8	7
SURVIVING PUPS/LITTER c				
DAY 1 d	MEAN±S.D.	13.7 ± 1.4	12.8 ± 3.0	13.8 ± 1.7
DAY 4 PRECULLING	MEAN±S.D.	13.3 ± 1.0	12.6 ± 3.0	13.4 ± 1.8
DAY 4 POSTCULLING	MEAN±S.D.	10.0 ± 0.0	9.4 ± 0.9	10.0 ± 0.0
DAY 7	MEAN±S.D.	10.0 ± 0.0	9.4 ± 0.9	10.0 ± 0.0
DAY 14	MEAN±S.D.	10.0 ± 0.0	9.4 ± 0.9	10.0 ± 0.0
DAY 21	MEAN±S.D.	10.0 ± 0.0	9.4 ± 0.9	10.0 ± 0.0
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED				
DAY 1 d	MEAN±S.D.	44.9 ± 17.9	46.6 ± 11.2	50.9 ± 9.7
DAY 4 PRECULLING	MEAN±S.D.	46.2 ± 17.9	47.3 ± 12.1	50.4 ± 10.9
DAY 4 POSTCULLING	MEAN±S.D.	48.6 ± 12.1	46.5 ± 5.2	48.6 ± 3.8
DAY 7	MEAN±S.D.	48.6 ± 12.1	46.5 ± 5.2	48.6 ± 3.8
DAY 14	MEAN±S.D.	48.6 ± 12.1	46.5 ± 5.2	48.6 ± 3.8
DAY 21	MEAN±S.D.	48.6 ± 12.1	46.5 ± 5.2	48.6 ± 3.8

DAY = DAY POSTPARTUM
a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Average number of live pups per litter, including litters with no surviving pups.
d. Includes pups born alive, found dead day 1 postpartum.

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TABLE 16 (PAGE 3): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	7	8	7
LIVE LITTER SIZE AT WEIGHING				
DAY 1	MEAN±S.D.	13.7 ± 1.4	12.8 ± 3.0	13.6 ± 1.7
DAY 4 PRECULLING	MEAN±S.D.	13.3 ± 1.0	12.6 ± 3.0	13.4 ± 1.8
DAY 4 POSTCULLING	MEAN±S.D.	10.0 ± 0.0	9.4 ± 0.9	10.0 ± 0.0
DAY 7	MEAN±S.D.	10.0 ± 0.0	9.4 ± 0.9	10.0 ± 0.0
DAY 14	MEAN±S.D.	10.0 ± 0.0	9.4 ± 0.9	10.0 ± 0.0
DAY 21	MEAN±S.D.	10.0 ± 0.0	9.4 ± 0.9	10.0 ± 0.0
PUP WEIGHT/LITTER (GRAMS)				
DAY 1	MEAN±S.D.	6.4 ± 0.4	6.5 ± 0.5	6.2 ± 0.6
DAY 4 PRECULLING	MEAN±S.D.	8.9 ± 0.9	8.5 ± 1.4	8.2 ± 0.8
DAY 4 POSTCULLING	MEAN±S.D.	9.0 ± 0.9	8.5 ± 1.4	8.3 ± 0.8
DAY 7	MEAN±S.D.	14.1 ± 1.3	13.2 ± 1.8	12.8 ± 1.3
DAY 14	MEAN±S.D.	29.8 ± 1.6	29.4 ± 2.4	26.6 ± 2.6
DAY 21	MEAN±S.D.	47.1 ± 3.6	47.5 ± 2.9	42.4 ± 3.9

DAY = DAY POSTPARTUM

a. Dosage occurred on day 1 of study until day 0 of presumed gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

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TABLE 17 (PAGE 1): CLINICAL OBSERVATIONS FROM BIRTH TO DAY 21 POSTPARTUM - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
LITTERS EXAMINED (N)		7	8	7
PERSISTENT CLINICAL OBSERVATIONS: c				
TIP OF TAIL, BLACK	N/N	0/ 0	15/ 1	0/ 0

N/N = TOTAL FREQUENCY (DAYS x PUPS)/LITTERS WITH OBSERVATIONS

a. Dosage occurred on day 1 of study until day 0 of gestation.

b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

c. Tabulation restricted to adverse observations; all other pups appeared normal.

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TABLE 18 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1b	1.6b
LITTERS EXAMINED (N)		7	8	7
TOTAL PUPS STILLBORN				
OR FOUND DEAD c	N	2	2	2
STILLBORN	N	1	1	0
FOUND DEAD	N	1	1	2
NO MILK IN STOMACH e	N(%)	0(0.0)	1(100.0)	2(100.0)
PUPS SACRIFICED AND NECROPSIED ON DAY 4 AND/OR 21 POSTPARTUM d				
LITTERS EXAMINED	N	7	8	7
PUPS EVALUATED	N	80	85	80
APPEARED NORMAL				
LITTER INCIDENCE	N(%)	7(100.0)	8(100.0)	7(100.0)
PUP INCIDENCE	N(%)	80(100.0)	85(100.0)	80(100.0)

a. Dosage occurred on day 1 of study until day 0 of gestation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation.
d. Refer to the individual pup clinical observations table (Table 31) for external clinical observations confirmed at necropsy.
e. Analysis restricted to pups found dead and necropsied.

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TABLE 19 (PAGE 1): CLINICAL OBSERVATIONS - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DOSAGE GROUP I		VEHICLE CONTROL	0 (VEHICLE) MG/KG/DAY
RAT #		DESCRIPTION	
13726		NO ADVERSE FINDINGS	
13727		NO ADVERSE FINDINGS	
13728	DL(22)	CHEST: SWOLLEN (RIGHT SIDE, 2.0 CM X 1.5 CM; LEFT SIDE, 2.0 CM X 1.0 CM)a	
13729	DS(44- 45)	CHROMODACRYORRHEA	
	DS(44- 45)	EXOPHTHALMOS	
	DS(44- 45)	RIGHT EYE: HEMORRHAGIC AREA	
	DG(0- 4)	CHROMODACRYORRHEA	
	DG(0- 4)	EXOPHTHALMOS	
	DG(0- 4)	RIGHT EYE: HEMORRHAGIC AREA	
	DG(4)	EXCLUDED FROM STUDY	
13730	DG(4)	EXCLUDED FROM STUDY	
13731	DG(15)	RED PERINASAL SUBSTANCE a	
	DG(15)	FORE AND HINDPAWS: BLUE	
	DG(15)	GASPING	
	DG(15)	ACCIDENTAL DEATH	
13732	DG(4)	EXCLUDED FROM STUDY	
13733	DG(4)	EXCLUDED FROM STUDY	
13734		NO ADVERSE FINDINGS	
13735		NO ADVERSE FINDINGS	
13736	DG(14)	CHROMORHINORRHEA	
	DL(16- 18)	SOFT OR LIQUID FECES	
	DL(20- 22)	RIGHT EAR: TORN a	
13737		NO ADVERSE FINDINGS	

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
DL = DAY OF LACTATION
a. Observation confirmed at necropsy.

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TABLE 19 (PAGE 2): CLINICAL OBSERVATIONS - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DOSAGE GROUP II		LOW DOSAGE	0.1 MG/KG/DAY a
RAT #		DESCRIPTION	
13738	DS(45)	URINE-STAINED ABDOMINAL FUR	
	DG(0- 1)	URINE-STAINED ABDOMINAL FUR	
	DG(4)	EXCLUDED FROM STUDY	
13739		NO ADVERSE FINDINGS	
13740	DG(11- 12)	CHROMORHINORRHEA	
	DL(2- 11)	CHROMORHINORRHEA	
13741		NO ADVERSE FINDINGS	
13742	DG(4- 6)	LOCALIZED ALOPECIA: LIMBS	
	DG(6)	EXCLUDED FROM STUDY	
13743	DS(1- 2)	LOCALIZED ALOPECIA: HEAD	
	DS(3)	ALOPECIA NO LONGER APPARENT	
	DS(50)	EXCLUDED FROM STUDY	
13744	DL(16)	CHROMORHINORRHEA	
	DL(16)	RED DRIED PERIORAL SUBSTANCE	
13745	DG(16- 19)	EXOPHTHALMOS	
	DG(16- 19)	RIGHT EYE: HEMORRHAGIC AREA	
	DG(16- 20)	CHROMODACRYORRHEA	
	DG(20)	RIGHT EYE: REMOVED	
	DL(1- 22)	RIGHT EYE: MISSING b	
	DL(1- 7)	CHROMODACRYORRHEA	
	DL(1- 7)	AROUND RIGHT EYE: SWOLLEN	
	DL(8)	SWELLING RESOLVED	
13746	DL(18- 20)	CHROMODACRYORRHEA	
	DL(18- 22)	CHROMORHINORRHEA b	
13747	DG(3- 4)	LOCALIZED ALOPECIA: LIMBS	
	DG(4)	EXCLUDED FROM STUDY	
13748	DG(9- 13)	CHROMODACRYORRHEA	
	DG(10- 16)	CHROMORHINORRHEA	
	DL(4- 7)	CHROMORHINORRHEA	
13749	DG(4- 5)	CHROMODACRYORRHEA	

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
DL = DAY OF LACTATION

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

b. Observation confirmed at necropsy.

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TABLE 19 (PAGE 3): CLINICAL OBSERVATIONS - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DOSAGE GROUP III		HIGH DOSAGE 1.6 MG/KG/DAY a
RAT #		DESCRIPTION
13750	DS(28- 32)	INCISORS: MISSING/BROKEN
	DS(33)	INCISORS: GROWN IN
	DS(28- 45)	INCISORS: MISALIGNED
	DS(34- 45)	CHROMODACRYORRHEA
	DG(0- 4)	CHROMODACRYORRHEA
	DG(0- 4)	INCISORS: MISALIGNED
	DG(4)	EXCLUDED FROM STUDY
13751		NO ADVERSE FINDINGS
13752	DL(16- 22)	RIGHT EYE: CORNEAL OPACITY b
13753		NO ADVERSE FINDINGS
13754		NO ADVERSE FINDINGS
13755		NO ADVERSE FINDINGS
13756	DG(9- 16)	LOCALIZED ALOPECIA: LIMBS
	DG(17)	ALOPECIA NO LONGER APPARENT
	DL(17)	CHROMODACRYORRHEA
13757	DS(36- 40)	FOREPAW: RIGHT, ABRASION (0.2 CM X 0.3 CM)
	DS(41)	ABRASION HEALED
	DG(4- 6)	LOCALIZED ALOPECIA: LIMBS
	DG(6)	EXCLUDED FROM STUDY
13758		NO ADVERSE FINDINGS
13759		NO ADVERSE FINDINGS
13760	DG(6)	EXCLUDED FROM STUDY
13761	DG(1- 3)	CHROMORHINORRHEA
	DG(3)	EXCLUDED FROM STUDY

DS = DAY OF STUDY DG = DAY OF PRESUMED GESTATION
DL = DAY OF LACTATION

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

b. Observation confirmed at necropsy.

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TABLE 20 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
I					
0 (VEHICLE)	13726	DL 22	P	45	ALL TISSUES APPEARED NORMAL.
	13727	DL 22	P	43	ALL TISSUES APPEARED NORMAL.
	13728	DL 22	P	43	CERVICAL MAMMARY GLAND: LARGE. ALL OTHER TISSUES APPEARED NORMAL.
	13731	DG 15	P	44	ACCIDENTAL DEATH ON DAY 15 OF GESTATION. THORACIC CAVITY: RED SUBSTANCE. ALL OTHER TISSUES APPEARED NORMAL. UTERINE CONTENTS: 17 IMPLANTATION SITES (16 DEAD FETUSES AND ONE EARLY RESORPTION).b
	13734	DL 22	P	45	ALL TISSUES APPEARED NORMAL.
	13735	DL 22	P	43	ALL TISSUES APPEARED NORMAL.
	13736	DL 22	P	46	ALL TISSUES APPEARED NORMAL.
	13737	DL 22	P	46	ALL TISSUES APPEARED NORMAL.
II					
0.1c	13739	DL 22	P	44	ALL TISSUES APPEARED NORMAL.
	13740	DL 22	P	43	ALL TISSUES APPEARED NORMAL.
	13741	DL 22	P	45	ALL TISSUES APPEARED NORMAL.
	13744	DL 22	P	46	ALL TISSUES APPEARED NORMAL.
	13745	DL 22	P	43	ALL TISSUES APPEARED NORMAL.
	13746	DL 22	P	46	ALL TISSUES APPEARED NORMAL.
	13748	DL 22	P	43	ALL TISSUES APPEARED NORMAL.
	13749	DL 22	P	43	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF GESTATION DL = DAY OF LACTATION
a. Refer to the individual clinical observations table (Table 19) for external observations confirmed at necropsy.
b. Viability of fetuses unable to be determined because of maternal death.
c. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 20 (PAGE 2): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	PREGNANCY STATUS	DOSAGES ADMINISTERED	OBSERVATIONS a
III					
1.6b	13751	DL 22	P	44	ALL TISSUES APPEARED NORMAL.
	13752	DL 22	P	45	ALL TISSUES APPEARED NORMAL.
	13753	DL 22	P	44	ALL TISSUES APPEARED NORMAL.
	13754	DL 22	P	46	ALL TISSUES APPEARED NORMAL.
	13755	DG 25	NP	46	ALL TISSUES APPEARED NORMAL.
	13756	DL 22	P	45	ALL TISSUES APPEARED NORMAL.
	13758	DL 22	P	46	ALL TISSUES APPEARED NORMAL.
	13759	DL 22	P	43	ALL TISSUES APPEARED NORMAL.

P = PREGNANT NP = NOT PREGNANT
DG = DAY OF GESTATION DL = DAY OF LACTATION
a. Refer to the individual clinical observations table (Table 19) for external observations confirmed at necropsy.
b. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 21 (PAGE 1): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
RAT #	DOSAGE GROUP I				VEHICLE CONTROL				0 (VEHICLE) MG/KG/DAY							
13726	203.	198.	206.	210.	213.	210.	215.	216.	214.	212.	218.	221.	224.	223.	229.	227.
13727	242.	248.	249.	251.	246.	244.	251.	252.	245.	247.	252.	255.	252.	253.	262.	260.
13728	229.	231.	226.	235.	238.	242.	238.	244.	243.	250.	244.	244.	252.	260.	259.	259.
13729	225.	219.	225.	227.	229.	228.	235.	238.	237.	237.	243.	245.	246.	247.	247.	253.
13730	219.	214.	219.	224.	221.	220.	226.	228.	226.	225.	230.	232.	233.	230.	238.	242.
13731	229.	237.	239.	239.	236.	241.	243.	244.	238.	241.	245.	251.	257.	257.	258.	263.
13732	218.	216.	222.	225.	228.	226.	221.	232.	228.	230.	228.	228.	236.	237.	236.	236.
13733	228.	237.	236.	236.	233.	242.	246.	240.	244.	247.	250.	246.	247.	257.	257.	260.
13734	222.	218.	218.	227.	232.	233.	234.	230.	235.	239.	236.	233.	235.	244.	245.	248.
13735	227.	229.	227.	220.	228.	230.	232.	224.	226.	232.	234.	229.	234.	239.	241.	233.
13736	219.	220.	216.	224.	222.	218.	221.	228.	222.	223.	224.	231.	232.	236.	234.	235.
13737	239.	248.	250.	253.	258.	247.	247.	249.	247.	242.	239.	245.	252.	252.	248.	256.

DAY	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
13726	229.	226.	232.	235.	235.	234.	239.	241.	237.	237.	243.	246.	244.	244.	249.	251.
13727	258.	258.	254.	260.	262.	257.	253.	249.	253.	258.	259.	260.	253.	256.	263.	267.
13728	252.	254.	266.	268.	266.	261.	271.	270.	273.	272.	266.	268.	271.	272.	273.	275.
13729	258.	256.	253.	254.	259.	259.	262.	262.	263.	265.	267.	269.	268.	269.	276.	274.
13730	241.	233.	239.	245.	242.	241.	242.	248.	250.	246.	255.	256.	258.	256.	258.	263.
13731	270.	268.	262.	262.	269.	273.	270.	265.	258.	266.	272.	273.	268.	267.	272.	280.
13732	236.	244.	243.	246.	241.	241.	247.	248.	250.	246.	248.	258.	255.	256.	254.	248.
13733	258.	256.	265.	269.	263.	267.	263.	263.	263.	262.	263.	273.	273.	272.	270.	271.
13734	244.	247.	251.	255.	255.	251.	252.	252.	258.	259.	266.	268.	260.	261.	258.	265.
13735	239.	241.	241.	234.	240.	243.	240.	233.	242.	244.	244.	238.	244.	247.	248.	243.
13736	236.	234.	226.	232.	236.	234.	225.	232.	239.	237.	234.	236.	241.	238.	232.	240.
13737	256.	256.	256.	260.	268.	266.	261.	270.	268.	269.	269.	277.	280.	280.	273.	283.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 21 (PAGE 2): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAY	33	34	35	36	37	38	39	40	41	42	43a
RAT #	DOSAGE GROUP I				VEHICLE CONTROL				0 (VEHICLE) MG/KG/DAY		
13726	248.	244.	248.	256.	255.	252.	257.	260.	258.	251.	251.
13727	260.	259.	268.	269.	267.	266.	262.	271.	280.	278.	274.
13728	276.	280.	278.	284.	290.	288.	289.	287.	288.	290.	289.
13729	277.	273.	279.	282.	284.	281.	284.	287.	290.	284.	287.
13730	261.	258.	267.	268.	263.	260.	267.	271.	272.	270.	272.
13731	280.	276.	276.	282.	291.	293.	284.	285.	289.	290.	288.
13732	255.	257.	260.	255.	255.	261.	262.	260.	261.	259.	270.
13733	271.	275.	271.	273.	280.	282.	276.	277.	284.	278.	294.
13734	269.	268.	268.	266.	272.	277.	274.	269.	271.	275.	278.
13735	251.	254.	250.	247.	250.	253.	255.	248.	256.	258.	254.
13736	242.	241.	234.	239.	243.	243.	238.	242.	248.	245.	241.
13737	285.	284.	285.	290.	292.	295.	292.	298.	298.	296.	296.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 21 (PAGE 3): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
RAT #	DOSAGE GROUP II				LOW DOSAGE				0.1 MG/KG/DAY a							
13738	208.	209.	208.	205.	214.	216.	216.	215.	213.	216.	219.	218.	221.	220.	225.	228.
13739	242.	240.	235.	239.	249.	253.	252.	251.	249.	256.	260.	259.	255.	266.	270.	266.
13740	233.	241.	235.	234.	236.	242.	240.	238.	238.	241.	244.	243.	248.	254.	254.	248.
13741	232.	228.	231.	240.	239.	239.	240.	247.	243.	240.	246.	252.	249.	245.	252.	257.
13742	218.	219.	219.	222.	229.	230.	224.	227.	236.	242.	242.	241.	246.	250.	254.	250.
13743	233.	235.	242.	235.	241.	249.	253.	248.	254.	258.	259.	255.	266.	274.	274.	268.
13744	225.	228.	226.	228.	234.	237.	241.	237.	239.	245.	247.	246.	248.	258.	256.	256.
13745	228.	233.	227.	226.	235.	241.	242.	237.	242.	244.	246.	244.	249.	254.	250.	249.
13746	221.	221.	219.	226.	229.	234.	232.	226.	234.	239.	242.	238.	236.	244.	247.	248.
13747	235.	231.	235.	242.	239.	236.	240.	245.	247.	242.	247.	251.	251.	248.	254.	255.
13748	224.	223.	220.	226.	228.	232.	227.	232.	234.	240.	241.	238.	239.	246.	253.	249.
13749	227.	219.	228.	237.	234.	229.	226.	236.	244.	242.	242.	237.	247.	250.	248.	247.

DAY	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
13738	229.	228.	225.	228.	236.	234.	233.	228.	233.	237.	237.	235.	232.	238.	243.	243.
13739	259.	268.	268.	266.	262.	272.	269.	268.	263.	271.	268.	272.	267.	273.	280.	275.
13740	254.	255.	257.	253.	255.	261.	260.	255.	258.	265.	263.	264.	264.	269.	271.	270.
13741	259.	256.	259.	265.	259.	258.	260.	266.	265.	261.	272.	272.	271.	269.	271.	278.
13742	247.	250.	252.	256.	253.	250.	255.	259.	258.	255.	255.	259.	264.	261.	262.	260.
13743	284.	275.	280.	278.	286.	290.	285.	280.	286.	290.	291.	283.	292.	295.	296.	288.
13744	261.	259.	263.	261.	263.	265.	264.	261.	267.	270.	273.	268.	276.	277.	280.	276.
13745	254.	255.	256.	251.	257.	263.	256.	255.	260.	264.	263.	261.	266.	270.	264.	264.
13746	246.	242.	247.	252.	252.	244.	243.	248.	251.	249.	243.	240.	245.	253.	253.	244.
13747	254.	250.	251.	257.	258.	253.	256.	258.	259.	256.	259.	264.	260.	256.	259.	263.
13748	255.	255.	252.	248.	250.	252.	246.	241.	244.	248.	244.	242.	247.	245.	247.	240.
13749	245.	254.	256.	257.	250.	248.	254.	259.	256.	256.	258.	260.	260.	261.	265.	270.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

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TABLE 21 (PAGE 4): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAY	33	34	35	36	37	38	39	40	41	42	43a
RAT #	DOSAGE GROUP II				LOW DOSAGE				0.1 MG/KG/DAYb		
13738	241.	240.	249.	252.	247.	251.	246.	251.	252.	251.	254.
13739	274.	286.	290.	283.	284.	291.	294.	288.	289.	302.	298.
13740	271.	279.	277.	273.	276.	281.	276.	276.	280.	282.	269.
13741	278.	276.	284.	281.	281.	281.	287.	291.	292.	287.	295.
13742	264.	268.	268.	265.	266.	267.	267.	265.	274.	274.	269.
13743	299.	302.	301.	296.	302.	308.	311.	306.	312.	316.	314.
13744	274.	283.	286.	284.	279.	280.	285.	293.	297.	290.	286.
13745	266.	270.	275.	269.	272.	275.	273.	266.	275.	271.	271.
13746	246.	252.	251.	249.	254.	251.	252.	259.	263.	255.	257.
13747	264.	260.	265.	272.	268.	268.	268.	272.	273.	269.	278.
13748	244.	247.	246.	242.	248.	252.	247.	242.	251.	252.	256.
13749	270.	266.	267.	269.	274.	272.	268.	273.	279.	276.	270.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 21 (PAGE 5): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
RAT #	DOSAGE GROUP III				HIGH DOSAGE				1.6 MG/KG/DAYa							
13750	213.	206.	214.	217.	215.	212.	218.	216.	214.	212.	217.	223.	218.	215.	221.	222.
13751	256.	260.	266.	263.	256.	259.	260.	261.	257.	259.	262.	268.	262.	274.	274.	271.
13752	225.	223.	227.	237.	231.	233.	239.	238.	237.	236.	244.	245.	245.	245.	252.	252.
13753	227.	233.	225.	239.	240.	237.	233.	237.	233.	238.	235.	247.	250.	244.	245.	250.
13754	219.	224.	217.	224.	227.	224.	222.	226.	227.	227.	228.	229.	232.	231.	230.	233.
13755	236.	235.	231.	238.	240.	239.	235.	240.	236.	241.	235.	238.	244.	240.	239.	225.
13756	218.	227.	228.	232.	230.	230.	236.	240.	238.	242.	239.	246.	248.	250.	250.	249.
13757	233.	241.	235.	235.	239.	248.	246.	247.	243.	246.	250.	254.	255.	252.	256.	260.
13758	219.	219.	218.	221.	226.	229.	223.	226.	223.	228.	227.	233.	239.	235.	238.	237.
13759	235.	238.	232.	232.	239.	240.	240.	231.	223.	233.	233.	236.	236.	236.	241.	240.
13760	212.	213.	214.	211.	220.	215.	215.	214.	215.	216.	215.	220.	224.	223.	226.	225.
13761	223.	227.	220.	226.	228.	228.	230.	231.	231.	231.	232.	236.	238.	241.	236.	241.

DAY	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
13750	221.	214.	222.	226.	223.	218.	223.	220.	216.	214.	221.	215.	221.	214.	220.	223.
13751	268.	277.	277.	278.	271.	274.	277.	274.	261.	267.	275.	277.	271.	276.	279.	283.
13752	250.	247.	252.	256.	253.	250.	252.	253.	252.	249.	255.	255.	256.	257.	262.	261.
13753	250.	246.	245.	248.	252.	254.	248.	251.	253.	254.	254.	257.	259.	262.	258.	255.
13754	237.	234.	228.	231.	235.	232.	232.	236.	235.	232.	233.	237.	234.	236.	232.	236.
13755	248.	244.	240.	242.	244.	244.	240.	240.	239.	241.	239.	240.	245.	240.	238.	239.
13756	254.	250.	252.	253.	247.	253.	254.	254.	251.	252.	257.	253.	253.	255.	251.	254.
13757	257.	260.	259.	260.	266.	265.	257.	256.	263.	267.	269.	265.	265.	273.	273.	274.
13758	239.	236.	238.	238.	241.	243.	232.	232.	234.	234.	228.	234.	241.	236.	233.	233.
13759	243.	239.	244.	241.	242.	245.	238.	239.	238.	237.	237.	241.	244.	239.	241.	244.
13760	229.	222.	225.	225.	232.	226.	226.	232.	231.	229.	230.	232.	231.	233.	234.	236.
13761	240.	238.	237.	242.	246.	239.	234.	240.	241.	241.	237.	243.	243.	242.	237.	243.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 21 (PAGE 6): BODY WEIGHTS - PRECOHABITATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAY	33	34	35	36	37	38	39	40	41	42	43a
RAT #	DOSAGE GROUP III				HIGH DOSAGE				1.6 MG/KG/DAYb		
13750	217.	219.	226.	223.	222.	219.	218.	224.	223.	223.	212.
13751	269.	279.	282.	286.	278.	288.	291.	289.	285.	284.	287.
13752	259.	257.	262.	265.	267.	265.	267.	267.	266.	262.	269.
13753	262.	264.	264.	259.	256.	261.	264.	265.	260.	263.	263.
13754	236.	235.	229.	232.	238.	233.	229.	233.	236.	234.	231.
13755	240.	239.	241.	246.	244.	246.	242.	244.	243.	242.	239.
13756	254.	255.	253.	253.	259.	260.	255.	254.	256.	262.	256.
13757	269.	266.	275.	277.	276.	272.	269.	280.	286.	282.	276.
13758	238.	235.	238.	238.	240.	238.	237.	239.	243.	240.	232.
13759	245.	243.	245.	244.	246.	246.	239.	244.	238.	242.	240.
13760	239.	239.	239.	237.	240.	236.	234.	236.	242.	242.	244.
13761	243.	243.	242.	244.	246.	243.	239.	246.	247.	247.	241.

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recored before cohabitation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 22 (PAGE 1): MATERNAL BODY WEIGHTS - PRESUMED GESTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT #	DOSAGE GROUP I	VEHICLE CONTROL	0 (VEHICLE) MG/KG/DAY										
PREGNANCY STATUS	DAY 0	1	2	3	4	5	6	7	8	9	10	11	12
13726 P	259.	265.	270.	275.	282.	283.	282.	281.	278.	283.	293.	291.	301.
13727 P	281.	292.	297.	296.	303.	304.	320.	305.	316.	316.	325.	333.	340.
13728 P	295.	289.	293.	308.	309.	314.	325.	323.	320.	320.	329.	335.	352.
13729 a	281.	297.	302.	302.	303.								
13730 a	281.	281.	285.	288.	296.								
13731 P	288.	291.	302.	306.	309.	323.	322.	310.	322.	328.	330.	347.	342.
13732 a	278.	282.	282.	288.	294.								
13733 a	298.	309.	311.	310.	311.								
13734 P	279.	283.	292.	300.	302.	303.	305.	316.	313.	312.	320.	317.	328.
13735 P	259.	262.	270.	272.	274.	277.	281.	285.	284.	289.	286.	294.	303.
13736 P	260.	273.	275.	284.	284.	288.	288.	295.	279.	293.	296.	304.	310.
13737 P	316.	318.	317.	331.	336.	329.	335.	332.	329.	341.	334.	355.	349.
DAY	13	14	15	16	17	18	19	20	21	22	23	24	25
13726 P	306.	308.	312.	315.	326.	337.	352.	360.	370.	372.			
13727 P	335.	348.	354.	348.	366.	379.	397.	413.	421.				
13728 P	342.	353.	364.	368.	384.	399.	427.	441.	464.				
13729 a													
13730 a													
13731 P	358.	357.	369.	ACCIDENTAL DEATH ON DAY 15 OF GESTATION									
13732 a													
13733 a													
13734 P	334.	332.	344.	347.	363.	367.	385.	399.	406.				
13735 P	298.	309.	314.	313.	324.	333.	346.	362.	373.	360.			
13736 P	314.	310.	318.	327.	340.	355.	371.	385.	409.				
13737 P	359.	359.	363.	370.	386.	400.	408.	426.	444.				

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Rat was not selected for continuation on study; values excluded from group averages.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 22 (PAGE 2): MATERNAL BODY WEIGHTS - PRESUMED GESTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT # DOSAGE GROUP II LOW DOSAGE 0.1 MG/KG/DAY a

RAT #	PREGNANCY STATUS	DAY 0	1	2	3	4	5	6	7	8	9	10	11	12
13738	b	261.	265.	269.	272.	282.								
13739	P	294.	308.	315.	313.	309.	316.	314.	305.	319.	322.	330.	335.	335.
13740	P	282.	288.	297.	298.	298.	300.	313.	317.	310.	310.	316.	320.	325.
13741	P	310.	314.	319.	325.	333.	331.	323.	331.	326.	327.	341.	339.	343.
13742	b	282.	284.	291.	296.	295.	291.	300.						
13743	b													
13744	P	302.	311.	311.	318.	318.	320.	321.	326.	326.	332.	334.	351.	357.
13745	P	280.	281.	288.	294.	292.	294.	307.	308.	297.	304.	316.	315.	328.
13746	P	252.	268.	275.	279.	278.	282.	276.	292.	281.	287.	290.	301.	311.
13747	b	293.	295.	296.	295.	305.								
13748	P	254.	267.	273.	270.	277.	283.	289.	286.	284.	288.	290.	297.	308.
13749	P	275.	289.	300.	305.	304.	302.	318.	311.	312.	322.	328.	332.	338.

RAT #	DAY 13	14	15	16	17	18	19	20	21	22	23	24	25
13738 b													
13739 P	347.	348.	364.	367.	379.	401.	415.	436.	455.				
13740 P	322.	334.	341.	337.	347.	356.	368.	374.	385.				
13741 P	349.	352.	364.	364.	377.	397.	415.	407.	437.				
13742 b													
13743 b													
13744 P	358.	363.	366.	374.	385.	402.	410.	425.	448.				
13745 P	322.	339.	350.	336.	347.	358.	369.	384.	392.				
13746 P	318.	314.	308.	332.	346.	362.	379.	385.	405.				
13747 b													
13748 P	303.	312.	326.	324.	344.	355.	374.	387.	369.	387.			
13749 P	333.	346.	358.	354.	365.	384.	391.	397.	416.				

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

b. Rat was not selected for continuation on study; values excluded from group averages.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 22 (PAGE 3): MATERNAL BODY WEIGHTS - PRESUMED GESTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT #	DOSAGE GROUP III			HIGH DOSAGE				1.6 MG/KG/DAY a					
PREGNANCY STATUS DAY	0	1	2	3	4	5	6	7	8	9	10	11	12
13750 b	232.	221.	229.	237.	241.								
13751 P	295.	302.	315.	314.	312.	309.	310.	305.	312.	321.	326.	342.	333.
13752 P	277.	287.	278.	279.	282.	278.	284.	289.	281.	289.	290.	296.	298.
13753 P	263.	274.	276.	278.	280.	287.	284.	270.	281.	289.	292.	308.	304.
13754 P	246.	254.	256.	259.	259.	263.	267.	264.	264.	275.	271.	281.	288.
13755 NP	252.	255.	258.	263.	264.	263.	266.	269.	265.	273.	266.	280.	282.
13756 P	261.	262.	271.	271.	276.	274.	274.	278.	282.	282.	298.	295.	306.
13757 b	284.	296.	302.	302.	305.	305.	321.						
13758 P	243.	254.	254.	256.	256.	263.	269.	265.	267.	275.	281.	283.	284.
13759 P	242.	248.	259.	258.	261.	265.	270.	269.	269.	278.	283.	287.	299.
13760 b	236.	243.	250.	250.	252.	258.	261.						
13761 b	252.	257.	255.	266.									

DAY	13	14	15	16	17	18	19	20	21	22	23	24	25
13750 b													
13751 P	352.	349.	361.	370.	380.	398.	395.	436.	432.	437.			
13752 P	297.	304.	324.	326.	348.	359.	371.	384.	411.				
13753 P	318.	321.	334.	335.	345.	367.	382.	405.	413.				
13754 P	290.	288.	294.	302.	321.	336.	337.	355.	366.				
13755 NP	279.	275.	276.	274.	276.	282.	278.	284.	287.	278.	289.	290.	298.
13756 P	306.	308.	319.	322.	336.	351.	364.	380.	394.				
13757 b													
13758 P	294.	302.	301.	321.	330.	348.	364.	381.	394.				
13759 P	296.	310.	320.	324.	331.	356.	373.	389.	406.				
13760 b													
13761 b													

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

b. Rat was not selected for continuation on study; values excluded from group averages.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 23 (PAGE 1): MATERNAL BODY WEIGHTS - LACTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT # DOSAGE GROUP I VEHICLE CONTROL 0 (VEHICLE) MG/KG/DAY

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13
13726	270.	270.	278.	280.	282.	284.	293.	299.	298.	303.	310.	325.	322.
13727	309.	325.	332.	325.	331.	338.	327.	335.	339.	336.	346.	351.	351.
13728	324.	318.	329.	327.	333.	333.	337.	348.	348.	352.	358.	358.	368.
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION												
13734	310.	313.	317.	320.	326.	323.	324.	332.	331.	341.	326.	360.	343.
13735	276.	265.	279.	275.	280.	286.	295.	293.	298.	308.	312.	309.	316.
13736	292.	299.	294.	289.	291.	290.	300.	306.	317.	316.	321.	327.	322.
13737	316.	328.	325.	321.	318.	319.	325.	334.	339.	346.	361.	363.	375.

DAY	14	15	16	17	18	19	20	21	22
13726	323.	321.	325.	336.	332.	332.	337.	328.	312.
13727	352.	360.	360.	358.	359.	357.	356.	342.	327.
13728	344.	367.	362.	356.	375.	358.	352.	354.	353.
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION								
13734	325.	337.	338.	343.	353.	350.	337.	327.	330.
13735	312.	328.	327.	319.	329.	341.	339.	328.	313.
13736	314.	325.	318.	303.	328.	332.	325.	333.	334.
13737	350.	360.	363.	357.	366.	352.	368.	362.	353.

DAY = DAY OF LACTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 23 (PAGE 2): MATERNAL BODY WEIGHTS - LACTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT #	DOSAGE GROUP II			LOW DOSAGE			0.1 MG/KG/DAY a						
	DAY 1	2	3	4	5	6	7	8	9	10	11	12	13
13739	316.	302.	317.	324.	325.	329.	323.	335.	337.	345.	350.	345.	351.
13740	310.	297.	303.	296.	307.	307.	313.	317.	324.	338.	337.	340.	348.
13741	320.	315.	333.	333.	323.	324.	317.	336.	342.	346.	343.	358.	356.
13744	327.	339.	340.	342.	336.	322.	343.	350.	358.	367.	364.	366.	351.
13745	313.	279.	253.	242.	282.	299.	312.	312.	325.	330.	338.	343.	354.
13746	284.	272.	293.	302.	298.	295.	307.	319.	325.	333.	348.	337.	344.
13748	281.	288.	296.	297.	301.	298.	308.	314.	320.	325.	319.	331.	306.
13749	338.	327.	330.	326.	330.	339.	331.	342.	339.	347.	350.	351.	328.

	DAY 14	15	16	17	18	19	20	21	22
13739	352.	356.	352.	347.	342.	355.	350.	362.	362.
13740	340.	334.	341.	343.	343.	338.	333.	335.	306.
13741	366.	359.	359.	366.	382.	364.	352.	340.	338.
13744	367.	375.	379.	378.	371.	378.	389.	386.	374.
13745	309.	357.	368.	363.	360.	365.	357.	362.	348.b
13746	324.	316.	331.	340.	339.	340.	327.	320.	316.
13748	336.	334.	342.	348.	346.	346.	342.	338.	331.
13749	359.	360.	352.	369.	374.	358.	364.	347.	333.

DAY = DAY OF LACTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.
b. Dead body weight.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 23 (PAGE 3): MATERNAL BODY WEIGHTS - LACTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT #	DOSAGE GROUP III			HIGH DOSAGE			1.6 MG/KG/DAY a						
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13
13751	333.	321.	323.	331.	333.	331.	341.	341.	342.	353.	355.	361.	389.
13752	278.	289.	298.	293.	292.	298.	312.	307.	315.	319.	331.	329.	338.
13753	299.	300.	290.	293.	300.	305.	300.	309.	320.	321.	325.	336.	336.
13754	264.	253.	272.	271.	281.	285.	297.	303.	319.	314.	325.	330.	315.
13755	NOT PREGNANT												
13756	311.	296.	292.	290.	292.	299.	298.	309.	305.	316.	321.	328.	342.
13758	296.	284.	283.	288.	291.	292.	289.	302.	309.	309.	322.	326.	325.
13759	274.	280.	286.	274.	291.	288.	294.	298.	302.	316.	325.	325.	334.

DAY	14	15	16	17	18	19	20	21	22
13751	370.	371.	380.	372.	389.	370.	376.	367.	386.
13752	333.	333.	342.	340.	343.	334.	325.	319.	330.
13753	340.	333.	321.	333.	339.	343.	344.	342.	326.
13754	322.	324.	340.	338.	334.	304.	333.	332.	336.
13755	NOT PREGNANT								
13756	340.	328.	344.	295.	323.	341.	342.	356.	337.
13758	322.	317.	320.	323.	329.	330.	340.	308.	311.
13759	338.	345.	343.	342.	347.	342.	362.	350.	339.

DAY = DAY OF LACTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 24 (PAGE 1): FEED CONSUMPTION VALUES - PRECOHABITATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAYS	1- 8	8- 15	15- 22	22- 29	29- 36	36- 43a
RAT #	DOSAGE GROUP I			VEHICLE CONTROL		0 (VEHICLE) MG/KG/DAY
13726	140.	b	138.	131.	137.	137.
13727	141.	129.	128.	106.	130.	137.
13728	135.	149.	139.	143.	141.	159.
13729	131.	143.	141.	136.	142.	142.
13730	140.	135.	146.	147.	154.	155.
13731	144.	138.	144.	120.	130.	146.
13732	127.	114.	120.	117.	118.	124.
13733	144.	138.	b	b	b	141.
13734	134.	129.	147.	136.	129.	144.
13735	b	b	b	b	b	b
13736	122.	119.	114.	107.	108.	120.
13737	151.	126.	155.	143.	150.	163.
RAT #	DOSAGE GROUP II			LOW DOSAGE		0.1 MG/KG/DAY c
13738	116.	114.	124.	114.	128.	128.
13739	b	147.	140.	122.	144.	144.
13740	138.	135.	138.	126.	146.	141.
13741	137.	130.	139.	132.	142.	153.
13742	131.	150.	137.	137.	142.	146.
13743	b	164.	b	155.	b	163.
13744	139.	143.	146.	130.	143.	139.
13745	139.	144.	133.	126.	126.	135.
13746	128.	133.	121.	99.	116.	120.
13747	142.	153.	b	b	b	168.
13748	135.	150.	148.	b	b	b
13749	119.	131.	121.	124.	122.	126.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.
b. Spilled feed precluded the calculation of this value.
c. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 24 (PAGE 2): FEED CONSUMPTION VALUES - PRECOHABITATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAYS	1- 8	8- 15	15- 22	22- 29	29- 36	36- 43a
RAT #	DOSAGE GROUP III			HIGH DOSAGE		1.6 MG/KG/DAY b
13750	114.	110.	111.	98.	100.	89.
13751	148.	134.	135.	111.	124.	133.
13752	134.	126.	124.	114.	117.	123.
13753	131.	128.	123.	122.	131.	171.
13754	123.	119.	121.	108.	125.	116.
13755	121.	113.	115.	105.	113.	114.
13756	129.	128.	121.	105.	107.	115.
13757	131.	133.	133.	124.	135.	134.
13758	124.	130.	133.	105.	108.	117.
13759	149.	141.	c	134.	c	137.
13760	109.	113.	108.	99.	111.	112.
13761	122.	122.	123.	107.	111.	110.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Last value recorded before cohabitation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Spilled feed precluded the calculation of this value.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 25 (PAGE 1): MATERNAL FEED CONSUMPTION VALUES - PRESUMED GESTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT #	DOSAGE GROUP I			VEHICLE CONTROL			0 (VEHICLE) MG/KG/DAY						
PREGNANCY STATUS DAYS	0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13
13726 P	25.	24.	28.	22.	23.	22.	41.	19.	22.	23.	27.	19.	26.
13727 P	26.	24.	21.	27.	25.	25.	24.	21.	24.	25.	29.	20.	31.
13728 P	18.	41.	14.	28.	28.	27.	23.	24.	25.	28.	30.	29.	31.
13729 a	b	24.	25.	18.									
13730 a	24.	25.	28.	25.									
13731 P	16.	28.	26.	27.	25.	27.	13.	29.	26.	27.	30.	29.	25.
13732 a	25.	22.	24.	20.									
13733 a	28.	27.	25.	21.									
13734 P	23.	27.	27.	22.	27.	28.	30.	27.	28.	24.	35.	22.	32.
13735 P	24.	25.	28.	23.	29.	24.	b	24.	22.	20.	23.	22.	28.
13736 P	28.	23.	23.	23.	25.	24.	24.	16.	20.	27.	19.	25.	26.
13737 P	24.	25.	26.	29.	24.	27.	28.	21.	25.	24.	24.	26.	29.

DAYS	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20	20 - 21	21 - 22	22 - 23	23 - 24	24 - 25
13726 P	22.	33.	22.	23.	23.	24.	20.	b	14.			
13727 P	18.	22.	19.	29.	27.	30.	26.	23.				
13728 P	20.	25.	26.	29.	29.	34.	29.	24.				
13729 a												
13730 a												
13731 P	27.	29.	ACCIDENTAL DEATH ON DAY 15 OF GESTATION									
13732 a												
13733 a												
13734 P	27.	27.	25.	27.	31.	31.	21.	26.				
13735 P	20.	32.	25.	29.	25.	24.	24.	29.	10.			
13736 P	24.	20.	25.	26.	23.	26.	20.	25.				
13737 P	24.	29.	24.	32.	26.	22.	25.	25.				

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Rat was not selected for continuation on study; values excluded from group averages.
b. Spilled feed precluded the calculation of this value.

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TABLE 25 (PAGE 2): MATERNAL FEED CONSUMPTION VALUES - PRESUMED GESTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT #	DOSAGE GROUP II			LOW DOSAGE			0.1 MG/KG/DAY a						
PREGNANCY STATUS DAYS	0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13
13738 b	24.	21.	22.	21.									
13739 P	22.	28.	26.	23.	20.	25.	19.	25.	24.	27.	22.	32.	20.
13740 P	24.	24.	27.	25.	26.	26.	23.		25.	25.	25.	24.	28.
13741 P	26.	27.	30.	26.	24.	24.	27.	20.	21.	23.	30.	18.	26.
13742 b	24.	24.	28.	27.	27.	24.							
13743 b													
13744 P	25.	23.	22.	22.	26.	24.	27.	26.	21.	32.	23.	28.	29.
13745 P	19.	23.	27.	23.	25.	23.	24.	21.	27.	26.	25.	26.	30.
13746 P	24.	22.	15.	16.	20.	22.	23.	20.	19.	25.	19.	30.	31.
13747 b	29.	24.	28.	23.									
13748 P	25.	23.	28.	39.	29.	23.	22.	21.	24.	20.	24.	25.	30.
13749 P	27.	22.	29.	26.	26.	24.	14.	22.	31.	28.	29.	25.	33.

DAYS	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20	20 - 21	21 - 22	22 - 23	23 - 24	24 - 25
13738 b												
13739 P	24.	34.	23.	27.	28.	27.	27.	39.				
13740 P	23.	25.	24.	26.	28.	26.	27.	20.				
13741 P	23.	26.	26.	27.	28.	28.	18.	28.				
13742 b												
13743 b												
13744 P	27.	22.	25.	29.	27.	22.	29.	27.				
13745 P	25.	30.	19.	24.	26.	27.	34.	25.				
13746 P	27.	17.	28.	34.	35.	30.	25.	28.				
13747 b												
13748 P	16.	34.	26.	32.	31.	30.	30.	10.	14.			
13749 P	22.	33.	21.	33.	33.	24.	30.	29.				

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

b. Rat was not selected for continuation on study; values excluded from group averages.

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TABLE 25 (PAGE 3): MATERNAL FEED CONSUMPTION VALUES - PRESUMED GESTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

RAT #	DOSAGE GROUP III			HIGH DOSAGE			1.6 MG/KG/DAY a						
PREGNANCY STATUS DAYS	0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13
13750 b	8.	18.	21.	12.									
13751 P	20.	29.	22.	23.	16.	21.	18.	20.	26.	23.	23.	28.	23.
13752 P	28.	15.	16.	16.	16.	17.	23.	17.	20.	15.	25.	15.	21.
13753 P	23.	21.	23.	23.	18.	18.	16.	19.	23.	23.	22.	27.	19.
13754 P	22.	22.	18.	20.	20.	20.	24.	20.	20.	24.	19.	25.	22.
13755 NP	22.	21.	18.	22.	22.	20.	29.	25.	23.	26.	22.	25.	19.
13756 P	17.	20.	18.	16.	16.	16.	26.	18.	20.	23.	25.	18.	20.
13757 b	26.	22.	25.	26.	26.	26.							
13758 P	23.	22.	18.	21.	22.	22.	45.	28.	22.	30.	17.	16.	25.
13759 P	20.	25.	22.	24.	25.	23.	28.	23.	23.	27.	24.	28.	30.
13760 b	15.	18.	19.	20.	19.	17.							
13761 b	20.	19.	20.										

DAYS	13 - 14	14 - 15	15 - 16	16 - 17	17 - 18	18 - 19	19 - 20	20 - 21	21 - 22	22 - 23	23 - 24	24 - 25
13750 b												
13751 P	25.	29.	33.	25.	25.	23.	35.	24.	23.			
13752 P	19.	32.	25.	25.	26.	22.	22.	30.				
13753 P	24.	32.	23.	29.	24.	27.	26.					
13754 P	22.	23.	24.	27.	29.	14.	23.	27.				
13755 NP	21.	21.	22.	25.	25.	15.	38.	27.	17.	20.	20.	22.
13756 P	20.	25.	24.	24.	24.	21.	21.	28.				
13757 b												
13758 P	27.	30.	28.	26.	24.	23.	23.	33.				
13759 P	24.	34.	28.	28.	30.	28.	26.	32.				
13760 b												
13761 b												

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAYS = DAYS OF PRESUMED GESTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

b. Rat was not selected for continuation on study; values excluded from group averages.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 26 (PAGE 1): MATERNAL FEED CONSUMPTION VALUES - LACTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAYS	1 - 4	4 - 7	7 - 10	10 - 14a
RAT #	DOSAGE GROUP I		VEHICLE CONTROL	0 (VEHICLE) MG/KG/DAY
13726	85.	144.	171.	281.
13727	106.	128.	152.	252.
13728	91.	136.	173.	282.
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION			
13734	93.	136.	170.	268.
13735	119.	c	161.	280.
13736	71.	110.	150.	c
13737	92.	204.	165.	271.
RAT #	DOSAGE GROUP II		LOW DOSAGE	0.1 MG/KG/DAY b
13739	49.	108.	146.	223.
13740	74.	130.	149.	233.
13741	99.	127.	169.	268.
13744	97.	109.	175.	265.
13745	20.	111.	166.	250.
13746	121.	206.	219.	c
13748	206.	c	159.	262.
13749	57.	93.	133.	218.

DAY = DAY OF LACTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. It is presumed that the pups begin to consume the maternal feed after day 14 of lactation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.
c. Spilled feed precluded the calculation of this value.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 26 (PAGE 2): MATERNAL FEED CONSUMPTION VALUES - LACTATION - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS

DAYS	1 - 4	4 - 7	7 - 10	10 - 14a
RAT #	DOSAGE GROUP III		HIGH DOSAGE	1.6 MG/KG/DAY b
13751	65.	110.	157.	234.
13752	72.	119.	140.	232.
13753	69.	115.	161.	244.
13754	70.	151.	181.	240.
13755	NOT PREGNANT			
13756	52.	99.	121.	228.
13758	72.	115.	150.	223.
13759	87.	133.	161.	264.

DAYS = DAYS OF LACTATION
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. It is presumed that the pups begin to consume the maternal feed after day 14 of lactation.
b. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 27 (PAGE 1): NATURAL DELIVERY, IMPLANTATION SITES, AND PUP VIABILITY AND SEX - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS/F1 GENERATION LITTERS

RAT/ LITTER NUMBER	DURATION OF GESTATION (DAYS) N	LITTER DELIVERED LIVE BORN N	STILL-BORN N	TOTAL BORN N	NUMBER OF LIVE PUPS AT COMPLETION OF DAY POSTPARTUM 1 M	1 F	4a M	4a F	4b M	4b F	7 M	7 F	14 M	14 F	21 M	21 F	TOTAL IMPLAN-TATIONS N
MATERNAL DOSAGE GROUP I		VEHICLE CONTROL							0 (VEHICLE) MG/KG/DAY								
13726	23	13	0	13	4	9	4	9	4	6	4	6	4	6	4	6	14
13727	23	14	0	14	7	7	7	7	5	5	5	5	5	5	5	5	16
13728	23	15	1	16	7	8	7	6	5	5	5	5	5	5	5	5	16
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION																
13734	23	13	0	13	7	6	7	6	5	5	5	5	5	5	5	5	15
13735	23	12	0	12	3	9	3	9	3	7	3	7	3	7	3	7	14
13736	c	13	0	13	10	3	10	3	7	3	7	3	7	3	7	3	15
13737	c	16	0	16	5	11	5	10	5	5	5	5	5	5	5	5	16
MATERNAL DOSAGE GROUP II		LOW DOSAGE							0.1 MG/KG/DAY d								
13739	23	16	0	16	9	7	9	7	5	5	5	5	5	5	5	5	16
13740	23	9	0	9	4	5	4	5	4	5	4	5	4	5	4	5	16
13741	23	16	0	16	7	9	7	9	5	5	5	5	5	5	5	5	16
13744	c	14	0	14	4	10	4	10	4	6	4	6	4	6	4	6	15
13745	c	12	0	12	7	5	7	4	4	4	4	4	4	4	4	4	13
13746	23	13	1	14	8	5	8	5	5	5	5	5	5	5	5	5	17
13748	23	14	0	14	6	8	6	8	5	5	5	5	5	5	5	5	15
13749	23	8	0	8	3	5	3	5	3	5	3	5	3	5	3	5	11

M = MALE F = FEMALE

a. Number of live pups preculling.

b. Number of live pups postculling.

c. Delivery observations were not recorded, values excluded from group averages.

d. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 27 (PAGE 2): NATURAL DELIVERY, IMPLANTATION SITES, AND PUP VIABILITY AND SEX - INDIVIDUAL DATA - Fo GENERATION FEMALE RATS/F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP III HIGH DOSAGE 1.6 MG/KG/DAY a

RAT/ LITTER NUMBER	DURATION OF GESTATION (DAYS) N	LITTER DELIVERED LIVE BORN N	STILL-BORN N	TOTAL BORN N	NUMBER OF LIVE PUPS AT COMPLETION OF DAY POSTPARTUM 1 M	1 F	4b M	4b F	4c M	4c F	7 M	7 F	14 M	14 F	21 M	21 F	TOTAL IMPLAN-TATIONS N
13751	23	13(1)	0	13	7	5	7	5	5	5	5	5	5	5	5	5	17
13752	22	15	0	15	6	9	5	9	5	5	5	5	5	5	5	5	17
13753	22	14	0	14	7	7	7	7	5	5	5	5	5	5	5	5	16
13754	d	11	0	11	4	7	4	7	4	6	4	6	4	6	4	6	11
13755	NOT PREGNANT																
13756	22	13	0	13	7	6	6	6	5	5	5	5	5	5	5	5	14
13758	22	15	0	15	9	6	9	6	5	5	5	5	5	5	5	5	17
13759	22	16	0	16	10	6	10	6	5	5	5	5	5	5	5	5	16

M = MALE F = FEMALE

( ) = NUMBER OF PUPS DYING PRIOR TO WEIGHING ON DAY 1 POSTPARTUM.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

b. Number of live pups preculling.

c. Number of live pups postculling.

d. Delivery observations were not recorded, values excluded from group averages.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 28 (PAGE 1): PUP BODY WEIGHT LITTER AVERAGES FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION LITTERS

RAT/ LITTER NUMBER	DAY 1			DAY 4			DAY 7			DAY 14			DAY 21		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
MATERNAL DOSAGE GROUP I				VEHICLE CONTROL						0 (VEHICLE) MG/KG/DAY					
13726	7.0	6.3	6.5	9.8	9.2	9.4	15.4	14.7	15.0	31.2	30.4	30.8	50.7	48.3	49.3
13727	6.2	6.0	6.1	9.4	9.0	9.2	15.2	14.1	14.7	30.3	28.7	29.5	48.6	48.0	48.3
13728	7.0	6.3	6.6	10.2	9.6	9.9	15.8	14.9	15.3	33.3	31.7	32.5	55.0	51.8	53.4
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION														
13734	6.3	5.7	6.0	9.0	8.3	8.7	15.3	13.9	14.6	29.6	28.2	28.9	44.3	41.1	42.7
13735	6.4	5.8	6.0	9.0	8.4	8.5	14.0	12.2	12.8	29.7	27.2	28.0	46.5	43.2	44.2
13736	7.3	6.8	7.2	9.6	9.1	9.4	14.9	14.3	14.7	30.8	29.0	30.3	46.4	45.6	46.2
13737	6.5	6.1	6.2	7.4	6.9	7.0	11.9	11.5	11.7	28.7	27.9	28.3	45.6	45.1	45.4
MATERNAL DOSAGE GROUP II				LOW DOSAGE						0.1 MG/KG/DAY a					
13739	6.4	6.2	6.3	7.8	7.5	7.7	11.6	11.6	11.6	25.9	26.2	26.0	42.8	42.2	42.5
13740	7.3	6.6	6.9	10.6	9.6	10.0	15.6	13.8	14.6	32.4	29.9	31.0	51.8	47.2	49.3
13741	6.4	6.0	6.2	9.4	9.2	9.3	16.0	15.3	15.7	32.6	31.6	32.1	49.9	47.7	48.8
13744	6.8	6.4	6.5	7.2	6.9	7.0	11.5	11.1	11.3	31.6	31.0	31.2	46.0	44.0	44.8
13745	5.7	5.1	5.4	6.5	6.3	6.4	11.3	10.8	11.0	28.8	27.8	28.3	50.2	48.6	49.4
13746	6.9	6.5	6.7	9.0	8.3	8.8	14.2	13.5	13.8	26.9	25.3	26.1	47.3	46.6	47.0
13748	7.0	6.5	6.7	8.7	8.4	8.5	13.1	13.0	13.0	30.0	28.5	29.2	48.2	45.2	46.7
13749	7.3	6.9	7.1	11.0	10.4	10.6	15.7	14.7	15.0	32.6	30.9	31.5	54.1	49.9	51.5

M = MALE F = FEMALE T = TOTAL (SUM OF PUP WEIGHTS/NUMBER OF LIVE PUPS) DAY = DAY POSTPARTUM
ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 28 (PAGE 2): PUP BODY WEIGHT LITTER AVERAGES FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION LITTERS

RAT/ LITTER NUMBER	DAY 1			DAY 4			DAY 7			DAY 14			DAY 21		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
MATERNAL DOSAGE GROUP III				HIGH DOSAGE						1.6 MG/KG/DAY a					
13751	7.3	6.6	7.0	10.0	8.9	9.5	15.0	13.4	14.2	30.9	28.0	29.4	45.3	48.5	46.9
13752	6.1	5.9	6.0	8.7	8.0	8.3	13.5	12.3	12.9	26.7	25.8	26.2	42.4	40.0	41.2
13753	6.5	6.4	6.5	8.6	8.7	8.7	13.1	13.6	13.3	28.6	28.7	28.6	45.4	45.2	45.3
13754	7.1	6.8	6.9	8.6	8.6	8.6	14.2	13.9	14.0	29.3	28.9	29.1	47.9	46.0	46.8
13755	NOT PREGNANT														
13756	6.1	5.7	5.9	7.9	7.3	7.6	10.8	10.3	10.5	23.7	20.8	22.3	38.5	35.3	36.9
13758	5.5	5.6	5.6	7.1	7.2	7.1	11.6	11.8	11.7	24.3	24.9	24.6	38.9	39.0	39.0
13759	6.0	5.6	5.8	8.0	7.5	7.8	12.9	12.4	12.7	26.4	25.3	25.8	42.0	40.2	41.1

M = MALE F = FEMALE T = TOTAL (SUM OF PUP WEIGHTS/NUMBER OF LIVE PUPS) DAY = DAY POSTPARTUM
ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 1): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP I					VEHICLE CONTROL				0 (VEHICLE) MG/KG/DAY						POSTPARTUM DAY 1				
13726	7.6	6.9	6.6	7.0	6.2	6.1	6.4	6.3	6.0	5.8	6.4	7.2	6.1							
13727	6.3	6.4	6.1	6.1	6.4	6.3	6.2	5.8	6.1	5.9	6.1	5.7	6.2	6.0						
13728	7.1	6.9	6.9	7.2	7.4	6.4	7.2	6.3	4.5	7.1	6.4	6.1	6.8	6.4	7.0	FS				
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION																			
13734	6.4	6.3	5.5	6.8	6.2	6.4	6.8	6.0	5.7	5.6	6.0	5.3	5.5							
13735	6.3	7.0	5.9	6.3	6.2	6.0	5.6	6.0	5.6	5.7	5.0	5.9								
13736	7.7	7.6	6.6	7.6	7.6	6.9	7.7	7.4	7.2	7.1	7.0	6.9	6.4							
13737	6.8	6.3	6.2	6.8	6.3	6.4	5.7	6.0	6.1	6.1	6.7	6.1	6.0	5.9	5.8	5.9				
	MATERNAL DOSAGE GROUP II					LOW DOSAGE				0.1 MG/KG/DAY a						POSTPARTUM DAY 1				
13739	6.3	6.8	5.7	5.9	6.7	6.2	6.8	6.3	6.5	6.1	6.2	6.4	6.3	6.4	6.1	6.0				
13740	7.2	7.8	6.8	7.5	6.8	6.6	6.6	6.9	6.2											
13741	6.1	6.1	6.7	6.7	6.3	6.5	6.2	5.6	6.2	6.2	5.7	6.3	6.0	5.7	6.4	6.3				
13744	7.0	7.1	6.8	6.5	6.0	6.2	6.8	6.4	6.3	6.0	6.2	6.8	6.5	6.6						
13745	5.8	5.8	5.8	5.7	5.8	5.7	5.1	4.0	5.3	5.4	5.4	5.5								
13746	6.6	6.8	7.2	7.4	6.9	6.8	7.5	5.9	MS	6.6	6.0	6.2	6.7	6.9						
13748	7.3	7.2	7.6	6.6	6.8	6.6	6.6	7.0	6.9	6.6	6.2	5.8	6.0	6.6						
13749	7.3	7.5	7.1	6.6	6.9	6.8	7.0	7.3												

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 2): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP III					HIGH DOSAGE				1.6 MG/KG/DAY a						POSTPARTUM DAY 1				
13751	7.0	7.9	7.2	7.0	7.6	7.4	7.2	6.3	6.6	6.4	6.6	7.0	FD 1							
13752	4.1	6.5	6.2	6.8	6.4	6.6	6.0	6.0	5.2	5.9	6.0	6.1	6.4	5.5	5.8					
13753	6.9	6.8	7.0	6.2	6.8	5.5	6.5	6.8	6.6	6.3	6.2	6.2	7.1	6.0						
13754	7.0	7.4	7.0	7.1	6.8	6.6	7.0	7.1	6.7	6.6	7.1									
13755	NOT PREGNANT																			
13756	6.1	6.4	5.8	5.9	6.0	6.2	6.2	5.7	6.3	5.7	5.4	5.5	5.7							
13758	5.4	5.7	5.4	5.6	5.5	5.6	5.5	5.3	MA	5.5	5.4	5.3	6.0	5.7	6.0					
13759	5.7	6.1	6.2	6.2	6.2	4.9	6.5	5.9	6.1	6.1	5.2	5.9	4.6	5.8	5.9	6.0				

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 3): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP I					VEHICLE CONTROL				0 (VEHICLE) MG/KG/DAY					POSTPARTUM DAY 4 (PRECULLING)					
13726	9.7	9.2	10.5	9.8	8.9	9.9	9.2	9.2	9.0	9.5	9.2	9.4	9.0							
13727	9.5	9.4	9.1	9.4	9.2	9.7	9.4	8.5	9.4	8.8	9.2	9.1	9.2	8.9						
13728	10.3	10.6	9.9	10.0	10.4	9.9	10.4	10.3	9.8	9.3	9.0	9.0	10.0	FD 3	FM 2	FS				
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION																			
13734	9.0	9.2	9.5	8.8	9.6	9.3	7.8	8.9	7.8	8.8	8.6	7.1	8.4							
13735	9.4	9.0	8.5	8.7	8.8	7.9	8.3	8.7	8.7	8.5	7.2	8.4								
13736	9.1	8.8	9.6	9.8	10.1	8.8	10.0	9.1	10.2	10.1	9.6	8.4	9.3							
13737	7.6	7.6	7.2	6.9	7.7	7.1	6.9	6.9	7.5	7.1	6.8	7.0	5.7	6.7	7.0	FM 4				
	MATERNAL DOSAGE GROUP II					LOW DOSAGE				0.1 MG/KG/DAY a					POSTPARTUM DAY 4 (PRECULLING)					
13739	8.1	8.4	8.5	7.9	7.7	7.2	7.8	7.5	7.4	7.9	7.7	7.3	7.9	7.0	7.4	7.3				
13740	10.7	10.8	10.2	10.9	9.5	9.4	9.7	9.5	9.8											
13741	9.7	9.1	9.9	8.8	9.0	9.2	10.2	8.6	9.3	9.8	9.8	8.3	9.8	9.5	8.2	9.4				
13744	6.1	7.5	7.5	7.5	7.7	7.5	6.0	7.0	6.7	6.8	7.4	6.7	5.9	7.0						
13745	6.6	5.8	6.5	6.8	6.6	6.8	6.6	6.3	6.2	6.4	6.3	FD 2								
13746	9.3	9.0	8.6	9.2	9.6	8.8	9.7	8.2	MS	8.4	9.0	9.1	7.4	7.6						
13748	9.3	9.7	8.8	7.8	8.5	7.9	8.3	8.1	7.9	9.0	8.9	9.3	7.1	8.6						
13749	10.9	11.2	10.8	10.6	10.8	10.2	10.2	10.4												

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.
a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 4): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP III					HIGH DOSAGE				1.6 MG/KG/DAY					POSTPARTUM DAY 4 (PRECULLING)					
13751	10.1	10.1	10.0	9.6	10.0	10.5	9.8	9.0	9.2	8.1	9.8	8.2	FD 1							
13752	9.2	7.6	8.5	8.9	9.1	MD 4	7.1	8.1	8.1	8.6	8.1	7.0	8.3	8.8	8.3					
13753	7.7	8.8	9.2	9.4	8.8	8.4	8.3	8.2	9.2	8.9	8.3	8.4	8.4	9.6						
13754	8.4	8.9	8.5	8.8	9.6	8.1	9.2	8.7	8.8	7.7	8.0									
13755	NOT PREGNANT																			
13756	8.2	7.7	7.8	8.0	8.0	7.8	MM 3	6.6	6.9	7.3	7.0	7.4	8.5							
13758	7.1	6.6	7.1	7.7	7.1	7.4	7.3	6.9	6.5	7.5	6.9	7.2	6.7	7.7	6.9					
13759	8.0	8.9	8.3	6.6	8.3	7.4	8.2	8.8	8.0	8.0	5.9	8.0	8.2	7.3	7.6	8.1				

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 5): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP I					VEHICLE CONTROL				0 (VEHICLE) MG/KG/DAY					POSTPARTUM DAY 4 (POSTCULLING)					
13726	9.7	9.2	10.5	9.8	FC 4	9.9	9.2	9.2	FC 4	9.5	9.2	FC 4	9.0							
13727	9.5	MC 4	9.1	9.4	9.2	9.7	MC 4	FC 4	9.4	8.8	9.2	9.1	FC 4	8.9						
13728	10.3	10.6	9.9	MC 4	MC 4	9.9	10.4	10.3	FC 4	9.3	9.0	9.0	10.0	FD 3	FM 2	FS				
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION																			
13734	9.0	9.2	MC 4	8.8	9.6	9.3	MC 4	8.9	FC 4	8.8	8.6	7.1	8.4							
13735	9.4	9.0	8.5	8.7	FC 4	7.9	8.3	8.7	8.7	8.5	7.2	FC 4								
13736	MC 4	MC 4	9.6	MC 4	10.1	8.8	10.0	9.1	10.2	10.1	9.6	8.4	9.3							
13737	7.6	7.6	7.2	6.9	7.7	7.1	6.9	FC 4	7.5	7.1	FC 4	7.0	FC 4	FC 4	FC 4	FM 4				
	MATERNAL DOSAGE GROUP II					LOW DOSAGE				0.1 MG/KG/DAY a					POSTPARTUM DAY 4 (POSTCULLING)					
13739	MC 4	MC 4	MC 4	7.9	MC 4	7.2	7.8	7.5	7.4	7.9	7.7	7.3	FC 4	7.0	7.4	FC 4				
13740	10.7	10.8	10.2	10.9	9.5	9.4	9.7	9.5	9.8											
13741	MC 4	9.1	9.9	8.8	MC 4	9.2	10.2	8.6	9.3	FC 4	FC 4	8.3	9.8	FC 4	FC 4	9.4				
13744	6.1	7.5	7.5	7.5	7.7	7.5	6.0	7.0	FC 4	6.8	7.4	FC 4	FC 4	FC 4						
13745	MC 4	MC 4	6.5	MC 4	6.6	6.8	6.6	6.3	6.2	6.4	6.3	FD 2								
13746	MC 4	MC 4	8.6	9.2	9.6	8.8	MC 4	8.2	MS	8.4	9.0	9.1	7.4	7.6						
13748	9.3	9.7	8.8	7.8	8.5	MC 4	FC 4	8.1	FC 4	9.0	FC 4	9.3	7.1	8.6						
13749	10.9	11.2	10.8	10.6	10.8	10.2	10.2	10.4												

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE, U-SEX UNDETERMINABLE
SECOND LETTER -- A-ALIVE, S-STILLBORN, U-UNCERTAIN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED), X-OTHER
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 6): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP III					HIGH DOSAGE				1.6 MG/KG/DAY					POSTPARTUM DAY 4 (POSTCULLING)					
13751	MC 4	10.1	MC 4	9.6	10.0	10.5	9.8	9.0	9.2	8.1	9.8	8.2	FD 1							
13752	9.2	7.6	8.5	8.9	9.1	MD 4	FC 4	FC 4	8.1	FC 4	FC 4	7.0	8.3	8.8	8.3					
13753	7.7	8.8	MC 4	9.4	MC 4	8.4	8.3	FC 4	9.2	8.9	8.3	FC 4	8.4	9.6						
13754	8.4	8.9	8.5	8.8	9.6	8.1	9.2	8.7	8.8	7.7	FC 4									
13755	NOT PREGNANT																			
13756	8.2	MC 4	7.8	8.0	8.0	7.8	MM 3	6.6	6.9	7.3	7.0	7.4	FC 4							
13758	MC 4	MC 4	7.1	MC 4	7.1	7.4	7.3	MC 4	6.5	7.5	6.9	7.2	FC 4	7.7	6.9					
13759	MC 4	8.9	8.3	MC 4	MC 4	MC 4	8.2	8.8	8.0	MC 4	FC 4	8.0	8.2	7.3	7.6	8.1				

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE, U-SEX UNDETERMINABLE
SECOND LETTER -- A-ALIVE, S-STILLBORN, U-UNCERTAIN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED), X-OTHER
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 7): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP I VEHICLE CONTROL 0 (VEHICLE) MG/KG/DAY POSTPARTUM DAY 7																			
13726	15.3	15.1	16.6	14.4	FC 4	14.7	15.1	14.3	FC 4	14.5	15.0	FC 4	14.6							
13727	15.6	MC 4	14.7	15.4	14.9	15.5	MC 4	FC 4	14.6	14.7	13.7	13.4	FC 4	14.3						
13728	15.1	15.6	15.9	MC 4	MC 4	16.0	16.2	15.6	FC 4	14.8	15.6	14.2	14.4	FD 3	FM 2	FS				
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION																			
13734	15.6	15.6	MC 4	14.5	15.1	15.6	MC 4	12.6	FC 4	14.4	14.3	13.6	14.7							
13735	14.2	13.2	14.5	11.7	FC 4	10.7	13.9	11.2	12.0	13.3	12.8	FC 4								
13736	MC 4	MC 4	15.6	MC 4	15.9	14.6	13.3	15.1	15.9	13.8	14.8	14.5	13.5							
13737	12.9	13.0	11.1	10.6	12.0	12.6	11.7	FC 4	11.3	10.5	FC 4	11.4	FC 4	FC 4	FC 4	FM 4				
	MATERNAL DOSAGE GROUP II 0.1 MG/KG/DAY a POSTPARTUM DAY 7																			
13739	MC 4	MC 4	MC 4	11.4	MC 4	12.3	12.2	11.4	10.8	10.9	12.4	10.8	FC 4	12.5	11.3	FC 4				
13740	16.1	14.7	15.2	16.2	14.4	13.6	13.1	13.8	13.9											
13741	MC 4	15.4	15.9	16.9	MC 4	16.4	15.5	14.8	15.9	FC 4	FC 4	14.4	14.8	FC 4	FC 4	16.6				
13744	12.0	11.9	10.3	11.7	11.1	11.6	12.2	11.2	FC 4	10.6	10.0	FC 4	FC 4	FC 4						
13745	MC 4	MC 4	11.2	MC 4	11.8	11.0	11.2	10.8	11.1	10.6	10.7	FD 2								
13746	MC 4	MC 4	14.1	14.3	14.2	15.3	MC 4	13.2	MS	11.9	12.4	14.4	14.9	13.8						
13748	12.7	14.0	12.8	10.9	15.1	MC 4	FC 4	13.1	FC 4	13.6	FC 4	10.2	14.5	13.5						
13749	15.5	15.4	16.1	14.2	14.4	15.0	14.4	15.3												

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED), X-OTHER
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 8): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP III					HIGH DOSAGE				1.6 MG/KG/DAY a						POSTPARTUM DAY 7				
13751	MC 4	15.7	MC 4	15.2	15.2	14.2	14.8	13.4	13.3	13.7	12.2	14.4	FD 1							
13752	14.0	14.0	12.1	14.2	13.3	MD 4	FC 4	FC 4	10.4	FC 4	FC 4	12.9	13.1	12.8	12.4					
13753	13.1	13.2	MC 4	14.1	MC 4	13.2	11.8	FC 4	14.4	12.5	13.9	FC 4	12.9	14.2						
13754	13.5	14.4	14.7	14.0	14.4	14.1	13.7	12.3	15.0	13.8	FC 4									
13755	NOT PREGNANT																			
13756	13.0	MC 4	12.3	8.8	11.6	8.4	MM 3	10.8	8.7	11.0	11.0	9.8	FC 4							
13758	MC 4	MC 4	11.5	MC 4	11.5	11.8	11.4	MC 4	12.0	12.6	11.6	12.1	FC 4	11.3	11.2					
13759	MC 4	12.5	12.4	MC 4	MC 4	MC 4	12.6	13.2	14.0	MC 4	FC 4	12.9	11.5	12.7	12.2	12.7				

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 9): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP I					VEHICLE CONTROL				0 (VEHICLE) MG/KG/DAY						POSTPARTUM DAY 14				
13726	31.2	29.8	31.1	32.9	FC 4	30.4	30.4	29.5	FC 4	30.6	31.1	FC 4	30.6							
13727	29.8	MC 4	30.5	29.0	31.7	30.5	MC 4	FC 4	28.9	27.5	29.3	28.6	FC 4	29.0						
13728	32.8	33.6	33.0	MC 4	MC 4	33.9	33.0	32.8	FC 4	30.8	30.0	32.9	31.8	FD 3	FM 2	FS				
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION																			
13734	30.7	30.3	MC 4	29.8	28.1	29.2	MC 4	28.6	FC 4	31.3	28.3	24.1	28.9							
13735	30.8	30.0	28.4	28.3	FC 4	27.7	24.5	30.1	27.7	26.3	26.1	FC 4								
13736	MC 4	MC 4	32.2	MC 4	32.1	29.1	29.8	30.2	31.6	31.0	29.1	28.4	29.6							
13737	30.1	29.7	26.3	28.2	29.0	27.9	27.5	FC 4	27.7	30.0	FC 4	26.4	FC 4	FC 4	FC 4	FM 4				
	MATERNAL DOSAGE GROUP II					LOW DOSAGE				0.1 MG/KG/DAY a						POSTPARTUM DAY 14				
13739	MC 4	MC 4	MC 4	25.0	MC 4	26.9	25.4	26.9	25.5	25.9	27.0	27.0	FC 4	24.9	26.0	FC 4				
13740	31.7	31.3	33.5	33.3	29.8	30.8	30.2	28.8	29.7											
13741	MC 4	32.9	33.4	33.8	MC 4	31.2	31.6	31.4	33.9	FC 4	FC 4	30.3	33.0	FC 4	FC 4	29.6				
13744	31.9	31.3	28.7	34.5	32.3	27.5	31.5	33.2	FC 4	28.9	32.6	FC 4	FC 4	FC 4						
13745	MC 4	MC 4	28.7	MC 4	29.2	28.4	28.7	28.5	28.1	26.5	28.2	FD 2								
13746	MC 4	MC 4	27.4	26.5	26.6	27.5	MC 4	26.6	MS	24.6	26.9	24.6	26.5	24.1						
13748	30.7	26.9	32.4	29.6	30.3	MC 4	FC 4	30.3	FC 4	29.4	FC 4	29.9	28.1	24.7						
13749	33.1	32.5	32.2	29.8	30.0	31.9	32.8	29.9												

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 10): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP III					HIGH DOSAGE				1.6 MG/KG/DAY a						POSTPARTUM DAY 14				
13751	MC 4	32.0	MC 4	30.9	31.2	30.6	29.6	28.4	27.6	30.0	26.3	27.8	FD 1							
13752	28.6	26.4	27.4	25.7	25.3	MD 4	FC 4	FC 4	26.2	FC 4	FC 4	27.0	26.2	23.0	26.5					
13753	29.8	29.7	MC 4	29.0	MC 4	27.8	26.7	FC 4	29.4	27.8	29.0	FC 4	29.6	27.6						
13754	29.9	30.2	28.2	28.8	29.9	30.2	29.0	28.7	27.2	28.6	FC 4									
13755	NOT PREGNANT																			
13756	18.5	MC 4	24.7	27.3	28.5	19.6	MM 3	19.5	26.2	21.4	17.7	19.3	FC 4							
13758	MC 4	MC 4	26.0	MC 4	25.6	23.8	22.3	MC 4	24.0	26.2	23.7	26.6	FC 4	24.9	23.3					
13759	MC 4	26.5	27.9	MC 4	MC 4	MC 4	26.5	25.7	25.4	MC 4	FC 4	23.4	25.1	25.2	26.9	25.9				

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 11): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP I					VEHICLE CONTROL				0 (VEHICLE) MG/KG/DAY						POSTPARTUM DAY 21				
13726	49.7	49.6	52.8	50.6	FC 4	48.7	48.0	48.1	FC 4	47.5	48.4	FC 4	49.3							
13727	48.2	MC 4	48.4	48.9	52.3	45.1	MC 4	FC 4	47.7	48.2	47.9	47.6	FC 4	48.4						
13728	54.4	55.6	54.1	MC 4	MC 4	55.0	55.9	52.2	FC 4	52.4	53.4	50.5	50.4	FD 3	FM 2	FS				
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION																			
13734	45.8	46.3	MC 4	41.8	42.4	45.4	MC 4	42.1	FC 4	41.0	43.3	40.4	38.6							
13735	48.2	45.3	46.1	44.3	FC 4	42.9	38.3	41.1	43.9	48.7	43.6	FC 4								
13736	MC 4	MC 4	47.8	MC 4	48.3	45.9	45.6	48.7	45.2	43.4	45.7	45.1	46.0							
13737	46.7	48.5	44.4	41.2	47.4	44.9	42.4	FC 4	45.5	49.0	FC 4	43.6	FC 4	FC 4	FC 4	FM 4				
	MATERNAL DOSAGE GROUP II					LOW DOSAGE				0.1 MG/KG/DAY a						POSTPARTUM DAY 21				
13739	MC 4	MC 4	MC 4	43.6	MC 4	46.1	40.5	42.2	41.5	39.2	41.8	44.1	FC 4	44.0	41.8	FC 4				
13740	48.6	53.7	54.2	50.9	47.5	44.8	49.7	47.6	46.4											
13741	MC 4	51.4	49.0	49.2	MC 4	51.0	49.0	46.7	49.5	FC 4	FC 4	51.9	45.1	FC 4	FC 4	45.5				
13744	45.0	46.6	45.9	46.4	41.9	42.6	44.2	47.1	FC 4	46.1	41.8	FC 4	FC 4	FC 4						
13745	MC 4	MC 4	49.7	MC 4	52.2	49.9	49.0	48.3	47.9	49.2	48.8	FD 2								
13746	MC 4	MC 4	50.9	47.7	43.3	47.6	MC 4	47.1	MS	42.4	46.4	50.4	50.4	43.3						
13748	48.5	47.6	44.8	49.7	50.4	MC 4	FC 4	40.8	FC 4	45.1	FC 4	47.2	46.7	46.2						
13749	53.8	55.4	53.2	47.3	51.0	52.5	50.9	47.7												

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 29 (PAGE 12): PUP BODY WEIGHTS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RAT/ LITTER #	MATERNAL DOSAGE GROUP III					HIGH DOSAGE				1.6 MG/KG/DAY a						POSTPARTUM DAY 21				
13751	MC 4	46.7	MC 4	48.4	43.4	44.0	44.1	51.6	48.3	46.3	50.9	45.4	FD 1							
13752	41.3	46.4	42.5	40.5	41.5	MD 4	FC 4	FC 4	40.4	FC 4	FC 4	35.4	40.4	40.8	42.9					
13753	46.5	45.3	MC 4	45.6	MC 4	47.8	41.9	FC 4	47.5	46.5	43.4	FC 4	43.4	45.2						
13754	46.8	50.4	46.4	48.1	46.8	44.2	44.9	47.5	46.1	46.7	FC 4									
13755	NOT PREGNANT																			
13756	39.7	MC 4	42.4	33.8	33.4	43.0	MM 3	33.9	41.6	30.7	34.1	36.4	FC 4							
13758	MC 4	MC 4	39.6	MC 4	38.7	39.0	38.4	MC 4	39.0	36.9	39.0	40.7	FC 4	41.3	37.3					
13759	MC 4	41.9	44.1	MC 4	MC 4	MC 4	39.8	40.8	43.2	MC 4	FC 4	41.8	39.3	36.7	41.2	42.2				

ALL WEIGHTS WERE RECORDED IN GRAMS (G). MEAN LITTER WEIGHTS INCLUDE ONLY WEIGHTS OF LIVE PUPS.
FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 30 (PAGE 1): PUP VITAL STATUS AND SEX FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RAT/ LITTER #	MATERNAL DOSAGE GROUP I							VEHICLE CONTROL					0 (VEHICLE) MG/KG/DAY										
13726	M A	M A	M A	M A	FC 4	F A	F A	F A	FC 4	F A	F A	FC 4	F A										
13727	M A	MC 4	M A	M A	M A	M A	MC 4	FC 4	F A	F A	F A	F A	FC 4	F A									
13728	M A	M A	M A	MC 4	MC 4	M A	M A	F A	FC 4	F A	F A	F A	F A	FD 3	FM 2	F S							
13731	ACCIDENTAL DEATH ON DAY 15 OF GESTATION																						
13734	M A	M A	MC 4	M A	M A	M A	MC 4	F A	FC 4	F A	F A	F A	F A										
13735	M A	M A	M A	F A	FC 4	F A	F A	F A	F A	F A	F A	FC 4											
13736	MC 4	MC 4	M A	MC 4	M A	M A	M A	M A	M A	M A	F A	F A	F A										
13737	M A	M A	M A	M A	M A	F A	F A	FC 4	F A	F A	FC 4	F A	FC 4	FC 4	FC 4	FM 4							
	MATERNAL DOSAGE GROUP II							LOW DOSAGE					0.1 MG/KG/DAY a										
13739	MC 4	MC 4	MC 4	M A	MC 4	M A	M A	M A	M A	F A	F A	F A	FC 4	F A	F A	FC 4							
13740	M A	M A	M A	M A	F A	F A	F A	F A	F A														
13741	MC 4	M A	M A	M A	MC 4	M A	M A	F A	F A	FC 4	FC 4	F A	F A	FC 4	FC 4	F A							
13744	M A	M A	M A	M A	F A	F A	F A	F A	FC 4	F A	F A	FC 4	FC 4	FC 4									
13745	MC 4	MC 4	M A	MC 4	M A	M A	M A	F A	F A	F A	F A	FD 2											
13746	MC 4	MC 4	M A	M A	M A	M A	MC 4	M A	M S	F A	F A	F A	F A	F A									
13748	M A	M A	M A	M A	M A	MC 4	FC 4	F A	FC 4	F A	FC 4	F A	F A	F A									
13749	M A	M A	M A	F A	F A	F A	F A	F A															

FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 30 (PAGE 2): PUP VITAL STATUS AND SEX FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

PUP #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RAT/ LITTER #	MATERNAL DOSAGE GROUP III							HIGH DOSAGE					1.6 MG/KG/DAY a										
13751	MC 4	M A	MC 4	M A	M A	M A	M A	F A	F A	F A	F A	F A	FD 1										
13752	M A	M A	M A	M A	M A	MD 4	FC 4	FC 4	F A	FC 4	FC 4	F A	F A	F A	F A								
13753	M A	M A	MC 4	M A	MC 4	M A	M A	FC 4	F A	F A	F A	FC 4	F A	F A									
13754	M A	M A	M A	M A	F A	F A	F A	F A	F A	F A	FC 4												
13755	NOT PREGNANT																						
13756	M A	MC 4	M A	M A	M A	M A	MM 3	F A	F A	F A	F A	F A	FC 4										
13758	MC 4	MC 4	M A	MC 4	M A	M A	M A	MC 4	M A	F A	F A	F A	FC 4	F A	F A								
13759	MC 4	M A	M A	MC 4	MC 4	MC 4	M A	M A	M A	MC 4	FC 4	F A	F A	F A	F A	F A							

FIRST LETTER -- M-MALE, F-FEMALE
SECOND LETTER -- A-ALIVE, S-STILLBORN, D-DIED, C-CULLED, M-MISSING (PRESUMED CANNIBALIZED),
NUMBER FOLLOWING "SECOND LETTER" INDICATES THE DAY POSTPARTUM THE EVENT OCCURRED.

a. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 31 (PAGE 1): CLINICAL OBSERVATIONS FROM BIRTH TO DAY 21 POSTPARTUM - INDIVIDUAL DATA - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)	LITTER NUMBER	DAY(S) POSTPARTUM	OBSERVATION a
I 0 (VEHICLE)	-	-	-
II 0.1b	13746	7-21	1/10 PUPS: TIP OF TAIL, BLACK. c
III 1.6b	-	-	-

a. Tabulation restricted to adverse observations; all other pups appeared normal.
b. As a result of an error in calculation, which resulted in an error in test article preparation, Group II rats were dosed with 0.125 mg/kg/day and Group III rats were dosed with 2 mg/kg/day on days 1 through 4 of study.
c. Observation confirmed at necropsy.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 32 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)	LITTER NUMBER	DAY(S) POSTPARTUM	OBSERVATIONS a
I 0 (VEHICLE)	13726	4, 21	ALL PUPS APPEARED NORMAL.
	13727	4, 21	ALL PUPS APPEARED NORMAL.
	13728	1	1 PUP: STILLBORN. ALL TISSUES APPEARED NORMAL.
		3	1 PUP: FOUND DEAD. ALL TISSUES APPEARED NORMAL.
		4, 21	ALL PUPS APPEARED NORMAL.
	13734	4, 21	ALL PUPS APPEARED NORMAL.
	13735	4, 21	ALL PUPS APPEARED NORMAL.
	13736	4, 21	ALL PUPS APPEARED NORMAL.
	13737	4, 21	ALL PUPS APPEARED NORMAL.

a. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation. Refer to the individual pup clinical observations table (Table 31) for external observations confirmed at necropsy.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 32 (PAGE 2): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)	LITTER NUMBER	DAY(S) POSTPARTUM	OBSERVATIONS a
II 0.1b	13739	4, 21	ALL PUPS APPEARED NORMAL.
	13740	21	ALL PUPS APPEARED NORMAL.
	13741	4, 21	ALL PUPS APPEARED NORMAL.
	13744	4, 21	ALL PUPS APPEARED NORMAL.
	13745	2	1 PUP: FOUND DEAD. NO MILK IN STOMACH. ALL OTHER TISSUES APPEARED NORMAL.
		4, 21	ALL PUPS APPEARED NORMAL.
	13746	1	1 PUP: STILLBORN. ALL TISSUES APPEARED NORMAL.
		4, 21	ALL PUPS APPEARED NORMAL.
	13748	4, 21	ALL PUPS APPEARED NORMAL.
	13749	21	ALL PUPS APPEARED NORMAL.

a. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation. Refer to the individual pup clinical observations table (Table 31) for external observations confirmed at necropsy.
b. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 0.125 mg/kg/day on days 1 through 4 of study.

PROTOCOL 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER: T-6295.14)

TABLE 32 (PAGE 3): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)	LITTER NUMBER	DAY(S) POSTPARTUM	OBSERVATIONS a
III 1.6b	13751	1	1 PUP: FOUND DEAD. NO MILK IN STOMACH. ALL OTHER TISSUES APPEARED NORMAL.
		4, 21	ALL PUPS APPEARED NORMAL.
	13752	4	1 PUP: FOUND DEAD. NO MILK IN STOMACH. ALL OTHER TISSUES APPEARED NORMAL. ALL OTHER PUPS APPEARED NORMAL.
		21	ALL PUPS APPEARED NORMAL.
	13753	4, 21	ALL PUPS APPEARED NORMAL.
	13754	4, 21	ALL PUPS APPEARED NORMAL.
	13756	4, 21	ALL PUPS APPEARED NORMAL.
	13758	4, 21	ALL PUPS APPEARED NORMAL.
	13759	4, 21	ALL PUPS APPEARED NORMAL.

a. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation. Refer to the individual pup clinical observations table (Table 31) for external observations confirmed at necropsy.
b. As a result of an error in calculation, which resulted in an error in test article preparation, rats were dosed with 2 mg/kg/day on days 1 through 4 of study.

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 418-015

SPONSOR'S STUDY NUMBER: T-6295.14

STUDY TITLE: Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PURPOSE: The purpose of this study is to evaluate the pharmacokinetics of PFOS in Fo generation and F1 generation rats during gestation and lactation following cessation of PFOS treatment of Crl:CD®BR VAF/Plus® female rats at confirmed mating.

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 Corporate Toxicology
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 (Synonym: FC-95).
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 (ATTACHMENT 2).

Storage:

Bulk Test Article:	Room temperature.
Vehicle Components:	Room temperature.
Prepared Vehicle:	Room temperature.
Prepared Formulations:	Room temperature.

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. Data verifying the stability of the test article in the vehicle for 48 hours under the conditions of administration are on file with the Sponsor.

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 frozen (-70°C or below) 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 Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat was selected as the Test System because: 1) this strain of rat was used in the reproductive and developmental toxicity studies; 2) historical data and experience exist at the Testing Facility[1-3]; and 3) the test article is pharmacologically active in the species and strain.

Number:

Initial population acclimated:	36 virgin female rats.
Population selected for study:	24 mated female rats (8 per dosage group).

Body Weight and Age:

Female rats will be ordered to have body weights of 200 g to 225 g each at receipt, at which time they will be expected to be at least 60 days of age. Actual body weights will be recorded the day after receipt and will be documented in the raw data. The weight range will be included in the final report.

Sex:

Female rats will be given the test article. Male rats of the same source and strain will be used only as breeders and are not considered part of the Test System.

Source:

Charles River Laboratories, Inc.

The rats will be shipped in filtered cartons by air freight and/or truck from Charles River Laboratories, Inc., to the Testing Facility.

Identification:

Fo Generation:

Rats are permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20101). Male rats are given unique permanent identification numbers upon assignment to the Testing Facility's breeder male rat population. Female rats are assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study.

F1 Generation:

Pups will not be individually identified during lactation; all parameters will be evaluated in terms of the litter.

ANIMAL HUSBANDRY:

All cage sizes and housing conditions are in compliance with the *Guide for the Care and Use of Laboratory Animals*[4].

Housing:

Fo Generation Rats/F1 Generation Litters:

Fo generation rats will be individually housed in stainless steel, wire-bottomed cages, except during the cohabitation and postpartum periods. During cohabitation, each pair of rats will be housed in the male rat's cage. Beginning no later than day 20 of presumed gestation, Fo generation female rats will be individually housed in nesting boxes, except during collection intervals for urine and fecal samples. During these collection intervals, the female rats will be housed individually in metabolism cages. Each dam and delivered litter will be housed in a common nesting box during the postpartum period.

Nesting Material:

Nesting material (bed-o'cobs®) will be provided.

Bedding will be changed as often as necessary to keep the animals dry and clean. Analyses for possible contamination are conducted annually and documented in the raw data.

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 (Airo Clean® room). Room temperature will be maintained at 64°F (18°C) to 79°F (26°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:

Rats will be given Certified Rodent Diet® #5002 (PMI Nutrition International) available *ad libitum* from individual feeders.

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, the drinking water or the nesting material 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.

RANDOMIZATION AND COHABITATION:

Upon arrival, male and female rats will be assigned to individual housing on the basis of computer-generated random units. After acclimation, 36 virgin female rats will be selected for study on the basis of physical appearance and body weights recorded during acclimation. The female rats will be assigned to dosage groups (12 female rats per dosage group) based on computer-generated (weight-ordered) randomization procedures and treated with either the vehicle or the test article for 42 days prior to cohabitation.

Within each dosage group, consecutive order will be used to assign female rats to cohabitation with breeder male rats, one male rat per female rat. The cohabitation period will consist of a maximum of five days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* will be considered to be at day 0 of presumed gestation and assigned to individual housing.

Eight mated female rats (those with confirmed evidence of mating) will be assigned to each dosage group. Any remaining female rats with or without confirmed evidence of mating that were assigned to either of the treated groups or the control group prior to cohabitation will be sacrificed and discarded without further evaluation at the discretion of the Study Director and the Study Monitor.

Day 1 of lactation (postpartum) is defined as the day of birth and is also the first day on which all pups in a litter are individually weighed (pup body weights will be recorded after all pups in a litter are delivered and groomed by the dam).

On day 4 postpartum, litters will be culled to five male pups and five female pups per litter, where possible. Pups not selected for continued evaluation will be sacrificed via decapitation; the lungs (saved in Bouin's solution) and the liver (saved in neutral buffered 10% formalin) will be collected from the first ten culled pups from each dosage group (irrespective of litter) determined to be at day 4 postpartum and preserved for possible future histopathological evaluation. Remaining culled pups will be sacrificed via decapitation and discarded without necropsy evaluation.

ADMINISTRATION:

Route and Reason for Choice:

The oral (gavage) route was selected for use because: 1) this was the route of administration in the developmental and reproductive toxicology studies; and 2) it is one of the possible routes of human exposure.

Method and Frequency:

Fo Generation Female Rats:

Female rats will be given the test article once daily beginning 42 days prior to cohabitation until day 0 of presumed gestation (confirmed evidence of mating, such as observation of spermatozoa in a smear of the vaginal contents or a copulatory plug *in situ*). Female rats will not be given the test article or the vehicle on day 0 of presumed gestation. Dosages will be adjusted daily for body weight changes and given at approximately the same time each day.

F1 Generation Pups:

F1 generation pups will not be directly given the test article, but may be possibly exposed to the test article during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period.

Rationale for Dosage Selection:

Dosages were selected on the basis of a previous study conducted with the test article (Argus Research Laboratories, Inc., Protocol 418-008).

Dosage Levels, Concentrations and Volumes:

Dosage Group	Number of Female Rats	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Argus Batch Number
I	8	0 (Vehicle)	0	5	B-418-015-A(Day.Month.Year)
II	8	0.1	0.02	5	B-418-015-B(Day.Month.Year)
III	8	1.6	0.32	5	B-418-015-C(Day.Month.Year)

The test article will be considered 100% pure for the purpose of dosage calculations.

TESTS, ANALYSES AND MEASUREMENTS - Fo GENERATION:

Viability:

All Periods: At least twice daily.

Clinical Observations and/or General Appearance:

Acclimation Period: At least once.

Dosage Period:	Twice daily. Prior to administration and once approximately one hour postdosage.
All Other Periods:	Once daily.
Maternal Behavior:	Days 1, 4, 7, 10, 14 and 21 postpartum. Observed abnormal behavior will be recorded daily.

Clinical observations may be recorded more frequently than cited above, if deemed appropriate by the Study Director and/or Study Monitor.

Body Weights:

Acclimation Period:	At least once.
Dosage Period:	Daily.
All Other Periods:	Daily.
Sacrifice:	Terminal weight.

Feed Consumption Values (recorded and tabulated):

Acclimation Period:	At least once.
Dosage Period:	Weekly to cohabitation.
All Other Periods:	Daily during presumed gestation and days 1, 4, 7, 10 and 14 postpartum. Feed consumption will not be tabulated after day 14 postpartum, when it is expected that pups will begin to consume maternal feed.

Feed consumption values may be recorded more frequently if it is necessary to replenish the feed. These intervals will not be tabulated.

Mating Performance:

Mating will be evaluated daily during the cohabitation period and confirmed by observation of spermatozoa in a smear of the vaginal contents and/or a copulatory plug observed *in situ*.

Natural Delivery:

Female rats will be evaluated for:

Duration of Gestation (day 0 of presumed gestation to the day the first pup is observed).

Litter Size (defined as all pups delivered).

Live Litter Size (live born pups only).

Pup Viability at Birth.

Pharmacokinetic Sample Collection:

Urine and Fecal Samples:

Female rats will be housed individually in metabolism cages for collection of urine and fecal samples for the following intervals: one day prior to initiation of cohabitation to the following morning and days 6 to 7, 14 to 15 and 20 to 21 of presumed gestation, as well as days 21 to 22 postpartum. Following each 24-hour collection interval, samples will be collected into centrifuge tubes, placed on dry ice and stored frozen (-70°C or below) until shipment for analysis.

In the event that a dam begins to deliver before completion of urine and fecal sample collection on day 20 to day 21 of presumed gestation, the dam will be removed from the metabolism cage and placed in a nesting box with sufficient bedding.

Blood Samples:

Blood samples will be collected from each of the maternal rats following removal from metabolism caging (prior to administration) on each of the following days: on the day cohabitation is initiated (prior to cohabitation), and on days 7, 15 and 21 of presumed gestation, as well as on days 14 and 22 postpartum. The time of blood collection will be recorded in the raw data.

On all days of collection except day 22 postpartum, blood samples (approximately 1 mL each) will be collected from the orbital sinus. If necessary, whole blood may be collected from an alternate site; if so, the alternate site will be documented in the raw data. On day 22 postpartum, blood samples (approximately 4 mL each) will be collected via the inferior vena cava. Blood will be collected and transferred into serum separator tubes. The samples will be spun in a refrigerated centrifuge. The serum will be transferred into polypropylene tubes labeled with the study number, animal identification, date of collection, study day and collection timepoint. All samples will be immediately frozen on dry ice and maintained frozen (-70°C or below) until shipment to the Sponsor for analysis.

Shipping Instructions:

All samples will be maintained frozen (-70°C or below) until shipment for analysis. Samples will be shipped on frozen on dry ice via overnight mail. A packing list will be included with the samples and sent to Kris J. Hansen, Ph.D., at the previously cited address. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

METHOD OF SACRIFICE - Fo GENERATION RATS:

Rats will be sacrificed by carbon dioxide asphyxiation.

NECROPSY - Fo GENERATION RATS:

Gross lesions will be retained in neutral buffered 10% formalin for possible future evaluation (a table of random units will be used to select one control group rat from which all tissues examined at necropsy will be retained, in order to provide control tissues for any possible histopathological evaluations of gross lesions). Unless specifically cited below, all other tissues will be discarded.

Rats Not Selected for Continued Evaluation:

Any remaining female rats with or without confirmed evidence of mating that were assigned to either of the treated groups or the control group prior to cohabitation will be sacrificed and discarded without further evaluation at the discretion of the Study Director and the Study Monitor.

Rats Not Delivering a Litter:

Rats that do not deliver a litter will be sacrificed on day 25 of presumed gestation and examined for gross lesions. Uteri will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5].

Scheduled Sacrifice:

On day 22 postpartum, following the final collection interval for urine and fecal samples and blood sample collection, female rats will be sacrificed, and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. The number and distribution of implantation sites will be recorded. A liver section (right lateral lobe) from each dam will be collected, frozen and stored (-70°C or below) until shipment to the Sponsor for analysis.

Dams with No Surviving Pups:

Dams with no surviving pups will be sacrificed after the last pup is found dead, missing or presumed cannibalized.

Prior to sacrifice, a blood sample (approximately 4 mL) will be collected from the maternal rat via the inferior vena cava and transferred into serum separator tubes. The sample will be spun in a refrigerated centrifuge. The serum will be transferred into a polypropylene tube labeled with the study number, animal identification, date of collection, study day and collection timepoint. The sample will be immediately frozen on dry ice and maintained frozen (-70°C or below) until shipment to the Sponsor for analysis.

A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. A liver section (right lateral lobe) from each dam will be collected, frozen and stored (-70°C or below) until shipment to the Sponsor for analysis. Postpartum data for these dams will be excluded from summary tables.

Rats Found Dead or Moribund:

Rats 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. The rats will be examined for gross lesions. A liver section (right lateral lobe) from each dam will be collected, frozen and stored (-70°C or below) until shipment to the Sponsor for analysis. Pregnancy status and uterine contents of female rats will be recorded. Aborted fetuses and/or delivered pups will be examined to the extent possible. Uteri of apparently nonpregnant rats will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5].

Shipping Instructions:

All samples will be maintained frozen (-70°C or below) until shipment for analysis. Samples will be shipped frozen on dry ice via overnight mail. A packing list will be included with the samples and sent to Kris J. Hansen, Ph.D., at the previously cited address. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

TESTS, ANALYSES AND MEASUREMENTS - F1 GENERATION:

Viability:

Postpartum Period:	Litters will be observed for dead pups at least twice daily. The pups in each litter will be counted once daily.

Clinical Observations and/or General Appearance:

Postpartum Period:	Once daily.

Clinical observations may be recorded more frequently than cited above, if deemed appropriate by the Study Director and/or the Study Monitor.

Body Weights:

Postpartum Period:	Days 1 (birth), 4, 7, 14 and 21 postpartum.
Sacrifice:	Terminal weight.

METHOD OF SACRIFICE - F1 GENERATION RATS:

As previously cited for Fo generation rats.

NECROPSY - F1 GENERATION RATS:

Gross lesions will be retained in neutral buffered 10% formalin for possible future evaluation (a table of random units will be used to select one control group rat of each sex from which all tissues examined at necropsy will be retained, in order to provide control tissues for any possible histopathological evaluations of gross lesions). Unless specifically cited below, all other tissues will be discarded.

Caps and labeled tubes will be weighed (combined weight, to the nearest .001 gram) before and after retention of pooled pup samples. These weights will be documented in the raw data, and copies of these weights will be included with the packing list prior to shipment.

Pups Found Dead on Day 1 Postpartum:

Pups that die before examination of the litter for pup viability will be evaluated for vital status at birth. The lungs will be removed and immersed in water. Pups with lungs that sink will be identified as stillborn; pups with lungs that float will be identified as liveborn, and to have died shortly after birth. Pups with gross lesions will be preserved in Bouin's solution for possible future evaluation. All lungs will be preserved in Bouin's solution for possible future evaluation.

Pups Found Dead or Moribund on Days 2 to 21 Postpartum:

Pups found dead or sacrificed because of moribundity will be examined for gross lesions and for the cause of death or the moribund condition. Pups with gross lesions found on days 2 to 4 postpartum will be preserved in Bouin's solution for possible future evaluation; gross lesions of pups found on days 5 to 21 postpartum will be preserved in neutral buffered 10% formalin.

For all pups found dead on days 2 to 4 postpartum, all lungs will be preserved in Bouin's solution for possible future evaluation. For all pups found dead on days 5 to 21 postpartum, all lungs will be preserved in neutral buffered 10% formalin for possible future evaluation.

Pups Not Selected for Continued Observation - Day 4 Postpartum:

All pups culled on day 4 postpartum will be sacrificed via decapitation. All lungs and livers will be collected from the first ten pups culled (irrespective of litter) from each dosage group; the lungs will be individually retained in Bouin's solution, and the livers will be individually retained in neutral buffered 10% formalin for possible future evaluation. Remaining culled pups will be sacrificed and discarded without evaluation.

Scheduled Sacrifice:

On day 21 postpartum, blood samples will be collected from all remaining pups on study.

Blood samples will be collected via the inferior vena cava from each pup, pooled (per litter) and transferred into serum separator tubes. The samples will be spun in a refrigerated centrifuge. The serum will be transferred into polypropylene tubes labeled with the study number, animal identification, date of collection, study day and collection timepoint. All samples will be immediately frozen on dry ice and maintained frozen (-70°C or below) until shipment to the Sponsor for analysis.

The pups will be examined for gross lesions. The liver from each pup will be collected, pooled (per litter), frozen and stored (-70°C or below) until shipment to the Sponsor for analysis.

Shipping Instructions:

All samples will be maintained frozen (-70°C or below) until shipment for analysis. Samples will be shipped frozen on dry ice via overnight mail. A packing list will be included with the samples and sent to Kris J. Hansen, Ph.D., at the previously cited address. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

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.
Mating History.
Treatment (if prescribed by Staff Veterinarian).
General Comments.
Clinical Observations and/or General Appearance.
Tissue and Sample Collection, Processing and Shipment.
Cap and Labeled Tube Weights.
Body Weights.
Feed Consumption Values.
Natural Delivery Observations.
Litter Observations.
Gross Necropsy Observations.
Organ Weights (if required).
Photographs (if required).
Study Maintenance (room and environmental records).
Feed, Water and Bedding 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.
Director of Study Management: Valerie A. Sharper, M.S.
Manager of Animal Operations and Chairperson, Institutional Animal Care and Use Committee: Dena C. Lebo, V.M.D.
Director of Operations and Compliance: Barbara J. Patterson, B.A.
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. and Voytek, P.E. (1982). *In Vivo Reproductive and Mutagenicity Tests*. Environmental Protection Agency, Washington, D.C. National Technical Information Service, U.S. Department of Commerce, Springfield, VA 22161.

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. Lang, P.L. (1988). *Embryo and Fetal Developmental Toxicity (Teratology) Control Data in the Charles River Crl:CD®BR Rat*. Charles River Laboratories, Inc., Wilmington, MA 01887-0630. (Data base provided by Argus Research Laboratories, Inc.)

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

Alan M. Hoberman, Ph.D., DABT
Director of Research

16-NOV-98
Date

Raymond G. York, Ph.D., DABT
Associate Director of Research
Study Director

16-NOV-98
Date

Dena C. Lebo, V.M.D.
Chairperson, Institutional Animal Care and Use Committee

16 Nov 98
Date

FOR THE SPONSOR

Marvin T. Case, D.V.M., Ph.D.
Study Monitor

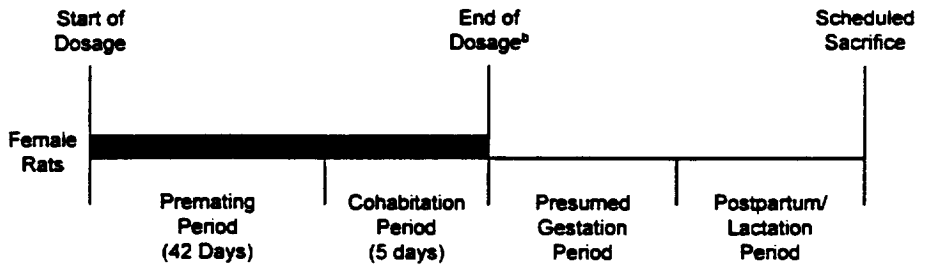
17 Nov 98
Date

ATTACHMENT 1

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE

STUDY SCHEMATIC

PHARMACOKINETIC RECOVERY STUDY[a]



Dosage Period.

a. For additional details see "Tests, Analyses and Measurements" section of the protocol.

b. Fo generation female rats will receive test article or vehicle until mating is confirmed (day 0 of presumed gestation).

SCHEDULE[a]

17 NOV 98	Animals Arrive - Acclimation Begins.
23 NOV 98	Dosage Period - Female Rats [42 days prior to cohabitation until confirmed mating (day 0 of presumed gestation)].
04 JAN 99 PM - 09 JAN 99 AM	Cohabitation Period.
05 JAN 99	First Possible Day 0 of Presumed Gestation.
09 JAN 99	Last Possible Day 0 of Presumed Gestation.
26 JAN 99	First Possible Delivery (Day 21 of presumed gestation).
03 FEB 99	Last Possible Delivery (Day 25 of presumed gestation).
29 JAN 99	First Possible Day 4 Postpartum Culling.
06 FEB 99	Last Possible Day 4 Postpartum Culling.
30 JAN 99	First Possible Day 25 of Presumed Gestation Female Sacrifice.
03 FEB 99	Last Possible Day 25 of Presumed Gestation Female Sacrifice.
15 FEB 99 - 23 FEB 99	Scheduled Sacrifice - F1 Generation Pups (Day 21 postpartum).
16 FEB 99 - 24 FEB 99	Scheduled Sacrifice - Fo Generation Dams (Day 22 postpartum).
29 JUN 99	Draft Final Report.

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a. The study initiation date is the day 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)

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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. 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 rats on Argus Study 418-015.

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 0.32 mg/mL, Group III 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 0.02 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.

4. 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: _____

Approved by: _____ Date: 16-NOV-98

Clarification: ✓ No ___ Yes (See attached clarification form.)

Initials/Date : Christopher K. Ruggeri 3/3/99

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

PROTOCOL 418-015

Oral (Gavage) Pharmacokinetc Recovery Study of PFOS in Rats

SPONSOR'S STUDY NUMBER: T-6295.14

Amendment 1 – 07 January 1999

1. Analyses of Prepared Formulations, Concentration of Test Article Formulations (Page 4 of the protocol):

[Effective date: 06 January 1999] Duplicate samples (2 mL each) will be taken from the first and last preparation rather than, the first and last preparation on the day prepared.

Reason for Change:

The preparation occurs the day before it is used for dosage administration and, according the to protocol, day 0 of gestation is the last day of dosage administration. Since day 0 of gestation is the day that the rats are confirmed pregnant, the sample cannot be taken of the last day of preparation.

07-JAN-99

Alan M. Hoberman, Ph.D., DABT Date
Director of Research

07-JAN-99

Raymond G. York, Ph.D., DABT Date
Associate Director ~~of~~ Research and
Study Director

Theresa A. Woodard for 07 Jan 99

Dena C. Lebo, V.M.D. Date
Chairperson, Institutional Animal Care and
Use Committee

7 Jan 99

Marvin T. Case, D.V.M., Ph.D. Date
Study Monitor

APPENDIX D

DEVIATIONS FROM THE PROTOCOL AND THE STANDARD OPERATING PROCEDURES OF THE TESTING FACILITY

DEVIATIONS FROM THE PROTOCOL AND STANDARD OPERATING PROCEDURES OF THE TESTING FACILITY

1. All rats were administered the test article or vehicle on premating day 43, 4 January 1999. The rats should have been placed into cohabitation on premating day 42 after dosage administration. This deviation did not adversely affect the outcome or interpretation of the study because no data were lost.

2. From 23 November 1998 to 26 November 1998 (days 1 to 4 of the premating period), the following Fo generation female rats received the incorrectly calculated amount of test article in vehicle, resulting in the rats receiving 25% more of the test article than required.

Dosage Group	Dosage (mg/kg/day)	Rat Numbers
II	0.1	13738 – 13749
III	1.6	13750 - 13761

These deviations did not adversely affect the outcome or interpretation of the study because the dosages were only for the first four days out of a total of 43 days of test article administration prior to mating.

All deviations are documented in the raw data.

23-Jul-99

Raymond G. York, Ph.D., DABT — Date
Associate Director of Research
and Study Director

APPENDIX E

TEMPERATURE AND RELATIVE HUMIDITY REPORTS

ARGUS

Temperature and Relative Humidity Report
Location: Room 15

Protocol Number: 418-015

Range of Dates: 17-Nov-1998 14:00 to 27-Nov-1998 08:55

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: rat				
Total Number of Days:	11		11	
Total Number of Hours:	234.72		234.72	
Total Number of Data Points:	235		235	
Mean (± SD):	67.0	(± 0.9)	44.6	(± 4.8)
Maximum:	68.9		53.4	
Median:	67.2		45.6	
Minimum:	63.4		30.3	
Number of Points in Range (%):	232	(98.7)	235	(100.0)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	3	(1.3)	0	(0.0)

Report Generated: 28-Apr-1999 at 12:33

COMMENTS: _____

REVIEWED BY: [signature] **DATE:** 5/19/99

ARGUS

Temperature and Relative Humidity Report
Location: Room 04

Protocol Number: 418-015

Range of Dates: 27-Nov-1998 08:55 to 30-Nov-1998 09:15

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: rat				
Total Number of Days:	4		4	
Total Number of Hours:	71.99		71.99	
Total Number of Data Points:	75		75	
Mean (± SD):	72.4	(± 0.9)	45.1	(± 4.8)
Maximum:	74.3		68.7	
Median:	72.3		44.1	
Minimum:	69.6		40.0	
Number of Points in Range (%):	75	(100.0)	75	(100.0)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 28-Apr-1999 at 12:35

COMMENTS: _____

_____

_____

_____

REVIEWED BY: AC [illegible] **DATE:** 5/19/99

ARGUS

Temperature and Relative Humidity Report
Location: Room 27

Protocol Number: 418-015

Range of Dates: 30-Nov-1998 09:15 to 09-Dec-1998 14:30

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: rat				
Total Number of Days:	10		10	
Total Number of Hours:	221.0		221.0	
Total Number of Data Points:	222		222	
Mean (± SD):	71.3	(± 0.5)	59.3	(± 6.7)
Maximum:	72.4		68.8	
Median:	71.3		61.2	
Minimum:	70.0		37.0	
Number of Points in Range (%):	222	(100.0)	222	(100.0)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 28-Apr-1999 at 12:37

COMMENTS: _____

REVIEWED BY: [signature] **DATE:** 5/15/99

ARGUS

Temperature and Relative Humidity Report
Location: Room 28-29

Protocol Number: 418-015

Range of Dates: 09-Dec-1998 14:30 to 15-Dec-1998 15:44

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: rat				
Total Number of Days:	7		7	
Total Number of Hours:	145.01		145.01	
Total Number of Data Points:	146		146	
Mean (± SD):	70.7	(± 1.1)	49.0	(± 3.5)
Maximum:	76.5		59.4	
Median:	70.4		48.8	
Minimum:	69.1		39.6	
Number of Points in Range (%):	146	(100.0)	146	(100.0)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 28-Apr-1999 at 12:39

COMMENTS: _____

REVIEWED BY: _____ **DATE:** 5/15/99

ARGUS

Temperature and Relative Humidity Report
Location: Room 27

Protocol Number: 418-015

Range of Dates: 15-Dec-1998 15:44 to 21-Dec-1998 13:45

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: rat				
Total Number of Days:	7		7	
Total Number of Hours:	141.77		141.77	
Total Number of Data Points:	143		143	
Mean (± SD):	70.6	(± 0.8)	59.9	(± 2.6)
Maximum:	72.5		64.8	
Median:	70.5		60.1	
Minimum:	69.0		49.1	
Number of Points in Range (%):	143	(100.0)	143	(100.0)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 28-Apr-1999 at 12:41

COMMENTS: _____

REVIEWED BY: [signature] **DATE:** 5/15/99

ARGUS

Temperature and Relative Humidity Report
Location: Room 28-29

Protocol Number: 418-015

Range of Dates: 21-Dec-1998 13:45 to 20-Feb-1999 15:00

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: rat				
Total Number of Days:	62		62	
Total Number of Hours:	1464.99		1464.99	
Total Number of Data Points:	1466		1466	
Mean (± SD):	69.0	(± 1.1)	49.0	(± 10.6)
Maximum:	71.9		81.6	
Median:	69.0		48.7	
Minimum:	66.9		10.0	
Number of Points in Range (%):	1466	(100.0)	1407	(96.0)
Number of Points High (%):	0	(0.0)	49	(3.3)
Number of Points Low (%):	0	(0.0)	10	(0.7)

Report Generated: 28-Apr-1999 at 12:46

COMMENTS: _____

REVIEWED BY: _____ **DATE:** 5/5/99

ARGUS

Temperature Deviations Report
Location: Room 15

Protocol Number: 418-015

Range of Dates: 17-Nov-1998 14:00 to 27-Nov-1998 08:55

Temperature Target Range: 64°F to 79°F
Species: rat

Date	Time	Temp.	Date	Time	Temp.
25-Nov-1998	03:00	63.8 L			
25-Nov-1998	06:00	63.7 L			
25-Nov-1998	07:00	63.4 L			

H = Value out of range - High L = Value out of range - Low
Temp. = Temperature °F

Report Generated: 28-Apr-1999 at 12:34

✓ These deviations did not adversely affect the outcome or interpretation of the study.

_____ The following deviation(s) impacted on the outcome of the study as described:

Study Director: Date: 30 APR 99

ARGUS

Relative Humidity Deviations Report
Location: Room 28-29

Protocol Number: 418-015

Range of Dates: 21-Dec-1998 13:45 to 20-Feb-1999 15:00

Humidity Target Range: 30% to 70%
Species: rat

Date	Time	R.H.	Date	Time	R.H.
21-Dec-1998	18:00	77.2 H	21-Jan-1999	18:00	76.4 H
29-Dec-1998	12:00	70.3 H	22-Jan-1999	03:00	70.9 H
04-Jan-1999	13:00	29.4 L	22-Jan-1999	05:00	71.7 H
05-Jan-1999	22:00	26.3 L	22-Jan-1999	07:00	70.4 H
05-Jan-1999	23:00	26.8 L	22-Jan-1999	09:00	70.4 H
06-Jan-1999	00:00	19.3 L	22-Jan-1999	21:00	70.5 H
06-Jan-1999	01:00	20.7 L	23-Jan-1999	06:00	71.4 H
06-Jan-1999	02:00	17.0 L	23-Jan-1999	09:00	72.3 H
06-Jan-1999	03:00	14.7 L	23-Jan-1999	13:00	73.9 H
06-Jan-1999	04:00	15.7 L	23-Jan-1999	18:00	78.2 H
06-Jan-1999	05:00	10.0 L	23-Jan-1999	19:00	75.1 H
06-Jan-1999	06:00	10.2 L	23-Jan-1999	23:00	77.9 H
18-Jan-1999	11:00	75.4 H	24-Jan-1999	00:00	73.1 H
18-Jan-1999	15:00	72.4 H	24-Jan-1999	03:00	80.7 H
18-Jan-1999	18:00	72.0 H	24-Jan-1999	04:00	72.4 H
18-Jan-1999	20:00	73.4 H	24-Jan-1999	05:00	72.2 H
18-Jan-1999	21:00	75.4 H	24-Jan-1999	06:00	73.4 H

H = Value out of range - High L = Value out of range - Low
R.H. = Relative Humidity (%)

Report Generated: 28-Apr-1999 at 12:53

✓ These deviations did not adversely affect the outcome or interpretation of the study.

_____ The following deviation(s) impacted on the outcome of the study as described:

Study Director: [signature] Date: 30-Apr-99

ARGUS

Relative Humidity Deviations Report
Location: Room 28-29

Protocol Number: 418-015

Range of Dates: 21-Dec-1998 13:45 to 20-Feb-1999 15:00

Humidity Target Range: 30% to 70%
Species: rat

Date	Time	R.H.	Date	Time	R.H.
24-Jan-1999	07:00	70.1 H	02-Feb-1999	19:00	77.7 H
24-Jan-1999	09:00	70.3 H	02-Feb-1999	20:00	77.7 H
24-Jan-1999	10:00	75.5 H	02-Feb-1999	21:00	70.7 H
24-Jan-1999	11:00	75.6 H	02-Feb-1999	23:00	77.7 H
24-Jan-1999	12:00	75.8 H	03-Feb-1999	01:00	76.7 H
24-Jan-1999	13:00	78.2 H	03-Feb-1999	03:00	75.4 H
24-Jan-1999	14:00	81.6 H	03-Feb-1999	07:00	75.2 H
24-Jan-1999	15:00	75.5 H	06-Feb-1999	12:00	72.9 H
24-Jan-1999	16:00	80.3 H			
24-Jan-1999	17:00	78.7 H			
24-Jan-1999	18:00	75.9 H			
26-Jan-1999	11:00	70.9 H			
26-Jan-1999	15:00	74.5 H			
26-Jan-1999	16:00	75.7 H			
26-Jan-1999	17:00	76.1 H			
26-Jan-1999	18:00	73.6 H			
26-Jan-1999	20:00	70.6 H			

H = Value out of range - High L = Value out of range - Low
R.H. = Relative Humidity (%)

Report Generated: 28-Apr-1999 at 12:54

✓ These deviations did not adversely affect the outcome or interpretation of the study.

_____ The following deviation(s) impacted on the outcome of the study as described:

Study Director: [signature] Date: 30-APR-99

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 418-015: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS
SPONSOR'S STUDY NUMBER: T-6295.14

STATEMENT OF THE STUDY DIRECTOR

This final report accurately reflects the raw data obtained during the performance of the study. No 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.

23 [illegible] - [illegible]

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 418-015: Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats
Sponsor's Study Number: T-6295.14

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 18 OCT 98.

Critical phases of this study were inspected 10 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 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 10 MAY 99 and 15 JUN 99, and for revisions requested by the Sponsor on 20 JUL 99, 22 JUL 99, and for finalization on 23 JUL 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.

Nancy J. Gongliewski 23 Jul 99

Nancy J. Gongliewski Date
Quality Assurance Manager

Nancy J. Gongliewski 23 Jul 99

for Lisa A. Zaborowski, B.S. Date
Senior Quality Assurance Associate
and Principal Auditor

TABLE 1

CRITICAL PHASES INSPECTED

Test Article Administration - Gavage

Date of inspection: 23 NOV 98

Date results reported to the Study Director and Management: 25 NOV 98

Test Article Preparation

Date of inspection: 01 DEC 98

Date results reported to the Study Director and Management: 02 DEC 98

Urine/Fecal Collection

Date of inspection: 04 JAN 99

Date results reported to the Study Director and Management: 04 JAN 99

Blood Collection

Dates of inspection: 04 JAN 99, 17 FEB 99

Dates results reported to the Study Director and Management: 04 JAN 99, 17 FEB 99

Cohabitation

Date of inspection: 05 JAN 99

Date results reported to the Study Director and Management: 07 JAN 99

Natural Delivery/Litter Watch

Date of inspection: 27 JAN 99

Date results reported to the Study Director and Management: 02 FEB 99

Culling, Pup Scrifice: Day 4

Date of inspection: 01 FEB 99

Date results reported to the Study Director and Management: 03 FEB 99

Necropsy, Fo Dams, F_1 Pups

Dates of inspection: 17 FEB 99, 17 FEB 99

Dates results reported to the Study Director and Management: 22 FEB 99, 22 FEB 99

TABLE 2

RAW DATA AUDIT(S)

The following study information and raw data were audited from 07 APR 99 to 13 APR 99:

Protocol.
Protocol amendment.
List of personnel and computer operator codes.
Error codes and codes for clinical sign observations.
Animal receipt, randomization, physical examination and acclimation.
In-life transaction record.
Feed consumption.
Cohabitation.
Natural delivery observations.
Litter observations.
Pup body weights and status.
Pup Randomization.
Necropsy.
Organ weights.
Tissue packing lists.
Male breeder colony records.
General comments.
Study maintenance records.
Temperature and relative humidity reports.
Feed, water and bedding analyses.
Edit requests.
Deviations.
Data review page.
Blood collection data and packing lists.
Key for testing facility computer backup record abbreviations.
Urine/Fecal collection data and packing lists.

The results of this audit were reported to the Study Director and Management on 15 APR 99.

The following study information and raw data were audited on 20 APR 99:

Vehicle receipt, preparation and use.
Test article receipt, preparation and use.
Test article packing lists.
Deviations.

The results of this audit were reported to the Study Director and Management on 23 APR 99.

3M

Study Title

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL

Author
Lisa Clemen

Date:
June 8, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
FACT-TOX-111
U2994

Exact Copy of Original
Initial
Date 4/19/2000

Study Identification

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

Test Material	Perfluorooctane sulfonic acid potassium salt (T-6295)
Sponsor	3M Toxicology Services - Medical Department 3M Center, Building 220-2E-02 St. Paul, MN 55144-1000
Sponsor Representative	Marvin T. Case, D.V.M., Ph.D. 3M Toxicology Services Telephone: 651-733-5180 Facsimile: 651-733-1773
Study Director	Kristen J. Hansen, Ph.D. 3M Environmental Technology and Safety Services Building 2-3E-09 651-778-6018
Study Location(s)	
In vivo Testing Facility	Argus Research Laboratories, Inc. 905 Sheehy Drive, Building A Horsham, PA 19044
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106

Exact Copy of Original
Initial
Date 4/19

Sub-Contract Laboratories	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850
	Battelle Memorial Institute 505 King Avenue Columbus Ohio 43201-2693

Proposed Study Timetable

Study Initiation Date	June 8, 1999
Study Completion Date	August 8, 2000

1. STUDY

Oral (gavage) pharmacokinetic recovery study of potassium perfluorooctane sulfonic acid (PFOS) in rats.

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rats. The in-life portion of this study was conducted at Argus Research Laboratories, study #418-015. All serum samples will be extracted and analyzed at Advanced Bioanalytical Services, Inc. and all liver samples extracted and analyzed at Battelle Memorial Institute. Additional analyses may be performed at the 3M Environmental Laboratory as methods are developed and validated. . If additional analyses are performed an amendment to this protocol will be written.

3. REGULATORY COMPLIANCE

This study will be conducted in accordance with the United States Food and Drug Administration, Good Laboratory Practices Standards, Final Rule 21 CFR 58, with the exception that analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor.

4. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will review the protocol and audit study conduct, data, and final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures.

The QA Unit at the sub-contract laboratory will audit their study conduct, data, and results report prior to submitting to the 3M Environmental Laboratory.

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5. TEST MATERIAL

5.1 Refer to Argus Research Laboratory protocol for study #418-015.

6. CONTROL MATRICES

6.1 Identification Rat liver and serum, and/or rabbit liver and serum traceability numbers will be recorded in the raw data and included in the final report

6.2 Source Argus Research and/or Sigma Chemical

6.3 Physical Description Rat liver and serum, and/or rabbit liver and serum

6.4 Purity and Stability Not applicable

6.5 Storage Conditions Frozen at -20 °C ± 10 °C or –50 °C ± 10 °C

6.6 Reserve Matrix A portion of the control matrix will be retained in the 3M archives for as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

6.7 Disposition Matrices will be retained at the 3M Environmental Laboratory per GLP regulation. Certain matrices (feces, urine, and blood) may be disposed after QAU verification.

6.8 Safety Precautions Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

7. REFERENCE MATERIAL

7.1 Identification Potassium perfluorooctanesulfonate (PFOS), lot #s 171, 215, or 217 (equivalent lots)

7.2 Source 3M Specialty Chemicals

7.3 Physical Description White powder

7.4 Purity and Stability Purity of PFOS is 99% or greater. Stability has not been determined.

7.5 Storage Conditions Room temperature

7.6 Reserve Material A reserve sample from each batch of PFOS used in this study will be retained as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

7.7 Disposition Unused reference material will be retained for use by the 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation.

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7.8 Safety Precautions Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

8. Test System

Rats were used as the test system and were maintained and dosed as described in Argus Research protocol #418-015. The female rats will be given the test material or control once daily beginning 42 days prior to cohabitation until day 0 of presumed gestation. See table 1 for more dosage information.

Table 1
Dosage Levels, Concentration, and Volumes

Dosage Group	Number of female rats	Dosage mg/kg/day	Concentration mg/kg/day	Dosage volume mL/kg
1	8	0	0	5
2	8	0.1	0.02	5
3	8	~~0.6~~	0.32	5

(r.e.) 1.6 kgh 9/22/99

9. Specimen and Sample Receipt

The 3M Environmental Laboratory will receive homogeneity samples and specimens of the following body tissues and fluids from the indicated points in the study. All specimens will be packed on dry ice for shipping. See table 2 for more specimen information.

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Pup animals	Dam-Predose, Days 7, 14, 15, 21, and 22 Pup-Day 21	144 Dam 24 Pup (pooled)
Urine and Feces – Dam and Pup animals	Predose, Days 7, 15, 21, and 22	120 Urine and 120 Feces
Liver – Dam and Pup animals	Dam-At the termination of the study Pup-Day21	24 Dam 24 Pup (pooled)

Total number of expected specimens: 456
Total number of test animals: 16
Total number of control animals: 8

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Specimens sent to 3M Environmental Laboratories will be received and tracked according to applicable Standard Operating Procedures.

10. Preparatory Methods

10.1 FACT-M-1.1, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.2 ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.3 If preparatory methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

10.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

11. Analytical Methods

11.1 FACT-M-2.1, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

11.2 ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry

11.3 If analytical methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

11.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

12. Data Quality Objectives

The number of spikes/duplicates, use of surrogates, and information on other data quality indicators are included in the analytical methods. In addition, the following criteria will be met:

12.1 ***Linearity*** $r^2 \geq 0.98$

12.2 ***Limits of detection / quantitation***

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

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12.2.2 Limit of Quantitation (LOQ) – Equal to the lowest acceptable standard in the calibration curve

12.3 ***Duplicate acceptable precision*** < 30% for the method

12.4 ***Spike acceptable recoveries*** 70% - 130%

12.5 ***Use of confirmatory methods*** Indeterminate samples will be re-analyzed using a confirmatory method. If a confirmatory method is used, an amendment to this protocol will be written.

12.6 ***Demonstration of specificity*** Chromatographic retention time, mass spectral daughter ion characterization.

13. Sub-Contracted Analysis

13.1 All analyses as detailed in this protocol will be performed at 3M Environmental Laboratories, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106, at Advanced Bioanalytical Services, Inc., 15 Catherwood Road, Ithaca, NY 14850, or at Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693.

13.2 An amendment to this protocol will be written if analyses are performed at laboratories other than the 3M Environmental Laboratories, Advanced Bioanalytical Services, Inc., or Battelle Memorial Institute.

14. Statistical Analysis

Averages and standard deviations will be calculated. The statistical methods that will be used are described below:

14.1 ***Data transformations and analysis*** Data will be reported as the concentration (weight/weight or weight/vol) of PFOS or metabolite per tissue or fluid.

14.2 ***Statistical analysis*** Statistics used may include regression analysis of concentrations over time, and standard deviations calculated for the concentrations within each dose group. If necessary, simple statistical tests, such as Student's t test, may be applied to evaluate statistical difference.

15. Report

A report containing all the results of the study will be prepared by the 3M Environmental Laboratory. If analyses are sub-contracted to other laboratories, each laboratory will prepare a report and submit it to the 3M Environmental Laboratory for inclusion in the 3M Environmental Laboratory report. Each report will include, but not be limited to, the following, when applicable:

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

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15.3 A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards

15.4 Objectives and procedures as stated in the approved protocol, including any changes in the original protocol

15.5 The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor

15.6 Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor

15.7 A description of the methods used to conduct the test(s)

15.8 A description of the test system

15.9 A description of any circumstances that may have affected the quality or the integrity of the data

15.10 The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study

15.11 A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses

15.12 Statistical methods used to evaluate the data, if applicable

15.13 The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable

15.14 The location where raw data and the final report are to be stored

15.15 A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management

If it is necessary to make corrections or additions to a report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

16. Location of Raw Data, Records, and Final Report

Original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

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16.1 The following raw data and records will be retained in the study folder in the study/project archives according to 3M Environmental Laboratory Standard Operating Procedures:

- ***16.1.1*** Approved protocol and amendments
- ***16.1.2*** Study correspondence
- ***16.1.3*** Shipping records
- ***16.1.4*** Raw data
- ***16.1.5*** Approved final report (original signed copy)
- ***16.1.6*** Electronic copies of data

16.2 The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory Standard Operating Procedures:

- ***16.2.1*** Training records
- ***16.2.2*** Calibration records
- ***16.2.3*** Instrument maintenance logs
- ***16.2.4*** Standard Operating Procedures, Equipment Procedures, and Methods

17. Specimen Retention

Specimens will be maintained in the 3M Environmental Laboratory specimen archives for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable), and as established by 3M Environmental Laboratory Standard Operating Procedures.

18. Protocol Amendments and Deviations

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and filed with the raw data.

19. Attachments

19.1 Attachment A Preparatory and analytical methods

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20. SIGNATURES

Marvin T. Case — 9 June 1999

Marvin T. Case, D.V.M., Ph.D., Sponsor Representative — Date

Kristen Hansen — 6/9/99

Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director — Date

Exact Copy of Original
Initial — Date 4/19

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:
August 12, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS FACT-TOX111
LIRN U2994

Exact Copy of Original
Initial 4/19
Date

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:*** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
FACT-M-2.1 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

AMEND TO READ: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to:

ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

REASON: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to ETS-8-6.0 and ETS-8-7.0. These methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix.
The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

2. ***PROTOCOL READS:*** Section 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.

AMEND TO READ: The extraction and analytical methods to follow at Advanced Bioanalytical Services will be attached to the protocol.

The extraction and analytical method to follow at Battelle Memorial Institute is:

"Method for Analysis of Perfluorooctane Sulfonate (PFOS) in Rat ~~Sera~~ Liver by LC/MS/MS, Version ~~1~~ 1.0"

REASON: The analytical methods at the sub-contract laboratories were not included in the original protocol.

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3. ***PROTOCOL READS:*** Section 12.2.1 b) lists liver method detection limit as 15 ppb.

AMEND TO READ: The liver method detection limit is 8.50 ppb (ng/g).

REASON: The validation supporting methods ETS-8-6.0 and ETS-8-7.0 included a lower method detection limit for PFOS.

Amendment Approval

Marvin T Case — 17 August 1999
Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kris Hansen — 8/18/99
Kris J. Hansen, Ph.D., Study Director — Date

Exact Copy of Original
Initial — Date 4/19

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 30, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS FACT-TOX111
LIRN U2994

Exact Copy of Original
Initial
Date 4/19

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
Section 2 states the study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and sera in rats.

AMEND TO READ:
The study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver, sera, and urine in rats.

REASON:
The urine analytical methods were validated and approved after approval of the original protocol.

2. ***PROTOCOL READS:***
Section 10.0 and 11.0 list the following methods to use for extraction and analysis:
ETS-8-4.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

AMEND TO READ::
ETS-8-4.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-96.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-97.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"

REASON: The extraction and analytical methods ETS-8-96.0 and ETS-8-97.0, were approved after approval of the original protocol.

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Date 4/19

3. ***PROTOCOL READS:***
Section 6.1 lists control matrices as rat liver and serum or rabbit liver and serum. Source Argus and/or Sigma Chemical.

AMEND TO READ:
Control matrices identification rat liver and serum, rabbit liver and serum, human urine and/or rat urine. Source Argus, Sigma Chemical, Lampire Biologicals, Biological Specialty Corp. and/or Golden West Biologicals.

REASON:
Addition of control urine specifications.

4. ***PROTOCOL READS:***
Section 12.2.1 a) lists sera method detection limit as 1.75 ppb and b) lists liver method detection limit as 15 ppb.

AMEND TO READ:
The method detection limits for all compounds and matrices will be taken from the methods used for extraction and analysis.

REASON:
The method detection limits listed are specific to the 3M Environmental Laboratory. Statement was added to allow for sub-contracted analyses and/or revised methods.

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Date 4/19

5. ***PROTOCOL READS:***
Section 16 states that the original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, electronic copies of data, training records, calibration records, instrument maintenance logs and standard operating procedures, equipment procedures, and methods will be retained in the archives of the 3M Environmental Laboratory.

AMEND TO READ:
Section 16 states that the original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis.

REASON:
Clarification of the disposition of archived records if analyses are performed at a sub-contract laboratory.

6. ***PROTOCOL READS:***
Section 17 states that specimens will be maintained in the 3M Environmental Laboratory specimen archives.

AMEND TO READ:
Specimens will be maintained in the 3M Environmental Laboratory specimen archives. All specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. The specimens will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.

REASON:
Clarification of the disposition of specimens and documentation for analyses performed by a sub-contract laboratory.

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Date 4/19

Amendment Approval

Marvin T Case — 4 Oct 1999

Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kristen Hansen — 5 Oct 1999

Kristen J. Hansen, Ph.D., Study Director — Date

Exact Copy of Original
Initial — Date 4/19

Study Title

Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number 2759-39-3) in the Serum, Liver, and Urine of Crl:CD®BR VAF/Plus® Rats Exposed to PFOS Via Gavage

PROTOCOL AMENDMENT NO. 3

Amendment Date:

20 January 2000

Performing Laboratories

Performing Laboratories

Urine Analyses	Liver Analyses	Serum Analyses
3M Environmental Technology and Safety Services Fluorine Analytical Chemistry Team Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850

Exact copy of Original
Initial
Date 4/19

Laboratory Project Identification

ET&SS LRN-U2994

FACT TOX-111

Argus Study: 418-015

3M Medical Department Study: T-6295.14

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
   The study director for the present study was identified in the protocol as Kristen J. Hansen, Ph.D.
   AMEND TO READ:
   The role of study director for the present study was reassigned to Marvin T. Case, D.V.M., Ph.D., as of 20 January 2000. The previous study director, Kristen J. Hansen, has been reassigned to the role of Principle Analytical Investigator.
   REASON:
   The role of study director was reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements (refer to 21 CFR Part 58).

2. ***PROTOCOL READS:***
   The sponsor for the present study was identified as Marvin T. Case, D.V.M., Ph.D.
   AMEND TO READ:
   The role of sponsor for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000.
   REASON:
   To ensure that the study director does not also carry the duties of study sponsor, the sponsor role was reassigned. In this manner, personnel responsibilities and workload are more evenly balanced.

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Date 4/8

Amendment Approval

John L. Butenhoff — February 10, 2000

John L. Butenhoff, Ph.D., Sponsor Representative — Date

Kristen Hansen — 11-Feb-2000

Kristen J. Hansen, Ph.D., Outgoing Study Director — Date

Marvin T Case — 10 February 2000

Marvin T. Case, D.V.M., Ph.D., Incoming Study Director — Date

Exact Copy of Original
Initial — Date

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 4

Amendment Date:
20 April 2000

Performing Laboratories

URINE ANALYSES	FECES ANALYSES
3M Environmental Technology and Safety Services Fluorine Analytical Chemistry Team Building 2-3E-09, 935 Bush Avenue St. Paul, MN 55106	**Centre Analytical Laboratories, Inc.** 3048 Research Drive State College, PA 16801
LIVER ANALYSES	**SERUM ANALYSES**
Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693	**Advanced Bioanalytical Services, Inc.** 15 Catherwood Road Ithaca, NY 14850

Laboratory Project Identification
ET&SS LRN-U2994
FACT TOX-111
Argus Study: 418-015
3M Medical Department Study: T-6295.14

Exact Copy of Original
Initial
Date 4/19/2000

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
   The amended section 2.0 text states that this study is designed to determine PFOS in specimens of rat liver, serum, and urine.
   AMEND TO READ:
   This study is designed to determine PFOS in specimens of rat liver, serum, feces, and urine.
   REASON:
   The analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the protocol. Feces extraction and analytical methods were not validated and approved prior to protocol approval.

2. ***PROTOCOL READS:***
   The amended section 6.0 lists rat or rabbit liver, serum, and urine.
   AMEND TO READ:
   Add: rat or rabbit feces with a physical description of rat or rabbit feces.
   REASON:
   Analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

3. ***PROTOCOL READS:***
   Section 13.1 lists all of the laboratories that will be conducting analyses for this study.
   AMEND TO READ:
   Add: Centre Analytical Laboratories, Inc., 3048 Research Drive, State College, PA 16801
   REASON:
   Feces analyses were added to the scope of this study. The sub-contract laboratory performing analyses was not in the original protocol.

4. ***PROTOCOL READS:***
   Sections 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.
   AMEND TO READ:
   The feces extraction and analytical method used by **Centre Analytical Laboratories** will be; 00M–023–003 (Revision 2), "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS."
   REASON:
   The sub-contract laboratory performing feces analyses was added to the scope of this study; this method was not validated and approved prior to protocol approval.

Exact Copy of Original
Initial
Date 4/14

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
   The amended section 2.0 text states that this study is designed to determine PFOS in specimens of rat liver, serum, and urine.
   AMEND TO READ:
   This study is designed to determine PFOS in specimens of rat liver, serum, feces, and urine.
   REASON:
   The analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the protocol. Feces extraction and analytical methods were not validated and approved prior to protocol approval.

2. ***PROTOCOL READS:***
   The amended section 6.0 lists rat or rabbit liver, serum, and urine.
   AMEND TO READ:
   Add: rat or rabbit feces with a physical description of rat or rabbit feces.
   REASON:
   Analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

3. ***PROTOCOL READS:***
   Section 13.1 lists all of the laboratories that will be conducting analyses for this study.
   AMEND TO READ:
   Add: Centre Analytical Laboratories, Inc., 3048 Research Drive, State College, PA 16801
   REASON:
   Feces analyses were added to the scope of this study. The sub-contract laboratory performing analyses was not in the original protocol.

4. ***PROTOCOL READS:***
   Sections 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.
   AMEND TO READ:
   The feces extraction and analytical method used by **Centre Analytical Laboratories** will be; 00M–023–003 (Revision 2), "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS."
   REASON:
   The sub-contract laboratory performing feces analyses was added to the scope of this study; this method was not validated and approved prior to protocol approval.

Exact Copy of Original
Initial
Date 4/15

Amendment Approval

John L. Butenhoff April 19, 2000

John L. Butenhoff, Ph.D., Sponsor Representative Date

Marvin T. Case 19 April 2000

Marvin T. Case, D.V.M., Ph.D., Study Director Date

Exact Copy of Original
Initial Date 4/15

Summary PFOS Rat Two-Generation Reproduction Study

Study Numbers: 3M **T-6295.9**, Argus 418-008 (in-life), FACT-TOX-012 (analytical).

Compound & Lot: PFOS (Perfluorooctanesulfonate) – Lot 217, 98.4% pure (SMD Analytical Request 53030).

CAS No. 2795-39-3

Study Title: Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats.

Report Date: 10 June 1999 (in-life); 19 April 1999 (analytical).

Study Year and GLP status: 1997-1999 and GLP study

Procedures: Groups of male and female rats (35/sex/group) were administered (daily oral intubation) PFOS at dose levels of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day. Dosing started when the animals were ≈ 70 days old. The male and female rats (F_0 generation) were dosed for 42 days prior to mating. After mating, dosing continued in the mated females throughout gestation. At day 10 of gestation, Caesarean section was done on 10 females/group to check for any effects in early gestation. The remaining 25 females/group were allow to deliver their litters. Dosing of the females continued during the 21-day lactation period. At weaning, 2 males and 2 females per litter were randomly selected for continuation on the study (F_1 generation). Note: Because of post-natal effects at high doses (see below for details) only three groups of F_1 animals continued the study, namely 0, 0.1 and 0.4 mg/kg/day.
Daily dosing began in the F_1 pups the day after weaning and continued throughout their growth to sexual maturity. At 24 days of age the F_1 rats were tested in a passive avoidance paradigm. The animals were also tested in a water-filled M-maze when they were 70 days old. Cohabitation for mating began when the F_1 animals were 90 days old – littermate matings were avoided. Dosing continued in the females through gestation and 21-day lactation after delivery of F_2 pups. The study ended upon weaning of the F_2 pups.

Results: No deaths occurred in any of the F_0 animals and there were no compound related clinical signs. Dose related reduced weight body weights occurred in both male and females at the higher two dose levels (3.2 & 1.6 mg/kg/day). Reduced food consumption correlated with these body weight effects. The 0.4 mg/kg/day males also had a slight, but statistically significant, reduced body weight gain during the pre-mating period. Dosing of PFOS as high as 3.2 mg/kg/day had no effect on estrous cycling; all mating and fertility parameters of the F_0 rats were unaffected. There were no significant differences at any dose level in regard to litter averages for corpora lutea, implanations, viable embryos or nonviable embryos from the day 10 of gestation Caesarean section females.
Upon littering, post-natal survival was reduced at the higher two dose levels (1.6 & 3.2 mg/kg/day). The post-natal survival effect was manifested by increased number of

apparent still-births and increased pup deaths during the first 24 to 72 hours after birth. Necropsy of dead pups revealed many had not nursed – no milk curd in the stomach.

Reflex and physical development (surface righting, pinna unfolding, eye opening, acoustic startle reflex, air right reflex, pupil constriction) was not affected in the 0.1 & 0.4 mg/kg/day F_1 pups. Some of these parameters were slightly delayed in the 1.6 mg/kg/day F_1 pups and they were not evaluated at the high dose (3.2 mg/kg/day) as no pups survived beyond four days post-natal.
Because of adverse effects in the higher two dose levels (1.6 & 3.2 mg/kg/day) F_1 pups, reduced survival and/or reduced growth, the decision was made, in conjunction with the attending laboratory veterinarian, not to continue these two dose levels into the second generation. It had been determined that dose of 1.6 mg/kg/day or higher produced compound toxicity. Continuance of these dose levels would not result in any additional useful information and would subject the animals to unnecessary stress.
Death did not occur post-weaning in the F_1 animals nor were there any compound related clinical signs. The male and female F_1 rats in the 0.4 mg/kg/day group had somewhat reduced body weight and food consumption; at some points in time (weeks of growth) the differences from controls were statistically significant. The appearance of external evidence of sexual development (preputial separation in males & vaginal patency in females) was not affected at either dose level. Also, the passive avoidance and water maze testing did not reveal any differences in regard to learning, short-term retention nor long-term memory. Likewise, none of the mating or reproductive performance measurements (parameters) were adversely affected in the 0.1 & 0.4 mg/kg/day F_1 male and female rats when they were mated and allowed to deliver litters. There were no significant differences in regard to F_2 pup survival or pup weights at weaning.

Conclusions: The results of the study indicated the following NOEL levels.

The F_0 maternal and paternal NOEL is 0.1 mg/kg/day. [higher dose levels had reduced body weight and food consumption]

The F_0 reproductive NOEL is > 3.2 mg/kg/day. [no effect on reproductive performance even at highest dose level]

The F_1 post-natal NOEL is 0.4 mg/kg/day. [higher dose levels had reduced pup viability and growth]

The F_1 maternal and paternal NOEL is 0.1 mg/kg/day. [0.4 mg/kg/day animals had reduced body weight and food consumption]

The F_1 reproductive NOEL is > 0.4 mg/kg/day. [no effect on reproductive performance at either 0.1 or 0.4 mg/kg/day]

The F_2 post-natal NOEL is 0.4 mg/kg/day. [no important toxicological effect on pup survival or growth at either 0.1 or 0.4 mg/kg/day]

Compound Level Samples: At the terminal sacrifice of the F_0 males (after mating was completed), serum and liver samples were collected from 5 males in each of the dose groups. Serum samples were collected from 5 F_0 females in all the dose groups except 3.2 mg/kg/day group at the terminal sacrifice the day after weaning the F_1 litters. Liver samples were collected from the same F_0 females and liver samples were also collected from the weaned F_1 pups of the same dams.
Analysis of the samples for PFOS (3M Analytical Report TOX012) yielded the following mean PFOS concentrations.
Male F_0 Serum: control – 0.024 ppm, 0.1 mg/kg/day – 10.5 ppm, 0.4 mg/kg/day – 45.4 ppm, 1.6 mg/kg/day – 152 ppm, 3.2 mg/kg/day – 273 ppm.
Male F_0 Liver: control – 0.665 ppm, 0.1 mg/kg/day – 84.9 ppm, 0.4 mg/kg/day – 176 ppm, 1.6 mg/kg/day – 323 ppm, 3.2 mg/kg/day – 1357 ppm.
Female F_0 Serum: control – 0.037 ppm, 0.1 mg/kg/day – 5.28 ppm, 0.4 mg/kg/day – 18.9 ppm, 1.6 mg/kg/day – 82.0 ppm.
Female F_0 Liver: control – 0.171 ppm, 0.1 mg/kg/day –14.8 ppm, 0.4 mg/kg/day – 58.0 ppm, 1.6 mg/kg/day – 184 ppm.
Pup F_1 Liver: control – 0.051 ppm, 0.1 mg/kg/day – 6.19 ppm, 0.4 mg/kg/day – 57.6 ppm, 1.6 mg/kg/day – 70.4 ppm.
Note: Serum and liver reflect PFOS concentrations of F_0 dams and F_1 pups at the end of 21-day lactation, i.e. when the samples were collected. Compound levels at parturition when reduced pup survival was observed could differ and those are being measured in a follow-up PK study.

Data Evaluation: A well conducted GLP study following established testing guidelines (OECD 416). Study evaluated reproductive parameters and post-natal pup development including neurological parameters. The study established valid NOEL values.

Serum and Liver Samples: At the terminal sacrifice of the F_0 males (after mating was completed), serum and liver samples were collected from 5 males in each of the dose groups. Serum samples were collected from 5 F_0 females in all the dose groups except 3.2 mg/kg/day group at the terminal sacrifice the day after weaning the F_1 litters (day 21 of lactation). Liver samples were collected from the same F_0 females. Liver samples were also collected from the weaned F_1 pups (liver samples were pooled by litter) of the same dams.

Milk Curd Samples: On day 4 of lactation, all litters, as stated in the protocol, were reduced to 8 pups per litter. At this time stomach contents (milk curd) samples, pooled by litter, were collected from 4 or 5 litters from three dose groups – control, low dose (0.1 mg/kg/day) and high dose (3.2 mg/kg/day). A validated analytical method for milk curd matrix has not been developed and these samples have not been analyzed.

PRIMEDICA Argus Division | 905 Sheehy Drive, Bldg. A | Horsham, PA 19044
Tel 215.443.8710 | Fax 215.443.8587

April 13, 2000

Marvin T. Case, D.V.M., Ph.D.
3M Toxicology Services
3M Center, Building 220-2E-02
St. Paul, Minnesota 55144-1000

Telephone: (612) 733-5180
Telefax: (612) 733-1773

RE: Final Report 418-008 - Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity of PFOS in Rats
Sponsor's Study Number: 6295.9

Dear Dr. Case:

Enclosed is one copy of the final report Amendment 1 to the above-referenced report.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Raymond G. York, Ph.D, DABT
Associated Director of Research
and Study Director

RGY:kgp
Attach.

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

REPORT AMENDMENT 1
13 APRIL 2000

PROTOCOL 418-008
SPONSOR'S STUDY NUMBER: 6295.9

COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS

FINAL REPORT

1. Page I-9, paragraph 4 has been revised to:

   Pregnancy occurred in 22 (95.6%), 21 (84.0%) and 24 (96.0) of the 23, 25 and 25 female rats assigned to cohabitation in the 0 (Vehicle), 0.1 and 0.4 mg/kg/day dosage groups, respectively. All pregnant dams delivered litters. The gestation index was comparable across the three dosage groups. ***Viability and growth of the second generation offspring (F2 pups) to weaning were also unaffected by the highest dosage tested, 0.4 mg/kg/day. There were no toxicologically important differences from the control group values.*** No clinical or necropsy observations in the F2 generation pups were attributable to dosages of the test article as high as 0.4 mg/kg/day.

2. Page I-10, paragraph 4 has been revised to:

   The F1 generation reproductive NOEL is greater than a dosage of 0.4 mg/kg/day; no effects on mating or fertility occurred. The NOEL for viability and growth in the F2 generation offspring is ***also 0.4*** mg/kg/day. ***There were no toxicologically important effects on pup survival or growth at the highest dosage tested, 0.4 mg/kg/day.***

3. Page V-8, paragraph 3 has been revised to:

   The viability of the second generation offspring (F2 pups) was unaffected at the highest dosage tested, 0.4 mg/kg/day. Small increases in the number of dams with stillborn pups and in the number of pup deaths after DL 2 in the 0.4 mg/kg/day dosage group were not toxicologically important because: 1) the values were not significantly different from the control group values; 2) the average for live litter sizes delivered and surviving to DL 21 were greater than or comparable to the control group values; and 3) neither the viability or lactation indices for the 0.4 mg/kg/day dosage group remarkably differed from the control group values.

4. V-8, paragraph 4 has been revised to:

Pup body weights of the second generation offspring (F2 pups) were unaffected at the highest dosage tested, 0.4 mg/kg/day. Small reductions in pup body weights in the 0.1 and 0.4 mg/kg/day dosage groups were not toxicologically important because: 1) the small differences between the pup body weights for the control and 0.1 mg/kg/day dosage groups were not statistically significant and were associated with the larger live litter sizes of the 0.1 mg/kg/day dosage group on DLs 1 through 4, as compared with the control group values, and the random selection of pups for continued observations on DL 4; 2) the small reductions in pup body weights in the 0.4 mg/kg/day dosage group were associated with a minimally larger live litter size at birth (DL 1) and on DL 4 preculling, as compared with the control group values, and the random selection of pups for continued observation on DL 4; the statistically significant reductions ($p \leq 0.05$ and $p \leq 0.01$, respectively) present on DLs 7 and 14, as compared to the control group values, were transient and disappeared by DL 21.

5. Page V-9: Revisions to paragraph 4 on page V-8 caused it to extend onto page V-9.

These revisions were made to clarify the interpretation of the observations reported and the NOEL for the second generation offspring (F2 generation).

13-APR-00 | 13 Apr 00

Raymond G. York, Ph.D., DABT — Date
Associate Director of Research
and Study Director

Nancy J. Gongliewski — Date
Quality Assurance Manager

feed consumption values were reduced during lactation in the 0.4 mg/kg/day dosage group.

Dosages of the test article as high as 0.4 mg/kg/day did not affect the average day of vaginal patency in the F1 generation female rats. There were no biologically important differences in the values for learning, short-term retention, long-term retention or response inhibition in the F1 generation female rats, as evaluated by performance in a passive avoidance or watermaze performance paradigm.

Dosages of the test article as high as 0.4 mg/kg/day did not affect any mating and fertility parameters evaluated in the F1 generation female rats.

All necropsy observations in the F1 generation female rats were considered unrelated to the test article.

Pregnancy occurred in 22 (95.6%), 21 (84.0%) and 24 (96.0) of the 23, 25 and 25 female rats assigned to cohabitation in the 0 (Vehicle), 0.1 and 0.4 mg/kg/day dosage groups, respectively. All pregnant dams delivered litters. The gestation index was comparable across the three dosage groups. Viability and growth of the second generation offspring (F2 pups) to weaning were also unaffected by the highest dosage tested, 0.4 mg/kg/day. There were no toxicologically important differences from the control group values. No clinical or necropsy observations in the F2 generation pups were attributable to dosages of the test article as high as 0.4 mg/kg/day

C. Conclusions

On the basis of these data, the Fo generation maternal and paternal no-observable-effect-level (NOEL) of PFOS is 0.1 mg/kg/day (0.4 mg/kg/day and higher dosages caused reductions in body weight gain and reduced feed consumption values).

The Fo generation reproductive NOEL is greater than 3.2 mg/kg/day; no effects on mating, fertility or estrous cycling occurred. The NOEL for viability and growth in the F1 generation offspring is 0.4 mg/kg/day (1.6 mg/kg/day and higher dosages caused preimplantation loss and reductions in litter size, pup viability, growth and survival).

The F1 generation maternal and paternal NOEL of PFOS is 0.1 mg/kg/day (0.4 mg/kg/day dosage caused reductions in body weight gain and reduced feed consumption values).

The F1 generation reproductive NOEL is greater than a dosage of 0.4 mg/kg/day; no effects on mating or fertility occurred. The NOEL for viability and growth in the F2 generation offspring is also 0.4 mg/kg/day. There were no toxicologically important effects on pup survival or growth at the highest dosage tested, 0.4 mg/kg/day.

13 Apr 00

Mildred S. Christian, Ph.D., Fellow, ATS Date
Executive Director of Research

for 13 APR 00

Alan M. Hoberman, Ph.D., DABT Date
Director of Research

13-APR-00

Raymond G. York, P.D., DABT Date
Associate Director of Research and Study Director

B.8. Necropsy Observations (Summary - Table E18; Individual Data - Table E34)

All necropsy observations in the F1 generation female rats were considered unrelated to the test article because: 1) the observations occurred in rats in the control group; and 2) the observation commonly occurs in this strain of rat. These observations included rough and/or pitted cortex of the kidneys with calculi in the kidney and/or urinary bladder of two control group rats. One of these rats also had a tan granular material in the renal cortex and thickened walls of the urinary bladder. Necropsy observations for the 0.4 mg/kg/day dosage group dam that died as the result of an intubation error were described previously.

B.9. Natural Delivery and Litter Observations (Summaries - Tables E19 and E20; Individual Data - Tables E35 through E38)

Pregnancy occurred in 22 (95.6%), 21 (84.0%) and 24 (96.0%) of the 23, 25 and 25 female rats assigned to cohabitation in the 0 (Vehicle), 0.1 and 0.4 mg/kg/day dosage groups, respectively. All pregnant dams delivered litters. The gestation index (the percentage of pregnant rats with live offspring) was comparable across the three dosage groups.

The viability of the second generation offspring (F2 pups) was unaffected at the highest dosage tested, 0.4 mg/kg/day. Small increases in the number of dams with stillborn pups and in the number of pup deaths after DL 2 in the 0.4 mg/kg/day dosage group were not toxicologically important because: 1) the values were not significantly different from the control group values; 2) the average for live litter sizes delivered and surviving to DL 21 were greater than or comparable to the control group values; and 3) neither the viability or lactation indices for the 0.4 mg/kg/day dosage group remarkably differed from the control group values.

Pup body weights of the second generation offspring (F2 pups) were unaffected at the highest dosage tested, 0.4 mg/kg/day. Small reductions in pup body weights in the 0.1 and 0.4 mg/kg/day dosage groups were not toxicologically important because: 1) the small differences between the pup body weights for the control and 0.1 mg/kg/day dosage groups were not statistically significant and were associated with the larger live litter sizes of the 0.1 mg/kg/day dosage group on DLs1 through 4, as compared with the control group values, and the random selection of pups for continued observations on DL 4; 2) the small reductions in pup body weights in the 0.4 mg/kg/day dosage group were associated with a minimally larger live litter size at birth (DL 1) and on DL 4 preculling, as compared with the control group values, and the random selection of pups for continued observation on DL 4; the statistically significant reductions

($p \leq 0.05$ and $p \leq 0.01$, respectively) present on DLs 7 and 14, as compared to the control group values, were transient and disappeared by DL 21.

Administration of the test article at dosages as high as 0.4 mg/kg/day did not adversely affect any other parameter evaluated at natural delivery or during the 21-day lactation period (duration of gestation, averages for implantations and live litter sizes, numbers of dams with all pups dying during lactation, viability and lactation indices, surviving pups per litter and pup sex ratios).

B.10. Clinical Observations from Birth to Day 21 Postpartum and Necropsy Observations (Summaries - Tables E21 and E22; Individual Data - Tables E39 and E40)

No clinical or necropsy observations were attributable to dosages of the test article as high as 0.4 mg/kg/day because: 1) the incidences were not dosage-dependent; and/or 2) the observation occurred in only one or two pups. These clinical observations included one control group pup that was not nesting or nursing, had decreased motor activity and was cold to the touch, one pup in each of the control and 0.4 mg/kg/day dosage groups that had a missing tip of tail and one 0.4 mg/kg/day dosage group pup that had an umbilical hernia.

No milk in stomach occurred in 2, 4 and 4 pups that were found dead in the three respective dosage groups. One control group pup had hydrocephaly and one 0.1 mg/kg/day dosage group pup had a raised tan area on the median and left lateral lobes of the liver at necropsy on DL 21.

Study Title

Analytical Laboratory Report on Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

REPORT AMENDMENT NO. 1

Amendment Date:

19 April 2000

Performing Laboratory

3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification

ET&SS LRN-U2006
ET&SS FACT Tox-012

This amendment modifies the following portion(s) of the final report:

1. ***FINAL REPORT:***
   Make the following addition to the final report text.
   ADD:
   After the final report was issued (signed and archived), it was discovered that the purity value stated for the analytical reference substance was inaccurate. This value was based only on NMR analyses. Subsequent chemical characterization is occurring to determine the actual purity of theses substances (PFOS Lot 193). If necessary, the final report will be amended to detail the analytical results using the purity value from the certificate of analysis when it is issued for PFOS Lot 193.
   REASON:
   To describe the changes that will be made to the final report when the certificate of analysis is issued for PFOS Lot 193.

2. ***FINAL REPORT:***
   Make the following addition to the exceptions list in the Statement of Compliance section.
   ADD:
   - The purity and stability of the analytical reference substance is unknown and was not determined prior to the initiation of this study.

   REASON:
   The Statement of Compliance section was incomplete.

3. ***FINAL REPORT:***
   The purity of the analytical reference substance is 99.28% (Lot 193), as listed in Table 2.
   AMEND TO READ:
   The purity of the analytical reference substance (PFOS Lot 193) listed in Table 2 is unknown.
   REASON:
   The purity value stated for the analytical reference substance is incorrect.

4. ***FINAL REPORT:***
   Make the following addition to the final report text under circumstances that may affect the quality of the data.
   ADD:
   - According to the Dilution Summary Sheet, the extracts for F0 liver samples 8243M, 8244M, 8249M, 8250M, and 8255M were diluted 0.001mL into 1.0mL (1:1000), but were calculated as 1:2000. On the same sheet, the extracts for F0 liver sample 8181M and F1 liver sample 8937 Pup were diluted 0.001mL into 1.0mL (1:1000), but were calculated as 1:500.
   - The result for 8249M liver sample was quantitated based on peak response that exceeds the highest calibration standard by 20%.

   REASON:
   To include the circumstances that were found after the final report for this study was issued.

5. *FINAL REPORT:*

On page 4 of 16, a print error occurred that replaced several lines of the Exceptions to GLP compliance text with an "X" character, without any change in the spacing between the subsequent bullets on the rest of the page. The incorrect page will be replaced in the final report and it will archived in the study folder.

AMEND TO READ:

The correct page 4 of 16 is attached to this amendment. The following list is the complete text lost in the print error:

- The identity, strength, purity, and composition defining the test or control article was not determined at the time of the study; however, these analyses were performed on the lot of PFOS used subsequent to the present study.
- Two separate study directors were assigned to the *in vivo* and the analytical portions of this study.
- From 15 September 1998 until 1 October 1998, the protocol was not sponsor approved and was during that time period not in compliance.
- Deviations were not consistently approved by the study director as required by GLP regulations.
- Data corrections were not always recorded as required by GLP regulations.
- Data were not always attributed to the individual responsible for recording the data.

REASON:

The final printed report with the incorrect page does not reflect the complete report document as reviewed by the study director and sponsor representative.

Amendment Approval

Marvin T. Case — 19 April 2000

Marvin T. Case, D.V.M., Ph.D., Sponsor Representative — Date

Kristen J. Hansen — 04/19/00

Kristen J. Hansen. Ph.D., Study Director — Date

STATEMENT OF COMPLIANCE

Study Title: Analytical Laboratory Report on Presence and Concentration of Potassium Perfluoro-octanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

Study Identification Number: FACT Tox-012

This study was conducted in compliance with Food and Drug Administration Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies [Data Requirement(s): 21 CFR (Part 58)], with the exceptions in the bulleted list below. In addition, the present study has been audited retrospectively by an independent quality assurance unit. Audit procedures and findings for audits performed at the 3M Environmental Laboratory and at participating contract laboratories are housed with documentation pertinent to this study in archives at the 3M laboratory and will be retained for at least 10 years. The analytical portion completed at the 3M Environmental Lab was performed in accordance with 3M Environmental Technology and Safety Services Standard Operating Procedures.

Exceptions to GLP compliance:

- Storage containers for the reference substance, PFOS, were not labeled with name, CAS number, batch number, expiration date, or storage conditions.
- Details of the preparation, maximum storage time, and stability properties of the reference substance, PFOS, are unknown.
- The identity, strength, purity, and composition defining the test or control article was not determined at the time of the study; however, these analyses were performed on the lot of PFOS used subsequent to the present study.
- Two separate study directors were assigned to the *in vivo* and the analytical portions of this study.
- From 15 September 1998 until 1 October 1998, the protocol was not sponsor approved and was during that time period not in compliance.
- Deviations were not consistently approved by the study director as required by GLP regulations.
- Data corrections were not always recorded as required by GLP regulations.
- Data were not always attributed to the individual responsible for recording the data.
- Records in electronic form do not meet the criteria set forth under 21 CFR(11).
- A finalized sample log-in /tracking system was not in place at the time of the study; however, draft documents did exist and were used to log in and track samples.

ANALYTICAL LABORATORY REPORT

ON THE

Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number: 2759-39-3) in the Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

Laboratory Report No. <U2006>
Requester Project No. <3M TOX 6295.9>

Study Dates

Study Initiation: 26 May 1998
Study Completion: At Signature

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Attachment A: Report Signature Page

Attachment B: Table of Results of Analysis of Liver Samples from F0 Male and Female Sprague-Dawley Rats Administered PFOS Daily by Gavage

Attachment C: Table of Results of Analysis of Sera Samples from F0 Male and Female Sprague-Dawley Rats Administered PFOS Daily by Gavage

Attachment D: Table of Results of Analysis of Liver Samples from F1 Male and Female Sprague-Dawley Rats Administered PFOS Daily by Gavage

Attachment E In-life Protocol and Analytical Study Protocol

Attachment F: Preparatory and Analytical Methods

Attachment G: Dose Analyses

STUDY PERSONNEL AND CONTRIBUTORS

Analytical Chemistry Laboratories

3M Environmental Technology
and Safety Services
3M Environmental Laboratory
Fluorine Analytical Chemistry Team (FACT)
2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Kris Hansen, Ph.D., *Analytical Study Director*
Lisa Clemen, *Analytical Chemist(s)*

In-life Testing Laboratory

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044

Raymond G. York, Ph.D., *Study Director*

Sponsor

3M Toxicology Services
3M Center
Building 220-2E-02
St. Paul, MN 55144

Marvin Case, *Sponsor Representative*

STATEMENT OF COMPLIANCE

Study Title: Analytical Laboratory Report on Presence and Concentration of Potassium Perfluoro-octanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

Study Identification Number: FACT Tox-012

This study was conducted in compliance with Food and Drug Administration Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies [Data Requirement(s): 21 CFR (Part 58)], with the exceptions in the bulleted list below. In addition, the present study has been audited retrospectively by an independent quality assurance unit. Audit procedures and findings for audits performed at the 3M Environmental Laboratory and at participating contract laboratories are housed with documentation pertinent to this study in archives at the 3M laboratory and will be retained for at least 10 years. The analytical portion completed at the 3M Environmental Lab was performed in accordance with 3M Environmental Technology and Safety Services Standard Operating Procedures.

Exceptions to GLP compliance:

- Storage containers for the reference substance, PFOS, were not labeled with name, CAS number, batch number, expiration date, or storage conditions.
- Details of the preparation, maximum storage time, and stability properties of the reference substance, PFOS, are unknown.
- The identity, strength, purity, and composition defining the test or control article was not determined at the time of the study; however, analyses to detail the characterization were ongoing at the time of this analytical study.
- Two separate study directors were assigned to the *in vivo* and the analytical portions of this study.
- From 15 September 1998 until 1 October 1998, the protocol was not sponsor approved and was during that time period not in compliance.
- Deviations were not consistently approved by the study director as required by GLP regulations.
- Data corrections were not always recorded as required by GLP regulations.
- Data were not always attributed to the individual responsible for recording the data.
- Records in electronic form do not meet the criteria set forth under 21 CFR(11).
- A finalized sample log-in /tracking system was not in place at the time of the study; however, draft documents did exist and were used to log in and track samples.

- Not all raw data were verified by the study director.
- No in-phase audits were conducted during the present study.
- At the time of the present study, personnel files did not always contain documentation of equipment and method training; however, personnel files have been updated, and this noncompliance has been resolved.

Signature of Study Director: Peter Hu 27 Oct 99

Signature of Study Sponsor: Marvin T. Case 20 October 1999

GLP STUDY
QUALITY ASSURANCE STATEMENT

Study Title: Analytical Laboratory Report on Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

Study ID Number: FACT Tox-012

This study has been inspected by the 3M Environmental Laboratory Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the study director and management.

INSPECTION DATES		PHASE	DATE REPORTED TO	
From	*To*		*Management*	*Study Director*
9 August 1999	27 September 1999	Final Report	4 October 1999	4 October 1999

Kathleen M Stock 10-15-99

QAU Representative Date

INTRODUCTION

Potassium Perfluorooctanesulfonate

CAS Number: 2795-39-3

Chemical Formula: $C_8F_{17}SO_3$-K+ Molecular Weight: 538

In the present study, groups of F0 rats were administered 0.1, 0.4, 1.6, or 3.2 mg PFOS kg/day in 0.5% Tween 80. (These doses correspond to concentrations of 0.02, 0.08, 0.32, and 0.64 mg/mL.) Groups of vehicle control F0 rats were administered only Tween 80. Male F0 animals were treated 42 days prior to mating and through the mating period; female F0 animals were administered PFOS daily 42 days prior to mating, through gestation, and up to 20 days following litter delivery. F1 male and female rats were exposed to the chemical *in utero* and during lactation. Following weaning at 21 days of age, selected F1 animals were treated during development and production of F2 animals. Various physical and biological parameters were monitored in F0, F1, and F2 animals.

In the analytical study reported here, liver and sera samples collected from the initial population of dosed animals (generation F0) and their offspring (generation F1) were analyzed for the presence of PFOS. (Analyses were performed to determine the presence of EtFOSE, PFOSA, POAA< PFOSEA, PFOSAA, and the monoester; however, these data were collected for informational purposes only, and were not reported.)

Liver samples were homogenized, and liver and sera samples were extracted by an ion-pairing extraction procedure. The extracts were quantitatively analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HLPC/ESMSMS), and PFOS levels were evaluated against extracted standards. Analytical details are included in this report; further details are available in the study binder maintained by the 3M Fluor Analytical Chemistry Team (FACT).

Analyses assessing the toxicological effects of PFOS in the livers and sera of Sprague-Dawley rats were conducted in compliance with Good Laboratory Practice Regulations (21 CFR 58). Validated methods and standard operating procedures were followed during the preparation and analysis of the samples associated with this study.

SAMPLE RECEIPT

Tissue samples were received from Argus Research Laboratories sporadically, from August, 1998, through January, 1999. Samples received were packaged in dry ice. Specimens were registered with the 3M Environmental Laboratory and transferred to a freezer for storage. Sample receipt, identification, storage, and chain of custody protocols and data are located in the study folder for this report; the folder is located in the 3M archives.

Specimens analyzed at the 3M Environmental Laboratory will be maintained for a period of 10 years and will be stored at the laboratory at –20°C ± 10°C.

MATERIALS AND METHODS

Chemical Characterization

Table 1 presents information regarding characterization of the test, control, and reference substances used in the analytical portion of this study.

	ANALYSES PERFORMED AT THE 3M ENVIRONMENTAL LABORATORY
Reference Substance	Potassium perfluorooctanesulfonate
Source	3M Specialty Chemical Division
Preparation	Details unknown
Maximum Storage Time	Unknown
Storage Conditions	In a sealed container at room temperature, exposed to light
Chemical Lot Number	193
Physical Description and Identity Analyses	White powder
Purity	99.28%
Stability	Unknown
Control Substance	Rat liver and serum matrices
Source	Rabbit liver—Argus Research Laboratories, Inc. Rabbit serum—Sigma Chemical Company
Preparation	Unknown
Maximum Storage Time	Unknown
Storage Conditions	Frozen at –20°C
Chemical Lot Number	Liver:F00012 Serum: 17H9306
Physical Description and Identity Analyses	Rabbit liver and serum
Purity	Unknown
Stability	Unknown

Table 1. Characterization and Treatment of the Control, Reference, and Test Materials in the Study of the Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

	ANALYSES PERFORMED AT THE 3M ENVIRONMENTAL LABORATORY
Test Material	Rat serum and liver
Source	Sprague-Dawley rats exposed to PFOS via gavage during the *in vivo* portion of 3M TOX 6295.9 at Argus Research Laboratories
Preparation	Liver: extraction per FACT M-1.0 (refer to Attachment F) Serum: extraction per FACT M-3.0 (refer to Attachment F)
Maximum Storage Time	1 week
Storage Conditions	Frozen at –20°C
Chemical Lot Number	NA
Physical Description and Identity Analyses	Rat liver and serum
Purity	NA
Stability	NA

Table 1. Characterization and Treatment of the Control, Reference, and Test Materials in the Study of the Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage (continued)

Following analysis, remaining original specimens (test and control material) were stored frozen at –20°C and will be maintained for a period of 10 years.

Method Summaries

Following is a brief description of the methods used during this analytical study by the 3M Environmental Laboratory. Detailed descriptions of these methods are located in Attachment F.

Preparatory Methods:

- Method FACT-M-1.0: Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for analysis Using HPLC/ESMS.

  In this method, an ion pairing reagent was added to the sample and the analyte ion pair was partitioned into MtBE. Four mLs of the MtBE extract was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was 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.

- Method FACT-M-3.0: Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry.

  Sera samples were extracted using an ion-pairing extraction procedure. In summary, an ion pairing reagent was added to the sample and the analyte ion pair was partitioned into methyl-*tert*-butyl ether (MtBE). Four mL of the MtBE extract was removed and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol and filtered through a 3-cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

Analytical Methods

- Method FACT-M-2.0: Analysis of Fluorochemicals in Liver Extract Using HPLC-Electrospray/Mass Spectrometry

  The analysis was performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, m/z = 499. Additionally, samples were analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the selected parent ion.

- Method FACT M-4.0: Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemical in Serum or other Fluid Using HPLC-Electrospray/Mass Spectrometry

  The analysis was performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, m/z= 499. Additionally, samples were analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the parent ion.

Confirmatory dose analyses were conducted following the end of the study, and the results of these analyses are presented in Attachment G.

Analytical Equipment

Following are typical analytical equipment settings for the procedures used in the present study. These settings vary somewhat during actual data collection. Exact settings during all phases of data collection are recorded and presented in the analysis section of the study binder for FACT Tox-12.

HPLC System: Hewlett-Packard Series 1100 Liquid Chromatograph

Column:	Keystone Betasil C18 Column 2X100 mm, 5µm particle size
Flow rate:	300 µL/min
Solvent A:	2.0 mM ammonium acetate
Solvent B:	Methanol
Solvent Gradient:	40% to 90% B in 8.5 minutes Hold at 90% B for 3.0 minutes Return to 40% B in 1.0 minute Hold at 40% B for 1.0 minute
Injection Volume:	10 µL
Run Time:	13.5 minutes

Electrospray Tandem Mass Spectrometer: Micromass Quattro II API Mass Spectrometer

Mass Lynx 3.1 Software

Cone Voltage:	30—60V
Collision Gas Energy:	40 eV
Mode:	Electrospray Negative
Source Block Temperature:	115°C
Desolvation Temperature:	250°C
Electrode:	Z-spray
Primary Ion/Daughter Ions:	499/80, 99, 130, 180, 230 amu

Protocol Deviations

There was one deviation to the protocol: The protocol called for the use of reference standard lot number 217; however, lot number 193 was used during the analytical portion of the study.

DATA SUMMARY, ANALYSES, AND RESULTS

Summary of Quality Control Analyses Results

- Calibration Check Standards: A mid-level, extracted matrix calibration check was analyzed at least every 10 samples to monitor instrument drift. Calibration check standards were compliant, and all calibration criteria were met (within ± 30%).
- Blanks: Four blanks were extracted concurrently with each batch of samples. Two extraction blanks were prepared with water as a surrogate matrix. Two additional samples of blank matrix were typically prepared with rabbit liver or sera and were used for extracted calibration curves. All blanks were nondetect for the compound(s) of interest.
- Duplicate matrix spike analyses including all target analytes were prepared and analyzed for one control animal. With a few exceptions, recoveries were within the acceptable range of 70-130%.

  Analysis of two liver matrix spike samples resulted in 51% and 61% recoveries of PFOS. These recoveries are below the lower boundary of the acceptable range; however, the second set of matrix spike samples were within the acceptable range. No action was taken to further characterize these matrix spikes.

  Analyses of both sets of sera matrix spikes resulted in PFOS recoveries greatly above the high limit of the acceptable range. Although 1 mL of sera was used for the analysis of PFOS levels in the sera of test animals, only a small amount of serum (500 µL) was available for the matrix spike preparation; this limited quantity of matrix may have been a source of error. As the extraction is scaled-down to accommodate the available matrix, the resulting extract is particularly susceptible to loss of solvent due to evaporation. These evaporative losses, which would not effect the PFOS concentration (PFOS has a low vapor pressure) in the extract, could result in a greater-than-expected PFOS concentration in the extract. When analyses of sera matrix spikes are performed with a full 1 mL of sera, high PFOS recoveries are unusual.
- Matrix Spikes: Matrix spikes and matrix spike duplicates were analyzed every 40 samples, with a minimum of two per batch during analytical analyses. The results of these analyses are located in the archived study binder.

Summary of Sample Results

- PFOS was detected in the livers of all control and exposed groups of F0 animals and F1 animals. PFOS was detected in the sera samples collected from all of the control and dosed F0 males and in sera samples collected from all dosed F0 females. The PFOS levels found in the samples from control animals were quite low—generally 100-less than levels detected in the low-dose group. PFOS concentration in exposed animals increased with increasing dose group, generally in a dose-related manner.

  PFOS levels in both sera and liver samples collected from female F0 animals were much less than levels detected in samples collected from the corresponding group of F0 males.

  PFOS concentrations measured in pooled liver samples collected from the exposed groups of F1 animals shortly after birth were roughly equal to or lower than concentrations measured in the corresponding groups of F0 females. No samples collected from F0 females that received 3.2 mg PFOS per kg/day were submitted for analysis. Table 1 summarizes results of the quantitative analyses performed on the F0 liver and sera samples examined.

Group	Dose (mg/kg/day)	Dose Concentration (mg/mL)	Average Concentration of PFOS in Serum (µg/mL)	Average Concentration of PFOS in Liver (µg/g)
0.0 mg/kg/day	0.0	0.0	Female: 0.0307 Male: 0.0244	Female: 0.171 Male: 0.665
0.1 mg/kg/day	0.1	0.02	Female: 5.28 Male: 10.5	Female: 14.8 Male: 84.9
0.4 mg/kg/day	0.4	0.08	Female: 18.9 Male: 45.4	Female: 58.0 Male: 176
1.6 mg/kg/day	1.6	0.32	Female: 82.0 Male: 152	Female: 184 Male: 323
3.2 mg/kg/day	3.2	0.64	Female: NR[a] Male: 273	Female: NR[a] Male: 1360

[a] Samples were not received.

Table 2. PFOS Concentrations in Serum and Liver Samples from F0 Male and Female Sprague-Dawley Rats

PFOS concentrations measured in pooled liver samples collected from the **control and exposed** groups of F1 animals shortly after birth were roughly the equal to or lower than concentrations measured in the corresponding groups of F0 females. No samples collected from F1 males or females that received 3.2 mg/kg/day were submitted for analysis. PFOS was detected in the liver of all other exposed groups of F1 animals. Table 2 summarizes results of the quantitative analyses performed on the F1 sera samples examined.

GROUP	DOSE (MG/KG/DAY)	DOSE CONCENTRATION (MG/ML)	AVERAGE CONCENTRATION OF PFOS IN LIVER (µG/G)
0.0 mg/kg/day	0.0	0.0	0.0511
0.1 mg/kg/day	0.1	0.020	6.19
0.4 mg/kg/day	0.4	0.080	57.6
1.6 mg/kg/day	1.6	0.320	70.4
3.2 mg/kg/day	3.2	0.640	NR[a]

[a] Samples were not received.

Table 3. PFOS Concentrations in Liver Samples from F1 Male and Female Sprague-Dawley Rats

Paper and electronic copies of raw data and study reports generated during the present studies will be kept for at least a period of time as established by regulation and by the 3M Standard Operating Procedures. Paper and electronic copies of reports, protocols, and methods generated by the 3M Environmental Technology and Safety Services laboratory will be retained with the study folder for at least a period of time as established by regulation and by the 3M Standard Operating Procedures.

STATISTICAL METHODS

For data generated by the 3M Environmental Laboratory, means and standard deviations were calculated using Microsoft Excel®, and relative standard deviations were calculated manually.

Standard deviation is a measurement of analytical precision; thus it is used to gauge the precision of the analyses. Relative standard deviation presents a measure of the magnitude of the standard deviation.

$$\sqrt{\frac{n\Sigma x^2-(\Sigma x)^2}{n(n-1)}}$$

Means are calculated by adding individual entities and dividing the resultant sum by the number of individual entities. Standard deviations were calculated using the equation:

Relative standard deviations were calculated by division of the standard deviation by the mean with subsequent multiplication of the quotient by 100.

DATA QUALITY OBJECTIVES AND DATA INTEGRITY

No circumstances existed during the present study that would have affected the quality or integrity of the data. The following data quality objectives (DQOs) were followed during the study:

No circumstances existed during the present study that would have affected the quality or integrity of the data. The following data quality objectives (DQOs) were followed during the study:

- Linearity— The correlation coefficient (R^2) of the standard curve was equal to or greater than 0.98 using 1/x weighting
- Limits of detection—PFOS in serum=1.75 ppb
- Limits of quantitation—Equal to the lowest acceptable standard in the calibration curve
- Duplicate frequency/acceptable precision—<30%
- Spike frequency/acceptable recoveries—70% to 130%
- Use of confirmatory methods—no confirmatory methods were used
- Demonstration of specificity—specificity was demonstrated by chromatographic retention time and mass spectral daughter ion characterization

STATEMENT OF CONCLUSION

Statement of Conclusion:

Under the conditions of the present studies, potassium perfluorooctanesulfonate was observed in the livers and sera of all groups of rats exposed to the chemical during the *in vivo* portion of the studies.

REFERENCES

None

ATTACHMENTS

- Attachment A: Report signature page
- Attachment B: Table of results of analysis of liver samples from F0 male and female Sprague-Dawley rats administered PFOS daily by gavage.
- Attachment C: Table of results of analysis of sera samples from F0 male and female Sprague-Dawley rats administered PFOS daily by gavage.
- Attachment D: Table of results of analysis of liver samples from F1 male and female Sprague-Dawley rats administered PFOS daily by gavage.
- Attachment E: In-life protocol and study protocol
- Attachment F: Preparatory and analytical methods
- Attachment G: Dose Analyses

ATTACHMENT A
REPORT SIGNATURE PAGE

Kristen J. Hansen, Ph.D., Study Director — Date: 10/27/99

Marvin T. Case, D.V.M., Ph.D., Study Sponsor — Date: 20 October 1999

Dale L. Bacon, Laboratory Director — Date: 10/27/99

ATTACHMENT B

Dose Group	Sample Number	Extracted Weight (g)	PPOS CALCULATED CONCENTRATION (mG/G)	Total Amount of PFOS (µg/g)	Mean PFOS (µg/g)	Relative Standard Deviation
Method Blank	H_2O Blank 1	N/A	<MDL	<MDL		
Method Blank	H_2O Blank 2	N/A	<MDL	<MDL	<MDL	<MDL
Matrix Blank	Rabbit Liver (Blank 1)	N/A	<MDL	<MDL		
Matrix Blank	Rabbit Liver (Blank 2)	N/A	<MDL	<MDL	<MDL	<MDL
QC — 100 ppb	8801F-MS	1.0169	60	50%		
	8801F-MSD	1.0169	72	60%	55%	18%
	8801M-MS	1.0525	100	87%		
	8801M-MSD	1.0525	93	81%	84%	7%
0.0 mg/kg/day	8801F	1.0169	674	0.674		
	8807F	1.0384	158	0.158		10.1[a]
	8808F	1.0177	165	0.165	0.171[a]	0.0172[a]
	8821F	1.0439	197	0.197		82.9
	8833F	1.0048	166	0.166	0.272	0.225
	8101M	1.0525	910	0.910		
	8103M	1.0407	600	0.600		
	8104M	1.0568	577	0.577		
	8107M	1.0104	593	0.593		21.0
	8108M	1.0321	643	0.643	0.665	0.139
0.1 mg/kg/day (0.02 mg/mL)	8838F	1.0374	17251	17.3		
	8840F	1.0168	14433	144		
	8842F	1.0489	15557	15.6		
	8864F	1.0608	13807	13.8		11.6
	8870F	1.0225	12784	12.8	14.8	1.71
	8138M	1.0617	80270	80.3		
	8140M	1.0509	76629	76.6		
	8142M	0.9961	92106	92.1		
	8145M	1.036	87938	87.9		7.39
	8146M	0.9788	87451	87.5	84.9	6.28

Table 4. Analyses of Liver Samples from F0 Sprague-Dawley Rats in the Study of the Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

ATTACHMENT B (CONTINUED)

DOSE GROUP	SAMPLE NUMBER	EXTRACTED WEIGHT (g)	PPOS CALCULATED CONCENTRATION (mG/G)	TOTAL AMOUNT OF PFOS (µG/G)	MEAN PFOS (µG/G)	RELATIVE STANDARD DEVIATION
0.4 mg/kg/day	8890F	1.0346	61853	61.9		
(0.08 mg/mL)	8893F	1.0247	62547	62.5		
	8895F	1.0154	49543	49.5		
	8902F	1.0039	64081	64.1		11.6
	8905F	1.0426	51922	51.9	58.0	6.73
	8172M	1.0234	137715	138		
	8174M	1.0067	189256	189		
	8175M	1.0471	183050	183		
	8176M	1.0335	173288	173		13.3
	8181M	1.0197	198173	198	176	23.4
1.6 mg/kg/day	8919F	1.0756	200313	200		
(0.32 mg/mL)	8921F	1.0544	212684	213		
	8926F	1.0219	282001	282		
	8934F	1.022	183576	184		48.0
	8937F	1.654	40958	41.0	184	88.3
	8209M	1.0016	374773	375		
	8213M	1.0211	325087	325		
	8219M	1.0421	279320	279		
	8221M	1.0153	335349	335		11.2
	8225M	1.0316	301145	301	323	36.1
3.2 mg/kg/day	NR	NR	NR	NR		
(0.64 mg/mL)	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		NR
	NR	NR	NR	NR	NR	NR
	8243M	1.0065	1271156	1271		
	8244M	1.0397	1116251	1116		
	8249M	1.0107	2077414	207 7		
	8250M	1.0522	1168784	1169		30.0
	8255M	0.9908	1149853	1150	1360	40.7

[a] Sample 8801F value was excluded from the calculations.

Table 4. Analyses of Liver Samples from F0 Sprague-Dawley Rats in the Study of the Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage (continued)

ATTACHMENT C

DOSE GROUP	SAMPLE NUMBER	PFOS CALCULATED CONCENTRATION (ng/g)	TOTAL AMOUNT OF PFOS (µg/g)	MEAN PFOS (µg/g)	RELATIVE STANDARD DEVIATION (RSD)
Method Blank	H_2O Blank 1	<MDL			
Method Blank	H_2O Blank 2	<MDL	<MDL	<MDL	<MDL
Matrix Blank	Rabbit Liver (Blank 1)	<MDL	<MDL		
Matrix Blank	Rabbit Liver (Blank 2)	<MDL	<MDL	<MDL	<MDL
QC — 100 ppb	8801F-MS	60	50%		
	8801F-MSD	72	60%	55%	18%
	8801M-MS	100	84%		
	8801M-MSD	93	78%	81%	7%
0.0 mg/kg/day	8801F/M	369	0.369		
	8807F/M	51.5	0.0515		5.58[a]
	8808F/M	53.2	0.0532	0.0511[a]	0.00285[a]
	8821F/M	52.7	0.0527		124
	8833F/M	46.9	0.0469	0.115	0.142
0.1 mg/kg/day (0.02 mg/mL)	8838F/M	5872	5.87		
	8840F/M	5149	5.15		
	8842F/M	7390	7.39		
	8864F/M	6745	6.74		14.2
	8870F/M	5788	5.79	6.19	0.879
0.4 mg/kg/day (0.08 mg/mL)	8890F/M	62050	62.1		
	8893F/M	61745	61.7		
	8895F/M	47738	47.7		
	8902F/M	63057	63.1		11.7
	8905F/M	53492	53.5	57.6	6.72
1.6 mg/kg/day (0.32 mg/mL)	8919F/M	78376	78.4		
	8921F/M	58893	58.9		
	8926F/M	91956	92.0		
	8934F/M	65023	65.0		20.6
	8937F/M	57897	57.9	70.4	14.5

[a] Sample 8801F value not included in this calculation

Table 5. Analyses of Liver Samples from F1 Sprague-Dawley Rats in the Study of the Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

ATTACHMENT D

DOSE GROUP	SAMPLE NUMBER	PPOS REPORTED (mg/mL)	MEAN PFOS (µg/mL)	RELATIVE STANDARD DEVIATION (RSD)
Method Blank	H_2O Blank 1	<MDL		
Method Blank	H_2O Blank 2	<MDL	<MDL	<MDL
Matrix Blank	Rabbit Liver (Blank 1)	<MDL		
Matrix Blank	Rabbit Liver (Blank 2)	<MDL	<MDL	<MDL
QC — 100 ppb	8801F-MS	136%		
	8801F-MSD	133%	135%	1%
	8801M-MS	119%		
	8801M-MSD	121%	120%	1%
0.0 mg/kg/day	8801F	0.127		
	8807F	0.0229		30.9[a]
	8808F	0.0291	0.037[a]	0.00950[a]
	8821F	0.0445		87.6
	8833F	0.0265	0.0500	0.044
	8101M	0.0306		
	8103M	0.0311		
	8104M	0.0177		
	8107M	0.0213		2.48
	8108M	0.0214	0.0244	0.00605
0.1 mg/kg/day (0.02 mg/mL)	8838F	5.14		
	8840F	4.93		
	8842F	5.24		
	8864F	5.23		6.78
	8870F	5.89	5.28	0.358
	8138M	11.8		
	8140M	9.54		
	8142M	9.65		
	8145M	11.0		9.02
	8146M	10.4	10.5	0.946
0.4 mg/kg/day (0.08 mg/mL)	8890F	20.9		
	8893F	18.6		
	8895F	18.4		
	8902F	17.4		6.89
	8905F	19.1	18.9	1.30
	8172M	40.7		
	8174M	54.1		
	8175M	41.6		
	8176M	47.4		12.1
	8181M	43.3	45.4	5.49

Table 6. Analyses of Sera Samples from F0 Sprague-Dawley Rats in the Study of the Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage

Dose Group	Sample Number	PPOS Reported (mg/mL)	Mean PFOS (µg/mL)	Relative Standard Deviation (RSD)
1.6 mg/kg/day	8919F	75.3		
(0.32 mg/mL)	8921F	79.2		
	8926F	113		
	8934F	74.4		21.4
	8937F	68.4	82.0	17.5
	8209M	144		
	8213M	151		
	8219M	148		
	8221M	165		5.20
	8225M	153	152	7.91
3.2 mg/kg/day	NR	NR		
(0.64 mg/mL)	NR	NR		
	NR	NR		
	NR	NR		NR
	NR	NR	NR	NR
	8243M	249		
	8244M	242		
	8249M	257		
	8250M	361		18.3
	8255M	255	273	49.8

[a] Sample 8801F value not included in this calculation

Table 6. Analyses of Sera Samples from F0 Sprague-Dawley Rats in the Study of the Presence and Concentration of Potassium Perfluorooctanesulfonate in Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage (continued)

ATTACHMENT E
IN-LIFE PROTOCOL AND ANALYTICAL STUDY PROTOCOL

ATTACHMENT F:
PREPARATORY AND ANALYTICAL METHODS

ATTACHMENT G:
RESULTS OF CONFIRMATORY DOSE ANALYSES

3M SPECIALTY ADHESIVES & CHEMICALS ANALYTICAL LABORATORY

Request #'s **53030**

To: Leo Gehlhoff - (309727) -3M Chemicals - 236-2A-01

From: Tom Kestner - (3-5633) SA&C Analytical Lab - 236-2B-11

Subject: ***Fluorochemical Isomer Distribution by ^{19}F-NMR Spectroscopy***

Date: December 1, 1997

SAMPLE DESCRIPTIONS:

- FC-95, lot # 217 (T-6295); Nominal product = C_8F_{17}-SO_3(-) K(+)

INTRODUCTION:

This sample was subjected to a ^{19}F-NMR spectral analysis method to determine the identities and relative concentrations of the fluorochemical isomers and as many other identifiable impurity components as possible.

EXPERIMENTAL:

A portion of the sample solid was totally dissolved in DMSO-d_6 and then a 376 MHz ^{19}F-NMR spectrum (F53030.401) was acquired using a Varian UNITYplus 400 FT-NMR spectrometer. Aida Robbins prepared the sample for analysis and she also acquired and plotted the NMR spectrum.

RESULTS:

The ^{19}F-NMR spectrum was used to determine the identities and relative concentrations of the nominal fluorochemical isomers and three other impurity components in this sample. The qualitative and quantitative compositional results which were derived from the single trial ^{19}F-NMR spectral analysis are summarized in **TABLE-1** on the following page.

A copy of the NMR spectrum and the spectral assignments data page are attached for your reference. If you have any questions about these NMR results, please let me know. I apologize for the delay in completing this work.

Tom Kestner

c: Jim Johnson - EE&PC - 2-3E-09

File Reference: LG53030.DOC/43

TABLE-1
Sample: T-6295 (FC-95, Lot 217)
^{19}F-NMR Compositional Results

Structural Assignments	^{19}F-NMR Relative Mole% Concentrations
$CF_3(CF_2)_x$-SO_3(-) K(+) (Normal chain, where x is mainly 7)	70.0%
$CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$- SO_3(-) K(+) (Internal monomethyl branch, where x+y is mainly 5, and $x \neq 0$, $y \neq 0$)	17.0%
$(CF_3)_2CF$-$(CF_2)_x$- SO_3(-) K(+) (Isopropyl branch, where x is mainly 5)	10.3%
C_xF_{2x+1}-$CF(CF_3)$- SO_3(-) K(+) (Alpha branch, where x is mainly 6)	1.6%
R_f-CF_2-SF_4-F	0.35%
$(CF_3)_3C$-$(CF_2)_x$- SO_3(-) K(+) (t-butyl branch, where x is mainly 4)	0.23%
CF_3-$(CF_2)_x$-$C(CF_3)_2$-$(CF_2)_y$- SO_3(-) K(+) (Internal gem-dimethyl branch, where x+y is mainly 4, and $x \neq 0$)	0.15%
Possible CF_3-SO_3(-) K(+)	0.25%
Possible CF_3-CO_2(-) K(+)	0.05%

Summary PFOS Rat Cross-Fostering

Study Numbers: 3M **T-6295.13**, Argus 418-014

Compound & Lot: PFOS (Perfluorooctanesulfonate) – Lot 217, 98.4% pure (SMD Analytical Request 53030)

C.A.S. Registry Number: 275-39-3

Study Title: Oral (Gavage) Cross-Fostering Study of PFOS in Rats

Report Date: 23 July 1999 (in-life); 11 February 2000 (serum analysis)

Study Year and GLP status: 1998 – 2000 and GLP study

Procedures: Groups of female rats (25/group) were administered Potassium Perfluorooctanesulfonate (PFOS) by daily oral intubation at dose levels of 0 or 1.6 mg/kg/day. Dosing started when the females were approximately 70 days old. The female rats were dosed for 42 days prior to mating to untreated males. After mating, dosing continued in the females throughout gestation and the 21 days of lactation.

At parturition, the control and treated groups were subdivided into additional groups for cross-fostering of litters and for obtaining pharmacokinetic samples. For cross-fostering, the litters were transferred to another dam which had delivered – no dam was allowed to raise its own litter. The four subgroups were as follows: control dams with litters from control dams (negative control); treated dams with litters from treated dams (positive control); control dams with litters from treated dams; and, treated dams with litters from control dams. On day of lactation 4, litters were culled to 10, with equal numbers per sex where possible.

On lactation day one (day of birth) lung and liver samples were collected from selected pups of control and treated litters not used for cross-fostering. These tissue samples were fixed for electron microscopic evaluation.

In addition to the cross-fostered litters, eight control dams and litters and two exposed dams and litters were assigned to pharmacokinetic sample collection on day 14 of lactation. Samples of milk, serum, liver and milk secreting glands were obtained from the dams. Pup serum and liver samples were obtained and pooled from the litters corresponding to the dams. All samples were frozen and sent to the 3M Environmental Analytical Laboratory for possible future analysis.

On days of lactation 21 and 22, serum and liver samples were obtained at term from dams and litters. Litter samples were pooled. All samples were frozen and shipped to the 3M Environmental Laboratory for possible future analysis.

Results: Mean body weights were reduced in the treated (1.6 mg/kg/day) females during the pre-mating and gestation periods. These body weight effects correlated with reduced food consumption. Reduced pup survival occurred in two of the four sub-groups as shown by the following data. Control dams X Control pups had 3/191 (1.6%) pup deaths (single death in three litters). Treated dams X Treated pups had 34/177 (19.2%) pup deaths (deaths in 8 of 12 litters). Control dams X Treated pups had 16/166 (9.6%) pup deaths (deaths in 10 of 11 litters). Treated dams X Control pups had 2/181 (1.1%) pup deaths (single death in two litters). These data indicate that reduced pup survival was a result of pup exposure *in utero* and not a result of effects on the dam from exposure.

Electron microscopy of the day-old rat pups' lungs showed no compound-related changes on Type II pneumocytes nor on the lamellar bodies or lung surfactant. Electron microscopy of the liver did reveal a compound related effect in the day-old rat pups. The liver of the pups from treated dams had increased number (2 to 3 X) of peroxisomes. Peroxisome proliferation is a known PFOS liver effect in rats. This finding indicates compound-related liver changes in the new-born rat pup delivered from dams treated with PFOS at 1.6 mg/kg/day.

Only the analysis of serum samples taken during the study is complete. On lactation day 14, control dams (n=8) and their litters (n=8) demonstrated low background levels of PFOS, with seven of the eight pairs (dams and litters) having serum concentrations of PFOS below the lower limit of quantitation (< 0.05 ppm). The eighth control pair (dam and litter) measured 0.08 ppm for the dam and 0.1 ppm for the pooled pup serum. The two exposed dams had serum PFOS levels of 98 and 218 ppm. For the dam with the 98 ppm value, the pooled pup serum was at 89 ppm PFOS. There was insufficient sample volume to determine the serum PFOS concentration from the pups associated with the dam having the 218 ppm serum PFOS.

From serum samples taken on days 21 and 22 of lactation, it is evident that exposure of pups can occur after birth via the milk. Pooled serum from pups in litters born from untreated dams and cross-fostered with treated dams (only exposed via milk) had 34 ppm PFOS on average (n = 4) compared to their treated foster-dam serum values, which averaged 72 ppm. Serum from litters born to treated dams and cross-fostered to untreated dams (only exposed *in utero*) had 54 ppm PFOS on average (n = 6) compared to their untreated foster-dam serum PFOS values, which averaged 2.54 ppm. The single-digit ppm PFOS levels observed in these untreated foster-dams is most likely from exposure to contaminated pup urine and feces, since all untreated dams which fostered pups from untreated dams had serum PFOS values at or below the lower limit of quantitation of 0.05 ppm. Litters from treated dams which were cross-fostered to other treated dams (exposed *in utero* and via milk) had the highest serum PFOS values, averaging 88 ppm (n = 6). In this latter case, the pooled litter serum PFOS concentrations were in the range of foster-dam serum values (88 ppm on average for litters vs. 98 ppm on average for their repective foster-dams). It should be noted that these samples are from the end of the lactation period (days 21 and 22), and that pups have likely started on feed around lactation days 14 and 15. Their only exposure would be *in utero* or via milk from

treated dams. These analyses clearly demonstrate that exposure occurs both *in utero* and via milk.

<u>Conclusions:</u> **Reduced pup survival observed in the PFOS rat reproduction study (see T-6295.9) was a result of pup exposure *in utero* and not a result of effects on the dam from exposure. In-utero exposure of PFOS (dam treated at 1.6 mg/kg/day) can result in liver effects (increased peroxisomes) being present in the new-born pup.** The production of pulmonary surfactant by pups does not appear to affected by exposure to PFOS *in utero*. Pups can be exposed to significant levels of PFOS from *in utero* exposure and via milk from exposed dams.

NOTE: Although samples of liver, urine, feces, milk, and milk-producing glands were taken for possible analysis of PFOS, these samples have not been analyzed. Two additional pharmacokinetic studies were undertaken to better characterize disposition of PFOS during pregnancy and lactation. The first study examines the disposition of body burden after pregnancy and through gestation. The second study examines the disposition through pregnancy and lactation. In-life is complete on these studies; however, analysis is not complete. Reports are expected by August 31, 2000. In addition, a mechanistic / pharmacokinetic study is planned to start in the second half of 2000. The objectives of this study are: 1) to better understand the possible mechanism of toxicity to the pups; 2) more precisely define the no-effect level for reduced viability; and, 3) obtain additional pharmacokinetic data. These three additional studies will enhance our complete understanding of the potential perinatal effects of PFOS as they relate to serum and tissue PFOS concentrations and potential mechanistic endpoints.

ANALYTICAL LABORATORY REPORT

ON THE

Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS)

(CAS Number: 2759-39-3)

in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage

Laboratory Report No. U2779
Requester Project No. 3M Tox 6295.13

Study Dates

Study Initiation: 10 June 1999
Sample Analysis Initiation: 22 June 1999
Sample Analysis Completion: 28 June 1999
Study Completion: At signing

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STUDY PERSONNEL AND CONTRIBUTORS

Analytical Chemistry Laboratories:

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John R. Perkins, Ph.D., *Assistant Scientific Director*

In-life Testing Laboratory

Argus Research Laboratory, Inc.
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Horsham, PA 19044

Raymond G. York, Ph.D. DABT, *Study Director*

Sponsor

3M Toxicology Services - Medical Department
3M Center, Building 220-2E-02
St. Paul, MN 55144-1000

Marvin T. Case, D.V.M., Ph.D., *Sponsor Representative*

STATEMENT OF COMPLIANCE

Study Title: Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage

Study Identification Number: FACT Tox-108

This study was conducted in compliance with Food and Drug Administration Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies [Data Requirement(s): 21 CFR (Part 58)], with the exceptions in the bulleted list below. In addition, the present study has been audited retrospectively by an independent quality assurance unit. Audit procedures and findings for audits performed at the 3M Environmental Laboratory and at participating contract laboratories are housed with documentation pertinent to this study in archives at the 3M laboratory and will be retained for a period not to exceed ten years. The analytical portion completed at the 3M Environmental Lab was performed in accordance with 3M Environmental Technology and Safety Services Standard Operating Procedures.

Exceptions to GLP compliance:

- Two separate study directors were assigned to the in-life phase and the analytical phase of this study
- The ABS final report does not have a Statement of Compliance
- The QAU statement in the ABS final report indicates compliance with EPA 40 CFR Part 792, rather than FDA 21 CFR Part 58
- Dose confirmation analyses were not conducted according to the GLP regulations; analytical method was not fully validated
- Not all raw data were verified by the group leader or designee

Kristen He — 11-Feb-00
Study Director — Date

Maureen T Case — 10 February 2000
Sponsor Representative — Date

GLP STUDY
QUALITY ASSURANCE STATEMENT

Study Title: Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage

Study Identification Number: FACT Tox-108

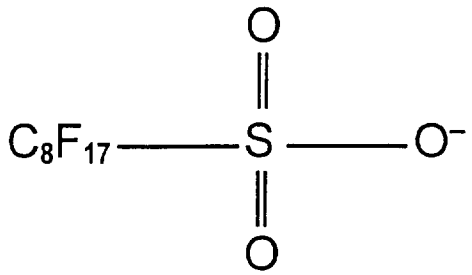
This study has been inspected by the 3M Environmental Laboratory Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the study director and management.

INSPECTION DATES		PHASE	DATE REPORTED TO	
From	*To*		*Management*	*Study Director*
12-30-99	01-06-00	Draft Final Report	01-11-00	01-07-00

Kathleen M Stock 2-11-00

QAU Representative Date

INTRODUCTION



Perfluorooctanesulfonate (PFOS)

CAS Number: 2759-39-3

Chemical Formula: $C_8F_{17}SO_3^-$ Molecular Weight: 499

Purpose

The purpose of the study is to determine the presence and concentration of PFOS ($C_8F_{17}SO_3^-$) in rat serum samples collected during the study of F1 offspring of F0 generation female rats exposed to potassium perfluorooctanesulfonate via gavage.

Data quality objectives for this analytical study (outlined in the 3M Environmental Technology and Safety Services protocol for FACT Tox-108) were met.

Test System

In the present study, two groups of female rats were used as the test system. Group I consisted of control F0 female rats that were administered only Tween 80 (vehicle). Group II F0 female rats were administered 1.6 mg of potassium perfluorooctanesulfonate per kg of body weight/day in 0.5% Tween 80 (test article). The dose corresponds to a concentration of 0.32 mg/mL. Table 1 outlines the F0 generation female rat population demographics for study T-6295.13. Group II F0 female rats were treated with the test article 42 days prior to cohabitation, and through day 14 (pharmacokinetic subgroup) or day 21 postpartum (lactation and delivery). F1 male and female rat pups were not directly administered the test article, but were exposed to the chemical through maternal gestation (*in utero* exposure) and during lactation. F1 pups were identified as being from Group I or II. Cross fostering was randomized; half of the pups from each group were cross fostered with Group I or II females creating four F1 animal subgroups (A-D). The identification and cross-fostering procedure is documented in the in-life study report (Final Report 418-014 for study T-6295.13 page II-10). Various physical and biological parameters were monitored in test animals.

Table 1. F0 Generation Rat Population Demographics for Study T-6295.13

POPULATION	TOTAL	SELECTED FOR STUDY	SELECTED FOR CROSS FOSTERING
Initial Quantity Acclimated	86 virgin female rats	78 virgin female rats	—
Control (Group I)	42 female rats	42 mated female rats	25 mated female rats
Exposed or Treated (Group II)	36 female rats	36 mated female rats	25 mated female rats
Pharmacokinetic Sample Subgroup	10 female rats	10 mated females (8 control, 2 treated rats)	None

The test system species and strain selected was the Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat received from Charles River Laboratories, Inc., permanently identified using a Monel® self-piercing ear tag. F0 generation rats were identified with ear tags. F1 generation rats were identified as part of either Group I or II litters, but were not individually tagged, and all parameters were evaluated in terms of the litter (dam and litter numbers are in Attachment E, Table 4). F0 virgin female rats were at least 60 days of age and weighed approximately 200–225 g when received (Weight data are included in Argus Research Laboratories, Inc., final report for study T-6295.13 on file at the 3M archives). Male rats of the same source and strain were used only as breeders and were not administered the test article or considered part of the test system.

Sample Collection and Analysis

F0 and F1 animals assigned to the control pool were culled on day 1 postpartum. On day 14 postpartum, samples were collected from the F0 mated female rats and F1 pups assigned to the pharmacokinetic subgroup. On day 22 postpartum, samples were collected from the remaining animals in the test system. All collected samples were immediately frozen on dry ice and maintained frozen (-70°C or below) until shipped to the 3M Environmental Laboratories.

In the analytical study reported here, sera samples collected from the population of exposed animals (generation F0) and their offspring (generation F1) were analyzed for the presence of PFOS.

Sera samples were extracted by a liquid-liquid extraction procedure using an ion pairing agent and ethyl acetate. The extracts were quantitatively analyzed using turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in selected ion monitoring (SIM) mode, and PFOS levels were evaluated against extracted standards. Analytical details are included in this report; further details are available in the study binder maintained by the 3M Fluorine Analytical Chemistry Team (FACT); the binder is located in the 3M archives.

Analyses assessing the presence and concentration of PFOS in the sera of Sprague-Dawley rats were conducted in compliance with Good Laboratory Practice Regulations (21 CFR 58). Validated methods and standard operating procedures were followed during the preparation and analysis of the samples associated with this study.

SAMPLE RECEIPT

Samples were received from Argus Research Laboratories sporadically, during and at the end of the in-life phase of study, from November 1998, through February, 1999. Samples received were packaged in dry ice. Specimens were registered with the 3M Environmental Laboratory and transferred to a freezer for storage.

Samples of body tissues and fluids other than rat serum were collected and received from Argus Research Laboratories (Protocol 418-014, Study T-6295.13), but were not part of the scope of analysis in the protocol determined by the study director.

Specimens sent to Advanced BioAnalytical Services, Inc., (ABS) for analysis were received and tracked according to the Sample Tracking System Standard Operating Procedure. Specimens sent to ABS will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the subcontract laboratory(s) final report. Specimens will be maintained in the 3M Environmental Laboratory specimen archives. Sample receipt, identification, storage, and chain of custody protocols and data are located in the study binder for this report (FACT Tox-108); the binder is located in the 3M archives.

MATERIALS AND METHODS

Chemical Characterization

Table 2 presents information regarding characterization of the test, control, and reference substances used in the in-life phase of this study.

Table 2. Characterization and Treatment of the Test Substance, Control, and Reference Materials in Study FACT Tox-108

	ANALYSES PERFORMED AT ABS
Test Substance	Potassium Perfluorooctanesulfonate
Source	3M Specialty Chemicals
Preparation	Daily—Records maintained in the raw data
Maximum Storage Time	Expiration May 2000
Storage Conditions	Room temperature
Chemical Lot Number	217
Physical Description and Identity Analyses	FC-95, White powder
Purity	99.28%
Stability	48-hr data of prepared formulations on file with the sponsor
Control Material	Deionized Water
Source	J.T. Baker, Phillipsburg NJ
Preparation	Suspensions were prepared daily
Maximum Storage Time	Unknown
Storage Conditions	Room temperature
Chemical Lot Number	Tween® 80—M03H05, L06662, AND M29477
Physical Description and Identity Analyses	0.5% Tween® 80 in Reverse Osmosis Deionized Water
Purity	There are no known contaminants in the diet that would interfere with this study.
Stability	48-hr data of prepared formulations on file with the sponsor

Table 2. Characterization and Treatment of the Test Substance, Control, and Reference Materials in Study FACT Tox-108 (continued)

	ANALYSES PERFORMED AT ABS
Analytical Reference Material	PFOS (Perfluorooctanesulfonate)
Source	3M Specialty Chemicals
Preparation	Analytical procedure in the ABS final report (Attachment F)
Maximum Storage Time	Unused reference material will be retained for use by the 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation
Storage Conditions	Room temperature
Chemical Lot Number	171
Physical Description and Identity Analyses	Light-colored powder
Purity	99.49%
Stability	Undetermined

Dose analyses were performed on test substance samples taken at various times during the in-life phase of the study: data are located in the study binder for this report (FACT Tox-108) and are presented in Attachment D.

Reserve samples of control materials from the 3M Environmental Laboratory analyses will be stored at the 3M Environmental Laboratory for a period not to exceed 10 years following the effective date of the final test rule, or until the quality of the preparation no longer affords evaluation, as will any reserve samples of test substance returned from the in-life phase of the study. Reserve samples of control material from ABS will be stored at the subcontract laboratories in such a manner that the control materials will be preserved for use in ensuing studies.

Method Summaries

Following is a brief description of the methods used during this analytical study by ABS. Detailed descriptions of these methods are presented in Attachment C.

- ABS Preparatory and Analytical Method: Method Validation for the Quantitation of Perfluorooctanesulfonate (PFOS) in Rat Serum by Turbo Ion Spray LC/MS.

  Analytical standard stock solution, standard spiking solutions, internal standard stock solutions, internal standard working solutions, control blanks, quality control stock solutions, quality control samples, and study samples are prepared in dilutions with appropriate volumes of methanol or purified water in preparation for extraction. Tetrabutyl ammonium hydrogen sulfate (1mL of 0.5 molar solution) is added to all quality control and study samples, along with 2mL of 0.25 molar sodium carbonate/ 0.25 molar sodium bicarbonate. Water (500µL) is added to the control blank, and 500µL of the internal standard working solution is added to all other tubes. Five mL of ethyl acetate is then added to each tube, and the tubes are placed on a reciprocal shaker at medium speed for 20 minutes. The tubes are then centrifuged, and the organic layer is transferred to a clean polypropylene tube. The organic layer is evaporated to dryness in a TurboVap™ at about 20°C under nitrogen. The dried extracts are then reconstituted with acetonitrile and water, and 200µL of the extract is transferred to an autosampler tube for analysis.

Equipment used at ABS:

LC/MS/MS System:

Autosampler: Waters 717plus
Mass spectrometer: PE SCIEX API 365 with TurboIonSpray™ interface
Analytical column: Keystone Betasil™ C_{18}, 2.1x50 mm (5µm)
Mobile phase components:
  Component A—10:90 methanol:2 mM ammonium acetate
  Component B—90:10 methanol:2 mM ammonium acetate
Injection volume: 3-5µL
Column temperature: Ambient
Curtain gas: UHP nitrogen
Nebulizer gas: UHP nitrogen
Ion spray voltage: -3000 V
Declustering potential: -35 V
Negative ions monitored:
  m/z=499.0 for PFOS $[M-K]^-$
  m/z=427.0 for tetra-H-PFOS $[M-H]^-$
Total run time: 8.0 minutes

Protocol Deviations

There were no deviations from the original protocol.

DATA SUMMARY, ANALYSES, AND RESULTS

Summary of Quality Control Analyses Results (ABS)

- The inter-assay precision (relative standard deviation–RSD) data from daily quality control samples ranged from 1.57–4.01% for PFOS, and the inter-assay accuracy (relative error–RE) ranged from -7.32–10.1%. Both are acceptable ranges.
- The inter-assay precision (RSD) of standards ranged from 1.11–5.83% for PFOS. The inter-assay accuracy (RE) from standards ranged from -5.86–11.0%. All are acceptable ranges.
- Coefficients of determination were ≥0.9954.
- Refer to the ABS final report (Attachment F, Table 2) for precision and accuracy data on the analyses of quality control partial volume (one-to-ten dilutions).

Summary of Sample Results (ABS)

Following is information regarding sample analyses in the present study:

- The samples in analytical run numbers 1 and 3 were reassayed because the measured concentrations exceeded the calibration range and upper limit of quantitation (ULQ) (refer to Table 6 in attachment F). Smaller volumes were reextracted from the samples and reanalyzed. Reassayed samples resulted in measured concentrations within the calibration range, and the data were reported (refer to Table 6 in attachment F).

Original data or copies thereof are available at the 3M Environmental Laboratory.

STATISTICAL METHODS

At ABS, data from peak areas were integrated by the PE SCIEX program MacQuan™, version 1.4, on a Macintosh computer. Following peak area integration, MacQuan results data were converted to text files, moved to the ABS file server, and analyzed with the Watson™ software package (version 5.3.1.01 from PSS, Inc., Wayne, PA). The Watson analysis applied a weighted ($1/y^2$) quadratic regression to the data, and calculations were performed on unrounded numbers; however, standard and quality control data were rounded to three significant figures prior to reporting the data.

DATA QUALITY OBJECTIVES AND DATA INTEGRITY

No circumstances existed during the present study that would have affected the quality or integrity of the data. The following data quality objectives (DQOs) were followed during the study:

- Linearity—The coefficient of determination (r^2) of the standard curve was equal to or greater than 0.98
- Limits of Quantitation (LOQ)—Equal to the lowest acceptable standard in the calibration curve
- Duplicate acceptable precision— <30%
- Spike acceptable recoveries— 70% to 130%
- Use of confirmatory methods—no confirmatory methods were used
- Demonstration of specificity—specificity was demonstrated by chromatographic retention time, mass spectral [M-K] product ion characterization

Assuming that spike recovery studies form a suitable indication of endogenous analyte recovery, data are quantitative to ± 30%. The validity of this assumption has not been verified by other techniques. In serum analyses, the limit of detection (LOD) for PFOS is approximately 1.75ng/mL and the limit of quantitation (LOQ) is 0.05µg/mL PFOS.

STATEMENT OF CONCLUSION

Statement of Conclusion:

Under the conditions of the present studies, PFOS was observed in the sera of F0 female rats exposed during the in-life phase of the study. Additionally, PFOS was observed in sera tissues taken from F1 generation pups from female rats exposed to the test substance, and in F1 generation pups exposed to the test substance via lactation, but not exposed *in utero*.

REFERENCES

None.

ATTACHMENTS

- Attachment A: Sample Receipt and Chain of Custody Documentation
- Attachment B: Protocol
- Attachment C: Extraction and Analytical Methods
- Attachment D: Dose Confirmation Analyses
- Attachment E: Data Summary and Tables
- Attachment F: Analytical Reports from Contributing Laboratories
- Attachment G: Report Signature Page

ATTACHMENT A
SAMPLE RECEIPT AND CHAIN OF CUSTODY DOCUMENTATION

Sample receipt and chain of custody information is documented in the binder for this report (FACT Tox-108); the binder is located in the 3M archives.

ATTACHMENT B
PROTOCOL

3M

Study Title

Oral (Gavage) Cross-Fostering Study of PFOS in Rats

PROTOCOL

Author
Lisa Clemen

Date:
June 8, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
FACT-TOX-108
U2779

Study Identification

Oral (Gavage) Cross-Fostering Study of PFOS in Rats

Test Material	Perfluorooctane sulfonic acid potassium salt (T-6295)
Sponsor	3M Toxicology Services - Medical Department 3M Center, Building 220-2E-02 St. Paul, MN 55144-1000
Sponsor Representative	Marvin T. Case, D.V.M., Ph.D. 3M Toxicology Services Telephone: 651-733-5180 Facsimile: 651-733-1773
Study Director	Kristen J. Hansen, Ph.D. 3M Environmental Technology and Safety Services Building 2-3E-09 651-778-6018
Study Location(s)	
In vivo Testing Facility	Argus Research Laboratories, Inc. 905 Sheehy Drive, Building A Horsham, PA 19044
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106

Sub-Contract Laboratory	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850 Battelle Memorial Institute 505 King Avenue Columbus, Ohio 43201-2693
Proposed Study Timetable	
Study Initiation Date	June 08, 1999
Study Completion Date	June 08, 2000

1. STUDY

Oral (gavage) cross-fostering study of potassium perfluorooctane sulfonic acid (PFOS) in rats.

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rats. The in-life portion of this study was conducted at Argus Research Laboratories, study #418-014. All serum samples will be extracted and analyzed at Advanced Bioanalytical Services, Inc. and all liver samples extracted and analyzed at Battelle Memorial Institute. Additional analyses may be performed at the 3M Environmental Laboratory as methods are developed and validated. If additional analyses are performed an amendment to this protocol will be written.

3. REGULATORY COMPLIANCE

This study will be conducted in accordance with the United States Food and Drug Administration, Good Laboratory Practices Standards, Final Rule 21 CFR 58, with the exception that analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor.

4. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will review the protocol and audit study conduct, data, and final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures.

The QA Unit at the sub-contract laboratory will audit their study conduct, data, and results report prior to submitting to the 3M Environmental Laboratory.

5. TEST MATERIAL

5.1 Refer to Argus Research Laboratory protocol for study #418-014.

6. CONTROL MATRICES

6.1 Identification Rat liver and serum and/or rabbit liver and serum, traceability numbers will be recorded in the raw data and included in the final report

6.2 Source Argus Research and/or Sigma Chemical

6.3 Physical Description Rat liver and serum and/or rabbit liver and serum

6.4 Purity and Stability Not applicable

6.5 Storage Conditions Frozen at -20 °C ± 10 °C or –50 °C ± 10 °C

6.6 Reserve Matrix A portion of the control matrix will be retained in the 3M archives for as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

6.7 Disposition Matrices will be retained at the 3M Environmental Laboratory per GLP regulation. Certain matrices (feces, urine, and blood) may be disposed after QAU verification.

6.8 Safety Precautions Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

7. REFERENCE MATERIAL

7.1 Identification Potassium perfluorooctanesulfonate (PFOS), lot #s 171, 215, or 217 (equivalent lots)

7.2 Source 3M Specialty Chemicals

7.3 Physical Description White powder

7.4 Purity and Stability Purity of PFOS is 99% or greater. Stability has not been determined.

7.5 Storage Conditions Room temperature

7.6 Reserve Material A reserve sample from each batch of PFOS used in this study will be retained as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

7.7 Disposition Unused reference material will be retained for use by the 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation.

7.8 Safety Precautions Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

8. TEST SYSTEM

Female rats were used as the test system and were maintained and dosed as described in Argus Research protocol #418-014. Group 1 control animals did not receive the test substance while the group 2 treated animals received the test substance at a concentration of 1.6 mg/kg/day for 6 weeks. Refer to Argus Research protocol #418-014 for tabular presentation of data. Dosing of the female animals in group 2 was continued during mating, gestation, and lactation. Cross-fostering of the pups born to the control dams were randomized, half of the pups were placed on control dams while the other half were placed on the PFOS dosed dams. Likewise, the same was done with the group 2 pups. Each of these pups will be marked to identify whether they are from control or PFOS dosed dams.

9. SPECIMEN AND SAMPLE RECEIPT

The 3M Environmental Laboratory will receive homogeneity samples and specimens of the following body tissues and fluids from the indicated points in the study. All specimens will be packed on dry ice for shipping.

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Pup animals	At lactation day 14 and termination of the study	60 Dam and 34 Pup
Urine and feces – Dam animals	After termination of the study	48 Urine and 48 Feces
Liver – Dam and Pup animals	At lactation day 14 and termination of the study	60 Dam and 34 Pup
Milk Secreting Glands – Dam animals	At lactation day 14 and termination of the study	58
Milk – Dam animals	At lactation day 14	10

Total number of expected specimens: 352
Total number of test animals: 36
Total number of control animals: 42

Specimens sent to 3M Environmental Laboratories will be received and tracked according to applicable Standard Operating Procedures.

10. Preparatory Methods

10.1 FACT-M-1.1, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.2 ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.3 If preparatory methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

10.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

11. Analytical Methods

11.1 FACT-M-2.1, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

11.2 ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry

11.3 If analytical methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

11.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

12. Data Quality Objectives

The number of spikes/duplicates, use of surrogates, and information on other data quality indicators are included in the analytical methods. In addition, the following criteria will be met:

12.1 ***Linearity*** $r^2 \geq 0.98$

12.2 ***Limits of detection / quantitation***

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

12.2.2 Limit of Quantitation (LOQ) – Equal to the lowest acceptable standard in the calibration curve

12.3 ***Duplicate acceptable precision*** < 30% for the method

12.4 ***Spike acceptable recoveries*** 70% - 130%

12.5 ***Use of confirmatory methods*** Indeterminate samples will be re-analyzed using a confirmatory method. If a confirmatory method is used, an amendment to this protocol will be written.

12.6 ***Demonstration of specificity*** Chromatographic retention time, mass spectral daughter ion characterization.

13. Sub-Contracted Analysis

13.1 All analyses as detailed in this protocol will be performed at 3M Environmental Laboratories, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106, at Advanced Bioanalytical Services, Inc., 15 Catherwood Road, Ithaca, NY 14850, or at Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693.

13.2 An amendment to this protocol will be written if analyses are performed at laboratories other than 3M Environmental Laboratories, Advanced Bioanalytical Services, Inc., or Battelle Memorial Institute.

14. Statistical Analysis

Averages and standard deviations will be calculated. The statistical methods that will be used are described below:

14.1 ***Data transformations and analysis*** Data will be reported as the concentration (weight/weight or weight/vol) of PFOS or metabolite per tissue or fluid.

14.2 ***Statistical analysis*** Statistics used may include regression analysis of concentrations over time, and standard deviations calculated for the concentrations within each dose group. If necessary, simple statistical tests, such as Student's t test, may be applied to evaluate statistical difference.

15. Report

A report containing all the results of the study will be prepared by the 3M Environmental Laboratory. If analyses are sub-contracted to other laboratories, each laboratory will prepare a report and submit it to the 3M Environmental Laboratory for inclusion in the 3M Environmental Laboratory report. Each report will include, but not be limited to, the following, when applicable:

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

15.3 A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards

15.4 Objectives and procedures as stated in the approved protocol, including any changes in the original protocol

15.5 The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor

15.6 Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor

15.7 A description of the methods used to conduct the test(s)

15.8 A description of the test system

15.9 A description of any circumstances that may have affected the quality or the integrity of the data

15.10 The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study

15.11 A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses

15.12 Statistical methods used to evaluate the data, if applicable

15.13 The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable

15.14 The location where raw data and the final report are to be stored

15.15 A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management

If it is necessary to make corrections or additions to a report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

16. Location of Raw Data, Records, and Final Report

Original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

16.1 The following raw data and records will be retained in the study folder in the study/project archives according to 3M Environmental Laboratory Standard Operating Procedures:

- ***16.1.1*** Approved protocol and amendments
- ***16.1.2*** Study correspondence
- ***16.1.3*** Shipping records
- ***16.1.4*** Raw data
- ***16.1.5*** Approved final report (original signed copy)
- ***16.1.6*** Electronic copies of data

16.2 The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory Standard Operating Procedures:

- ***16.2.1*** Training records
- ***16.2.2*** Calibration records
- ***16.2.3*** Instrument maintenance logs
- ***16.2.4*** Standard Operating Procedures, Equipment Procedures, and Methods

17. SPECIMEN RETENTION

Specimens will be maintained in the 3M Environmental Laboratory specimen archives for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable), and as established by 3M Environmental Laboratory Standard Operating Procedures.

18. PROTOCOL AMENDMENTS AND DEVIATIONS

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and filed with the raw data.

19. ATTACHMENTS

19.1 Attachment A Preparatory and analytical methods

20. SIGNATURES

Marvin T. Case, D.V.M., Ph.D., Sponsor Representative — Date: 9 June 1999

Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director — Date: 6/10/99

Study Title
Oral (Gavage) Cross-Fostering Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:
August 12, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS FACT-TOX108
LIRN U2779

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:*** Section 2.0 states that all liver samples will be extracted and analyzed at Battelle Memorial Institute.

AMEND TO READ: All liver samples will be extracted and analyzed at the 3M Environmental Laboratory.

REASON: Due to time constraints, the liver extraction and analysis will be performed at the 3M Environmental Laboratory.

2. ***PROTOCOL READS:*** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
FACT-M-2.1 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

AMEND TO READ: The extraction and analytical methods to follow at the 3M Environmental Laboratory are:

ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

REASON: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to ETS-8-6.0 and ETS-8-7.0. These methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix.
The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

3. ***PROTOCOL READS:*** Section 10.4 and 11.4 state that if analyses are sub-contracted to other laboratories an amendment will be written to include their methods.

AMEND TO READ: The extraction and analytical methods to follow at Advanced Bioanalytical Services will be attached to the protocol.

REASON: The analytical methods at the sub-contract laboratory were not included in the original protocol.

4. ***PROTOCOL READS:*** Section 12.2.1 b) lists the liver method detection limit as 15 ppb.

AMEND TO READ: The liver method detection limit is 8.50 ppb (ng/g).

REASON: The validation supporting methods ETS-8-6.0 and ETS-8-7.0 includes a lower method detection limit for PFOS.

Amendment Approval

Marvin T Case — 17 August 1999

Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kris Hansen — 8/18/99

Kris J. Hansen, Ph.D., Study Director — Date

Study Title
Oral (Gavage) Cross-Fostering Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 30, 1999

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS FACT-TOX108
LIRN U2779

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
   Section 2.0 states that all liver samples will be extracted and analyzed at the 3M Environmental Laboratory.

   AMEND TO READ:
   No liver samples will be extracted and analyzed.

   REASON:
   Liver results are no longer required.

2. ***PROTOCOL READS:***
   Section 16 states that the original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, electronic copies of data, training records, calibration records, instrument maintenance logs and standard operating procedures, equipment procedures, and methods will be retained in the archives of the 3M Environmental Laboratory.

   AMEND TO READ:
   Section 16 states that the original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis.

   REASON:
   Clarification of the disposition of archived records if analyses are performed at a sub-contract laboratory.

3. ***PROTOCOL READS:***
Section 17 states that specimens will be maintained in the 3M Environmental Laboratory specimen archives.

AMEND TO READ:
Specimens will be maintained in the 3M Environmental Laboratory specimen archives. All specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. The specimens will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.

REASON:
Clarification of the disposition of specimens and documentation for analyses performed by a sub-contract laboratory.

Amendment Approval

Marvin T Case — 4 Oct 1999
Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kristen Hansen — 10/5/99
Kristen J. Hansen, Ph.D., Study Director — Date

ATTACHMENT C
EXTRACTION AND ANALYTICAL METHODS

ADVANCED
BIOANALYTICAL
SERVICES, INC.

METHOD VALIDATION REPORT

TITLE: METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

DATE: 26 August 1999

REPORT: 99VDJA01.MI.DOC

AUTHORS: David J. Anderson, M.S.
Amie J. Prince, B.S.
Holly D. Ross, M.S.

PREPARED FOR: 3M Environmental Technology and Safety Services
St. Paul, MN 55133-3331

NUMBER OF PAGES: 50

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AUTHORS: David J. Anderson, M.S.
Amie J. Prince, B.S.
Holly D. Ross, M.S.

FOR: 3M Environmental Technology and Safety Services

DATE: 26 August 1999

TITLE: METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

ABSTRACT

A sensitive, specific, accurate, and reproducible analytical method was developed by Advanced BioAnalytical Services, Inc., Ithaca, New York to quantitate perfluorooctanesulfonate (PFOS) in rat serum samples. Serum samples (50 µL) were extracted by a liquid-liquid extraction procedure to isolate the analyte from rat serum. Sample extracts were reduced to dryness, reconstituted, and analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode. The assay demonstrated a lower limit of quantitation (LLQ) of 0.05 µg/mL using 50-µL sample aliquots. The calibration curves were fit from 0.05 µg/mL to 20 µg/mL for PFOS by a weighted ($1/y^2$) quadratic equation. The coefficients of determination of the calibration curves ranged from 0.9962 to 0.9965.

Precision and accuracy quality control (QC) samples were prepared at concentrations of 0.2, 6, and 18 µg/mL PFOS. Quality control (QC) samples were prepared at a concentration of 100 µg/mL PFOS for partial volume analysis. The intra- and inter-assay precision (RSD) results calculated from all QC samples ranged from 1.92% to 4.87% for PFOS. The intra- and inter-assay accuracies (RE) calculated from QC samples ranged from -3.58% to 5.58% for PFOS. The mean extraction recoveries were from 87.6% to 100% for PFOS and 89.9% for the internal standard (IS).

PFOS was measured as stable in rat serum for up to 24 hours at ambient temperature. PFOS was measured as stable in rat serum at –20 °C, currently for up to 33 days, and

after three freeze/thaw cycles. Reliable results were obtained for sample extracts reinjected 27 hours after initial reconstitution.

QAU STATEMENT

Periodic inspections of the method validation for the quantitation of PFOS in rat serum were conducted by the Quality Assurance Unit of Advanced BioAnalytical Services (ABS) for compliance with EPA GLP regulations (40 CFR Part 792).

The study was inspected on the following dates:
13, 21 May 1999; 7, 8, 14, 15 June 1999; 1, 2 July 1999; 3, 4 August 1999.

Results of the inspections were reported to ABS Management on:
13, 21 May 1999; 7, 8, 14, 15 June 1999; 1, 2 July 1999; 3, 4 August 1999.

Results of the inspections were reported to the Study Director on 5 August 1999.

Based on the inspections and the data reviewed, this report is a complete and accurate representation of the data.

Kathleen Cormack
Kathleen Cormack, B.S.
Quality Auditor

26Aug99
Date

SIGNATURE PAGE

TITLE: METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

Report Number: 99VDJA01.MI.DOC

Reported by: _____ 26 AUGUST 1999
David J. Anderson, M.S. — Date
Research Scientist

Reviewed by: Kathleen Cormack 26 Aug 99
Kathleen Cormack, B.S. — Date
Associate Auditor

Authorized for Release by: _____ 26 AUGUST 99
John R. Perkins, Ph.D. — Date
Assistant Scientific Director

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1. INTRODUCTION

The purpose of this report is to describe the method validation for the quantitative determination of perfluorooctanesulfonate (PFOS) in rat serum samples after modification of a previous method (1). The objectives of the method validation were to validate a simple extraction procedure, determine the lower limit of quantitation for routine analysis, determine the extraction recoveries of the analyte and internal standard (IS), determine the analyte stability in rat serum at ambient temperature, long-term freezer storage, and over three freeze/thaw cycles, and to provide specific, accurate, and reproducible quantitative results by turbo ion spray liquid chromatography/mass spectrometry (LC/MS).

2. EXPERIMENTAL

2.1. CHEMICALS AND MATERIALS

Perfluorooctanesulfonate (PFOS, Lot# 171) was obtained from 3M, Inc. The internal standard (IS) for PFOS was 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS). The internal standard (Lot# 59909) was obtained from 3M, Inc. A detailed list of chemicals and materials is found in Appendix A.

Stock and working solutions which were used to prepare the calibration curves for the analytes were prepared as described in Appendix A. Stock solutions used in the preparation of quality control (QC) samples were prepared separately from those used in preparation of the calibration curves.

Preparation of all solutions used during extraction and analysis are described in Appendix A.

2.2. LC/MS INSTRUMENTATION

The liquid chromatography/mass spectrometry system consisted of two LC-10AD pumps (Shimadzu, Columbia, MD 21046), a SCL-10A pump controller (Shimadzu,

Columbia, MD 21046), a WISP 717plus autosampler (Waters Associates, Millipore Corporation, Milford, MA 01757), a Betasil C_{18} (2 x 50 mm, 5 μm) column (Keystone Scientific, Inc., Bellefonte, PA 16823), and a PE SCIEX API 365 mass spectrometer (PE SCIEX, Concord, Ontario). A detailed list of the instrumentation and instrument conditions is found in Appendix A.

2.3. SAMPLE PREPARATION AND EXTRACTION PROCEDURE

For each analytical run (tray), duplicate 50-μL aliquots of the calibration curve samples were prepared as described in Appendix A. The nominal (theoretical) concentrations of PFOS in the calibration curves were 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 8, 16, and 20 μg/mL.

Rat serum quality control samples were prepared in advance of the validation study at nominal (theoretical) concentrations of 0.2, 6, and 18 μg/mL for QC1, QC2, and QC3, respectively, as detailed in Appendix A. A dilution QC (QC4, 100 μg/mL) was prepared at a concentration exceeding the upper limit of the calibration curve range (20 μg/mL), and was assayed using a 10-fold dilution for partial volume analysis.

Calibration standards and QC samples were extracted by the procedure detailed in Appendix A.

2.4. RAT SERUM VALIDATION DATA

The data were collected using selected ion monitoring (SIM) turbo ion spray LC/MS in the negative ion mode. Peak areas were integrated by the PE SCIEX program MacQuan, version 1.4, residing on a Macintosh computer. Following peak area integration, the results tables from MacQuan were saved as text files and uploaded to the Advanced BioAnalytical Services (ABS) file server where a weighted ($1/y^2$) quadratic regression was performed using the software package Watson v 5.3.1.01 (PSS, Inc., Wayne, PA 19087). All data were rounded to no less than three significant figures by ABS prior to reporting in Tables 1 through 11. The data for the rat serum validation are stored in ABS Notebook 2304.

All calculations were based on the peak area ratio of the analyte to the internal standard. Concentrations of each analyte in quality control samples were determined by inverse prediction from the calibration curve.

2.5. ASSAY EVALUATION

2.5.1. Intra- and Inter-Assay Accuracy

The intra- and inter-assay accuracy of the method was assessed by determining the relative error observed in the analysis of quality control samples. The mean concentration for each quality control level was divided by the theoretical concentration. One was subtracted from the result, converted to percent and expressed as RE. QC1 through QC3 were assayed in replicates of five. In addition, the RE was reported for standards at all levels over three runs.

2.5.2. Intra- and Inter-Assay Precision

The intra- and inter-assay precision of the method was assessed by determining the Relative Standard Deviation (RSD) observed for quality control sample data. The mean concentration and RSD were calculated for the first run and over three runs for intra-assay and inter-assay precision, respectively. QC1 through QC3 were assayed in replicates of five. In addition, the RSD was reported for standards at all levels over three runs.

2.5.3. Partial Volume Analysis

The effect of dilution on the analysis of PFOS in rat serum was determined by partial volume analysis. QC4 (100 μg/mL) was prepared containing PFOS at approximately five times the upper limit of quantitation (ULQ). Five replicates of QC4 were diluted ten-fold and analyzed. The RSD and RE were reported.

2.5.4. Lower Limit of Quantitation (LLQ)

Rat serum control samples from six separate individuals were spiked with PFOS at a concentration of 0.05 μg/mL. The LLQ was determined by obtaining the back-calculated concentrations from these control samples. The overall mean, RSD, and RE were also calculated.

2.5.5. Selectivity

The selectivity of the assay was determined by LC/MS. To monitor for interference from the biological matrix, rat serum samples containing neither the analyte nor the internal standard (control blank) were assayed with all experiments.

2.5.6. Carryover Evaluation

The carryover of analyte from one injection to the next was assessed by analyzing a control blank injected immediately after a high calibration standard (STD10, 20 μg/mL).

2.6. STABILITY OF PFOS IN QUALITY CONTROL SAMPLES

QC1 through QC3 were used to determine the stability of the analyte during sample storage, extraction, and analysis.

2.6.1. Ambient-Temperature Stability of PFOS in Rat Serum

The stability of PFOS was evaluated by storing QC samples at each concentration level at ambient temperature (ca. 25 °C) for nominal timepoints of 0, 2.5, 6, and 24 hours after thawing. All QCs were assayed in replicates of five.

2.6.2. Freezer Stability of PFOS in Rat Serum at –20 °C

The freezer stability of PFOS was evaluated by storing QC samples at each concentration level at –20 °C for 7, 13, and 33 days. Additional freezer stability timepoints will be reported in an addendum to this report. All QCs were assayed in replicates of four.

2.6.3. Freeze/Thaw Stability in Rat Serum

The stability of PFOS was evaluated after three freeze/thaw cycles. The mean concentrations of the QC samples after three freeze/thaw cycles (i.e., at least 2 hours frozen storage at the nominal temperature of –20 °C followed by thawing at ambient temperature for 30 minutes) were compared to the mean concentrations of freshly thawed QC samples. All QCs were assayed in replicates of five.

2.7. REPRODUCIBILITY OF REINJECTING EXTRACTED SAMPLES

The reproducibility of reinjecting reconstituted serum extracts was investigated by reinjecting a set of previously-assayed standards and QC samples which had been stored after injection at approximately 25 °C for 27 hours. The RE of the QC samples was used to assess processed sample stability in the reconstitution solution. All QCs were assayed in replicates of five.

2.8. EXTRACTION RECOVERY

The extraction recovery of PFOS from rat serum was determined by comparing the peak area ratio (PAR) of samples (0.25, 4, and 16 µg/mL) spiked after extraction (post-extract) with the PAR of samples spiked before extraction (pre-extract). The internal standard was spiked post-extraction for all samples. The recovery of the internal standard (4 µg/mL) was assessed following a similar approach using PFOS as the reference. The extraction recovery (% Recovery) was determined by dividing the pre-extract PAR by the post-extract PAR and expressing the result as a percentage. Five replicates were used at each concentration level.

3. RESULTS AND DISCUSSION

A quantitative analytical procedure using turbo ion spray LC/MS in the negative ion mode was developed to meet the high sensitivity, specificity, and reproducibility requirements for the determination of PFOS in rat serum. The full-scan single MS mass spectrum of PFOS showed an abundant $[M-K]^-$ ion at m/z = 499 (Figure 1). The full-scan single MS mass spectrum of Tetra-H-PFOS showed an abundant $[M-H]^-$ ion at m/z = 427 (Figure 2).

The following selected ion monitoring (SIM) was used to quantify the analytes in rat serum:

PFOS	m/z = 499.0
Tetra-H-PFOS (IS)	m/z = 427.0

The peak labeling of full-scan data does not accurately represent the performance of the instrument in SIM mode. The SIM ions were derived from separate experiments using narrow-range scanning, which more accurately depicts the operation of the instrument in the SIM mode. The mass chromatograms of a representative control blank rat serum extract are shown in Figure 3. Mass chromatograms from a representative control rat serum extract containing only the internal standard (zero sample), are shown in Figure 4.

Figures 5 and 6 are mass chromatograms of representative extracts from calibration standards 1 and 10, respectively.

3.1. ASSAY EVALUATION RESULTS

3.1.1. Intra- and Inter-Assay Accuracy

The intra-assay accuracy (RE) data from QC samples ranged from -2.35 to 5.58% for PFOS (Table 1). The inter-assay accuracy ranged from -3.58 to 4.95% for PFOS (Table 2). These data indicate acceptable intra- and inter-assay accuracy for the determination of PFOS in rat serum.

3.1.2. Intra- and Inter-Assay Precision

The intra-assay precision data (RSD) ranged from 1.99 to 3.28% for PFOS at all QC concentration levels (Table 1). The inter-assay precision results from QC samples ranged from 1.92 to 4.87% for PFOS (Table 2). The RSD ranged from 0.613 to 4.38% for calibration standards at all levels (Table 3). These data indicate acceptable intra-and inter-assay precision for the determination of PFOS in rat serum.

3.1.3. Linearity

The calibration curves were fit by a weighted ($1/y^2$) quadratic regression. Coefficients of determination (r^2) were ≥0.9962 for PFOS in rat serum. The calibration curve statistics are shown in Table 4.

3.1.4. Partial Volume Analysis

The results of partial volume analysis of QC4 is shown in Table 5. The precision (RSD) of QC4 samples diluted 1 in 10 was 2.71%. The accuracy was 5.89%. These data indicate acceptable accuracy and precision for partial volume analysis for the determination of PFOS in rat serum.

3.1.5. Lower Limit of Quantitation (LLQ)

Table 6 shows the lower limit of quantitation (LLQ) data. The serum LLQ experiment demonstrated that 0.05 μg/mL is an acceptable LLQ for PFOS. The precision (RSD) was 4.95% for PFOS. The RE was 4.57%.

3.1.6. Selectivity

The assay was specific for PFOS. No chromatographic interferences were observed in any of the control serum samples analyzed (Figure 3). The control blanks and zero samples did show some evidence of small chromatographic peaks at the retention times of the analyte. These peaks were not quantifiable as they were below the lower limit of quantitation (LLQ).

3.1.7. Carryover Evaluation

Carryover of PFOS and the IS was evaluated by injection of an extracted rat serum control blank following an injection of an extracted high standard (STD 10). The response for the small chromatographic peak at the retention time for PFOS was comparable to the response of a rat serum control blank injected before the high standard. Thus, carryover is negligible for PFOS. There was no evidence of carryover for the internal standard.

3.2. STABILITY OF PFOS IN QUALITY CONTROL SAMPLES

3.2.1. Ambient Temperature Stability of PFOS in Rat Serum

The results of the ambient stability of PFOS in QC samples are shown in Table 7. The mean predicted concentrations deviated from -4.45 to 10.5% from the 0-hour values for all QC levels and time points. Based on these data, PFOS was stable in rat serum for up to 24 hours at ambient temperature.

3.2.2. Freezer Stability of PFOS in Rat Serum at –20 °C

The results of the long-term stability of PFOS in QC samples stored at –20 °C are presented in Table 8. The PFOS concentrations deviated from 0-hour values from -2.76 to 12.3% for all QC levels stored up to 33 days.

Results of freezer storage after two, four, and eight months will be reported as an addendum to this report.

3.2.3. Freeze/Thaw Stability of PFOS in Rat Serum

The results of the freeze/thaw stability of PFOS in QC samples after three freeze/thaw cycles is shown in Table 9. PFOS was stable after three freeze/thaw cycles with deviations ranging from –1.38% to 10.1% from the 0-cycle samples for all QC levels (Table 9).

3.3. REPRODUCIBILITY OF REINJECTING EXTRACTED SAMPLES

The results of reinjecting reconstituted samples containing PFOS after storage for 27 hours at approximately 25 °C in reconstitution solution are shown in Table 10. Reinjecting processed samples after 27 hours in reconstitution solution was appropriate with RE values ranging from -6.33 to 4.59% at 27 hours for all QC levels.

3.4. EXTRACTION RECOVERY

The mean recoveries of PFOS and Tetra-H-PFOS are shown in Table 11. The mean recoveries ranged from 87.6 to 100% for PFOS. The mean recovery for Tetra-H-PFOS was 89.9%.

4. CONCLUSIONS

The turbo ion spray LC/MS assay procedure for the determination of PFOS in rat serum has proven to be sensitive, specific, accurate, and reproducible. Its high sensitivity allows reliable and reproducible quantitation of PFOS down to a level of 0.05 μg/mL in rat serum based on 50-μL samples.

5. DATA RETRIEVAL

The data for the rat serum validation are stored in ABS Notebook 2304 and in the ABS Archives.

6. REFERENCES

1. Advanced BioAnalytical Services, Inc. Report 98AGKP02.MMM. Analytical Report for the Determination of Perfluorooctanoate and Perfluorooctanesulfonate in Human Serum by LC/MS. Grace K. Poon, Ph.D., David Hardwick, B.S., and Ellen Pace, M.A.T., 6 February 1998.

7. TABLES

Table 1: Intra-Assay Accuracy and Precision of the PFOS Assay in Rat Serum for QC Samples

	PFOS Concentration (µg/mL)		
	QC1	QC2	QC3
Theoretical Conc.	0.2	6	18
Run 9	0.210	6.52	17.9
	0.202	6.31	18.0
	0.206	6.17	16.7
	0.205	6.32	18.0
	0.194	6.35	17.3
Mean	0.204	6.34	17.6
RSD (%)	2.93	1.99	3.28
RE (%)	1.77	5.58	-2.35

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 2: Inter-Assay Accuracy and Precision of the PFOS Assay in Rat Serum for QC Samples from Three Validation Runs

	PFOS Concentration (µg/mL)		
	QC1	QC2	QC3
Theoretical Conc.	0.2	6	18
Run 9	0.210	6.52	17.9
	0.202	6.31	18.0
	0.206	6.17	16.7
	0.205	6.32	18.0
	0.194	6.35	17.3
Run 10	0.187	6.44	16.9
	0.186	6.44	17.3
	0.182	6.32	17.5
	0.189	6.19	18.0
	0.182	6.31	17.4
Run 11	0.194	6.17	18.2
	0.197	6.13	17.1
	0.184	6.41	17.6
	0.192	6.15	17.7
	0.184	6.24	17.0
Mean	0.193	6.30	17.5
RSD (%)	4.87	1.92	2.66
RE (%)	-3.58	4.95	-2.73

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 3: Accuracy and Precision of the PFOS Assay in Rat Serum for Calibration Standards from Three Validation Runs

	PFOS Calibration Standard Concentration (µg/mL)									
Theoretical Conc.	STD1 0.05	STD2 0.1	STD3 0.25	STD4 0.5	STD5 1	STD6 2	STD7 4	STD8 8	STD9 16	STD10 20
Run 9	0.0483	0.0972	0.251	0.480	0.942	2.10	4.48	7.61	15.8	20.0
	0.0530	0.101	0.252	0.483	0.938	2.13	4.55	7.70	16.2	19.9
Run 10	0.0491	0.0951	0.239	0.469	0.954	2.16	4.58	8.05	15.9	19.6
	0.0542	0.101	0.251	0.479	0.942	2.11	4.43	7.87	15.7	20.0
Run 11	0.0510	0.0960	0.251	0.469	0.945	2.21	4.40	7.64	15.8	20.3
	0.0510	0.0995	0.252	0.477	0.948	2.13	4.53	7.78	15.6	20.0
Mean	0.0511	0.0983	0.249	0.476	0.945	2.14	4.50	7.78	15.8	20.0
RSD (%)	4.38	2.65	2.07	1.15	0.613	1.90	1.62	2.13	1.22	1.08
RE (%)	2.20	-1.67	-0.207	-4.78	-5.53	7.05	12.4	-2.80	-1.00	-0.0764

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 4: Calibration Curve Parameters for PFOS in Rat Serum

Run #	Quadratic Coefficient (A)[a]	Coefficient Linear (B)	Intercept (C)	Coefficient of Determination (r^2)
9	-0.0010769	0.13730	0.00042009	0.9965
10	-0.0010516	0.14535	0.0014910	0.9962
11	-0.0012359	0.15173	0.00088515	0.9964
Mean	-0.0011215	0.14479	0.00093206	0.9964

a: $y = Ax^2 + Bx + C$, weighted $1/y^2$

Table 5: Partial Volume Analysis of PFOS in Rat Serum

	PFOS Conc. (µg/mL)
Theoretical Conc.	QC4 100 (1 in 10 dilution)
Run 9	111 105 105 104 104
Mean	106
RSD (%)	2.71
RE (%)	5.89

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 6: Lower Limit of Quantitation of PFOS in Rat Serum

Run 11 Theoretical Concentration	PFOS Conc. Found (µg/mL)
LLQ (0.05 µg/mL)	0.0548
	0.0489
	0.0499
	0.0554
	0.0527
	0.0520
Mean	0.0523
RSD (%)	4.95
RE (%)	4.57

RSD = (SD/Mean) x 100

RE = [(Mean/Theoretical)-1] x 100

Table 7: Ambient-Temperature Stability of PFOS in Rat Serum After 24 Hours

Run 11	PFOS (µg/mL)			
QC (Theoretical Conc.)	0 Hour	2.5 Hour	6 Hour	24 Hour
	0.194	0.187	0.177	0.190
QC 1	0.197	0.181	0.179	0.187
(0.2 µg/mL)	0.184	0.184	0.180	0.191
	0.192	0.180	0.183	0.182
	0.184	0.178	0.189	0.190
Mean	0.190	0.182	0.182	0.188
RSD (%)	3.03	1.91	2.52	2.01
%DEV from 0 Hour	NA	-4.20	-4.45	-1.16
	6.17	6.17	6.36	6.55
QC 2	6.13	6.30	6.38	6.53
(6 µg/mL)	6.41	6.26	6.35	6.85
	6.15	6.28	6.22	6.66
	6.24	6.24	6.11	6.81
Mean	6.22	6.25	6.28	6.68
RSD (%)	1.82	0.797	1.86	2.16
%DEV from 0 Hour	NA	0.525	1.05	7.42
	18.2	18.0	18.1	19.1
QC 3	17.1	18.0	18.0	19.4
(18 µg/mL)	17.6	17.7	17.6	19.2
	17.7	17.9	18.2	19.4
	17.0	18.0	18.0	19.7
Mean	17.5	17.9	18.0	19.4
RSD (%)	2.84	0.719	1.26	1.19
%DEV from 0 Hour	NA	2.33	2.39	10.5

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Hour = [(X Hour Conc. - 0 Hour Conc.)/0 Hour Conc.] x 100

Table 8: Freezer Stability of PFOS in Rat Serum at -20 °C

QC Nominal Concentration	PFOS (ug/mL)			
	0 Hour (Run 2)*	7 Days (Run 6)*	13 Days (Run 3)**	33 Days (Run 16)
QC 1 (0.2 ug/mL)	0.213	0.225	0.211	0.245
	0.209	0.222	0.210	0.234
	0.208	0.218	0.217	0.228
	0.208	0.219	0.214	0.232
	0.207			
Mean	0.209	0.221	0.213	0.235
RSD (%)	1.24	1.32	1.55	3.17
%DEV from 0 Hour	NA	5.72	1.84	12.3
QC 2 (6 ug/mL)	6.02	6.22	5.97	6.45
	5.71	6.15	5.84	6.13
	6.00	6.39	6.07	6.58
	6.06	6.27	5.99	6.37
	5.69			
Mean	5.89	6.26	5.97	6.38
RSD (%)	3.08	1.62	1.58	2.97
%DEV from 0 Hour	NA	6.19	1.20	8.28
QC 3 (18 ug/mL)	20.2	19.1	18.8	18.6
	18.6	19.5	18.2	18.8
	18.9	19.8	18.8	18.7
	18.2	19.3	17.9	18.7
	18.9			
Mean	18.9	19.4	18.4	18.7
RSD (%)	3.96	1.37	2.42	0.395
%DEV from 0 Hour	NA	2.52	-2.76	-1.30

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Hour = [(Conc. - 0 Hour Conc.)/0 Hour Conc.] x 100
*From Protocol FACT-TOX-110
**From Protocol FACT-TOX-111

Table 9: Stability of PFOS in Rat Serum After Three Freeze/Thaw Cycles

Run 10 QC (Theoretical Conc.)	PFOS (µg/mL)	
	0 Cycle	3 Cycles
QC 1 (0.2 µg/mL)	0.187	0.177
	0.186	0.177
	0.182	0.185
	0.189	0.185
	0.182	0.189
Mean	0.185	0.182
RSD (%)	1.70	3.12
%DEV from 0 Cycle	NA	-1.38
QC 2 (6 µg/mL)	6.44	6.59
	6.44	6.61
	6.32	6.44
	6.19	6.39
	6.31	6.40
Mean	6.34	6.49
RSD (%)	1.66	1.63
%DEV from 0 Cycle	NA	2.35
QC 3 (18 µg/mL)	16.9	19.2
	17.3	19.1
	17.5	19.2
	18.0	19.6
	17.4	18.7
Mean	17.4	19.2
RSD (%)	2.31	1.80
%DEV from 0 Cycle	NA	10.1

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Cycle = [(3 Cycle - 0 Cycle)/0 Cycle] x 100

Table 10: Reproducibility of Reinjecting Extracted Samples Containing PFOS after 27 Hours in Reconstitution Solution

	PFOS (µg/mL)	
	t=0 Hours (Run 10)	t=27 Hours (Run 12)
QC 1 (0.2 µg/mL)	0.187	0.187
	0.186	0.182
	0.182	0.188
	0.189	0.188
	0.182	0.190
Mean	0.185	0.187
RSD (%)	1.70	1.61
RE (%)	-7.50	-6.33
QC 2 (6 µg/mL)	6.44	6.39
	6.44	6.02
	6.32	6.19
	6.19	6.55
	6.31	6.22
Mean	6.34	6.28
RSD (%)	1.66	3.23
RE (%)	5.62	4.59
QC 3 (18 µg/mL)	16.9	17.3
	17.3	18.2
	17.5	18.1
	18.0	17.3
	17.4	17.6
Mean	17.4	17.7
RSD (%)	2.31	2.40
RE (%)	-3.29	-1.73

NA: Not Applicable
RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 11: Extraction Recovery of PFOS from Rat Serum

Theoretical Conc. in serum (µg/mL)	Pre-Extraction Response (Area Ratio)	Post-Extraction Response (Area Ratio)	% Recovery*
PFOS Spike 1 (0.25 µg/mL) Run 14	0.03160	0.02880	110
	0.02850	0.03010	94.7
	0.02930	0.02950	99.3
	0.02790	0.02930	95.2
	0.03050	0.02960	103
Mean	0.02956	0.02946	100
PFOS Spike 2 (4 µg/mL) Run 14	0.4597	0.5270	87.2
	0.4751	0.5304	89.6
	0.4776	0.5335	89.5
	0.4724	0.5298	89.2
	0.4772	0.5392	88.5
Mean	0.4724	0.5320	88.8
PFOS Spike 3 (16 µg/mL) Run 14	1.649	1.874	88.0
	1.679	1.857	90.4
	1.719	1.869	92.0
	1.535	1.887	81.4
	1.612	1.864	86.5
Mean	1.639	1.870	87.6
Tetra-H-PFOS Spike IS (4 µg/mL) Run 13	0.4068	0.4460	91.2
	0.3884	0.4423	87.8
	0.4043	0.4445	91.0
	0.3921	0.4512	86.9
	0.4078	0.4412	92.4
Mean	0.3999	0.4450	89.9

*(Individual Response for Pre-Ext./Individual Response for Post-Ext.) x 100

8. FIGURES

Figure 1: Full-Scan Single MS Mass Spectrum of PFOS

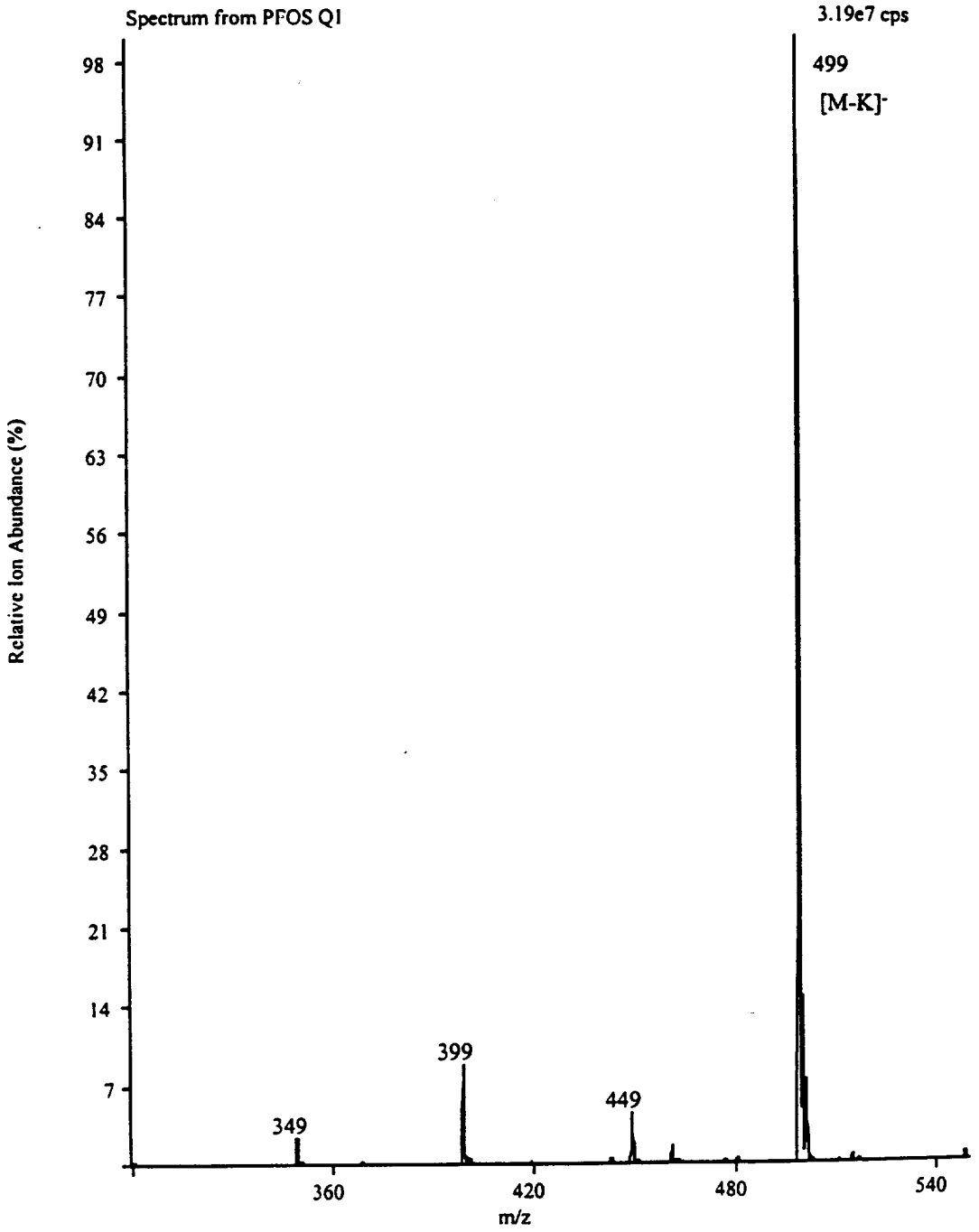


Figure 2: Full-Scan Single MS Mass Spectrum of Tetra-H-PFOS

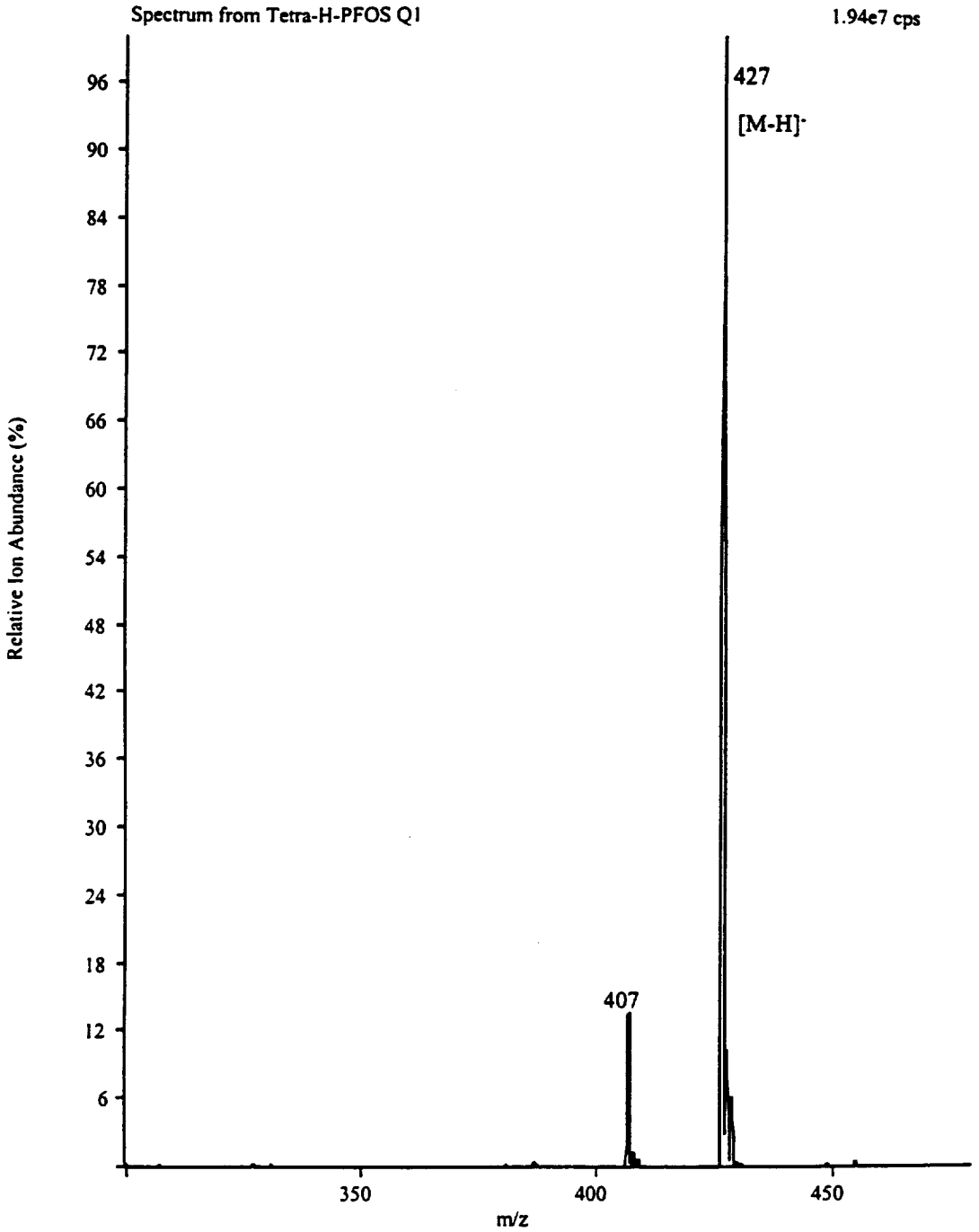
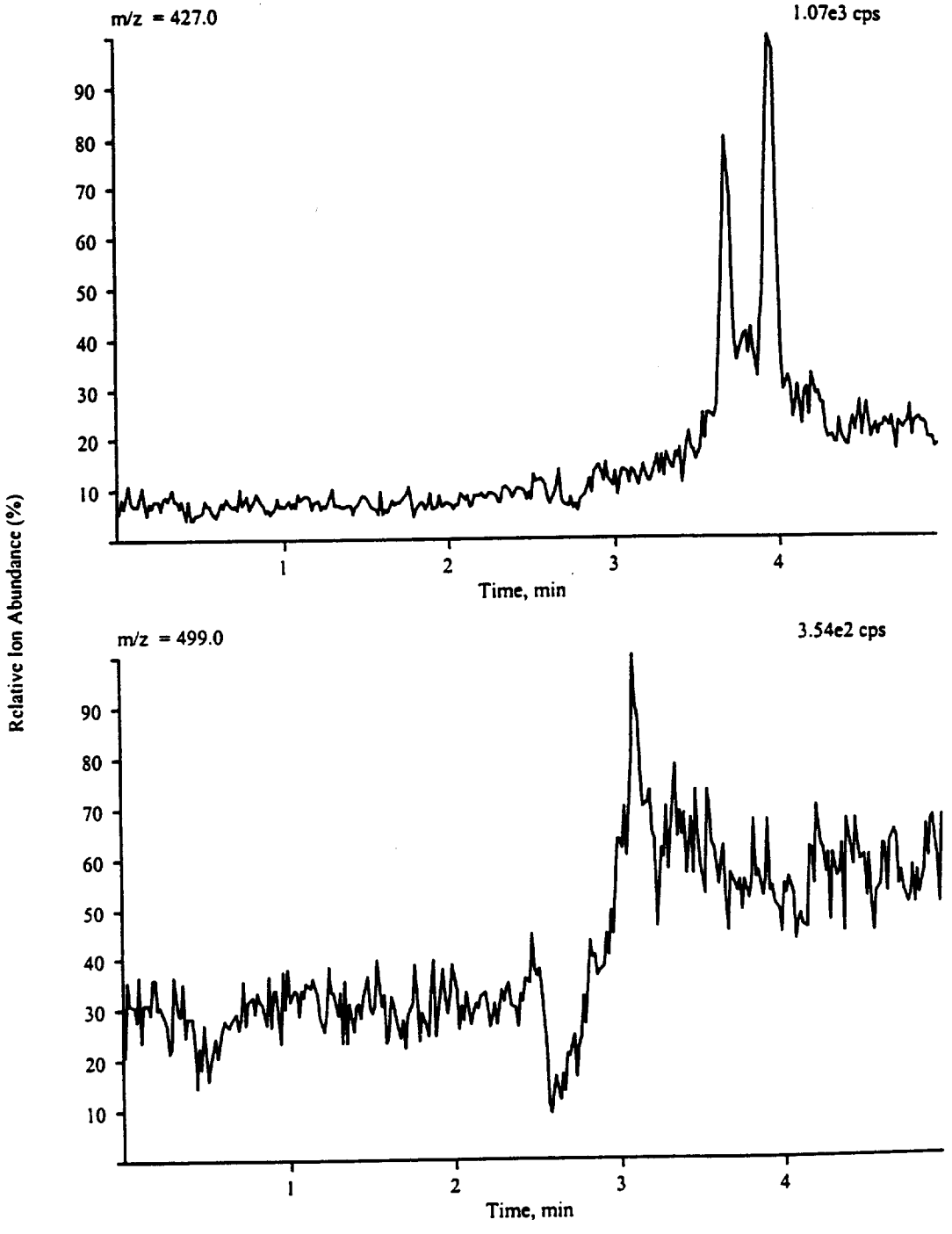


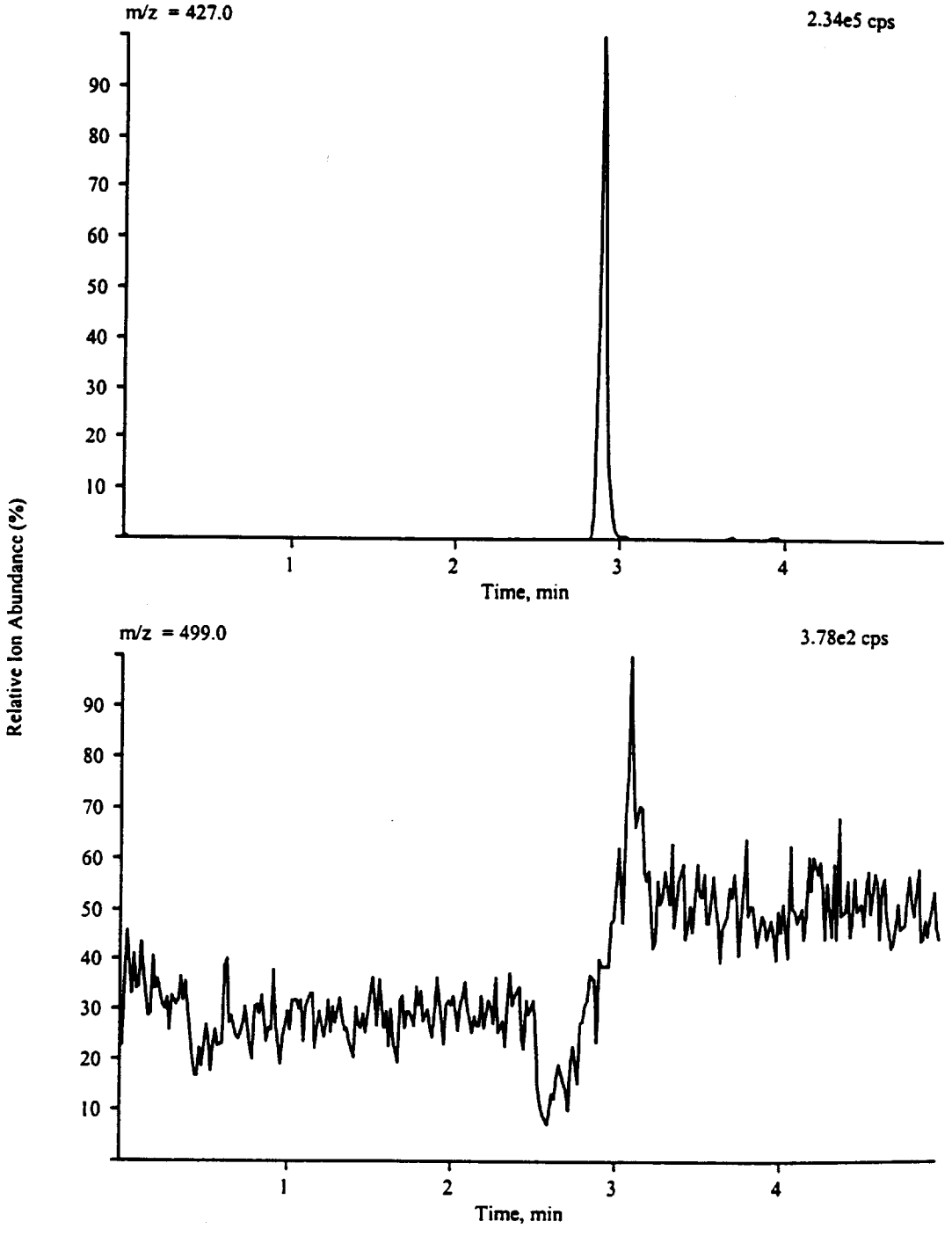
Figure 3: Mass Chromatograms of PFOS and its Internal Standard in Control Blank Rat Serum Extract



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

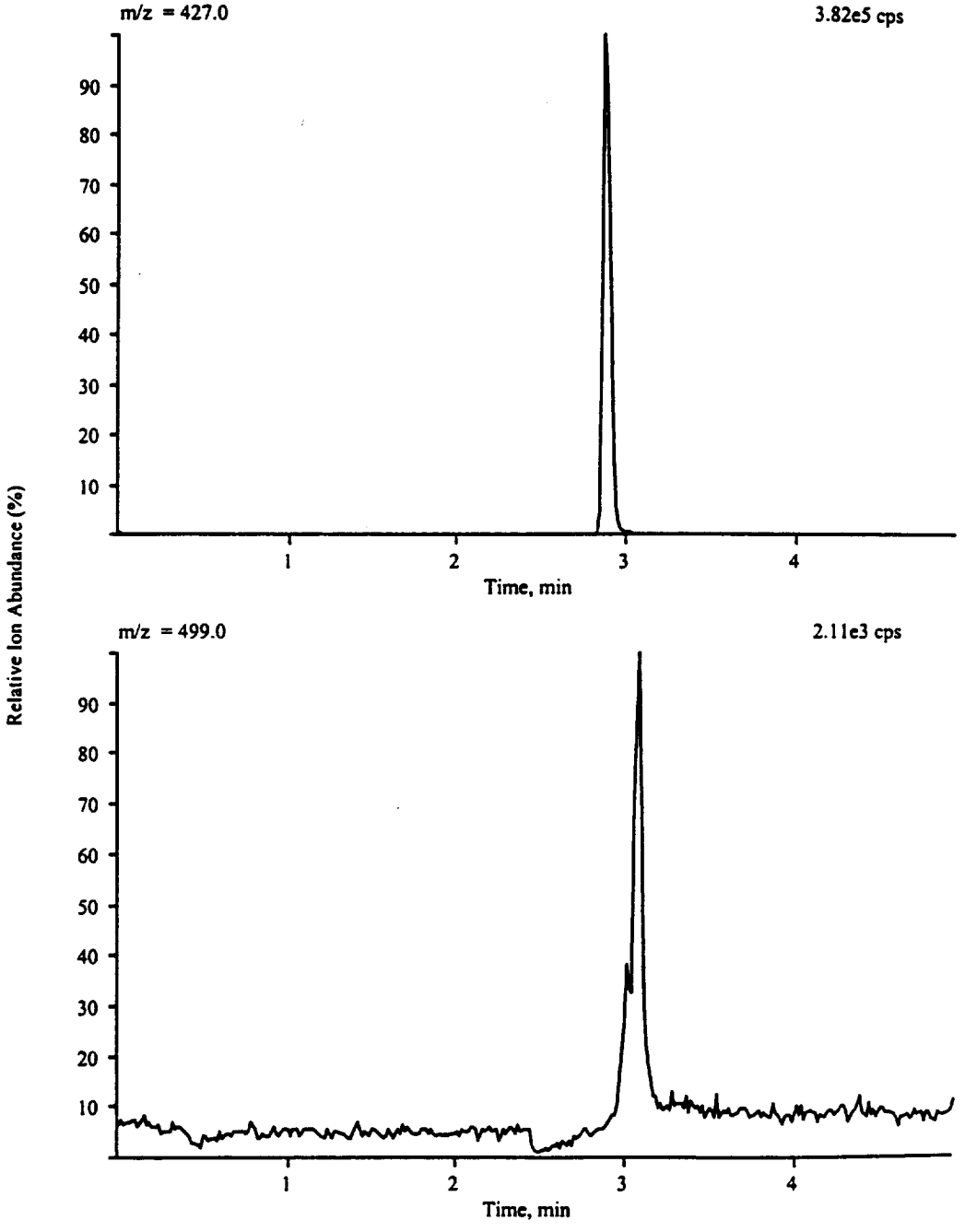
Figure 4: Mass Chromatograms of PFOS in a Rat Serum Extract Sample Containing Internal Standard Only (Zero Sample)



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

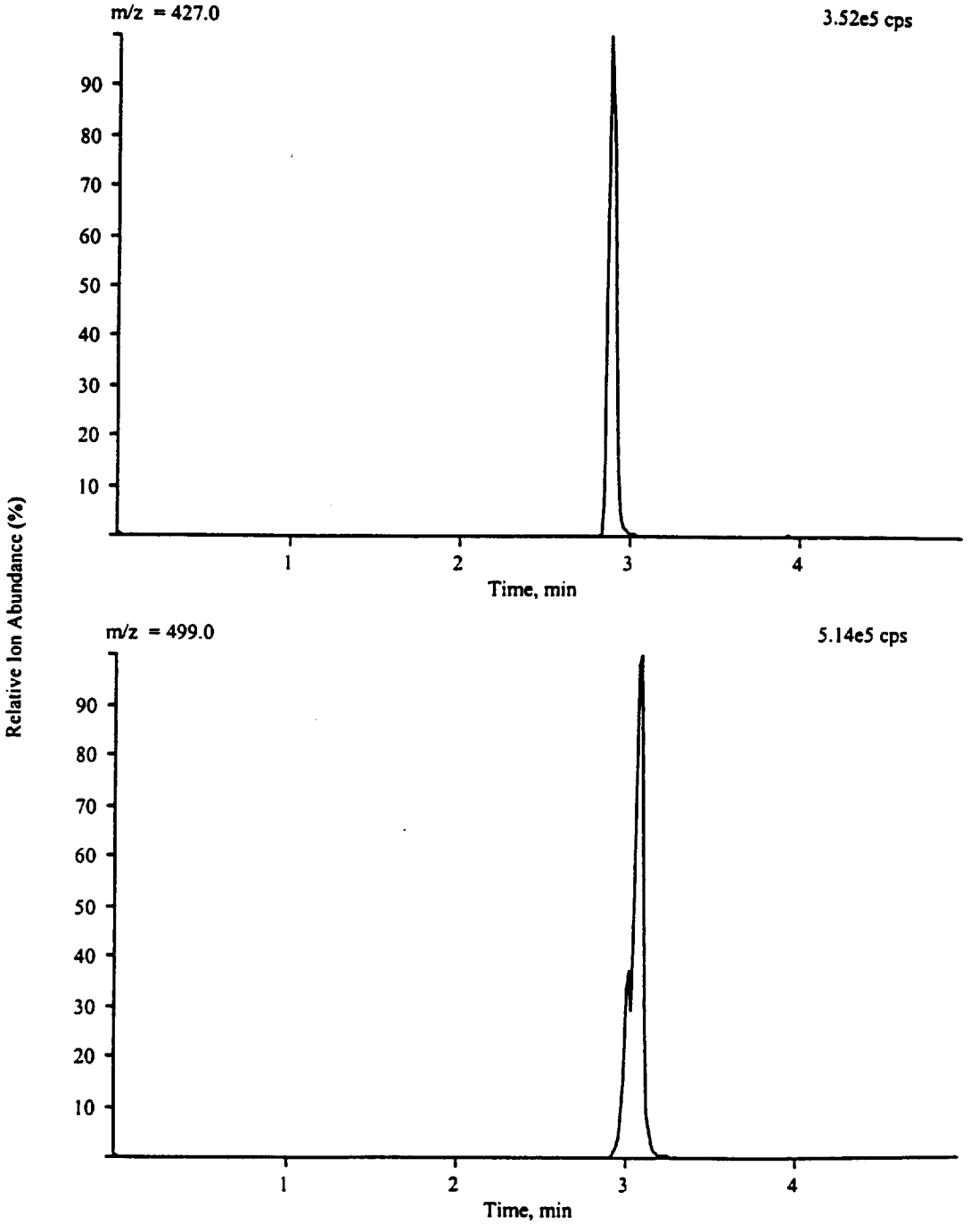
Figure 5: **Mass Chromatograms of Calibration Standard 1 in Rat Serum Extract Containing PFOS (0.05 µg/mL) and the Internal Standard**



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

Figure 6: Mass Chromatograms of Calibration Standard 10 in Rat Serum Extract Containing PFOS (20 µg/mL) and the Internal Standard



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

9. APPENDIX A: QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

AUTHORS: **David J. Anderson, M.S.**
Amie J. Prince, B.S.
Holly D. Ross, M.S.

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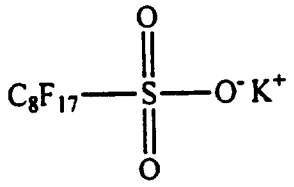
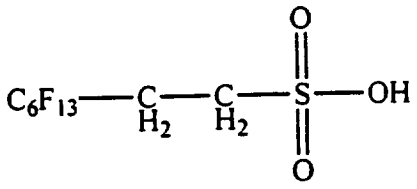
A.1.0 CHEMICAL STRUCTURE(S)



Potassium Perfluorooctanesulfonate
MW = 538



1H, 1H, 2H, 2H-Perfluorooctane Sulfonic Acid
MW = 428, Internal Standard

A.2.0 SPECIMEN(S)

This assay uses 50-µL aliquots of rat serum. Rat serum samples are stored at -20 °C.

A.3.0 ASSAY PRINCIPLE

PFOS and its internal standard, Tetra-H-PFOS, are extracted from rat serum samples (50 µL) using a liquid-liquid extraction procedure. The organic layer is evaporated to dryness and then reconstituted. An aliquot is then analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode.

A.4.0 COMPOUNDS

PFOS: Lot# 171, 99.976% purity, 3M, Inc., Minneapolis, MN.

Tetra-H-PFOS: Lot# 59909, 90% purity, 3M, Inc., Minneapolis, MN.

A.5.0 CHEMICALS

All chemicals may be substituted with that of an equivalent manufacturer and grade of chemical.

Acetonitrile	Cat# 015-4, Burdick & Jackson, Muskegon, MI 49442
Ammonium Acetate	Cat# 24,019-2, Aldrich, Milwaukee, WI 53201
Ethyl Acetate	Cat# 100-4, Burdick & Jackson, Muskegon, MI 49442
Methanol	Cat# 230-4, Burdick & Jackson, Muskegon, MI 49442
Rhodapex mass calibration solution	A mixture of 0.11 mM trifluoroacetic acid (J.T. Baker, Phillipsburg, NJ 08865), 0.52 mM Rhodapex CO-436, 1 mM ABEX EP-110 and 1 mM ABEX EP-120 (all three supplied by Rhone-Poulenc, Inc., Cranbury, NJ 08512) in 1:1 methanol:water.
Sodium Bicarbonate	Cat# 3509-01, J.T. Baker, Phillipsburg, NJ 08865
Sodium Carbonate, Annhydrous	Cat# 3604-01, J.T. Baker, Phillipsburg, NJ 08865
Tetrabutylammonium Hydrogen Sulfate	HPLC Grade, Cat# J360-07, J.T. Baker, Phillipsburg, NJ 08865
Water	High Purity (NANOpure), Barnstead Model D7331 Ultrapure Water System, Dubuque, IA 52001
Rat Serum	Lampire Biological Laboratories, Piperville, PA 18947
Rat Serum	Harlan Bioproducts for Science, Inc., Indianapolis, IN 46229

A.6.0 MATERIALS AND EQUIPMENT

Mass Spectrometer	PE SCIEX API 365 atmospheric pressure ionization tandem triple quadrupole mass spectrometer equipped with TurboIonSpray™ interface, PE SCIEX, Concord, Ontario L4K 4V8
Data system	API Standard Software, MacQuan v 1.4, LC2Tune v 1.3, MacDAD v 1.3, Bundler v 1.3, Multiview v 1.3,

	and Sample Control v 1.3, on a Power Macintosh, PE SCIEX, Concord, Ontario L4K 4V8
HPLC Pump	Shimadzu LC-10AD pump, Shimadzu Co., Columbia, MD 21046
LC Pump Controller	Shimadzu SCL-10A, Shimadzu Co., Columbia, MD 21046
Autosampler	Waters 717plus, Waters Associates, Millipore Corporation, Milford, MA 01757
HPLC Column	Betasil C_{18}, 5 µm particle size, 2.1 x 50 mm, Cat #852055-701, Keystone Scientific, Inc., Bellefonte, PA 16823
Harvard syringe pump 11	Harvard Apparatus Inc., South Natick, MA 01760
Multi-tube vortexer	Cat# 58816-115, VWR Scientific, West Chester, PA 19380
Balance	Model FX-300, AND Ltd., Tokyo, Japan
pH Meter	Model 340, Corning Inc., Corning, NY 14830
Mechanical shaker	Cat# 6000, Eberbach Corp., Ann Arbor, MI 48106
NANOpure-UV water purification system	Barnstead, Dubuque, IA 52001
Microbalance	Model MT-5, Mettler-Toledo Inc., Hightstown, NJ 08520
Micro weigh boats	Cat# 0219-0041, Perkin-Elmer Corp., Norwalk, CT 06859
Weigh boats	Cat# 12577-025, VWR Scientific, West Chester, PA 19380
Sorvall RT-6000D refrigerated centrifuge	Cat# 83071, DuPont Co., Wilmington, DE 19898
Beckman GS6KR Refrigerated centrifuge	Beckman, Palo Alto, CA 94304

TurboVap LV Evaporator	Cat# 43750, Zymark Instruments, Hopkinton, MA 01748
Pipettes	Cat# P-5000, P-1000, P-200, P-100, Rainin Instrument Co., Woburn, MA 01888
Pipette tips	RT-20, RT-200, C-5000, CP-25, CP-50, CP-250, Rainin Instrument Co., Woburn, MA 01888
Polypropylene tubes	13 mm x 100 mm, Cat# 15070-574, VWR Scientific, West Chester, PA 19380
Screw-capped Polypropylene vials	16.5 x 57 mm, Cat# 60.542, Sarstedt, Inc., Newton, SC 28658
Screw-capped Polypropylene vials	16.5 x 101 mm, Cat# 60.541, Sarstedt, Inc., Newton, SC 28658
Plug Tite Multipurpose Caps	Cat# 78-127-0019-100, Elkay Products, Inc., Worcester, MA 01607
Pyrex volumetric flask	Cat# 28014P-10, 28014P-25, 28014P-100, 28014P-1000, Kimble/Kontes, Vineland, NJ 08360
Solvent Filtration Apparatus	Cat# 953781-0000, 953751-0000, 953753-0000, 953826-0000, 953827-0000, Kimble/Kontes, Vineland, NJ 08360
Nylon Titan Membrane Filters	0.45 µm Scientific Resources Inc., Cat# 74547-NN, Eatontown, NJ 07724
PEEK tubing	0.005" i.d. x 1/16" o.d., Cat# 1535, Upchurch Scientific Inc., Oak Harbor, WA 98277
Liquid Nitrogen	Supplied in-house from bulk tank, BOC Gasses, Buffalo, NY 14210-2005
Gastight Glass Syringes	Cat# 1750, Hamilton Company, Reno, NV 89510

Other general laboratory glassware and supplies were used.

A.7.0 PREPARATION OF SOLUTIONS

NANOpure water (18 megaohm-cm.) or equivalent should be used wherever water is called for. Mix all solutions well.

A.7.1 Preparation of Analytical Standard Stock Solutions

Analytical Standard Stock Solution, PFOS (1 mg/mL): Weigh approximately 5 mg of PFOS (after correction for purity) on a microbalance and transfer it to a polypropylene vial. Dilute with the appropriate volume of methanol to yield a 1 mg/mL solution. Prepare a fresh solution every three months. Store the solution at 4 °C and bring to ambient temperature before use.

Standard Spiking Solutions

Prepare Standard Working Solutions A through J in 5-mL class "A" volumetric flasks according to the following dilution scheme. Dilute to the 5-mL mark with water to yield the final concentration. Prepare fresh solutions every three months. Store the solutions at 4 °C and bring to ambient temperature before use.

Standard Working	Volume Spiked (µL)	Solution Used	PFOS Final Conc. (µg/mL)
A	1000	Stock	200
B	800	Stock	160
C	400	Stock	80
D	200	Stock	40
E	100	Stock	20
F	250	200 µg/mL (A)	10
G	125	200 µg/mL (A)	5
H	62.5	200 µg/mL (A)	2.5
I	500	10 µg/mL (F)	1.0
J	250	10 µg/mL (F)	0.5

A.7.2 Preparation of Internal Standard Stock Solutions

Internal Standard Stock Solution, Tetra-H-PFOS (1 mg/mL): Weigh approximately 5 mg of Tetra-H-PFOS on a microbalance and transfer it to a polypropylene vial. Dilute with an appropriate volume of methanol to yield a 1 mg/mL solution. Prepare a fresh solution every three months. Store the solution at 4 °C and bring to ambient temperature before use.

A.7.3 Preparation of Internal Standard Working Solution

Internal Standard Working Solution (4 µg/mL Tetra-H-PFOS): Add 400 µL of Internal Standard Stock Solution to a 100-mL class "A" volumetric flask. Dilute to the mark with water to give a 4 µg/mL Tetra-H-PFOS solution. Store the solution at 4 °C and bring to ambient temperature before use. Prepare fresh solution as needed.

A.7.4 Preparation of Standard Curve and Control Blank

Prepare fresh calibration standards for each analytical run by combining 360 µL of rat control serum and 40 µL of the analytical standard working solution (see table below) in labeled 1.7-mL microtubes. Prepare control blanks and zero samples by combining 360 µL of rat control serum with 40 µL of water. Vortex each for 60 seconds.

Analytical Standard Dilution Table:

Standard #	Volume Spiked (µL)	Solution to use	µL control serum	Final Conc. (µg/mL)
10	40	A	360	20
9	40	B	360	16
8	40	C	360	8
7	40	D	360	4
6	40	E	360	2
5	40	F	360	1
4	40	G	360	0.5
3	40	H	360	0.25
2	40	I	360	0.1
1	40	J	360	0.05

A.7.5 Preparation of Other Solutions

0.25 M Sodium Carbonate/0.25 M Sodium Bicarbonate: Weigh 26.5 g of sodium carbonate and 21 g of sodium bicarbonate and dissolve in approximately 900 mL of HPLC-grade water. Transfer the solution to a 1000-mL volumetric flask and dilute to the mark with HPLC-grade water. Mix the solution thoroughly. Store at ambient temperature. Prepare a fresh solution every three months.

1 M Ammonium Acetate: Add 7.7 g of ammonium acetate to a 100-mL volumetric flask, dissolve in NANOpure water, and stir until completely dissolved. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

2 mM Ammonium Acetate: Combine 2 mL of 1M ammonium acetate and 900 mL NANOpure water in a 1000-mL volumetric flask; stir until completely mixed. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

10 M Sodium Hydroxide: Add 40 g of sodium hydroxide to a 100-mL volumetric flask, dissolve in NANOpure water, and stir until completely dissolved. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

1 M Sodium Hydroxide: Add 10 mL of 10 M sodium hydroxide to a 100-mL volumetric flask. Dilute to the mark with NANOpure water; stir until completely mixed. Store at ambient temperature. Prepare a fresh solution every three months.

0.5 M Tetrabutylammonium Hydrogen Sulfate, pH 10: Weigh 16.98 g of tetrabutylammonium hydrogen sulfate and dissolve in 40 mL of NANOpure water. Adjusted the pH to 10.0 with 10 M and 1 M sodium hydroxide. Transfer the solution to a 100-mL volumetric flask and dilute to the mark with NANOpure water.

10:90 Methanol:2 mM Ammonium Acetate (Mobile Phase Eluent A): Add 100 mL of methanol and 900 mL of 2 mM ammonium acetate to a 1000-mL Pyrex bottle. Mix the solution thoroughly and filter the solution through a 0.45 µM filter with a vacuum flask. Store at ambient temperature. Prepare a fresh solution every three months.

90:10 Methanol:2 mM Ammonium Acetate (Mobile Phase Eluent B): Add 900 mL of methanol and 100 mL of 2 mM ammonium acetate to a 1000-mL Pyrex bottle. Mix the solution thoroughly and filter the solution through a 0.45 µM filter with a vacuum flask. Store at ambient temperature. Prepare a fresh solution every three months.

A.8.0 PREPARATION OF QUALITY CONTROL SAMPLES

A.8.1 Preparation of Quality Control Stock Solutions

QC Stock Solution, PFOS (1 mg/mL): Weigh approximately 5 mg of PFOS (after correction for purity) on a microbalance and transfer it to polypropylene vial. Dilute

with the appropriate volume of methanol to yield a 1 mg/mL solution. Store the solution at 4 °C and bring to ambient temperature before use. Prepare a fresh solution every three months.

A.8.2 Preparation of Serum QC Samples

QC4 (Dilution QC, 100 µg/mL PFOS): Add 1000 µL QC Stock Solution to a 10-mL class "A" volumetric flask to yield a 100-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.2 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC3 (18 µg/mL PFOS): Add 450 µL QC Stock Solution to a 25-mL class "A" volumetric flask to yield a 18-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC2 (6 µg/mL PFOS): Add 150 µL of QC Stock Solution to a 25-mL class "A" volumetric flask to yield a 6-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC1 (0.2 µg/mL PFOS): Add 111.1 µL of QC3 to a 10-mL class "A" volumetric flask to yield a 0.2-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

A.9.0 LIQUID-LIQUID EXTRACTION PROCEDURE

1. Prepare fresh calibration standards for each run according to Section A.7.4.
2. Thaw QC samples.
3. Prepare QC4 samples (diluted 1 in 10) in two steps:
   a) Add 180 µL of control serum and 20 µL of QC4 into 1.7-mL microtubes and

vortex.

b) Aliquot 50 µL of diluted QC4 into labeled 16 x 100 mm polypropylene tubes.

4. Aliquot 50 µL of each control blank, zero sample, calibration standard, and quality control samples 1-3 into labeled 16 x 100 mm polypropylene tubes. Standards and blanks are analyzed in duplicate. All QC samples are analyzed in replicates of five.
5. Add 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate, pH 10 to each tube.
6. Add 2 mL of 0.25 M sodium carbonate/0.25 M sodium bicarbonate to each tube.
7. Add 500 µL of the Internal Standard Working Solution to each tube (except control blank, add 500 µL water).
8. Add 5 mL ethyl acetate to each tube.
9. Shake the tubes on a reciprocal shaker at medium speed for 20 minutes.
10. Centrifuge the tubes at 3000 rpm for 20 minutes at 20 °C.
11. Freeze the aqueous phase in an acetone/dry ice bath and keep the tubes in the bath an additional 5 minutes.
12. Transfer the ethyl acetate layer to a fresh, labeled 13 x 100 mm polypropylene tube.
13. Evaporate the ethyl acetate layer to dryness in a TurboVap™ at approximately 20 °C under nitrogen.
14. Reconstitute the dried extracts in two steps:
    Add 500 µL acetonitrile and vortex for 60 seconds.
    Then add 500 µL water and vortex for 60 seconds.
15. Transfer 200 µL of the extract to a labeled polypropylene autosampler vial.

A.10.0 INSTRUMENT CONDITIONS

<u>HPLC Conditions</u>:

Eluent (gradient)	Mobile Phase A: 10:90 methanol:2 mM ammonium acetate (v/v) Mobile Phase B: 90:10 methanol:2 mM ammonium acetate (v/v)

Eluent Gradient Conditions:

Time (min)	%B
0	45
1.0	100
5	100
5.5	45%
8	Stop

Flow Rate	300 µL/min.
Autosampler	Waters 717plus
Autosampler Temperature	Ambient
Injection Volume	3 to 5 µL
HPLC Column	Keystone Betasil C_{18}, 5 µm particle size, 2.1 mm x 50 mm
HPLC Column Temperature	Ambient
Typical Initial Column Pressure	90 bar
Autosampler Run Time	8.0 minutes

Analyte	Representative Retention Time (minutes)
PFOS	3.1
Tetra-H-PFOS (IS)	2.9

Mass Spectrometer Conditions:

Curtain Gas	UHP Nitrogen
Nebulizer Gas	UHP Nitrogen
TurboIonSpray™ Temperature	400 °C
TurboIonSpray™ Auxiliary Gas	UHP Nitrogen at 8 L/min

Ions Monitored:

Analyte	Ion Monitored	Dwell Time
PFOS	m/z = 499.0	500 ms
Tetra-H-PFOS (IS)	m/z = 427.0	500 ms

Ionization Mode	Negative Ion
Ion Spray Voltage	-3000 V
Declustering Potential	-35 V
MS Acquisition Time	5 minutes
Pause Time	2 ms

Calibrate the mass axis of the instrument by infusion of Rhodapex mass calibration solution (Section A.5.0) at a flow rate of 10 µL/min. Optimize the sensitivity of the instrument using an infusion of a 10 µg/mL solution of the analyte at 10 µL/min into a flow of 190 µL/min of mobile phase using the gradient condition at which the analyte elutes from the LC column (100% eluent B). Mass spectral peak widths should be approximately 0.6 amu at half-height.

One set of calibration standards was injected at the beginning and one set of calibration standards was injected at the end of each analytical run (tray).

A.11.0 CALCULATIONS

Calculated concentrations are based on peak area ratios of PFOS to Tetra-H-PFOS. The peak area for the analyte ion is divided by the peak area for the corresponding internal standard ion.

Data generated from samples in this study were acquired and integrated using PE SCIEX software applications Sample Control (v. 1.3) and MacQuan (v. 1.4). The validation data were formatted in the ABS laboratory information management system (Watson, v.5.3.1.01). All data were rounded to no less than three significant figures by ABS prior to reporting.

Acceptance Criteria for Sample Analysis

Calibration standards: The coefficient of determination (r^2) must be ≥0.98. At least three-fourths of the individual calibration standard replicates will have deviations within ±15% from their nominal concentrations.

LLQ acceptance criteria: At least one replicate at the LLQ must exhibit a deviation within ±20% from its nominal concentration. If this criterion is not met, both replicates are rejected and the standards at the next higher level are subjected to the same test.

Quality control samples: At least two-thirds of the individual QC sample replicates will have deviations within ±15% from their nominal concentrations, with at least one replicate at each QC concentration meeting this criterion.

ATTACHMENT D
DOSE CONFIRMATION ANALYSES

Study:	Argus 418-014, Oral (Gavage) Cross-Fostering Study of PFOS in Rats		
Product Number(Test Substance):	PFOS (T-6295.9)		
Matrix:	Tween Dosing Vehicle	See list to right	0
Method/Revision:	ETS-8-4.1 & ETS-8-5.1	See Attachments	0
Analytical Equipment System Number:	Madeline041098	See Attachments	0
Instrument Software/Version:	MassLynx 3.2	See Attachments	Grp 1
Date of Extraction/Analyst:	9/13/99 IAS		
Date of Analysis/Analyst:	9/15/99 IAS		
Date of Data Reduction/Analyst:	9/16/99 IAS		

Sample Data

Tween Dosing Confirmation

Group Dose	Sample #	Expected Conc. PFOS ng/ml	PFOS Conc. ng/ml	PFOS % Recovery Accuracy
QC	B-418-014-B, 11/1/98 (302ppb MS)	201	199	99%
	B-418-014-B, 1/27/99 (302ppb MS)	201	141	70%
Group 1 Control 0.0 mg/kg/day	B-418-014-A (11/01/98) Diluted 1/1	0	0	<LOD
	B-418-014-A (1/27/99) Diluted 1/1	0	0	<LOD
Group 2 1.6 mg/kg/day 0.32 mg/ml	B-418-014-B (11/01/98) Diluted 1/808	320000	252516	79%
	B-418-014-B (1/27/99) Diluted 1/808	320000	276069	86%

Limit of Quantitation Limit (LOQ) = PFOS = 30 ng/g
Method Detection Limit (MDL): PFOS = 15 ng/g
PFOS = Perfluorooctanesulfonate

NR = Sample not received nor reported.
NA = Not Applicable

Date Entered/Analyst: 9/27/99 GML
Date Verified/Analyst:

ATTACHMENT E
DATA SUMMARY TABLES

Table 1. Concentrations of PFOS in Rat Serum Samples from Study FACT Tox-108
<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL)

RAT NUMBER (RAT NUMBER WITH A "P" INDICATES A PUP SAMPLE)	PFOS CONCENTRATION (µg/mL)
18901	0.0507
18901P	<LLQ[0.0323]<(0.05)
18903	4.80
18903P	35.7
18904	<LLQ[0.0462]<(0.05)
18906	<LLQ[0.0329]<(0.05)
18907	1.35
18907P	33.5
18908	2.41
18909	1.06
18910	<LLQ[0.0316]<(0.05)
18910P	<LLQ[0.0382]<(0.05)
18911	0.0798
18911P	0.123
18912	<LLQ[0.0376]<(0.05)
18913	<LLQ[0.0341]<(0.05)
18913P	<LLQ[0.0398]<(0.05)
18915	<LLQ[0.0221]<(0.05)
18915P	<LLQ[0.0242]<(0.05)
18917	<LLQ[0.0355]<(0.05)
18917P	<LLQ[0.0221]<(0.05)
18918	<LLQ[0.0323]<(0.05)
18918P	<LLQ[0.0247]<(0.05)
18919	<LLQ[0.0340]<(0.05)
18920	1.96
18922	<LLQ[0.0412]<(0.05)
18922P	<LLQ[0.0367]<(0.05)
18923	<LLQ[0.0291]<(0.05)
18923P	<LLQ[0.0331]<(0.05)
18924	2.72
18924P	30.7
18926	<LLQ[0.0323]<(0.05)
18926P	<LLQ[0.0229]<(0.05)
18927	<LLQ[0.0305]<(0.05)
18927P	<LLQ[0.0318]<(0.05)

Table 1. Concentrations of PFOS in Rat Serum Samples from Study FACT Tox-108 (continued)
<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL)

RAT NUMBER (RAT NUMBER WITH A "P" INDICATES A PUP SAMPLE)	PFOS CONCENTRATION (µg/mL)
18929	2.40
18931	<LLQ[0.0382]<(0.05)
18931P	<LLQ[0.0196]<(0.05)
18932	<LLQ[0.0399]<(0.05)
18934	1.25
18934P	34.1
18935	<LLQ[0.0316]<(0.05)
18935P	<LLQ[0.0206]<(0.05)
18936	<LLQ[0.0302]<(0.05)
18936P	<LLQ[0.0230]<(0.05)
18937	1.80
18938	<LLQ[0.0359]<(0.05)
18939	<LLQ[0.0311]<(0.05)
18939P	<LLQ[0.0308]<(0.05)
18940	<LLQ[0.0330]<(0.05)
18940P	<LLQ[0.0231]<(0.05)
18941	5.34
18942	1.12
18943	65.1
18943P	82.2
18946	157
18947	86.9
18948	96.1
18950	66.4
18951	72.4
18951P	59.2
18952	90.8
18952P	48.3
18953	67.0
18953P	53.8
18956	97.5
18956P	89.3
18957	69.1
18957P	93.2
18958	68.3
18958P	56.2

Table 1. Concentrations of PFOS in Rat Serum Samples from Study FACT Tox-108 (continued)

<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL)

RAT NUMBER (RAT NUMBER WITH A "P" INDICATES A PUP SAMPLE)	PFOS CONCENTRATION (µg/mL)
18959	59.2
18960	78.0
18960P	93.6
18961	69.1
18961P	47.6
18963	83.6
18964	85.6
18964P	92.9
18965	75.9
18967	74.2
18968	80.9
18969	78.6
18970	99.3
18970P	96.9
18971	218
18972	66.9
18972P	58.2
18973	81.9
18973P	79.5
18974	172
18976	77.9
18977	124

Table 2. Repeat Analysis of Rat Serum Samples from Study FACT Tox-108

<ULQ: Greater than the Upper Limit of Quantitation (20.0µg/mL)

Rat Number (Rat Number with a "P" indicates a pup sample)	Treatment	Time	Original Concentration (µg/mL)	Original Curve Number	Reason for Reassay	Reassay Conc. (µg/mL)	Reassay Curve Number	Reported Conc. (ug/mL)	Reason for Reported Conc.
18903P	0	Day 14	>ULQ-28.5284>(20)	3	1	35.7	5	35.7	1
18907P	0	Day 14	>ULQ-30.8172>(20)	3	1	33.5	5	33.5	1
18924P	0	Day 14	>ULQ-27.5496>(20)	3	1	30.7	5	30.7	1
18934P	0	Day 14	>ULQ-36.8737>(20)	3	1	34.1	5	34.1	1
18971	1	Day 14	>ULQ-219.5172>(200)	1	2	218	3	218	2

Reasons for Reassay:
1) Run 3 concentration exceeded calibration range
2) Run 1 concentration exceeded calibration range

Reasons for Reported Conc:
1) Run 5 concentration within calibration range
2) Run 3 concentration within calibration range

Table 3. Pharmacokinetic Subgroup—Concentrations of PFOS in Dam and Litter Serum Samples from Study FACT Tox-108

<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL)

Dosage Group	Dam Number	PFOS Concentration (µg/mL)	Litter Number	PFOS Concentration (µg/mL)
I	18910	<LLQ[0.0316]<(0.05)	18910P	<LLQ[0.0382]<(0.05)
I	18911	0.0798	18911P	0.123
I	18913	<LLQ[0.0341]<(0.05)	18913P	<LLQ[0.0398]<(0.05)
I	18915	<LLQ[0.0221]<(0.05)	18915P	<LLQ[0.0242]<(0.05)
I	18926	<LLQ[0.0323]<(0.05)	18926P	<LLQ[0.0229]<(0.05)
I	18927	<LLQ[0.0305]<(0.05)	18927P	<LLQ[0.0318]<(0.05)
I	18939	<LLQ[0.0311]<(0.05)	18939P	<LLQ[0.0308]<(0.05)
I	18940	<LLQ[0.0330]<(0.05)	18940P	<LLQ[0.0231]<(0.05)
II	18956	97.5	18956P	89.3
II	18971	218	18971P	NA

NA–Sample not assayed. Insufficient sample volume for analysis.

Table 4. PFOS Concentrations in Dam and Cross-Fostered Litter Serum Samples by Dosage Group

<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL) —: Sample not received

Dosage Group	Dam Number	PFOS Concentration (µg/mL)	Subset Number	Cross Foster Litter Number	PFOS Concentration (µg/mL)
I Untreated Dams			A Treated Litters		
I	18903	4.80	A	18976P	—
I	18907	1.35	A	18963P	—
I	18908	2.41	A	18953P	53.8
I	18909	1.06	A	18961P	47.6
I	18918	<LLQ[0.0323]<(0.05)	A	18946P	—
I	18920	1.96	A	18952P	48.3
I	18924	2.72	A	18972P	58.2
I	18929	2.40	A	18950P	—
I	18934	1.25	A	18959P	—
I	18936	<LLQ[0.0302]<(0.05)	A	18977P	—
I	18937	1.80	A	18958P	56.2
I	18941	5.34	A	18951P	59.2
I	18942	1.12	A	18965P	—
I Untreated Dams			B Untreated Litters		
I	18901	0.0507	B	18931P	<LLQ[0.0196]<(0.05)
I	18904	<LLQ[0.0462]<(0.05)	B	18932P	—
I	18906	<LLQ[0.0329]<(0.05)	B	18938P	—
I	18912	<LLQ[0.0376]<(0.05)	B	18935P	<LLQ[0.0206]<(0.05)
I	18917	<LLQ[0.0355]<(0.05)	B	18922P	<LLQ[0.0367]<(0.05)
I	18919	<LLQ[0.0340]<(0.05)	B	18923P	<LLQ[0.0331]<(0.05)
I	18922	<LLQ[0.0412]<(0.05)	B	18917P	<LLQ[0.0221]<(0.05)
I	18923	<LLQ[0.0291]<(0.05)	B	18919P	—
I	18931	<LLQ[0.0382]<(0.05)	B	18901P	<LLQ[0.0323]<(0.05)
I	18932	<LLQ[0.0399]<(0.05)	B	18904P	—
I	18935	<LLQ[0.0316]<(0.05)	B	18912P	—
I	18938	<LLQ[0.0359]<(0.05)	B	18906P	—
II Treated Dams			C Treated Litters		
II	18943	65.1	C	18968P	—
II	18947	86.9	C	18973P	79.5
II	18948	96.1	C	18970P	96.9
II	18957	69.1	C	18969P	—
II	18960	78.0	C	18974P	—
II	18964	85.6	C	18967P	—
II	18967	74.2	C	18964P	92.9
II	18968	80.9	C	18943P	82.2
II	18969	78.6	C	18957P	93.2
II	18970	99.3	C	18948P	—
II	18973	81.9	C	18947P	—
II	18974	172	C	18960P	93.6
II Treated Dams			D Untreated Litters		
II	18946	157	D	18918P	<LLQ[0.0247]<(0.05)
II	18950	66.4	D	18929P	—
II	18951	72.4	D	18941P	—
II	18952	90.8	D	18920P	—
II	18953	67.0	D	18908P	—
II	18958	68.3	D	18937P	—
II	18959	59.2	D	18934P	34.1
II	18961	69.1	D	18909P	—
II	18963	83.6	D	18907P	33.5
II	18965	75.9	D	18942P	—
II	18972	66.9	D	18924P	30.7
II	18976	77.9	D	18903P	35.7
II	18977	124	D	18936P	<LLQ[0.0230]<(0.05)

ATTACHMENT F
ANALYTICAL REPORTS FROM CONTRIBUTING LABORATORIES

ADVANCED
BIOANALYTICAL
SERVICES, INC.

BIOANALYTICAL REPORT

Title:	Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-108 Using Turbo Ion Spray LC/MS
Date:	27 August 1999
Authors:	David J. Anderson, M.S. Amie J. Prince, B.S. Holly D. Ross, M.S.
Prepared For:	3M Environmental Technology and Safety Services St. Paul, MN 55133-3331
ABS Report No.:	99ADJA01.MI.DOC
Study Protocol:	FACT-TOX-108
Number of Pages:	16

Summary and Conclusions

The concentration of perfluorooctanesulfonate (PFOS) was determined in rat serum samples collected during the study #FACT-TOX-108 using a sensitive, specific, accurate, and reproducible analytical method developed by Advanced BioAnalytical Services, Inc., Ithaca, NY.

Rat serum samples (50 µL) were extracted by a liquid-liquid extraction procedure to isolate PFOS and the internal standard, 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS). Following evaporation and reconstitution, sample extracts were analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode.

All samples were successfully analyzed within four runs. The lower limit of quantitation was 0.05 µg/mL for PFOS. The precision of this assay (RSD) as determined from the analysis of quality control samples for PFOS was ≤4.01%. The precision of this assay (RSD), as determined from the calibration standards for PFOS was ≤5.83%. The relative error (RE) of the assay, as determined from the analysis of the quality control samples ranged from -7.32 to 10.1%. The RE of the assay, as determined from the analysis of calibration standards ranged from -5.86 to 11.0%.

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SIGNATURE PAGE

Title: Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-108 Using Turbo Ion Spray LC/MS

ABS Report No.: 99ADJA01.MI.DOC

Reported by: _____ 27 August 1999
David J. Anderson, M.S. — Date
Research Scientist

Reviewed by: _____ 27 Aug 99
Kathleen Cormack, B.S. — Date
Associate Auditor

Authorized for Release by: _____ 27. AUG. 99
John R. Perkins, Ph.D. — Date
Assistant Scientific Director

QAU STATEMENT

Periodic inspections of the bioanalytical portion of study #FACT-TOX-108 were conducted by the Quality Assurance Unit of Advanced BioAnalytical Services (ABS) for compliance with EPA GLP regulations (40 CFR Part 792).

The study was inspected on the following dates:

22 June 1999; 1, 2, 7, July 1999; 12 August 1999.

Results of the inspection were reported to ABS Management and Project Team Leader on:

22 June 1999; 1, 2, 7, July 1999; 12 August 1999.

Results of the inspections were reported to the Study Director on 12 August 1999.

Based on the inspections and the data reviewed, this report is a complete and accurate representation of the data.

Kathleen Cormack 27Aug99

Kathleen Cormack, B.S. Date
Associate Auditor

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1. INTRODUCTION

1.1. Study Description and Objective

The objective of this study was to determine the concentrations of perfluorooctanesulfonate (PFOS) in rat serum samples collected during the study #FACT-TOX-108, "Oral (Gavage) Cross-Fostering Study of PFOS in Rats." Rat serum samples were analyzed using a liquid-liquid extraction and turbo ion spray liquid chromatography/mass spectrometry (LC/MS) assay.

2. METHODS

2.1. Analytical Procedure(s)

PFOS concentrations were determined in rat serum according to the validated LC/MS method (1). Serum samples (50 µL) were extracted by a liquid-liquid extraction procedure using ethyl acetate to isolate PFOS and the internal standard (IS), 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS) from rat serum. Following evaporation to dryness and subsequent reconstitution, sample extracts were separated by reversed-phase chromatography on a 2.1 x 50 mm (5 µm) Betasil™ C_{18} column (Keystone Scientific, Inc., Bellefonte, PA) with an initial mobile phase of 55% Eluent A (10:90 methanol:2 mM ammonium acetate) and 45% Eluent B (90:10 methanol:2 mM ammonium acetate). PFOS concentrations were determined by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in selected ion monitoring (SIM) mode. The negative ions monitored were:

m/z = 499.0 for PFOS $[M-K]^-$
m/z = 427.0 for Tetra-H-PFOS $[M-H]^-$

Study sample concentrations were determined from a weighted ($1/y^2$), quadratic regression of peak area ratios (peak area of PFOS/peak area of Tetra-H-PFOS) versus nominal concentration of ten calibration standards. The nominal concentrations of the calibration standards were 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 8, 16, and 20 µg/mL PFOS.

The lower limit of quantitation (LLQ) for this assay was determined to be 0.05 µg/mL PFOS. Quality control (QC) serum samples at three different concentrations (0.2, 6, and 18 µg/mL PFOS) were analyzed with each assay batch in replicates of four. Dilution QC samples (QC4, 100 µg/mL) were prepared at each dilution that was used during a particular assay and were analyzed with each assay batch in replicates of four as needed.

The acceptance criteria for calibration standards stipulated that the back-calculated concentrations of at least three-fourths of the individual calibration standard replicates must not deviate more than ±15% from their nominal concentration (except at the LLQ). At least one duplicate at the LLQ must exhibit a deviation within ±20% of the nominal

concentration . The coefficient of determination (r^2) must be ≥0.98. The acceptance criteria followed are in accordance with ABS Standard Operating Procedures.

The acceptance criteria for the quality control samples stipulated that at least two-thirds of the individual QC sample replicates must not deviate more than ±15% from their nominal concentrations. At least one replicate at each QC concentration must exhibit a deviation within ±15%.

A summary of the sample and assay information for study #FACT-TOX-108 is given in Table 1.

2.2. Assay Site

All samples were analyzed at Advanced BioAnalytical Services, Inc., Ithaca, NY.

2.3. Data Processing

The data were collected using turbo ion spray LC/MS selected ion monitoring (SIM) in the negative ion mode. Peak areas were integrated by the PE SCIEX program MacQuan, version 1.4, residing on a Macintosh computer. Following peak area integration, the results tables from MacQuan were saved as text files and uploaded to the ABS file server where a weighted ($1/y^2$) quadratic regression was performed using the software package Watson (v 5.3.1.01 PSS, Inc., Wayne, PA 19087). Calculations were performed on unrounded numbers. All calibration standard and QC results were rounded to no less than three significant figures before reporting.

3. RESULTS

3.1. Assay Performance

The performance of the assay for PFOS as determined from the analysis of daily quality control samples is documented in Table 2. The inter-assay precision (RSD) of quality control samples ranged from 1.57 to 4.01% for PFOS. There was no marked inaccuracy in the results from these quality control samples; the relative error (RE) ranged from -7.32 to 10.1%.

The performance of daily calibration curves is documented in Table 3. The inter-assay precision (RSD) of the standards ranged from 1.11 to 5.83% for PFOS. There was no marked inaccuracy in the results from these standards; the relative error (RE) ranged between -5.86 to 11.0%.

The slope, y-intercept, and coefficient of determination (r^2) for each analytical run are presented in Table 4. The r^2 values ranged from 0.9954 to 0.9982 for PFOS in rat serum.

All ABS personnel assigned to analyze samples for this project were required to successfully extract quality control (QC) samples and calibration standards. Results of the extraction were reviewed by a trained analyst against the pre-defined acceptance criteria for this assay. Results are maintained in the study records of ABS.

3.2. Analytical Results for Study #FACT-TOX-108

PFOS concentrations in rat serum from Study #FACT-TOX-108 are presented in Table 5. All data were rounded to no less than three significant figures before reporting in Table 5. Study samples requiring repeat analysis for Study #FACT-TOX-108 are presented in Table 6.

4. SUMMARY AND CONCLUSIONS

The concentration of PFOS was determined in rat serum samples collected during the study #FACT-TOX-108.

PFOS was isolated from serum by a liquid-liquid extraction procedure and determined by LC/MS.

The quality of the determinations was satisfactory throughout. The LLQ was 0.05 µg/mL PFOS. The precision of the assay, as determined from the analysis of quality control samples was ≤4.01% for PFOS.

5. DATA RETRIEVAL

The calculated concentration data from the analysis of rat serum samples for Study #FACT-TOX-108 are maintained on file in the archives of the Advanced BioAnalytical Services, Inc., Ithaca, NY in ABS Notebook 2319.

6. REFERENCES

1. Advanced BioAnalytical Services Validation Report 99VDJA01.MI.DOC. Method Validation for the Quantitation of Perfluorooctanesulfonate (PFOS) in Rat Serum by Turbo Ion Spray LC/MS. David J. Anderson, M.S. Amie J. Prince, B.S., and Holly D. Ross, M.S. 26 August 1999.

7. TABLES

Table 1: Summary of Sample and Assay Information for Protocol FACT-TOX-108

Species/Matrix:	Rat/Serum
Sample Collection and Storage Information:	
Anticoagulant/Stabilizer:	None
Reported Sample Collection Dates:	7 January 1999 to 29 January 1999
Dates Received at ABS:	16 June 1999
Storage Temperature at ABS:	–20 °C
Assay Information:	
Assay Period:	22 June 1999 to 28 June 1999
Analyte:	**Potassium perfluorooctanesulfonate (PFOS)**
Analytical Standard:	PFOS
Lot No:	171
Source:	3M
Internal Standard:	1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS)
Lot No:	59909
Source:	3M
Calibration Range:	
Regression Method:	quadratic
Weighting Factor:	$1/y^2$
Lower Limit of Quantitation:	0.05 µg/mL
Upper Limit of Quantitation:	20 µg/mL

Table 2: Inter-Assay Precision and Accuracy for PFOS Quality Control Samples in Rat Serum for Study #FACT-TOX-108

	PFOS Concentration (µg/mL)				
Run No.	QC1 0.2	QC2 6	QC3 18	QC4 100 (1 in 10 Dilution)	QC4 100 (1 in 20 Dilution)
1	0.187	6.19	17.5	110	NA
	0.192	6.22	17.3	109	NA
	0.191	6.22	17.5	110	NA
	0.182	6.14	17.3	108	NA
2	0.191	6.13	16.9	NA	NA
	0.191	5.91	16.8	NA	NA
	0.190	6.20	16.5	NA	NA
	0.192	6.19	16.7	NA	NA
3	0.172	6.02	17.0	114	107
	0.174	6.22	16.7	111	106
	0.175	5.94	17.0	110	100
	0.177	6.08	17.1	109	103
5	0.184	6.37	17.3	109	NA
	0.187	6.36	17.5	113	NA
	0.197	6.39	17.5	111	NA
	0.183	6.33	17.1	109	NA
Mean	0.185	6.18	17.1	110	104
RSD (%)	4.01	2.33	1.92	1.57	2.92
RE	-7.32	3.04	-5.00	10.1	3.97

RSD = (SD/Mean) x 100%
RE = [(Mean-Nominal)/Nominal] x 100%
NA: Not Applicable.

Table 3: Inter-Assay Precision and Accuracy for PFOS Calibration Standards in Rat Serum for Study #FACT-TOX-108

	PFOS Concentration (µg/mL)									
Run No.	STD1 0.05	STD2 0.1	STD3 0.25	STD4 0.5	STD5 1	STD6 2	STD7 4	STD8 8	STD9 16	STD10 20
1	0.0479	0.105	0.240	0.488	0.891	2.03	4.24	7.64	15.4	20.5
	0.0490	0.112	0.258	0.509	0.935	2.12	4.51	8.03	16.1	20.0
2	0.0508	0.102	0.245	0.488	0.973	2.07	4.37	7.97	15.4	19.8
	0.0503	0.0985	0.240	0.483	0.970	2.05	4.41	7.93	15.9	20.4
3	0.0474	0.105	0.237	0.476	0.944	2.12	4.52	7.91	15.6	20.1
	0.0513	0.109	0.245	0.479	0.934	2.07	4.54	7.84	15.8	20.0
5	0.0498	0.0936	0.231	0.483	0.952	2.19	4.45	7.79	15.8	19.9
	0.0553	0.0982	0.244	0.507	0.933	2.21	4.47	7.89	15.6	20.1
Mean	0.0502	0.103	0.242	0.489	0.941	2.11	4.44	7.88	15.7	20.1
RSD (%)	4.90	5.83	3.20	2.54	2.71	3.09	2.23	1.52	1.62	1.11
RE	0.450	2.82	-3.04	-2.21	-5.86	5.34	11.0	-1.55	-1.86	0.446

RSD = (SD/Mean) x 100%
RE = [(Mean-Nominal)/Nominal] x 100%

Table 4: Calibration Curve Statistics for the Determination of PFOS in Rat Serum for Study #FACT-TOX-108

Run No.	Quadratic Coefficient (A)	Linear Coefficient (B)	Intercept (C)	Coefficient of Determination (r^2)
1	-0.0007277	0.1225	0.001121	0.9959
2	-0.0005896	0.1392	0.0009484	0.9982
3	-0.0006758	0.1401	0.001454	0.9959
5	-0.0004928	0.1345	0.001190	0.9954
Mean	-0.0006215	0.1341	0.001178	0.9964

$y = Ax^2 + Bx + C$, weighted $1/y^2$

Table 5: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-108

Rat Number*	PFOS Concentration (µg/mL)
18901	0.0507
18901P	<LLQ[0.0323]<(0.05)
18903	4.80
18903P	35.7
18904	<LLQ[0.0462]<(0.05)
18906	<LLQ[0.0329]<(0.05)
18907	1.35
18907P	33.5
18908	2.41
18909	1.06
18910	<LLQ[0.0316]<(0.05)
18910P	<LLQ[0.0382]<(0.05)
18911	0.0798
18911P	0.123
18912	<LLQ[0.0376]<(0.05)
18913	<LLQ[0.0341]<(0.05)
18913P	<LLQ[0.0398]<(0.05)
18915	<LLQ[0.0221]<(0.05)
18915P	<LLQ[0.0242]<(0.05)
18917	<LLQ[0.0355]<(0.05)
18917P	<LLQ[0.0221]<(0.05)
18918	<LLQ[0.0323]<(0.05)
18918P	<LLQ[0.0247]<(0.05)
18919	<LLQ[0.0340]<(0.05)
18920	1.96
18922	<LLQ[0.0412]<(0.05)
18922P	<LLQ[0.0367]<(0.05)
18923	<LLQ[0.0291]<(0.05)
18923P	<LLQ[0.0331]<(0.05)
18924	2.72
18924P	30.7
18926	<LLQ[0.0323]<(0.05)
18926P	<LLQ[0.0229]<(0.05)
18927	<LLQ[0.0305]<(0.05)
18927P	<LLQ[0.0318]<(0.05)
18929	2.40

*Rat Number with a "P" indicates a pup sample.
<LLQ: Less than the Lower Limit of Quantitation (0.05 µg/mL)

Table 5: **(Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-108**

Rat Number*	PFOS Concentration (µg/mL)
18931	<LLQ[0.0382]<(0.05)
18931P	<LLQ[0.0196]<(0.05)
18932	<LLQ[0.0399]<(0.05)
18934	1.25
18934P	34.1
18935	<LLQ[0.0316]<(0.05)
18935P	<LLQ[0.0206]<(0.05)
18936	<LLQ[0.0302]<(0.05)
18936P	<LLQ[0.0230]<(0.05)
18937	1.80
18938	<LLQ[0.0359]<(0.05)
18939	<LLQ[0.0311]<(0.05)
18939P	<LLQ[0.0308]<(0.05)
18940	<LLQ[0.0330]<(0.05)
18940P	<LLQ[0.0231]<(0.05)
18941	5.34
18942	1.12
18943	65.1
18943P	82.2
18946	157
18947	86.9
18948	96.1
18950	66.4
18951	72.4
18951P	59.2
18952	90.8
18952P	48.3
18953	67.0
18953P	53.8
18956	97.5
18956P	89.3
18957	69.1
18957P	93.2
18958	68.3
18958P	56.2

*Rat Number with a "P" indicates a pup sample.
<LLQ: Less than the Lower Limit of Quantitation (0.05 µg/mL)

Table 5: **(Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-108**

Rat Number*	PFOS Concentration (µg/mL)
18959	59.2
18960	78.0
18960P	93.6
18961	69.1
18961P	47.6
18963	83.6
18964	85.6
18964P	92.9
18965	75.9
18967	74.2
18968	80.9
18969	78.6
18970	99.3
18970P	96.9
18971	218
18972	66.9
18972P	58.2
18973	81.9
18973P	79.5
18974	172
18976	77.9
18977	124

*Rat Number with a "P" indicates a pup sample.

Table 6: Repeat Analysis of Rat Serum Samples from Study #FACT-TOX-108

Rat Number*	Treatment	Time	Original Conc. ug/ml	Original Curve Number	Reason for Reassay	Reassay Conc. ug/ml	Reassay Curve Number	Reported Conc. ug/ml	Reason for Reported Conc.
18903P	0	Day 14	>ULQ-28.5284>(20)	3	1	35.7	5	35.7	1
18907P	0	Day 14	>ULQ-30.8172>(20)	3	1	33.5	5	33.5	1
18924P	0	Day 14	>ULQ-27.5496>(20)	3	1	30.7	5	30.7	1
18934P	0	Day 14	>ULQ-36.8737>(20)	3	1	34.1	5	34.1	1
18971	1	Day 14	>ULQ-219.5172>(200)	1	2	218	3	218	2

>ULQ: Greater than the Upper Limit of Quantitation.
*Rat Number with a "P" indicates a pup sample.

REASONS FOR REASSAY:
1). Run 3 concentration exceeded calibration range.
2). Run 1 concentration exceeded calibration range.
REASONS FOR REPORTED CONC:
1). Run 5 concentration within calibration range.
2). Run 3 concentration within calibration range.

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SERVICES, INC.

ADDENDUM TO THE BIOANALYTICAL REPORT

Title:	**Addendum - Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-108 Using Turbo Ion Spray LC/MS**
Date:	**13 October 1999**
Addendum Report:	**99ADJA01.MI.ADD.DOC**
Original Report:	**99ADJA01.MI.DOC**
Authors:	**David J. Anderson, M.S.** **Amie J. Prince, B.S.** **Holly D. Ross, M.S.**
Prepared For:	**3M Environmental Technology and Safety Services** **St. Paul, MN 55133-3331**
Number of Pages:	**3**

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Title: Addendum - Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-108 Using Turbo Ion Spray LC/MS

ABS Report No.: 99ADJA01.MI.ADD.DOC

Reported by: _____ 13 OCT 99
David J. Anderson, M.S. — Date
Research Scientist

Reviewed by: Kathleen Cormack 13 Oct 99
Kathleen Cormack, B.S.. — Date
Associate Auditor

Authorized for Release by: _____ 13.OCT.99
John R. Perkins, Ph.D. — Date
Assistant Scientific Director

Accepted by: _____ 14 Oct 99
Kris Hanson, Ph.D. — Date
3M Study Director

ADDENDUM SUMMARY

ADDENDUM

Addition of Study Director signature on the Signature Page. The signature page of this addendum indicates acceptance of the original report.

REASON FOR ADDENDUM

Original GLP report requires the signature of the Study Director.

ATTACHMENT G
REPORT SIGNATURE PAGE

11-Feb-2000

Kristen J. Hansen, Ph.D., Study Director — Date

10 February 2000

Marvin T. Case, D.V.M., Ph.D., Sponsor Representative — Date

2/14/00

Dale L. Bacon, Laboratory Manager — Date

Study Title

Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage

REPORT AMENDMENT NO. 1

Amendment Date:

19 April 2000

Performing Laboratory

3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification

ET&SS LRN-U2779
ET&SS FACT Tox-108

This amendment modifies the following portion(s) of the final report:

1. ***FINAL REPORT:***
   Make the following addition to the final report text.
   ADD:
   After the final report was issued (signed and archived), it was discovered that the purity values stated for the analytical reference material and test substance were inaccurate. These values were based only on NMR analyses. Subsequent chemical characterization is occurring to determine the actual purity of theses substances (PFOS Lot 171 and 217). The final report will be amended to detail the analytical results using the purity values from the certificates of analysis when they are issued for PFOS Lot 171 and 217.
   REASON:
   To describe the changes that will be made to the final report when the certificate of analysis is issued for PFOS Lot 171 and 217.

2. ***FINAL REPORT:***
   Make the following addition to the exceptions list in the Statement of Compliance section.
   AMEND TO READ:
   - The purity and stability of the test substance and analytical reference material are unknown and was not determined prior to the initiation of this study.

   REASON:
   The Statement of Compliance section was incomplete.

3. ***FINAL REPORT:***
   The purity of the test substance is 99.28% (Lot 217), and analytical reference material is 99.49% (Lot 171) as listed in Table 2.
   AMEND TO READ:
   The purity of the test substance and analytical reference material listed in Table 2 is unknown.
   REASON:
   The purity values stated for the test substance and analytical reference material listed in Table 2 are incorrect.

Amendment Approval

Marvin T Case — 19 April 2000

Marvin T. Case, D.V.M., Ph.D., Sponsor Representative — Date

Kristen Hansen — 04/19/00

Kristen J. Hansen, Ph.D., Study Director — Date