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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

FINAL REPORT DATE: 10 JUNE 1999

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TITLE: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS

ARGUS RESEARCH LABORATORIES, INC.
PROTOCOL NUMBER: 418-008
SPONSOR'S STUDY NUMBER: 6295.9

I. SUMMARY AND CONCLUSION

A. Methods[a]

Text Figure 1 provides a schematic of the study design.

A.1. Fo Generation Rats/F1 Generation Litters:

One hundred seventy-five male and female Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats were assigned to five dosage groups (Groups I through V), 35 rats per sex per dosage group. The rats were administered the test article, PFOS, or the vehicle, 0.5% Tween 80®, orally (via gavage). The dosages were 0 (Vehicle), 0.1, 0.4, 1.6 and 3.2 mg/kg/day. The male rats were dosed once daily beginning 42 days before cohabitation and continuing through the day before sacrifice. The female rats were dosed once daily beginning 42 days before cohabitation and continuing through DG[b] 9 (rats assigned to Caesarean-sectioning), DG 24 (rats assigned to natural delivery that did not deliver a litter), or DL[c] 20 (rats that did deliver a litter). The dosage volume was 5 mL/kg.

All Fo generation rats were observed for viability at least twice daily during the study and for clinical signs of effects of the test article twice daily during the dosage period. Body weights and feed consumption values for male rats were recorded weekly during the dosage period and at sacrifice. Body weights for female rats were recorded weekly to cohabitation, daily during the gestation period, on DLs 1, 4, 7, 10 and 14 (rats assigned to natural delivery) and at sacrifice. Feed consumption values were recorded weekly to cohabitation, daily during the gestation period and on DLs 1, 4, 7, 10 and 14 (rats assigned to natural delivery).

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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 F (PROTOCOL AND AMENDMENTS).
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.

The first ten female rats per dosage group with a confirmed date of mating were assigned to Caesarean-sectioning on DG 10. The remaining female rats were permitted to naturally deliver litters. These rats were evaluated for clinical observations during parturition, duration of gestation, litter size and pup viability at birth. Maternal behavior of the dams was evaluated daily when the pups were examined during the 21-day postpartum period.

Each litter was evaluated for viability at least twice each day during the 21-day postpartum period. Pups in each litter were counted once daily. Physical signs in the pups were recorded once daily for 21 days postpartum. Pup body weights and observed nursing behavior were recorded on DLs 1 (birth), 4, 7, 14 and 21. Surface righting reflex, pinna unfolding, eye opening, acoustic startle response and air righting reflex were monitored during the 21-day postpartum period until all pups in the litter reached the criterion for the specific test. Pupil constriction was evaluated once on DL 21. On DL 4, a table of random units was used to cull litters to four male and four female pups, where possible. On DL 21, a table of random units was used to select two male and two female pups from each litter from Groups I, II and III for continued evaluation.

Fo generation male rats were sacrificed after completion of the cohabitation period and necropsied; gross lesions were retained. The testes, epididymides, prostate and seminal vesicles (with and without fluid) were excised, individually weighed and retained.

Fo generation female rats assigned to Caesarean-sectioning were sacrificed on DG 10 and necropsied; pregnancy status was confirmed. Ovaries and gross lesions were retained. The rats were examined for the number and distribution of corpora lutea in each ovary and implantation sites, and viable and nonviable embryos. Embryos were discarded after examination.

Fo generation female rats assigned to natural delivery were sacrificed on DL 21 and necropsied. Ovaries and gross lesions were retained. The number and distribution of implantation sites was recorded.

At scheduled sacrifice after completion of the cohabitation period (male rats that sired litters of dams allowed to naturally deliver a litter) and on DL 21 (female rats allowed to naturally deliver a litter), blood samples (approximately 4 mL per rat) were collected from the inferior vena cava from five male and five female rats per dosage group and shipped to the Sponsor for pharmacokinetic analysis.

Pups not selected for continued evaluation on DL 4 were sacrificed and necropsied. The stomach contents (milk curd) were collected from all culled pups from five of the largest litters in Groups I, II and III, frozen and shipped to the Sponsor for analysis.

The liver from each rat was excised, weighed, and a sample section (lateral lobe) was frozen and shipped to the Sponsor for analysis. The livers of the pups from the litters of the five dams in Groups I through IV selected for pharmacokinetic sample collection were excised, pooled per litter, frozen and shipped to the Sponsor for analysis. Dams in the 3.2 mg/kg/day dosage group (Group V) did not have surviving pups on DL 21.

A.2. F1 Generation Rats/F2 Generation Litters:

Only the 0.1 and 0.4 mg/kg/day dosage groups were continued into the second generation because of the excessive toxicity seen in the 1.6 and 3.2 mg/kg/day F1 generation pups.

There were 150 male and female F1 generation rats in the three dosage groups (Groups I through III), 25 rats per sex per dosage group. F1 generation male and female rats were given appropriate dosages of the test article orally (gavage) beginning on DL 22 and continuing through the day before sacrifice.

Beginning at 24 days of age, one male rat and one female rat from each litter in each dosage group were tested in a passive avoidance paradigm. Female rats were evaluated for the age of vaginal patency beginning on DL 28. Male rats were evaluated for the age of preputial separation beginning on DL 34. On postpartum day 70, one male rat and one female rat from each litter were evaluated in a water-filled M-maze. On approximately DL 90, rats within each dosage group were assigned to cohabitation.

F1 generation male rats were sacrificed after completion of the cohabitation period and necropsied, as previously described for the Fo generation male rats. All F1 generation female rats were permitted to naturally deliver litters. All dams that delivered litters were sacrificed on DL 21 and necropsied, as previously described for the Fo generation female rats.

On DL 21, all F2 generation pups were sacrificed and examined for gross lesions. Necropsy procedures were the same as those described for the F1 generation pups.

Text Figure 1

Protocol 418-008: Combined Oral (Gavage) Fertility, Development and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats

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B. Results

B.1. Fo Generation Male Rats:

No Fo generation male rats died during this study as a result of treatment with PFOS. All clinical observations (other than normal) were considered unrelated to the test article and not signs of compound toxicity.

Groups administered 0.4 mg/kg/day and higher dosages of the test article had reduced body weight gains for the entire study.

Absolute and relative feed consumption values were reduced in the 1.6 and 3.2 mg/kg/day dosage groups for the entire precohabitation period. After the cohabitation period, absolute feed consumption values were significantly reduced in the 0.4 and 1.6 mg/kg/day dosage groups.

Dosages of the test article as high as 3.2 mg/kg/day did not affect any mating and fertility parameters evaluated.

A significantly increased number of male rats in the 3.2 mg/kg/day dosage group had a light brown or brown liver, an observation attributed to effects of PFOS.

The 1.6 and 3.2 mg/kg/day dosage groups had significantly reduced terminal body weights. The absolute weights of the seminal vesicles with fluid and the prostate were significantly reduced in the 3.2 mg/kg/day dosage group.

B.2. Fo Generation Female Rats/F1 Generation Litters:

No Fo generation female rats died during this study as a result of treatment with PFOS.

Observations of localized alopecia were increased during the precohabitation, gestation and lactation periods in the 0.4, 1.6 and 3.2 mg/kg/day dosage groups.

Groups administered 1.6 mg/kg/day and higher dosages of the test article had significantly reduced body weight gains for the entire precohabitation period. The 1.6 and 3.2 mg/kg/day dosages of PFOS continued to reduce body weights and body weight gains during gestation. The 3.2 mg/kg/day dosage group had significantly reduced body weight on DL 1. Body weight gains tended to be reduced in the 0.4 mg/kg/day dosage group on DLs 1 to 4, when significant weight loss occurred in the 1.6 mg/kg/day dosage group (the 3.2 mg/kg/day dosage group was precluded from further evaluation because all pups died before DL 2).

Absolute and relative feed consumption values were reduced in the 1.6 and 3.2 mg/kg/day dosage groups during the precohabitation period. The 1.6 and 3.2 mg/kg/day dosage groups continued to have reduced absolute and relative feed consumption values early in the gestation period. Absolute and relative maternal feed consumption values tended to be reduced in the 0.4 mg/kg/day dosage group and were significantly reduced in the 1.6 mg/kg/day dosage group for the entire lactation period, and at most intervals within this period. The 3.2 mg/kg/day dosage group was precluded from evaluation because there were no surviving pups after DL 1.

Dosages of the test article as high as 3.2 mg/kg/day did not affect estrous cycling in the 15 rats per dosage group that were evaluated. Mating and fertility parameters were unaffected by the 3.2 mg/kg/day dosage of PFOS.

All necropsy observations were considered unrelated to treatment.

There were no biologically important or statistically significant differences in the litter averages for corpora lutea, implantations, viable embryos or nonviable embryos at Caesarean-sectioning on DG 10.

The duration of gestation was significantly reduced in the 3.2 mg/kg/day dosage group, an observation associated with preimplantation loss [the average number of implantation sites per dam was significantly reduced, resulting in a significantly reduced litter size]. Pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dosage groups. Reflecting these effects, the viability and lactation indices were significantly reduced in the 1.6 and 3.2 mg/kg/day dosage groups, the lactation index was also significantly reduced in the 1.6 mg/kg/day dosage group, as also were the averages for surviving pups in the 1.6 mg/kg/day dosage group beginning on DL 4 postculling, and in the 3.2 mg/kg/day dosage group on DL 1.

A dosage-dependent pattern of reduced pup body weight was evident in each group administered the test article. The 0.1 mg/kg/day dosage group tended to have reduced pup weights on DL 4 (pre- and postculling), compared to the control group value. The 0.4 mg/kg/day dosage group tended to have reduced pup body weights on DLs 1 through 4 (postculling), compared to the control group value. However, none of the differences in the 0.1 and 0.4 mg/kg/day dosage groups were statistically significant and may represent normal biological variations. The 1.6 mg/kg/day dosage group had significantly reduced pup body weights on all weighing days. The 3.2 mg/kg/day dosage group had a significantly reduced pup body weight on DL 1 (no pups survived to subsequent scheduled weighings).

Clinical and necropsy observations associated with reduced pup viability and potential reduction in maternal care occurred in the 3.2 mg/kg/day dosage group.

Adverse clinical observations include three litters with pups that were not nursing (two of these litters had pups from which the placenta had not been removed, and one had pups that were not nested). Apparent maternal cannibalization was also evident in this dosage group (missing tail in one pup and a cannibalized forelimb in another pup). It was not possible to determine whether these occurred before or after these pups had died.

Necropsy observations in pups that were stillborn or found dead indicated many pups with no milk in the stomach in the 1.6 and 3.2 mg/kg/day dosage group pups. The 3.2 mg/kg/day dosage group also had evidence of increased maternal cannibalization at necropsy of stillborn and found dead pups. Nine of these pups had missing hindlimbs and/or portion of the tail.

Reversible delays in reflex and physical development that are highly correlated with body weight occurred in the 0.4 and 1.6 mg/kg/day dosage groups. Surface righting was delayed in the 1.6 and 3.2 mg/kg/day dosage groups. The time of development for pinna unfolding, eye opening, acoustic startle reflex and ability to air right were delayed in the 1.6 mg/kg/day dosage group (no 3.2 mg/kg/day dosage group pups were evaluated for this parameter). All live pups in the 0 (Vehicle), 0.1, 0.4 and 1.6 mg/kg/day dosage groups had the pupil constriction response when testing was done on DL 21 (end of lactation).

Upon weaning of the litters (DL 21), a decision was made regarding the F1 generation pups in the 1.6 mg/kg/day dosage group. The 1.6 mg/kg/day dosage group pups were small (they had gained 20% less weight during lactation than the control pups) and in the opinion of the attending laboratory veterinarian, the rats were not suitable for further experimentation. It had been determined that the dosage of 1.6 mg/kg/day was a level which produced compound toxicity. Continued dosing of 1.6 mg/kg/day most likely would not result in any additional information and would subject the pups to additional distress. Therefore, consultation with the Sponsor lead to the decision not to continue the 1.6 mg/kg/day dosage group F1 generation pups. Only the 0 (Vehicle), 0.1 and 0.4 mg/kg/day dosage group pups were continued to a second generation.

B.3. <u>F1 Generation Male Rats</u>:

No deaths in the F1 generation male rats at 0.1 and 0.4 mg/kg/day dosages were attributed to PFOS. All clinical observations were considered unrelated to the test article.

The 0.1 and 0.4 mg/kg/day dosage groups tended to weigh less than the control group on day 1 postweaning and generally gained one or two grams less body weight per week than the control group rats. Reflecting this pattern of weight gain, weight gains in the 0.1 and 0.4 mg/kg/day dosage groups for the precohabitation period were 98.4% and 97.6% of the control group value,

respectively. Body weight gains in the 0.1 and 0.4 mg/kg/day dosage groups for day 1 to termination were 98.5% and 96.6% of the control group value, respectively. However, none of these body weight gain differences were statistically significant from control values.

Absolute feed consumption values for the 0.1 and 0.4 mg/kg/day dosage groups were significantly reduced on postweaning days 1 through 8. Relative feed consumption values tended to be reduced in the 0.1 and 0.4 mg/kg/day dosage groups during the first week of intubation but none were statistically significant.

Dosages of the test article as high as 0.4 mg/kg/day did not affect the average day of preputial separation in the F1 generation male 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 male 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 male rats.

All necropsy observations in the F1 generation male rats were considered unrelated to the test article because. No statistically significant differences occurred in the absolute or relative values for the weights of the right or left testis, seminal vesicles, right epididymis or prostate. Terminal body weights tended to be reduced in the 0.1 and 0.4 mg/kg/day dosage groups but were not statistically significant from control values.

B.4. **F1 Generation Female Rats/F2 Generation Litters:**

No deaths in the F1 generation female rats were attributed to PFOS. All adverse clinical observations that occurred during the precohabitation, gestation and lactation periods were considered unrelated to the test article.

Body weights tended to be reduced in the 0.4 mg/kg/day dosage group on day 1 postweaning. Maternal body weights and body weight gains during the gestation period were unaffected by dosages of the test article as high as 0.4 mg/kg/day. Significant maternal body weight loss occurred on DLs 1 to 4 in the 0.4 mg/kg/day dosage group.

The 0.4 mg/kg/day dosage of the test article was associated with a significant reduction in absolute feed consumption and a tendency for reduced relative feed consumption on days 1 to 8 postweaning. Absolute and relative feed consumption values during the gestation period were unaffected by dosages of the test article as high as 0.4 mg/kg/day. The absolute and relative maternal

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. As occurred in the previous generation, there was a tendency for reduced pup viability in the 0.4 mg/kg/day dosage group. The number of dams with stillborn pups was increased, and the number of pup deaths after DL 2 also were increased in this dosage group. As also occurred in the previous generation, pup body weights tended to be reduced in the 0.1 mg/kg/day dosage group on DL 4 (pre-and postculling) and DL 7. By DL 14, body weights were comparable to control group values. Pup body weights in the 0.4 mg/kg/day dosage group tended to be reduced, though not significantly, on DLs 4 through 21, with significant reductions present on DLs 7 and 14. 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 0.1 mg/kg/day (0.4 mg/kg/day dosage caused stillbirths and reductions in litter size, pup viability, growth and survival).

George E Deardorff 10-JUN-99

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

George E Deardorff 10-JUN-99

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

Raymond G York 10-JUN-99

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

II. DESCRIPTION OF TEST PROCEDURES

A. Conduct of Study:

A.1. Sponsor:

3M Toxicology Services, 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:

418-008

A.4. Sponsor's Study Number:

6295.9

A.5. Purpose of the Study:

The purpose of this study was to test for toxic effects/disturbances resulting from PFOS treatment of Crl:CD®BR VAF/Plus® male and female rats before cohabitation through mating, gestation and lactation. This study was designed to evaluate ICH Harmonised Tripartite Guideline stages A through F of the reproductive process and detect effects on the estrous cycle, tubal transport, implantation, gestation, parturition, lactation and maternal behavior in female rats, on the development of the offspring of the treated male and female rats, and permit detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be detected by histological examinations of male rat reproductive organs. Because manifestations of effects induced during this period may be delayed in the offspring, observations were continued through production of F2 generation litters.

A.6. Study Design:

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

A.7. Regulatory Compliance:

The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. Food and Drug Administration (FDA)[2], the Japanese Ministry of Health and Welfare (MHW)[3] and the European Economic Community (EEC)[4]. There were no significant deviations from the GLP regulations that affected the quality or integrity of the study. Quality Assurance Unit findings derived from the inspections during the conduct of this study are documented and have been provided to the Study Director and the Testing Facility Management.

A.8. Ownership of the Study:

The Sponsor owns the study. All raw data, analyses, reports and all preserved tissues are the property of the Sponsor.

A.9. Study Monitor:

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

A.10. Alternate Study Monitor:

Andrew M. Seacat, Ph.D.

A.11. Study Director:

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

A.12. Technical Performance:

John F. Barnett, B.S. (Director of Laboratory Operations)
Kristen Iandola Sherer, B.S. (Research Associate/Fetal Evaluation)
Joseph W. Lech, B.S. (Team Leader – General Laboratory)
Sharon Adamski (Laboratory Technician)

A.13. Report Preparation

Raymond G. York, Ph.D., DABT
Michelle R. Rzaca, B.S. (Study Coordinator)
Heidi M. Green, M.S. (Study Coordinator)
Erin Hagan, B.A. (Data Management Specialist)
Karen G. Parker, A.A. (Report Administrator)

A.14. Report Review:

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

A.15. Date Protocol Signed:

21 May 1998

A.16. Dates of Technical Performance:

Fo Generation Male Rats

Rat Arrival Date	12 MAY 98
Dosage Period (42 days before cohabitation, through a 14-day cohabitation period and until sacrifice)	26 MAY 98 – 30 JUL 98
Scheduled Sacrifice	31 JUL 98

Fo Generation Female Rats

Rat Arrival Date	12 MAY 98
Dosage Period – Female Rats Assigned to Caesarean-Sectioning (42 days before cohabitation and continuing through DG[a] 9)	26 MAY 98 – 17 JUL 98
Dosage Period – Female Rats Assigned to Natural Delivery [42 days before cohabitation through DG 24 (rats that did not deliver a litter) or DL[b] 20 (rats that delivered a litter)	26 MAY 98 – 30 AUG 98
Dosage Period Estrous Cycle Evaluation	09 JUN 98 – 06 JUL 98

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

(Fo Generation Female Rats Continued)

Cohabitation Period	
Male 1	06 JUL 98 PM – 13 JUL 98 AM
Male 2	13 JUL 98 PM – 20 JUL 98 AM
DG 10 Caesarean-Sectioning	17 JUL 98 – 30 JUL 98
Natural Delivery Period (DL 1)	28 JUL 98 – 10 AUG 98
DG 25 Sacrifice (rats that did not deliver a litter)	01 AUG 98 – 07 AUG 98
DL 21 Sacrifice (dams and pups not selected for continued study)	17 AUG 98 – 30 AUG 98

F1 Generation Rats

Dosage Period (Male Rats)	19 AUG 98 – 16 NOV 98
Dosage Period (Female Rats)	19 AUG 98 – 27 DEC 98
Passive Avoidance Testing	20 AUG 98 – 10 SEP 98
Watermaze Testing	07 OCT 98 – 19 OCT 98
Cohabitation Period (Initiated when the rats are approximately 90 days of age)	
Male 1	02 NOV 98 PM – 09 NOV 98 AM
Male 2	09 NOV 98 PM – 16 NOV 98 AM
Male Rats Sacrificed	17 NOV 98
Natural Delivery Period (DL 1)	24 NOV 98 – 08 DEC 98
DL 21 Sacrifice	14 DEC 98 – 28 DEC 98

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 mailing the draft final report, after which time the Sponsor will decide their final disposition. All unused test article suspensions were discarded at the Testing Facility. Unused bulk test article was returned to the Study Monitor.

B. Test Article Information:

B.1. Description:

PFOS – an off-white powder

B.2. Lot Number:

217 (Expiration Date: May 2000)

B.3. Date Received and Storage Conditions:

The test article was received on 20 May 1998, and stored at room temperature. Prepared suspensions were stored at room temperature overnight.

B.4. Special Handling Instructions:

Standard safety precautions (use of protective clothing, gloves, dust-mist respirator and safety goggles) were taken when handling the bulk test article and prepared suspensions.

B.5. Analysis of Activity:

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

C. Vehicle Information:

C.1. Description:

0.5% Tween® 80 in reverse osmosis membrane processed deionized water (R.O. deionized water).

C.2. Lot Numbers:

M03H05, K03737, M29477 and L06662

C.3. Dates Received and Storage Conditions:

The Tween® 80 was received from J.T. Baker, Phillipsburg, New Jersey, on 15 August 1997 (Lot K03737), 22 May 1998 (Lot M03H05), 17 September 1998 (Lot M29477), 3 December 1998 (Lot M03H05) and 1 September 1998 (Lot L06662), and was stored at room temperature. R.O. deionized water is available from a continuous source at the Testing Facility and is maintained at room temperature. The vehicle was prepared weekly and stored at room temperature.

C.4. Special Handling Instructions:

Standard safety precautions (use of protective clothing, gloves, dust-mist respirator, safety goggles or safety glasses and a face-shield) were taken when handling the vehicle.

C.5. Analysis of Purity:

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to be present in the vehicle that would interfere with the results of this study.

D. Test Article Preparation:

Suspensions of PFOS were prepared daily one day prior to the day of dosing at concentrations of 0, 0.02, 0.08, 0.32 and 0.64 mg/mL. The test article was considered 100% pure for the purpose of dosage calculations.

D.1 Sample Information:

Sample Type	Size	Date Retained	Shipping/Storage Conditions	Shipped To	Date Shipped
Concentration (all levels)	5 mL[a] 5 mL 5 mL 5 mL	26 MAY 98 29 JUN 98 21 DEC 98 27 DEC 98	Room temperature	Sponsor	26 MAY 98 29 JUN 98 04 JAN 98 04 JAN 98
Homogeneity	5 mL[b]	26 MAY 98	Room temperature	Sponsor	26 MAY 98
Vehicle Component Reserve Tween® 80 R.O. Water	 5 mL 5 mL	 25 MAY 98 10 SEP 98 10 NOV 98 25 MAY 98	Room temperature	 Testing Facility Archives	 09 JUL 98 01 OCT 98 25 NOV 98 09 JUL 98

a. A syringe was used to withdraw samples from each concentration during the first and sixth week of dosage administration for the Fo generation and during the first[a] and last weeks of dosage administration for the F1 generation. Each sample was divided into two aliquots (2 mL and 3 mL, respectively). One aliquot (2 mL) was shipped for analysis. The other aliquot (3 mL) was retained at the Testing Facility as a backup.

b. A syringe was used to withdraw samples from the top, middle and bottom of the highest concentration on the first day of preparation. Each sample was divided into two aliquots (2 mL and 3 mL, respectively). One aliquot (2 mL) was shipped for analysis. The other aliquot (3 mL) was retained at the Testing Facility as a backup.

Stability data for prepared formulations bracketing the range of concentrations are on file with the Sponsor. The Sponsor confirmed a 48-hour stability on the test article in 0.5% Tween® 80 solutions.

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

D.2. Analytical Results:

Results of the concentration and homogeneity 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:

Male and Female

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 has been demonstrated to be sensitive to reproductive and developmental toxins and has been widely used throughout industry for reproductive and developmental toxicity evaluations; 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:

	Male Rats	Female Rats
Number of Rats	195	205
Approximate Date of Birth	12 MAR 98	12 MAR 98
Approximate Age at Arrival	62 days	62 days
Weight (g) on the Day after Arrival	282 - 338	172 - 239
Weight (g) at Study Assignment	334 - 396	209 - 242

E.7. Method of Randomization:

E.7.a. Fo Generation Rats

Upon arrival, Fo generation rats were assigned to individual housing on the basis of computer-generated random units. After acclimation, male and female rats were selected for study on the basis of physical appearance and body weights recorded during acclimation. Rats were assigned to five dosage groups (Groups I through V), 35 rats per sex per dosage group, using a computer-generated (weight-ordered) randomization procedure.

The first ten female rats per dosage group with a confirmed mating date were assigned to Caesarean-sectioning on DG 10. The remaining rats were permitted to naturally deliver litters.

A table of random units was used to assign five rats per group to pharmacokinetic sample collection either at scheduled sacrifice after completion of the cohabitation period (male rats siring litters with dams allowed to naturally deliver a litter) or on DL 21 (female rats allowed to naturally deliver a litter).

E.7.b. F1/F2 Generation Pups

On DL 4, a table of random units was used to select the pups to be culled and litters were reduced to eight pups each. Whenever possible, the same number of male and female pups per litter were continued on study.

At weaning of the F1 generation pups on DL 21, a table of random units was used to select 25 male and 25 female pups in each of Groups I, II and III, resulting in a total of 150 F1 generation rats (75 per sex) chosen for continued evaluation. At least one male pup and one female pup per litter were selected. There were no surviving pups in Group V after DL 2; pups in Group IV were not continued on the study because of severe toxic effects in the pups (death and retarded growth) during lactation. This decision was made in consultation with the study veterinarian and the Sponsor.

E.8. System of Identification:

E.8.a. Fo Generation

Rats were permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20101). Male and female rats were assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study before administration of the first dosage of the test article.

E.8.b. F1/F2 Generation Pups and Rats

Pups were not individually identified during lactation; all parameters were evaluated in terms of the litter both before and after culling on DL 4. At weaning, each F1 generation rat selected for continued observation was identified with a Monel® self-piercing ear tag.

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 H (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].

F.3.a. Fo Generation Rats/F1 Generation Litters

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 assigned to natural delivery were individually housed in nesting boxes. Each dam and delivered litter were housed in a common nesting box during the postpartum period.

F.3.b. F1 Generation Rats/F2 Generation Litters

After weaning, the F1 generation rats were individually housed before cohabitation, housed in pairs (one male rat per female rat) during cohabitation, and individually housed after cohabitation. The same type of caging was used as described for the Fo generation rats. Beginning no later than DG 20, F1 generation female rats were individually housed in nesting boxes. 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. Bedding was changed as often as necessary to keep the rats dry and clean.

F.6. Feed:

Rats were given *ad libitum* access to Certified Rodent Diet® #5002 (PMI Nutrition International, Inc., 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 concentrations 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 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, Inc., 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.

F.10. Bedding:

Bed-o'cobs® was used as nesting material (The Andersons Industrial Products Group, Maumee, Ohio).

F.11. Bedding Analysis:

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to be present in the bedding that would interfere with the results of this study. Analyses for possible contamination are conducted annually and documented in the raw data.

G. Methods:

G.1. Dosage Administration:

Dosage Group	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Fo Generation Rats Per Sex	Number of F1 Generation Rats Per Sex
I	0 (Vehicle)	0	5	35	25
II	0.1	0.02	5	35	25
III	0.4	0.08	5	35	25
IV	1.6	0.32	5	35	-
V	3.2	0.64	5	35	-

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

G.2. Assigned Rat Numbers:

Dosage Group	Assigned Fo Generation Rat Numbers		Assigned F1 Generation Rat Numbers	
	Male	Female	Male	Female
I	8101-8135	8801-8835	13101 – 13125	13201 – 13225
II	8136-8170	8836-8870	13126 – 13150	13226 – 13250
III	8171-8205	8871-8905	13151 – 13175	13251 – 13275
IV	8206-8240	8906-8940	a	A
V	8241-8275	8941-8975	b	B

a. On DL 21, all surviving pups in the 1.6 mg/kg/day dosage group (Group IV) were sacrificed because of severe toxic effects in the pups during lactation.
b. There were no surviving pups after DL 2 in the 3.2 mg/kg/day dosage group (Group V).

G.3. Rationale for Dosage Selection:

Dosages were selected by the Sponsor on the basis of previous studies conducted with the test article.

G.4. Route of Administration:

Oral (gavage)

G.5. Rationale for Route of Administration:

The oral (gavage) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one of the possible routes of human exposure.

G.6. Frequency of Administration:

The Fo generation male rats were given appropriate dosages of the test article once daily beginning 42 days before cohabitation (which continued for a maximum of 14 days) and continuing through the day before sacrifice.

The Fo generation female rats were given the appropriate dosages of the test article beginning 42 days before cohabitation (which continued for a maximum of 14 days) and continuing through DG 9 (rats assigned to Caesarean-sectioning), DG 24 (rats assigned to natural delivery that did not deliver a litter), or DL 20 (rats that did deliver a litter). The dosage volume (5 mL/kg) was adjusted daily on the basis of the most recently recorded body weight and given at approximately the same time each day[a].

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a. See APPENDIX G, item 2.

Dams in the process of delivering pups were not dosed until completion of parturition, in order to preclude possible disruption of maternal behavior and/or cannibalization of the pups. Consequently, some dams were not administered one daily dosage during the delivery period. No dam missed more than one daily dosage.

F1 generation male and female rats were given appropriate dosages of the test article orally (gavage), beginning on day 1 postweaning (DL 22)[a] and continuing through the day before sacrifice. F2 generation pups were not directly given the test article, but may have been possibly exposed to the test article during gestation (*in utero* exposure) or via maternal milk during the lactation period.

G.7. Length of Study:

Approximately 7 months

G.8. Method of Study Performance:

G.8.a Fo Generation Rats

All Fo generation rats were observed for viability at least twice daily during all periods of the study. Rats were also observed for general appearance at least once during the acclimation period and for clinical observations of effects of the test article, abortions, premature deliveries, prior to and approximately one hour after dosage and on the day sacrificed.

Body weights for Fo generation male rats were recorded at least once during the acclimation period, weekly during the dosage period and at sacrifice. Feed consumption values for male rats were recorded weekly during the dosage period.

Body weights for Fo generation female rats were recorded at least once during the acclimation period, weekly to cohabitation, daily during the gestation period, on DLs 1, 4, 7, 10 and 14 (rats assigned to natural delivery) and at sacrifice. Feed consumption values were recorded weekly to cohabitation, daily during the gestation period and on DLs 1, 4, 7, 10 and 14 (rats assigned to natural delivery). Feed consumption values were not recorded after DL 14, when it was expected that the pups would begin to consume maternal feed.
A table of random units was used to select 15 female rats per dosage group for evaluation of estrous cycling by examination of vaginal cytology for 28 days before the start of the cohabitation period.

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a. See APPENDIX G, item 3.

Within each dosage group, consecutive order was used to assign rats to cohabitation, one male rat per female rat. The cohabitation period consisted of a maximum of 14 days. During cohabitation, all female rats were evaluated daily until observation of spermatozoa in a smear of the vaginal contents and/or a copulatory plug *in situ* (DG 0), following which they were assigned to individual housing. Female rats that did not mate within the first seven days of cohabitation were assigned alternate male rats that had mated (within the same dosage group) and remained in cohabitation for a maximum of seven additional days.

The first ten female rats per dosage group with a confirmed mating date were assigned to Caesarean-sectioning on DG 10. The female rats assigned to Caesarean-sectioning were examined for the number and distribution of corpora lutea, implantation sites and viable and nonviable fetuses. The remaining female rats were permitted to naturally deliver litters. These rats were evaluated for clinical observations during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter size (all pups delivered) 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. Each litter was subsequently examined daily for pup viability. 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, 14 and 21. Variations from expected maternal behavior were recorded, if and when present, on all other days of the postpartum period.

Fertility parameters were assessed for all dams assigned to natural delivery. These parameters included the fertility index (percentage of matings that resulted in pregnancies), gestation index (percentage of pregnancies that resulted in the birth of live litters), number of offspring per litter (live and dead pups), number of implantation sites, general condition of the dam and litter during the postpartum period, viability indices (percentage of pups born that survived 4 and 7 days), and lactation index (percentage of pups born that survived 21 days).

G.8.b F1/F2 Generation Pups – Preweaning Observations

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).

Vital status at birth was determined for pups that either appeared stillborn or that died before initial examination of the litter for viability. Pups that either appeared

stillborn or that died before initial examination of the litter for viability were examined for vital status at birth, as previously described.

Each litter was evaluated for viability at least twice each day during the 21-day postpartum period. Pups in each litter were counted once daily. Physical signs (including variations from expected nursing behavior and gross external physical anomalies) in the pups were recorded once daily for 21 days postpartum. Dead pups observed at these times were removed from the nesting box. When not precluded by autolysis or cannibalization by the dam, any pup found dead was necropsied and examined for the cause of death. Pup body weights were recorded on DLs 1 (birth), 4, 7, 14 and 21.

Reflex and physical development parameters in the F1 generation pups only were monitored during the 21-day postpartum period. Surface righting reflex [ability to right in 5 seconds (from DL 1)], pinna unfolding (from DL 2), eye opening (from DL 12), acoustic startle response (from DL 13) and air righting reflex (from DL 14) were monitored until all pups (100%) in the litter reached the criterion for the specific test. Pupil constriction was evaluated once on DL 21; the number of pups per litter with this reflex present was recorded.

G.8.c. <u>F1 Generation Rats – Postweaning Observations</u>

"Postweaning day" observations were recorded beginning on DL 22. All F1 generation rats were observed for viability at least twice daily during all periods of the study. Rats were also observed for general appearance at least once during the acclimation period and for clinical observations of effects of the test article, abortions, premature deliveries prior to and approximately one hour after dosage and on the day sacrificed.

Body weights for F1 generation female rats were recorded weekly during the postweaning periods and at sacrifice. Feed consumption values were recorded weekly except during cohabitation and at sacrifice.

Body weights for F1 generation female rats were recorded once weekly to cohabitation, daily during presumed gestation, on DLs 1, 4, 7 and 14 (rats assigned to natural delivery) and at sacrifice. Feed consumption values were recorded weekly to cohabitation, on DGs 0, 7, 10, 14, 17 and 20 and DLs 1, 4, 7, 10 and 14.

Beginning at 24 days of age, one male rat and one female rat from each litter in each dosage group were tested for learning, short-term retention and long-term retention in a passive avoidance paradigm. Each rat was tested on two days, separated by a one-week interval; the criterion for learning was the same for both days of testing. The passive avoidance apparatus consisted of a two-compartment chamber with hinged Plexiglas® lids. One compartment was

outfitted with a bright light and Plexiglas® floor. The other compartment was outfitted with a grid floor to which a brief (1 second) pulse of mild electric current (1 mA) was delivered. The two compartments were separated by a sliding door.

During each trial, the rat was placed into the "bright" compartment, the sliding door was opened and the light was turned on. The rat was allowed to explore the apparatus until it entered the "dark" compartment. The sliding door was then immediately closed, the light was turned off, and the brief pulse of current was delivered to the grid floor. The rat was then removed from the apparatus and placed into a holding cage for 30 seconds before the start of the next trial. Trials were repeated until the rat remained in the "bright" compartment continuously for 60 seconds on each of two consecutive trials (the criterion for learning) or until 15 trials were completed. The latency to enter the dark compartment or the maximum 60-second interval was recorded for each trial.

Dosage groups were compared on the following dependent measures: the number of trials to the criterion in the first session (overall learning performance); the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 of the first test session (activity level and exploratory tendency in a novel environment); the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session (short-term retention); the number of trials to the criterion in the second test session (long-term retention); and the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the second test session (long-term retention).

On postpartum day 70, one male rat and one female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory. Each rat was tested in a watertight 16-gauge stainless steel modified M-maze. The maze was filled with water to a depth of approximately nine inches, and the water was monitored for temperature (range of 21°C ± 1°C). On each test trial, the rat was placed into the starting position (base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals of the M-maze, in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial 1 was designated the incorrect goal during the remaining trials. Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal and were then removed from the water. A 15-second intertrial interval separated each trial. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15. Latency (measured in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Each rat was tested twice. The test sessions were separated by a one-week interval; the correct goal and the criterion were the same for both test sessions. Dosage groups were compared for the following dependent measures: the number of trials to criterion on the first day of testing; the average number of errors (incorrect turns in the maze) for each trial on the first day of testing; the latency (in seconds) to reach the correct goal on trial 2 of the first day of testing; the number of trials to criterion on the second day of testing; the average number of errors for each trial on the second day of testing; and the latency (in seconds) to reach the correct goal on trial 1 of day 2 of testing.

Female rats were evaluated for the age of vaginal patency, beginning on DL 28. Male rats were evaluated for the age of preputial separation, beginning on DL 34.

On DLs 85 to 98, the F1 generation rats within each dosage group were assigned to cohabitation, one male rat per female rat, based on random unit tables, with the exclusion of sibling matings. The cohabitation period consisted of a maximum of 14 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at DG 0 and assigned to individual housing. Female rats that did not mate within the first 7 days of cohabitation were assigned alternate male rats from the same dosage group that had mated. Female rats were allowed to naturally deliver and maintain litters through a 21-day postpartum period.

These rats were evaluated for clinical observations during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter size (all pups delivered) 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. Each litter was subsequently examined daily for pup viability. 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, 14 and 21. Variations from expected maternal behavior were recorded, if and when present, on all other days of the postpartum period.
Fertility parameters were assessed for all dams assigned to natural delivery. These parameters included the fertility index (percentage of matings that resulted in pregnancies), gestation index (percentage of pregnancies that resulted in the birth of live litters), number of offspring per litter (live and dead pups), number of implantation sites, general condition of the dam and litter during the postpartum period, viability indices (percentage of pups born that survived 4 and 7 days), and lactation index (percentage of pups born that survived 21 days).

G.9. Gross Necropsy[a]:

G.9.a Fo Generation Male and Female Rats Assigned to Pharmacokinetic Sample Collection

At scheduled sacrifice after completion of the cohabitation period (male rats that sired litters of dams allowed to naturally deliver a litter) and on DL 21 (female rats allowed to naturally deliver a litter) blood samples (approximately 4 mL per rat) were collected from five rats per dosage group from the inferior vena cava, into serum separator tubes and centrifuged. The resulting serum (approximately 2 mL) was immediately frozen on dry ice and maintained frozen (-70°C) until shipment to the Sponsor for analysis. The liver was excised, weighed, and a sample section (lateral lobe) was frozen and retained at –70°C until shipment to the Sponsor for analysis.

The liver of each pup from the litters of the five dams in each of Groups I through IV that were selected for pharmacokinetic sample collection was excised, pooled per litter, frozen and retained at -70°C, until shipment to the Sponsor for analysis. The dams in the 3.2 mg/kg/day dosage group (Group V) did not have surviving pups on DL 21.

G.9.b. Fo and F1 Generation Male Rats

Male rats were sacrificed by carbon dioxide asphyxiation after completion of the cohabitation period, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Gross lesions were retained in neutral buffered 10% formalin for possible future evaluation. Representative photographs of gross lesions are available in the raw data. The following organs were excised, individually weighed and retained for possible histologic evaluation: testes, epididymides, prostate and seminal vesicles (with and without fluid). The testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin for possible histopathological evaluation. The remaining organs were retained in neutral buffered 10% formalin.

G.9.c. Fo Generation Female Rats Assigned to Caesarean-Sectioning

Female rats assigned to Caesarean-sectioning were sacrificed on DG 10, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Uteri of apparently nonpregnant rats were stained with 10% ammonium sulfide to confirm the absence of implantation sites[9]. All ovaries and gross lesions were

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a. A table of random units was used to select one control group Fo and F1 generation rat from each sex from which all tissues examined at necropsy were retained, in order to provide control tissues for possible histopathological evaluations of gross lesions.

retained in neutral buffered 10% formalin for possible future evaluation. Representative photographs of gross lesions are available in the raw data. The rats were examined for the number and distribution of corpora lutea in each ovary and implantation sites, and viable and nonviable embryos. A viable embryo was oval or crescent shaped, pink, firm and enclosed in an amniotic sac filled with clear fluid. A nonviable embryo was 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. Embryos were discarded after examination.

G.9.d. Fo Generation Female Rats Assigned to Natural Delivery and F1 Generation Female Rats

At the completion of the 21-day postpartum period, all dams that delivered litters were sacrificed on DL 21, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites was recorded. Female rats assigned to natural delivery that did not deliver a litter were sacrificed on DG 25 and examined for gross lesions. To confirm the pregnancy status, uteri from rats that appeared nonpregnant were stained with 10% ammonium sulfide[9]. Dams with no surviving pups were sacrificed after the last pup was found dead, missing or presumed cannibalized. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. All ovaries were retained in neutral buffered 10% formalin for possible future evaluation.

Rats that died were examined for the cause of death on the day the observation was made. The rats were examined for gross lesions. Pregnancy status and uterine contents were recorded. Ovaries were retained in neutral buffered 10% formalin.

G.9.e. F1/F2 Generation Pups

On DL 21, F1 generation pups not continued on study and all F2 generation pups were sacrificed and examined for gross lesions. Necropsy procedures were the same as those used for pups culled on DL 4.

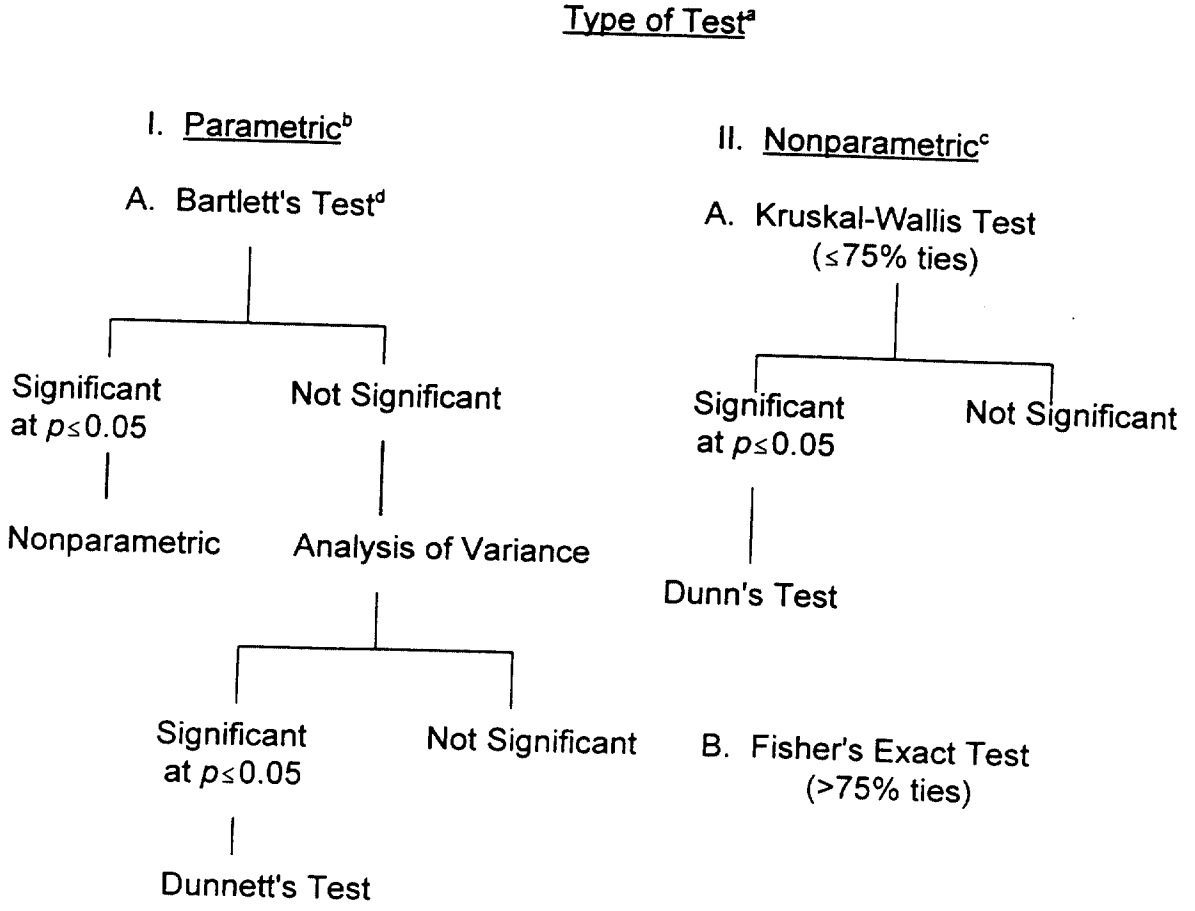
Pups that died before examination of the litter for pup viability were evaluated for vital status at birth, as described previously. Pups found dead were examined for gross lesions and for the cause of death. Pups with gross lesions found on DLs1 to 4 were preserved in Bouin's solution. Gross lesions from pups found on DLs 5 to 21 were preserved in neutral buffered 10% formalin. Representative photographs of pup gross lesions are available in the raw data.

Pups not selected for continued evaluation on DL 4 were sacrificed by carbon dioxide asphyxiation and examined for gross lesions. Necropsy included a single cross-section of the head at the level of the frontal and parietal suture and examination of the cross-sectioned head for apparent hydrocephaly. The

stomach contents (milk curd) were collected from culled pups from Groups I, II and III. Samples were collected from all pups from five of the largest litters in these three dosage groups. Individual pup samples were combined by litter into polypropylene tubes and frozen at –20°C. After completion of sample collection, samples were shipped (frozen on dry ice) to the Sponsor for analysis.

G.10. Statistical Analyses:

The following schematic represents the statistical analyses of the data:



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a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
b. Used only to analyze data with homogeneity of variance.
c. Proportion data are not included in this category.
d. Test for homogeneity of variance.

Proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[10].

Continuous data (e.g., body weights, body weight changes and feed consumption data) were analyzed using Bartlett's Test of Homogeneity of Variances[11] and the Analysis of Variance[12], when appropriate [i.e., Bartlett's Test was not significant ($p>0.05$)]. If the Analysis of Variance was significant ($p\leq 0.05$), Dunnett's Test[13] was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ($p\leq 0.05$)], the Kruskal-Wallis Test[14] was used (≤75% ties). In cases where the Kruskal-Wallis Test was statistically significant ($p\leq 0.05$), Dunn's Method of Multiple Comparisons[15] was used to identify the statistical significance of the individual groups. If there were greater than 75% ties, Fisher's Exact Test[16] was used. Fisher's Exact Test[16] was also used to evaluate necropsy data for the pups which were stillborn or found dead.

Data obtained at Caesarean-sectioning, natural delivery, preweaning reflex/physical developmental data and postweaning behavioral data involving discrete data (e.g., number of corpora lutea, number of pups per litter, trials to a criterion), were evaluated by the Kruskal-Wallis Test[14], as described above.

III. RESULTS - Fo GENERATION MALE RATS

A. Clinical Observations (Summary - Table B1; Individual Data - Table B10)

No Fo generation male rats died during this study.

All clinical observations were considered unrelated to the test article because the incidences were not dosage-dependent and/or the observation occurred in only one or two rats. These observations included localized alopecia on the limbs, corneal opacity, dental problems (missing, broken or misaligned incisors), abrasion on the neck, hyperactivity, excess salivation, chromodacryorrhea, swollen snout, chromorhinorrhea and dyspnea.

B. Body Weights and Body Weight Changes (Figure 1; Summaries - Tables B2 and B3; Individual Data - Table B11)

Groups administered 0.4 mg/kg/day and higher dosages of the test article had reduced body weight gains. The values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 0.4 mg/kg/day dosage group on days 56 to 63 of study (DSs 56 to 63); in the 1.6 mg/kg/day dosage group on DSs 29 to 36; and in the 3.2 mg/kg/day dosage group on DSs 15 to 22, 29 to 36, 36 to 42 and 56 to 63. Reflecting these effects of the test article, body weight gains were significantly reduced ($p \leq 0.01$) for the entire precohabitation period (DSs 1 to 42) in the 1.6 and 3.2 mg/kg/day dosage groups. The 0.4, 1.6 and 3.2 mg/kg/day dosage groups had significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) body weight gains for the entire study, calculated from DSs 1 to 63 or from DS 1 to termination.

Body weights were significantly reduced ($p \leq 0.05$) in the 1.6 mg/kg/day dosage group on DSs 56, 63 and at study termination. The 3.2 mg/kg/day dosage group had significantly reduced ($p \leq 0.01$) body weights on DSs 36, 42, 56, 63 and at study termination.

Body weights and body weight changes 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 B4 and B5; Individual Data - Table B12)

Relative feed consumption values were significantly reduced ($p \leq 0.05$) in the 1.6 and 3.2 mg/kg/day dosage groups on DSs 8 to 15. Absolute (g/day) and relative (g/kg/day) feed consumption values were significantly reduced ($p \leq 0.01$) in the 3.2 mg/kg/day dosage group on DSs 15 to 22, 29 to 36 and 36 to 42. As a result of these effects of the test article, absolute and relative feed consumption values were reduced and/or significantly reduced in these two dosage groups for

the entire precohabitation period (DSs 1 to 42). After the cohabitation period (DSs 56 to 63), absolute feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 0.4 and 1.6 mg/kg/day dosage groups. Absolute and relative feed consumption values were significantly reduced in the 3.2 mg/kg/day dosage group during this period.

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

D. **Mating and Fertility (Summary - Table B6; Individual Data - Table B13)**

Dosages of the test article as high as 3.2 mg/kg/day did not affect any mating and fertility parameters evaluated. Values for the fertility and pregnancy indices (number of pregnancies per number of rats in cohabitation and rats that mated, respectively), the number of days to inseminate, the number of rats that mated and the number of rats with confirmed mating dates during the first week of cohabitation were comparable among the five dosage groups.

E. **Necropsy Observations (Summary - Table B7; Individual Data - Table B14)**

A significantly increased ($p \leq 0.01$) number of male rats in the 3.2 mg/kg/day dosage group had a light brown or brown liver, an observation attributed to effects of PFOS. The gross lesions of the liver were considered related to the test article because the incidences were dosage-dependent.

One 1.6 mg/kg/day dosage group rat (8214) and three 3.2 mg/kg/day dosage group rats (8245, 8252, 8257) had small, flaccid and/or purple testes[a]. All four of these male rats mated but did not impregnate a female rat. One 3.2 mg/kg/day dosage group male rat (8243) had a small prostate; this rat impregnated a female rat. The gross lesions observed in the testes and prostate were not considered test article-related as they are common findings in control group male rats and did not vary from Historical Control incidence at the Testing Facility. No other male rats had gross lesions of the reproductive organs.

All other necropsy observations were considered unrelated to the test article because: 1) the observation occurred in only one rat in a group; or 2) the incidences were not dosage-dependent. These observations included a large liver, a liver with a rough surface, constricted papillary process of the liver with

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a. In 2079 control group rats from 91 studies conducted at the Testing Facility from August, 1992 to April, 1999, there were 21 rats with small, flaccid and/or purple testes.

pinpoint raised tan areas, opaque lens of the eye, large kidneys, kidneys with cysts in the parenchyma, dilation of the renal pelvis and a large spleen.

F. **Terminal Body Weights, Organ Weights and Ratios (%) of Organ Weight to Terminal Body Weight (Summaries - Tables B8 and B9; Individual Data - Table B15)**

The 1.6 and 3.2 mg/kg/day dosage groups had significantly reduced ($p \leq 0.05$ and $p \leq 0.01$, respectively) terminal body weights. The absolute weights of the seminal vesicles with fluid and the prostate were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 3.2 mg/kg/day dosage group, compared to the control group. Epididymides and testes weights and weights of the seminal vesicles without fluid were unaffected by dosages of the test article as high as 3.2 mg/kg/day.

The ratios of the weight of the left and right testes to terminal body weights were significantly increased ($p \leq 0.01$) in the 3.2 mg/kg/day dosage group, as compared to the control group values. These observations were associated with the significantly reduced ($p \leq 0.01$) terminal body weights in this dosage group. The ratios of the weights of the epididymides, seminal vesicles and prostate were generally comparable among the five dosage groups.

IV. RESULTS - Fo GENERATION FEMALE RATS

A. Clinical Observations (Summary - Table C1; Individual Data - Table C22)

A.1. Mortality

No deaths were attributable to PFOS. The only death occurred in a 1.6 mg/kg/day dosage group rat, an event attributable to an intubation accident.

Female rat 8938 in the 1.6 mg/kg/day dosage group was moribund sacrificed on study day (DS) 36 after administration of 35 daily dosages. Adverse clinical observations included chromodacryorrhea and a clear oral exudate on DSs 35 to 36, and ptosis, hunched posture and labored breathing on DS 36. Observations of chromodacryorrhea and ptosis were confirmed at necropsy; all other tissues appeared normal at necropsy. This rat lost body weight and had reduced feed consumption on DSs 29 to 36. The clinical observations indicated this rat was misintubated on or about DS 35. All other Fo generation female rats survived to scheduled sacrifice.

A.2. Clinical Observations

Observations of localized alopecia were increased during the precohabitation, gestation and lactation periods in the 0.4, 1.6 and 3.2 mg/kg/day dosage groups. The incidence of localized alopecia at any area was significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in the 0.4, 1.6 and 3.2 mg/kg/day dosage groups during the gestation period. The incidence of localized alopecia on the back was significantly increased ($p \leq 0.01$) in the 3.2 mg/kg/day dosage group during the lactation period.

All other clinical observations during the precohabitation, gestation and lactation 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 rat. These observations included chromodacryorrhea and dental problems (missing, broken or misaligned incisors). Clear oral exudate, ptosis, hunched posture and labored breathing occurred during the precohabitation period in the 1.6 mg/kg/day rat that was moribund sacrificed, as previously described.

B. Body Weights and Body Weight Changes (Figure 2;Summaries - Tables C2 through C7; Individual Data - Tables C23 through C25)

B.1. Precohabitation

Groups administered 1.6 mg/kg/day and higher dosages of the test article had significantly reduced ($p \leq 0.01$) body weight gains for the entire precohabitation

period (DSs 1 to 42). During this period, values were significantly reduced ($p \leq 0.01$) in the 1.6 mg/kg/day dosage group on DSs 29 to 36; and in the 3.2 mg/kg/day dosage group on DSs 8 to 15, 22 to 29 and 29 to 36, as compared with the control group value. Body weights were significantly reduced ($p \leq 0.01$) in the 3.2 mg/kg/day dosage group on DSs 15, 22, 29, 36 and 42, as compared with the control group values.

Body weights and body weight gains during the precohabitation period were unaffected by dosages of the test article as high as 0.4 mg/kg/day.

B.2. Gestation

The 1.6 and 3.2 mg/kg/day dosages of PFOS continued to reduce body weights and body weight gains during gestation. Reflecting effects that occurred during the precohabitation period, the 1.6 mg/kg/day dosage group tended to weigh less and the 3.2 mg/kg/day dosage group weighed significantly less ($p \leq 0.01$) than the control group on day 0 of gestation (DG 0). Weight gain was significantly reduced ($p \leq 0.05$) in the 1.6 mg/kg/day dosage group on DGs 0 to 7 and then essentially comparable to the control group values at all intervals throughout the remainder of gestation, beginning with DGs 10 to 12. Weight gains were significantly reduced in the 3.2 mg/kg/day dosage group on DGs 0 to 7 and 10 to 12, after which this group also had weight gains comparable to the control group. Reflecting these effects, body weight gains were significantly reduced ($p \leq 0.01$) in the 3.2 mg/kg/day dosage group for the entire gestation period (DGs 0 to 20) and body weights were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 and 3.2 mg/kg/day dosage groups on DGs 3 through 11 and 0 through 20, respectively.

Body weights and body weight gains during gestation were unaffected by dosages of the test article as high as 0.4 mg/kg/day.

B.3. Lactation

The 3.2 mg/kg/day dosage group had significantly reduced ($p \leq 0.01$) body weight on DL 1, as compared with the control group value. Body weight gains tended to be reduced in the 0.4 mg/kg/day dosage group on DLs 1 to 4, when significant weight loss ($p \leq 0.01$) occurred in the 1.6 mg/kg/day dosage group (the 3.2 mg/kg/day dosage group was precluded from further evaluation because all pups died before DL 2). A significant increase ($p \leq 0.05$) in body weight gain on DLs 10 to 14 in the 1.6 mg/kg/day dosage group was possibly associated with reduced litter size and associated with reduced milk demand and production.

Body weights and body weight gains during lactation 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 C8 through C13; Individual Data - Tables C26 through C28)

C.1. Precohabitation

Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced in the 1.6 and 3.2 mg/kg/day dosage groups during the precohabitation period. These effects were significant ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 mg/kg/day dosage group on DSs 22 to 29 and 29 to 36 (absolute only), and in the 3.2 mg/kg/day dosage group at most tabulated intervals. Reflecting these effects of the test article, the 3.2 mg/kg/day dosage group had significantly reduced ($p \leq 0.01$) absolute and relative feed consumption values for the entire precohabitation period (DSs 1 to 42).

Feed consumption values during the precohabitation period were unaffected by dosages of the test article as high as 0.4 mg/kg/day.

C.2. Gestation

The 1.6 and 3.2 mg/kg/day dosage groups continued to have reduced absolute and relative feed consumption values early in the gestation period. Absolute and relative feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 mg/kg/day dosage group on DGs 0 to 7 (absolute only) and 7 to 10, and in the 3.2 mg/kg/day dosage group on DGs 0 to 7, 7 to 10, 10 to 12 (absolute only). Significantly increased ($p \leq 0.05$ or $p \leq 0.01$) feed consumption values occurred in the 1.6 and 3.2 mg/kg/day dosage groups on DGs 15 to 18 (absolute and relative) and DGs 18 to 20 (relative only), observations interrelated with reduced body weights and the later stages of gestation, when maternal feed consumption tends to increase. Despite the increases in relative feed consumption values, the 3.2 mg/kg/day dosage group had a significantly reduced ($p \leq 0.01$) absolute feed consumption value for the entire gestation period (DGs 0 to 20).

Feed consumption values during gestation were unaffected by dosages of the test article as high as 0.4 mg/kg/day.

C.3. Lactation

Absolute and relative maternal feed consumption values tended to be reduced in the 0.4 mg/kg/day dosage group and were significantly reduced ($p \leq 0.01$) in the 1.6 mg/kg/day dosage group for the entire lactation period (DLs 1 to 14), and at most intervals within this period. The 3.2 mg/kg/day dosage group was precluded from evaluation because there were no surviving pups after DL 1.

Feed consumption values during lactation were unaffected by the 0.1 mg/kg/day dosage of the test article.

D. **Estrous Cycling, Mating and Fertility (Summary - Table C14; Individual Data - Table C29)**

Dosages of the test article as high as 3.2 mg/kg/day did not affect estrous cycling (number of estrus stages per 28-days) in the 15 rats per dosage group that were evaluated. All female rats mated except one in the 0.4 mg/kg/day dosage group. The number of days in cohabitation, the fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), and the number of rats with confirmed mating dates during the first and second week of cohabitation were comparable in the five dosage groups. Pregnancy occurred in 33, 32, 28, 29 and 30 female rats in Groups I through V, respectively.

E. **Necropsy Observations (Summary - Table C15; Individual Data - Table C30)**

All necropsy observations were considered unrelated to treatment. One rat in the 0.4 mg/kg/day dosage group had moderate dilation of the pelvis of the right kidney, an observation that is common in this species and strain. One rat in the 1.6 mg/kg/day dosage group had adhesion of abdominal adipose tissue and the spleen. No other gross lesions occurred in the female rats in this study.

F. **Caesarean-Sectioning and Litter Observations (Summary - Table C16; Individual Data - Tables C31 through C33)**

Caesarean-sectioning observations on DG 10 were based on 10 (100%), 7 (70.0%), 6 (60.0%), 9 (90.0%) and 9 (90.0%) pregnant dams in each of the five respective dosage groups.

There were no biologically important or statistically significant differences in the litter averages for corpora lutea, implantations, viable embryos or nonviable embryos. No dam had litter in which there were no live embryos.

G. **Natural Delivery and Litter Observations (Summaries - Tables C17 and C18; Individual Data - Tables C34 through C37)**

Although 25 presumed pregnant rats in each dosage group were assigned to natural delivery, because one rat (1.6 mg/kg/day dosage group) was moribund sacrificed during the premating period (on DS 36), as previously described, this dosage group had only 24 rats assigned to natural delivery. Of these rats, 20 to 25 were pregnant and delivered litters.

The duration of gestation was significantly reduced ($p \leq 0.01$) in the 3.2 mg/kg/day dosage group, an observation associated with preimplantation loss [the average number of implantation sites per dam was significantly reduced ($p \leq 0.01$), resulting in a significantly reduced ($p \leq 0.01$), litter size] and an effect of the test article.

Pup viability was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 and 3.2 mg/kg/day dosage groups, as described in the following information. Although the gestation index (the percentage of pregnant rats with live offspring) was comparable across all five dosage groups, the 1.6 mg/kg/day dosage group had increased numbers of pups that died or were presumed cannibalized on DL 1, and significantly increased ($p \leq 0.05$ or $p \leq 0.01$) numbers of pups dead or presumed cannibalized on DLs 2 to 4 and 5 to 7. The 3.2 mg/kg/day dosage group had postimplantation loss evident as reduced pup viability at birth [one litter had no liveborn pups, the number of dams with stillborn pups was significantly increased ($p \leq 0.01$), the litter average and number of stillborn pups were significantly increased ($p \leq 0.01$), and the litter average and number of liveborn pups were significantly reduced ($p \leq 0.01$)], as well as peripartum deaths [significant numbers ($p \leq 0.05$ or $p \leq 0.01$) of dead and presumed cannibalized pups on DLs 1 and 2 to 4 (all liveborn pups died by DL 2) and significant numbers ($p \leq 0.01$) of dams had all pups dead or presumed cannibalized on DLs 1 to 4]. Reflecting these effects, the viability index was significantly reduced ($p \leq 0.01$) in the 1.6 and 3.2 mg/kg/day dosage groups, the lactation index was also significantly reduced ($p \leq 0.01$) in the 1.6 mg/kg/day dosage group, as also were the averages for surviving pups ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 mg/kg/day dosage group beginning on DL 4 postculling, and in the 3.2 mg/kg/day dosage group on DL 1.

A dosage-dependent pattern of reduced pup body weight was evident in each group administered the test article. The 1.6 mg/kg/day dosage group had significantly reduced ($p \leq 0.01$) pup body weights on all weighing days. The 3.2 mg/kg/day dosage group had a significantly reduced ($p \leq 0.01$) pup body weight on DL 1 (no pups survived to subsequent scheduled weighings).

The percentage of male pups was comparable across all five dosage groups.

H. Clinical Observations from Birth to Day 21 Postpartum and Necropsy Observations (Summaries - Tables C19 and C21; Individual Data - Tables C38 and C45)

Adverse clinical and necropsy observations associated with reduced pup viability and potential reduction in maternal care occurred in the 3.2 mg/kg/day dosage group. Adverse clinical observations include three ($p \leq 0.01$) litters with pups that were not nursing (two of these litters had pups from which the placenta had not been removed, and one had pups that were not nested). Apparent maternal

cannibalization was also evident in this dosage group (missing tail in one pup and a cannibalized forelimb in another pup), which may have occurred after these pups died.

Necropsy observations in pups that were stillborn or found dead included significant increases ($p \leq 0.05$ or $p \leq 0.01$) in the numbers of pups with no milk in the stomach in the 1.6 and 3.2 mg/kg/day dosage group pups. No pups ($p \leq 0.01$) in the 0.1 mg/kg/day dosage group had this observation, as compared with two control group pups. The 3.2 mg/kg/day dosage group also had evidence of increased maternal cannibalization at necropsy of stillborn and found dead pups. Nine of these pups had missing hindlimbs and/or portion of the tail.

All other clinical and necropsy observations in the F1 generation pups were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; or 2) the observation occurred in only one litter. Clinical observations included a domed head, portion of the tail missing, umbilical hernia, missing digits and bent tail. One 1.6 mg/kg/day dosage group stillborn pup had anasarca (edema of the entire body). Necropsy on DL 21 revealed two 0.4 mg/kg/day dosage group littermates with moderate or severe dilation of the lateral ventricles of the brain, and one pup from a different 0.4 mg/kg/day dosage group litter with slight dilation of the renal pelvis.

I. Reflex and Physical Development (Summary - Table C20; Individual Data - Tables C39 through C44)

Reversible delays in reflex and physical development that are highly correlated with body weight[1,17] occurred in the 0.4 and 1.6 mg/kg/day dosage groups, as described below.

I.1. Surface Righting

Surface righting was delayed in the 1.6 and 3.2 mg/kg/day dosage groups, as described below. The percentage of pups that could surface right was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 mg/kg/day dosage group on DLs 3, 4, 5, 6, 7, 8 and 10, and in the 3.2 mg/kg/day dosage group on DL 1, after which there were no surviving pups in this dosage group. The average day that at least 50% of the pups in a dosage group had the ability to surface right was significantly increased ($p \leq 0.05$) in the 1.6 mg/kg/day dosage group. All surviving pups ultimately attained the ability to surface right.

The ability to surface right was unaffected by the 0.4 mg/kg/day dosage of the test article.

I.2. Pinna Unfolding

Pinna unfolding was delayed in the 0.1, 0.4 and 1.6 mg/kg/day dosage groups (no 3.2 mg/kg/day dosage group pups were evaluated for this parameter). The effect was transient (evident on only one day) in the 0.1 and 0.4 mg/kg/day dosage groups, and considered of no toxicological importance [the percentages of pups per litter with pinna unfolding were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 0.1 and 0.4 mg/kg/day dosage groups on DL 3 only]. The percentage of pups with an unfolded pinna was significantly reduced ($p \leq 0.01$) on DLs 3, 4 and 5) in the 1.6 mg/kg/day dosage group, and the average day that at least 50% of the pups in a dosage group had an unfolded pinna was significantly increased ($p \leq 0.01$) in this dosage group. All surviving pups ultimately had unfolded pinnae.

I.3. Eye Opening

Eye opening was delayed in the 0.1, 0.4 and 1.6 mg/kg/day dosage groups (no 3.2 mg/kg/day dosage group pups were evaluated for this parameter). The effect was transient (evident on only one day) in the 0.1 and 0.4 mg/kg/day dosage groups, and considered of no toxicological importance [the percentages of pups per litter with open eye lids were significantly reduced ($p \leq 0.05$) in the 0.1 and 0.4 mg/kg/day dosage groups on DL 14 only]. The percentage of pups with at least one open eye was significantly reduced ($p \leq 0.01$) in the 1.6 mg/kg/day dosage group on DLs 14, 15 and 16. The day that at least 50% of the pups had at least one open eye was significantly increased ($p \leq 0.01$) in the 0.4 and 1.6 mg/kg/day dosage groups. All pups had open eyelids by DL 21, when pupil constriction was tested.

I.4. Acoustic Startle

The time of development of the acoustic startle reflex was delayed ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 mg/kg/day dosage group; no 3.2 mg/kg/day dosage group pups survived to be tested for this reflex. The percentage of pups in the 1.6 mg/kg/day dosage group with this reflex was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) on DLs 13, 14, 16, 17, 18, 19 and 20, resulting in a tendency for an increase in the average day that at least 50% of the pups had the acoustic startle reflex.

The development of the acoustic startle reflex was unaffected by the 0.1 and 0.4 mg/kg/day dosages of the test article.

I.5. Air Righting

The ability to air right was delayed ($p \leq 0.01$) in the 0.4 and 1.6 mg/kg/day dosage groups; no 3.2 mg/kg/day dosage group pups survived to be tested for this

reflex. The effect was transient and not of toxicological importance in the 0.4 mg/kg/day dosage group, occurring only on DL 16. The percentage of pups in the 1.6 mg/kg/day dosage group that air righted was significantly reduced ($p \leq 0.01$) on DLs 14 through 21, resulting in a significant increase ($p \leq 0.01$) in the day that at least 50% of the pups could air right.

I.6. Pupil Constriction

All live pups in the 0 (Vehicle), 0.1, 0.4 and 1.6 mg/kg/day dosage groups had the pupil constriction response present on DL 21.

V. RESULTS – F1 GENERATION MALE AND FEMALE RATS

A. F1 Generation Male Rats

A.1. Mortality and Clinical Observations (Summary - Table D1; Individual Data - Table D13)

A.1.a. Mortality

No deaths in the F1 generation male rats were attributed to PFOS. Two control group rats were found dead on days 86 and 87 postweaning. These deaths were attributed to intubation errors. One 0.1 mg/kg/day dosage group rat was found dead on day 66 postweaning; this death was considered unrelated to the test article because it was a single, nondosage-dependent effect. Observations for these rats that died are provided below.

Control group male rat 13105 was found dead on day 87 postweaning, one hour and 11 minutes after the 87th daily dosage. No other adverse clinical observations occurred in this rat, and its body weight gains and feed consumption values were comparable to other rats in this dosage group. Necropsy of the rat revealed that all lung lobes were dark red, which in association with the time death occurred, is compatible with an intubation accident.

Control group male rat 13116 was found dead on day 86 postweaning, after it had been administered 85 daily dosages. Additional adverse clinical observations in this rat were limited to chromorhinorrhea (days 67 to 72 and 79 postweaning). Body weight gains and feed consumption values in this rat were comparable to those of other rats in this dosage group. Necropsy of the rat revealed dark red clotted material (presumed to be blood) in the thoracic cavity and a red perinasal substance, observations compatible with an intubation accident.

Male rat 13138 (0.1 mg/kg/day dosage group) was found dead on day 66 postweaning, after it had been administered 65 daily dosages. No other adverse clinical observations occurred in this rat, and its body weight gains and feed consumption values were comparable to those of other rats in this dosage group. All tissues appeared normal at necropsy.

A.1.b. Clinical Observations

All adverse clinical observations 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. A significant increase ($p \leq 0.01$) in the incidence of localized alopecia on the back in the 0.1 mg/kg/day dosage

group was not considered treatment-related because the value was not dosage-dependent. No other statistically significant increases in an adverse clinical observation occurred. Additional adverse clinical signs observed and considered unrelated to the test article included dental problems (missing, broken and/or misaligned incisors), chromorhinorrhea, abrasion on the head, near the eye, forelimb or back, chromodacryorrhea, laceration at the base of the tail, a swollen snout, a red perioral substance, a red substance on penis, localized alopecia on the back, limbs and/or head, a scab on the back, urine-stained abdominal fur, soft or liquid feces, swollen and purple ears, rales, gasping, excess salivation and dilated pupils.

A.2. Body Weights and Body Weight Changes (Figure 2; Summaries - Tables D2 and D3; Individual Data - Table D14)

The 0.1 and 0.4 mg/kg/day dosage groups tended to weigh less than the control group on day 1 postweaning and generally gained one or two grams less body weight per week than the control group rats, with the exception of days 71 to 78, when these groups had significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in body weight gains, as compared with the control group value. Reflecting this pattern of weight gain, weight gains in the 0.1 and 0.4 mg/kg/day dosage groups for the precohabitation period (day 1 postweaning to precohabitation) were 98.4% and 97.6% of the control group value, respectively. Body weight gains in the 0.1 and 0.4 mg/kg/day dosage groups for day 1 to termination were 98.5% and 96.6% of the control group value, respectively.

A.3. Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables D4 and D5; Individual Data - Table D15)

Absolute (g/day) feed consumption values were significantly reduced ($p \leq 0.05$ and $p \leq 0.01$, respectively) and relative (g/kg/day) feed tended to be reduced in the 0.1 and 0.4 mg/kg/day dosage groups during the first week of intubation (days 1 to 8 postweaning), as compared to the control group values. These observations commonly occur and were associated with the tendency for reduced body weight in these two dosage groups on day 1 postweaning. A significant increase ($p \leq 0.05$) in the relative feed consumption value in the 0.1 mg/kg/day dosage group on days 22 to 29 postweaning was considered unrelated to the test article because the value was not dosage-dependent. No other statistically significant differences occurred in the feed consumption values for this portion of the study.

A.4. Sexual Maturation (Summary - Table D6; Individual Data - Table D16)

Dosages of the test article as high as 0.4 mg/kg/day did not affect the average day of preputial separation in the F1 generation male rats. No significant differences occurred among the three dosage groups.

A.5. Passive Avoidance Performance (Summary - Table D7; Individual Data - Table D17)

There were no biologically important differences in the values for learning, short-term retention, long-term retention or response inhibition in the F1 generation male rats, as evaluated by performance in a passive avoidance paradigm. No statistically significant differences occurred in the number of trials to criterion, trial latencies or numbers of rats that failed to learn.

A.6. Watermaze Performance (Summary - Table D8; Individual Data - Table D18)

No biologically important dosage-dependent differences occurred in watermaze performance of the F1 generation male rats regarding learning, short-term retention, long-term retention or response inhibition. No statistically significant differences occurred in the number of trials to criterion, trial latencies or numbers of rats that failed to learn.

A.7. Mating and Fertility (Summary - Table D9; Individual Data - Table D19)

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 male rats. Values for the fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), the number of days to inseminate, the number of rats that mated and the number of rats with confirmed mating dates during the first week of cohabitation were comparable among the three dosage groups. Of the male rats assigned to cohabitation, 82.6%, 83.3% and 96.0% impregnated the cohort female rat.

A.8. Necropsy Observations (Summary - Table D10; Individual Data - Table D20)

All necropsy observations in the F1 generation male rats were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; and/or 2) the observation commonly occurs in this strain of rat.

Gross lesions of male reproductive organs included flaccid, small and/or purple testes and epididymides in three ($p \leq 0.01$) 0.1 mg/kg/day dosage group male rats (13134, 13145, 13148), each of which sired a litter). These observations were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; and 2) these findings are known to be genetically mediated and common in this rat strain. No other gross lesion occurred at a statistically significant incidence.

Additional necropsy observations considered unrelated to the test article included red abdominal adipose tissue with firm areas (one 0.4 mg/kg/day dosage group male rat; 13162), and urinary tract lesions, as described in the following information. One 0.1 mg/kg/day dosage group male rat (13143) had a dilated ureter; another rat in this dosage group (13134) had slight dilation of the renal pelvis. One 0.4 mg/kg/day dosage group male rat (13173) had large kidneys with numerous fluid-filled cysts in the parenchyma; the left kidney had slight dilation of the pelvis, in which there were six calculi, and thickened urinary bladder walls associated with the presence of numerous calculi. Necropsy observations in the control group rats that died as the result of an intubation error were described previously.

A.9. Terminal Body Weights and Organ Weights and Ratios (%) of Organ Weight to Terminal Body Weight (Summaries - Tables D11 and D12; Individual Data - Table D21)

No statistically significant differences occurred in the absolute or relative (to body weight) values for the weights of the right or left testis, seminal vesicles (with and without fluid), right epididymis or prostate. Terminal body weights tended to be reduced in the 0.1 and 0.4 mg/kg/day dosage groups; the terminal body weights in the 0.1 and 0.4 mg/kg/day dosage groups were 98.3% and 96.4%, respectively, of the control group value.

B. F1 Generation Female Rats

B.1. Mortality and Clinical Observations (Summary - Table E1; Individual Data - Tables E23)

B.1.a. Mortality

No deaths in the F1 generation female rats were attributed to PFOS. One control group rat was sacrificed on day 7 postweaning, because it was a male rat that had been incorrectly identified as a female rat. One control group rat was found dead on day 28 postweaning; no cause of death was identified for this rat. One rat in the 0.4 mg/kg/day dosage group was found dead on day 10 of lactation (DL 10) as the result of an intubation accident. Observations for these rats are provided below.

No gross lesions were revealed by necropsy of control group rat 13205 on day 7 postweaning.

The control group female rat (13221) that was found dead on day 28 postweaning, after it had been administered 27 daily dosages did not have any other adverse clinical observations, and its body weight gains and feed

consumption values were comparable to other rats in this dosage group. All tissues appeared normal at necropsy.

Female rat 13257 (0.4 mg/kg/day dosage group) was found dead on the morning of DL 10, after it had been administered a total of 106 daily dosages. No other adverse clinical observations occurred in this rat, although it lost weight on DLs 1 to 7. Its feed consumption values were unremarkable. This rat delivered 15 liveborn pups, of which seven were culled on DL 4 and eight were sacrificed after the death of the dam. Necropsy of the dam revealed gross lesions compatible with an intubation accident (white foam in the trachea, approximately 2 mL of a light orange fluid in the thoracic cavity, and dark red lungs).

B.1.b. Clinical Observations

A red perivaginal substance occurred in one and two dams in the 0.1 and 0.4 mg/kg/day dosage groups, respectively, on days 21 or 22 of gestation, and red perivaginal substance was also observed on DL 1 in five ($p \leq 0.01$) dams in the 0.4 mg/kg/day dosage group. These observations probably were associated with parturition, rather than an effect of the test article, and did not appear to adversely affect the health of the dams or their offspring. No other adverse clinical observation occurred at a statistically significant incidence.

All other adverse clinical observations that occurred during the precohabitation, gestation and lactation periods were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; 2) the observation occurred in only one or two rats; and/or 3) the observation is common in the laboratory environment. These observations included dental problems (misaligned, missing and/or broken teeth), chromodacryorrhea, swollen and purple ears, localized alopecia on the back, head, limbs and/or underside, abrasion or scab on the back, urine-stained abdominal fur, missing hindpaw digits, perivaginal swelling, swollen forepaw, chromorhinorrhea, soft or liquid feces, missing tail sheath, tip of tail black or missing and abrasion on the chest.

B.2. Maternal Body Weights and Body Weight Changes (Figure 4; Summaries - Tables E2 through E7; Individual Data - Tables E24 through E26)

B.2.a. Precohabitation

Body weights tended to be reduced in the 0.4 mg/kg/day dosage group on day 1 postweaning. No statistically significant or dosage-dependent differences in precohabitation body weight gains or body weights occurred in the F1 generation female rats during the precohabitation period.

B.2.b. Gestation

Maternal body weights and body weight gains during the gestation period were unaffected by dosages of the test article as high as 0.4 mg/kg/day. All values were comparable among the three dosage groups and did not significantly differ.

B.2.c. Lactation

Significant ($p \leq 0.05$) maternal body weight loss occurred on DLs 1 to 4 in the 0.4 mg/kg/day dosage group, as compared to the control group value, after which body weight gains were greater than, or weight loss less than the control group values.

Maternal body weights and body weight gains during the lactation period were unaffected by the 0.1 mg/kg/day dosage of the test article.

B.3. Maternal Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables E8 through E13; Individual Data - Tables E27 through E29)

B.3.a. Precohabitation

The 0.4 mg/kg/day dosage of the test article was associated with a significant reduction ($p \leq 0.05$) in absolute (g/day) feed consumption and a tendency for reduced relative (g/kg/day) feed consumption on days 1 to 8 postweaning, observations that were associated with the tendency for smaller body weight in this group on day 1 postweaning. Absolute and relative feed consumption values for the remainder of the precohabitation period were comparable and did not significantly differ among the three dosage groups.

B.3.b. Gestation

Absolute and relative feed consumption values during the gestation period were unaffected by dosages of the test article as high as 0.4 mg/kg/day. All values were comparable among the three dosage groups and did not significantly differ.

B.3.c. Lactation

The absolute maternal feed consumption value was significantly reduced ($p \leq 0.05$) on DLs 1 to 4 in the 0.4 mg/kg/day dosage group, as compared to the control group value. The relative feed consumption value was also reduced, however not significantly ($p > 0.05$). These observations were associated with the significant weight loss that occurred in this group on DLs 1 to 4 and attributed to the test article.

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

B.4. Sexual Maturation (Summary - Table E14; Individual Data - Table E30)

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. No significant differences occurred among the three dosage groups.

B.5. Passive Avoidance Performance (Summary - Table E15; Individual Data -Table E31)

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 paradigm. No statistically significant differences occurred in the trial latencies or numbers of rats that failed to learn.

The number of trials to criterion was significantly reduced ($p \leq 0.05$) for the female rats in the 0.4 mg/kg/day dosage group, as compared to the control group value. This observation was not considered an effect of the test article because an increase in the number of trials to criterion, rather than a decrease, would be expected as an indication of toxicity.

B.6. Watermaze Performance (Summary - Table E16; Individual Data - Table E32)

No biologically important dosage-dependent differences occurred in watermaze performance of the F1 generation female rats regarding learning, short-term retention, long-term retention or response inhibition. No statistically significant differences occurred in the number of trials to criterion, trial latencies or numbers of rats that failed to learn.

B.7. Mating and Fertility (Summary - Table E17; Individual Data - Table E33)

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. Values for the number of days in cohabitation, the number of rats that mated, the fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), and the number of rats with confirmed mating dates during the first and second week of cohabitation were comparable among the three dosage groups.

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.

As occurred in the previous generation, there was a tendency for reduced pup viability in the 0.4 mg/kg/day dosage group. The number of dams with stillborn pups was increased, albeit not significantly, and the number of pup deaths after DL 2 also were increased in this dosage group.

As also occurred in the previous generation, pup body weights tended to be reduced in the 0.1 mg/kg/day dosage group on DL 4 (pre- and postculling) and DL 7, but by DL 14 were comparable to control values. Pup body weights in the 0.4 mg/kg/day dosage group tended to be reduced, though not significantly, on DLs 4 through 21, with 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.
F2 generation pups body weights were comparable among all groups.

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.

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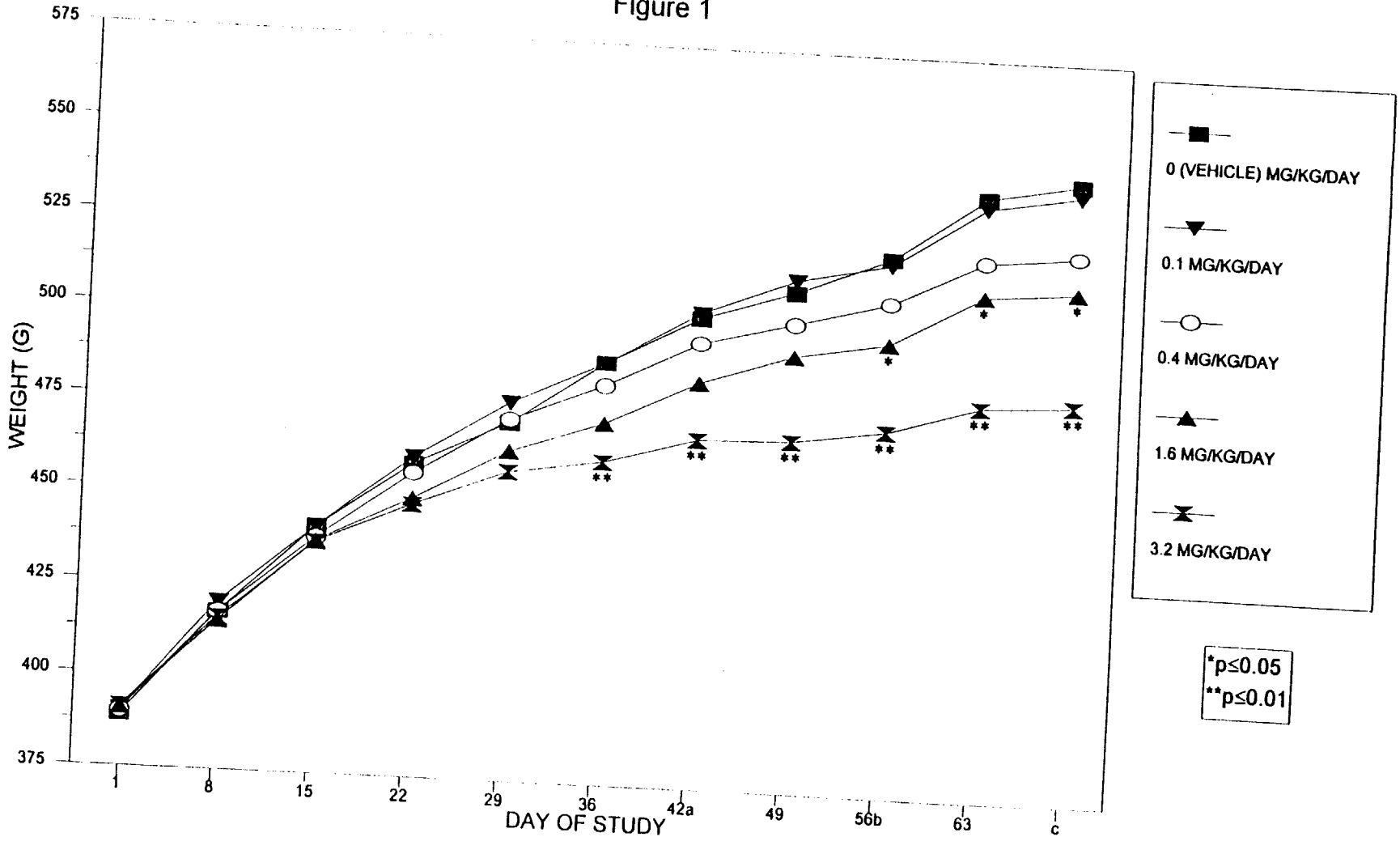
APPENDIX A

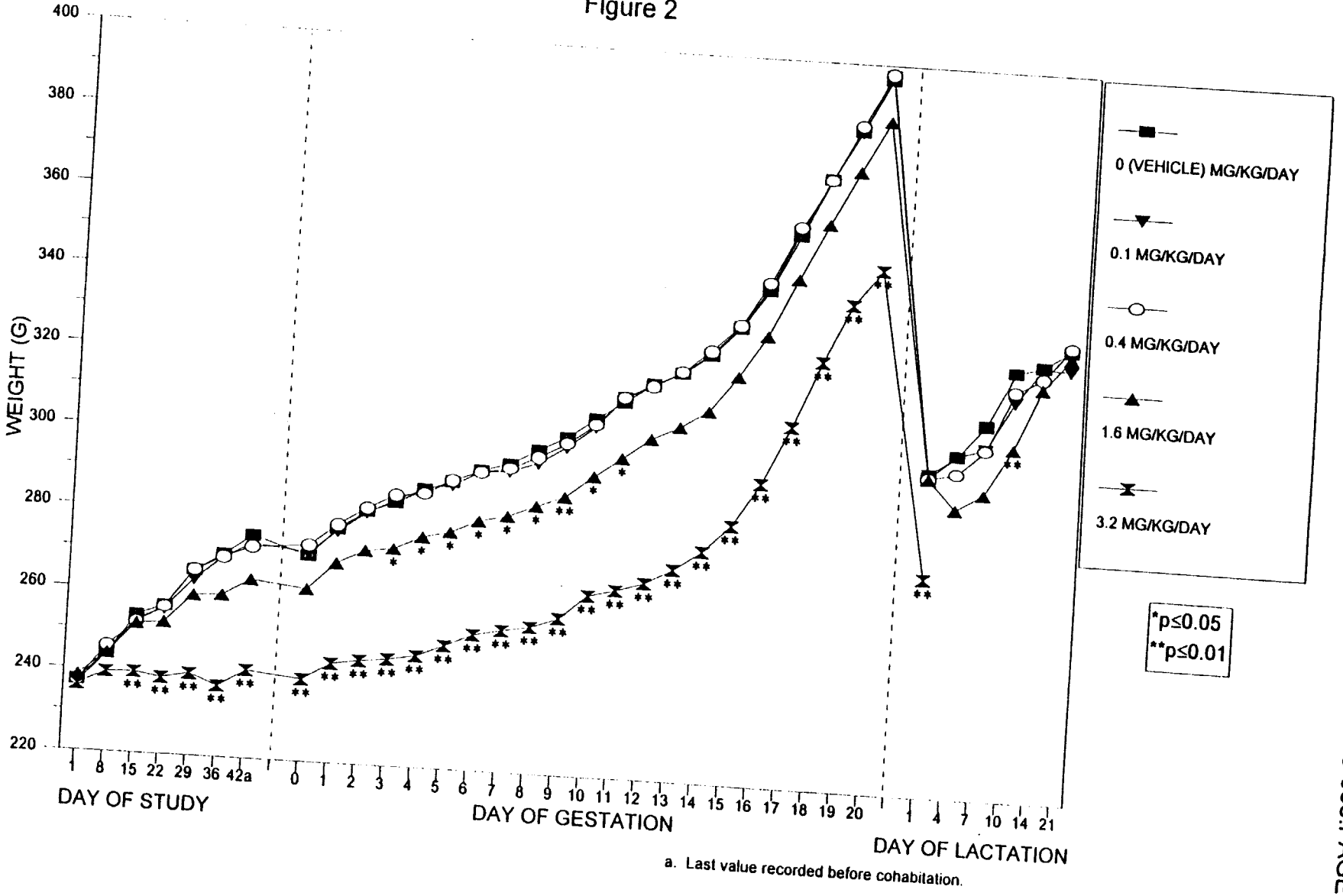
REPORT FIGURES

BODY WEIGHTS
Fo GENERATION MALE RATS
Figure 1



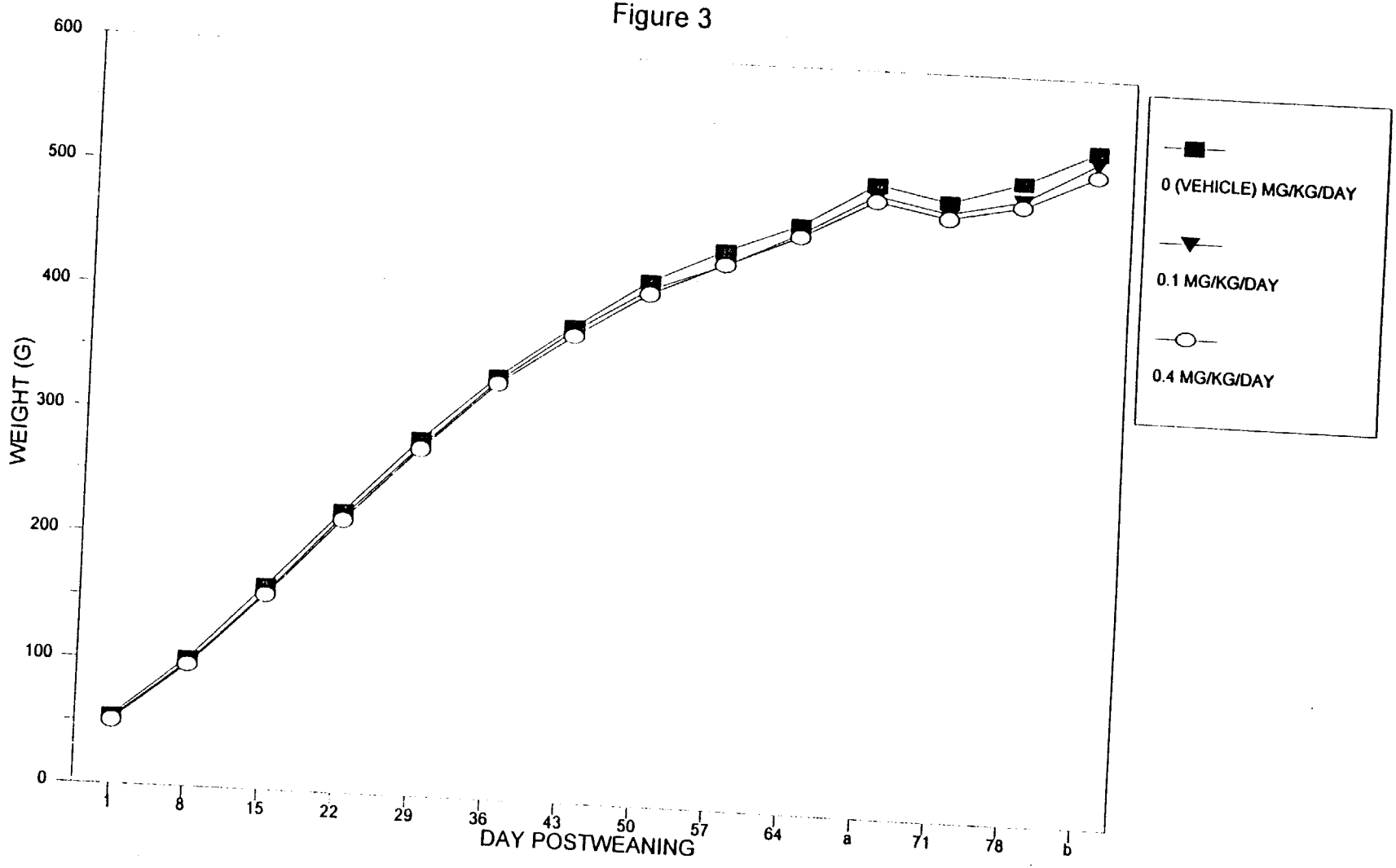
a. Last value recorded before cohabitation.
b. First value recorded after cohabitation.
c. Terminal body weight.

BODY WEIGHTS
Fo GENERATION FEMALE RATS
Figure 2



a. Last value recorded before cohabitation.

BODY WEIGHTS
F1 GENERATION MALE RATS
Figure 3



a. Precohabitation body weights.
b. Terminal body weights.

BODY WEIGHTS
F1 GENERATION FEMALE RATS
Figure 4

a. Precohabitation body weight.

APPENDIX B

REPORT TABLES - Fo GENERATION MALE RATS

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 B1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
MAXIMUM POSSIBLE INCIDENCE	2345/ 35	2345/ 35	2345/ 35	2345/ 35	2345/ 35
MORTALITY	0	0	0	0	0
LOCALIZED ALOPECIA: LIMBS	102/ 4	54/ 2	37/ 2	100/ 4	3/ 2
LEFT EYE: CORNEAL OPACITY	0/ 0	0/ 0	0/ 0	0/ 0	34/ 1
INCISORS: TOTAL	78/ 3	2/ 1	102/ 4	66/ 2	12/ 1
MISSING/BROKEN	45/ 3	2/ 1	68/ 4	19/ 1	12/ 1
MISALIGNED	51/ 1	0/ 0	80/ 2	47/ 1	0/ 0
NECK: ABRASION	0/ 0	0/ 0	0/ 0	0/ 0	3/ 1
HYPERACTIVITY	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
EXCESS SALIVATION	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
CHROMODACRYORRHEA	24/ 1	0/ 0	34/ 2	41/ 1	0/ 0
SNOUT: SWOLLEN	0/ 0	0/ 0	27/ 1	43/ 1	0/ 0
CHROMORHINORRHEA	0/ 0	1/ 1	0/ 0	4/ 1	0/ 0
DYSPNEA	0/ 0	0/ 0	0/ 0	4/ 1	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.

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

TABLE B2 (PAGE 1): BODY WEIGHTS - SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	35	35
BODY WEIGHT (G)						
DAY 1	MEAN±S.D	388.9 ± 22.0	390.1 ± 25.7	389.8 ± 23.6	390.8 ± 16.4	391.1 ± 18.0
DAY 8	MEAN±S.D	417.2 ± 25.8	419.8 ± 24.9	417.1 ± 24.0	414.8 ± 21.6	415.6 ± 21.8
DAY 15	MEAN±S.D	441.0 ± 30.1	441.1 ± 28.4	438.1 ± 28.4	437.2 ± 25.2	436.9 ± 25.8
DAY 22	MEAN±S.D	459.1 ± 36.3	460.8 ± 32.1	456.4 ± 32.1	449.6 ± 30.9	448.0 ± 30.2
DAY 29	MEAN±S.D	471.3 ± 41.9	476.7 ± 36.5	472.1 ± 34.3	463.5 ± 29.4	458.0 ± 34.3
DAY 36	MEAN±S.D	488.4 ± 42.4	488.5 ± 36.4	482.3 ± 36.1	472.1 ± 30.4	461.7 ± 36.1**
DAY 42a	MEAN±S.D	501.7 ± 45.3	503.1 ± 40.8	494.8 ± 34.0	484.2 ± 33.8	468.8 ± 37.7**
DAY 49	MEAN±S.D	509.8 ± 46.2	513.2 ± 42.3	501.2 ± 33.6	492.5 ± 35.2	469.4 ± 37.8**
DAY 56b	MEAN±S.D	520.2 ± 48.8	518.6 ± 45.0	507.9 ± 37.2	496.9 ± 36.5*	473.2 ± 37.9**
DAY 63	MEAN±S.D	538.0 ± 52.1	535.2 ± 48.6	520.2 ± 39.7	510.9 ± 39.4*	480.8 ± 41.8**
TERMINAL BODY WEIGHT	MEAN±S.D	542.6 ± 52.3	539.3 ± 49.2	522.6 ± 43.2	512.8 ± 40.5*	482.1 ± 41.5**

DAY = DAY OF STUDY
a. Last value recorded before cohabitation.
b. First value recorded after cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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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 B3 (PAGE 1): BODY WEIGHT CHANGES - SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	35	35
BODY WEIGHT CHANGE (G)						
DAYS 1 - 8	MEAN±S.D.	+28.2 ± 16.1	+29.7 ± 11.4	+27.3 ± 12.7	+23.9 ± 8.4	+24.5 ± 6.5
DAYS 8 - 15	MEAN±S.D.	+23.8 ± 7.8	+21.3 ± 6.6	+21.0 ± 7.4	+22.5 ± 6.8	+21.3 ± 7.3
DAYS 15 - 22	MEAN±S.D.	+18.1 ± 9.3	+19.8 ± 7.8	+18.3 ± 8.1	+12.3 ± 16.6	+11.1 ± 10.7*
DAYS 22 - 29	MEAN±S.D.	+12.2 ± 17.2	+15.9 ± 7.5	+15.7 ± 6.5	+13.9 ± 8.8	+10.0 ± 8.2
DAYS 29 - 36	MEAN±S.D.	+17.1 ± 15.7	+11.8 ± 5.6	+10.2 ± 5.7	+8.6 ± 7.4**	+3.6 ± 6.7**
DAYS 36 - 42a	MEAN±S.D.	+13.3 ± 7.8	+14.6 ± 6.9	+12.4 ± 12.1	+12.1 ± 7.2	+7.2 ± 6.3**
DAYS 1 - 42a	MEAN±S.D.	+112.8 ± 33.7	+113.0 ± 25.8	+105.0 ± 24.6	+93.4 ± 22.4**	+77.8 ± 25.2**
DAYS 56b- 63	MEAN±S.D.	+17.7 ± 5.8	+16.6 ± 7.2	+12.2 ± 8.8*	+14.0 ± 6.6	+7.7 ± 11.4**
DAYS 1 - 63	MEAN±S.D.	+149.0 ± 40.3	+145.1 ± 33.6	+130.3 ± 30.8*	+120.0 ± 28.9**	+89.8 ± 29.6**
DAY 1 - TERMINATION	MEAN±S.D.	+153.6 ± 41.5	+149.2 ± 34.5	+132.8 ± 34.0*	+121.9 ± 30.2**	+91.0 ± 29.9**

DAY(S) = DAY(S) OF STUDY
a. Last value recorded before cohabitation.
b. First value recorded after cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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

TABLE B4 (PAGE 1): ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	35	35
FEED CONSUMPTION (G/DAY)						
DAYS 1 - 8	MEAN±S.D.	26.6 ± 3.0	26.7 ± 2.2	26.8 ± 2.1	26.4 ± 2.4	26.8 ± 2.3
DAYS 8 - 15	MEAN±S.D.	27.0 ± 2.8	27.0 ± 2.2 [34]a	26.8 ± 2.4	25.9 ± 2.0	25.8 ± 2.3
DAYS 15 - 22	MEAN±S.D.	27.8 ± 3.4 [34]a	27.9 ± 3.0	27.4 ± 2.8	26.3 ± 2.8 [34]a	25.1 ± 3.4**
DAYS 22 - 29	MEAN±S.D.	28.0 ± 3.6	28.5 ± 2.4	27.9 ± 2.2	26.8 ± 2.4	26.4 ± 2.5
DAYS 29 - 36	MEAN±S.D.	29.5 ± 3.7	29.2 ± 2.4 [34]a	28.7 ± 2.4 [34]a	27.8 ± 2.6 [33]a	25.6 ± 2.5** [34]a
DAYS 36 - 42b	MEAN±S.D.	27.8 ± 3.2	27.4 ± 2.3	27.0 ± 2.9	26.6 ± 3.7	24.2 ± 2.1**
DAYS 1 - 42b	MEAN±S.D.	27.8 ± 2.8	27.8 ± 2.1	27.4 ± 1.9	26.6 ± 2.0*	25.7 ± 2.0**
DAYS 56c- 63	MEAN±S.D.	28.0 ± 2.7 [34]a	27.6 ± 2.4	26.6 ± 2.2*	25.8 ± 2.3**	23.7 ± 2.4**

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values that were incorrectly recorded, as well as those associated with spillage or wet feed.
b. Last value recorded before cohabitation.
c. First value recorded after cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 418-008: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS (SPONSOR'S STUDY NUMBER 6294.9)

TABLE B5 (PAGE 1): RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	35	35
FEED CONSUMPTION (G/KG/DAY)						
DAYS 1 - 8	MEAN±S.D.	65.8 ± 5.4	65.9 ± 4.6	66.4 ± 4.6	65.5 ± 4.2	66.3 ± 3.8
DAYS 8 - 15	MEAN±S.D.	63.0 ± 4.0	62.8 ± 3.6 [34]a	62.7 ± 3.9	60.9 ± 3.3*	60.6 ± 3.6*
DAYS 15 - 22	MEAN±S.D.	61.5 ± 4.4 [34]a	61.9 ± 4.8	61.4 ± 4.9	59.2 ± 5.2 [34]a	56.7 ± 6.3**
DAYS 22 - 29	MEAN±S.D.	60.2 ± 6.7	60.9 ± 3.6	60.2 ± 4.0	58.9 ± 4.4	58.3 ± 4.7
DAYS 29 - 36	MEAN±S.D.	61.5 ± 5.8	60.2 ± 3.1 [34]a	59.9 ± 3.3 [34]a	59.3 ± 4.7 [33]a	55.8 ± 3.1** [34]a
DAYS 36 - 42b	MEAN±S.D.	56.2 ± 4.2	55.2 ± 2.4	55.3 ± 5.7	55.6 ± 5.9	52.0 ± 3.0**
DAYS 1 - 42b	MEAN±S.D.	61.4 ± 3.3	61.2 ± 2.4	61.0 ± 2.7	59.9 ± 2.8*	58.4 ± 2.3**
DAYS 56c- 63	MEAN±S.D.	52.8 ± 2.5 [34]a	52.4 ± 3.4	51.7 ± 3.3	51.2 ± 3.3	49.6 ± 2.6**

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values that were incorrectly recorded, as well as those associated with spillage or wet feed.
b. Last value recorded before cohabitation.
c. First value recorded after cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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

TABLE B6 (PAGE 1): MATING AND FERTILITY - SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS IN COHABITATION	N	35	35	35	34a	35
DAYS IN COHABITATION b	MEAN±S.D	2.7 ± 1.8 [34]	2.6 ± 1.3	2.5 ± 1.8	2.1 ± 1.2 [31]	2.7 ± 1.8
RATS THAT MATED c	N(%)	35(100.0)	35(100.0)	33(94.3)	34(100.0)	32(91.4)
FERTILITY INDEX d,e	N/N (%)	33/35 (94.3)	32/35 (91.4)	27/33 (81.8)	29/34 (85.3)	28/32 (87.5)
RATS WITH CONFIRMED MATING DATES f	N	34	35	33	31	32
RATS MATING g DAYS 1-7	N(%)	34(100.0)	35(100.0)	33(100.0)	31(100.0)	32(100.0)
RATS PREGNANT/RATS IN COHABITATION f	N/N (%)	33/35 (94.3)	32/35 (91.4)	27/35 (77.1)	29/34 (85.3)	28/35 (80.0)

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rat 8238, which was not assigned to cohabitation because there were no available female rats.
b. Restricted to rats with a confirmed mating date on days 1 to 7 of cohabitation and rats that did not mate.
c. Includes only one mating for each male rat.
d. Number of pregnancies/number of rats that mated.
e. Includes only one pregnancy for each rat that impregnated more than one female rat.
f. Includes only one confirmed mating for each male rat.
g. Restricted to rats with a confirmed mating date on days 1 to 7 of cohabitation.

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TABLE B7 (PAGE 1): NECROPSY OBSERVATIONS SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS EXAMINED a	N	35	35	35	35	35
MORTALITY	N	0	0	0	0	0
APPEARED NORMAL	N	33	35	34	32	29
TESTES:						
BILATERAL, SMALL, PURPLE AND/OR FLACCID	N	0	0	0	1	3
LIVER:						
LIGHT BROWN OR BROWN	N	0	0	0	0	3**
LARGE	N	1	0	0	1	0
ROUGH SURFACE	N	0	0	0	1	0
PAPILLARY PROCESS, CONSTRICTED AND NUMEROUS TAN, RAISED AREAS	N	0	0	0	1	0
PROSTATE:						
SMALL	N	1	0	0	0	1
EYES:						
LEFT, LENS OPAQUE	N	0	0	0	0	1
KIDNEYS:						
BILATERAL, LARGE	N	1	0	0	1	0
PARENCHYMA, CONTAINED MULTIPLE, PALE, FLUID-FILLED CYSTS	N	0	0	0	1	0
RIGHT, PELVIS, SLIGHT DILATION	N	0	0	1	0	0
SPLEEN:						
LARGE	N	1	0	0	0	0

a. Refer to the individual clinical observations table (Table B10) for external observations confirmed at necropsy.

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TABLE B8 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	35	35
TERMINAL BODY WEIGHT	MEAN±S.D.	542.6 ± 52.3	539.3 ± 49.2	522.6 ± 43.2	512.8 ± 40.5*	482.1 ± 41.5**
EPIDIDYMIS LEFT	MEAN±S.D.	0.70 ± 0.08	0.72 ± 0.08	0.70 ± 0.06	0.70 ± 0.07	0.68 ± 0.07
TESTIS LEFT	MEAN±S.D.	1.72 ± 0.19 [33]b	1.76 ± 0.14	1.72 ± 0.15	1.74 ± 0.13 [34]b	1.79 ± 0.22 [32]b
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	2.07 ± 0.34	2.11 ± 0.32	1.97 ± 0.33 [34]b	2.01 ± 0.35 [34]b	1.77 ± 0.28** [34]b
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.99 ± 0.23	1.01 ± 0.21	0.95 ± 0.18	0.98 ± 0.17	0.92 ± 0.19
EPIDIDYMIS RIGHT	MEAN±S.D.	0.72 ± 0.08	0.74 ± 0.07	0.72 ± 0.06	0.70 ± 0.08	0.69 ± 0.08
TESTIS RIGHT	MEAN±S.D.	1.75 ± 0.16	1.75 ± 0.15	1.71 ± 0.16	1.74 ± 0.14 [34]b	1.78 ± 0.22 [32]b
PROSTATE	MEAN±S.D.	1.18 ± 0.27 [34]b	1.28 ± 0.28	1.15 ± 0.31	1.13 ± 0.29	1.02 ± 0.22* [34]b

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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TABLE B9 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT SUMMARY - Fo GENERATION MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	35	35	35	35	35
TERMINAL BODY WEIGHT	MEAN±S.D.	542.6 ± 52.3	539.3 ± 49.2	522.6 ± 43.2	512.8 ± 40.5*	482.1 ± 41.5**
EPIDIDYMIS LEFT	MEAN±S.D.	0.131 ± 0.018	0.134 ± 0.016	0.134 ± 0.016	0.136 ± 0.016	0.141 ± 0.018
TESTIS LEFT	MEAN±S.D.	0.319 ± 0.043	0.327 ± 0.032	0.331 ± 0.037	0.339 ± 0.032 [34]b	0.369 ± 0.046** [32]b
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.382 ± 0.062 [33]b	0.391 ± 0.054	0.380 ± 0.067 [34]b	0.394 ± 0.068 [34]b	0.368 ± 0.061 [34]b
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.183 ± 0.046	0.186 ± 0.036	0.182 ± 0.035	0.192 ± 0.035	0.191 ± 0.040
EPIDIDYMIS RIGHT	MEAN±S.D.	0.134 ± 0.018	0.138 ± 0.016	0.137 ± 0.015	0.137 ± 0.016	0.142 ± 0.016
TESTIS RIGHT	MEAN±S.D.	0.324 ± 0.038	0.327 ± 0.038	0.328 ± 0.034	0.340 ± 0.035 [34]b	0.370 ± 0.046** [32]b
PROSTATE	MEAN±S.D.	0.218 ± 0.053 [34]b	0.239 ± 0.057	0.220 ± 0.062	0.219 ± 0.054	0.212 ± 0.045 [34]b

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) x 100.
a. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

APPENDIX C

REPORT TABLES - Fo GENERATION FEMALE RATS

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 C1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP	I	II	III	IV	V
DOSAGE (MG/KG/DAY)	0 (VEHICLE)	0.1	0.4	1.6	3.2
PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION):					
MAXIMUM POSSIBLE INCIDENCE	1470/ 35	1470/ 35	1470/ 35	1464/ 35	1470/ 35
MORIBUND SACRIFICED	0	0	0	1a	0
LOCALIZED ALOPECIA: TOTAL	0/ 0	0/ 0	83/ 4	43/ 3	65/ 3
LIMBS	0/ 0	0/ 0	83/ 4	32/ 2	60/ 2
UNDERSIDE	0/ 0	0/ 0	0/ 0	11/ 1	5/ 1
CHROMODACRYORRHEA	16/ 1	2/ 1	0/ 0	2/ 1a	3/ 1
INCISORS: MISSING/BROKEN	0/ 0	47/ 2	11/ 1	0/ 0	6/ 1
CLEAR ORAL EXUDATE	0/ 0	0/ 0	0/ 0	2/ 1a	0/ 0
PTOSIS	0/ 0	0/ 0	0/ 0	1/ 1a	0/ 0
HUNCHED POSTURE	0/ 0	0/ 0	0/ 0	1/ 1a	0/ 0
LABORED BREATHING	0/ 0	0/ 0	0/ 0	1/ 1a	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Rat 8938 was moribund sacrificed on day 36 of study.

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TABLE C1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP	I	II	III	IV	V
DOSAGE (MG/KG/DAY)	0 (VEHICLE)	0.1	0.4	1.6	3.2
PRESUMED GESTATION: a					
MAXIMUM POSSIBLE INCIDENCE	649/ 34	660/ 35	647/ 34	581/ 31	666/ 35
MORTALITY	0	0	0	0	0
LOCALIZED ALOPECIA: TOTAL	0/ 0	19/ 1	75/ 4*	45/ 4**	83/ 8**
LIMBS	0/ 0	19/ 1	75/ 4	38/ 3	40/ 3
BACK	0/ 0	0/ 0	0/ 0	7/ 1	18/ 3
UNDERSIDE	0/ 0	0/ 0	4/ 1	0/ 0	31/ 3
INCISORS: TOTAL	0/ 0	22/ 1	2/ 1	0/ 0	33/ 2
MISSING/BROKEN	0/ 0	22/ 1	2/ 1	0/ 0	21/ 1
MISALIGNED	0/ 0	0/ 0	0/ 0	0/ 0	12/ 1
CHROMODACRYORRHEA	4/ 2	0/ 0	0/ 0	0/ 0	12/ 1

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Restricted to rats with a confirmed mating date.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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

TABLE C1 (PAGE 3): CLINICAL OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
LACTATION:						
MAXIMUM POSSIBLE INCIDENCE		483/ 23	525/ 25	462/ 22	384/ 20	31/ 21
MORTALITY		0	0	0	0	0
LOCALIZED ALOPECIA:	TOTAL	41/ 3	22/ 2	65/ 4	65/ 5	7/ 5
	BACK	0/ 0	0/ 0	0/ 0	21/ 1	4/ 3**
	UNDERSIDE	0/ 0	0/ 0	21/ 1	1/ 1	2/ 2
	LIMBS	21/ 2	22/ 2	65/ 4	43/ 3	2/ 1
	HEAD	20/ 1	0/ 0	0/ 0	0/ 0	0/ 0
INCISORS:	TOTAL	0/ 0	21/ 1	0/ 0	0/ 0	3/ 2
	MISSING/BROKEN	0/ 0	21/ 1	0/ 0	0/ 0	2/ 1
	MISALIGNED	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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TABLE C2 (PAGE 1): BODY WEIGHTS - PRECOHABITATION SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP			I	II	III	IV	V
DOSAGE (MG/KG/DAY)			0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED		N	35	35	35	35	35
BODY WEIGHT (G)							
DAY 1		MEAN±S.D	237.2 ± 12.2	236.4 ± 9.4	237.1 ± 11.8	238.4 ± 11.7	236.0 ± 10.2
DAY 8		MEAN±S.D	243.8 ± 14.5	243.8 ± 11.3	245.7 ± 13.5	243.9 ± 13.6	239.4 ± 12.3
DAY 15		MEAN±S.D	253.6 ± 17.3	252.4 ± 11.8	251.9 ± 15.5	251.5 ± 13.6	239.6 ± 13.6**
DAY 22		MEAN±S.D	256.2 ± 19.0	255.4 ± 12.4	255.8 ± 15.9	252.1 ± 17.8	238.6 ± 15.0**
DAY 29		MEAN±S.D	265.2 ± 20.4	263.0 ± 14.1	265.3 ± 18.2	259.2 ± 18.0	239.9 ± 15.6**
DAY 36		MEAN±S.D	269.5 ± 22.9	268.7 ± 13.5	268.8 ± 19.3	259.5 ± 19.9	237.2 ± 14.5**
DAY 42a		MEAN±S.D	274.4 ± 24.1	272.4 ± 15.1	271.6 ± 18.8	263.6 ± 19.1 [34]b	241.4 ± 15.7**

DAY = DAY OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Last value recorded before cohabitation.
b. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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TABLE C3 (PAGE 1): BODY WEIGHT CHANGES - PRECOHABITATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	35	35
BODY WEIGHT CHANGE (G)						
DAYS 1 - 8	MEAN±S.D.	+6.6 ± 7.3	+7.5 ± 6.4	+8.6 ± 5.7	+5.5 ± 5.7	+3.4 ± 5.6
DAYS 8 - 15	MEAN±S.D.	+9.8 ± 6.3	+8.5 ± 4.5	+6.2 ± 10.6	+7.6 ± 4.5	+0.2 ± 5.2**
DAYS 15 - 22	MEAN±S.D.	+2.6 ± 6.7	+3.0 ± 5.7	+3.9 ± 9.0	+0.6 ± 7.0	-1.0 ± 5.5
DAYS 22 - 29	MEAN±S.D.	+9.0 ± 5.6	+7.6 ± 6.3	+9.5 ± 6.6	+7.0 ± 7.0	+1.3 ± 5.8**
DAYS 29 - 36	MEAN±S.D.	+4.3 ± 6.0	+5.8 ± 5.1	+3.4 ± 5.2	+0.3 ± 6.7**	-2.7 ± 6.5**
DAYS 36 - 42a	MEAN±S.D.	+4.8 ± 6.8	+3.7 ± 4.8	+2.8 ± 4.1	+2.9 ± 4.5 [34]b	+4.3 ± 4.7
DAYS 1 - 42a	MEAN±S.D.	+37.1 ± 15.8	+36.0 ± 10.5	+34.5 ± 12.9	+25.0 ± 11.9** [34]b	+5.4 ± 10.2**

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Last value recorded before cohabitation.
b. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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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 C4 (PAGE 1): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	35	35	35	34a	35
PREGNANT	N	33	32	28	29	30
INCLUDED IN ANALYSES	N	32b	32	28	26b	30
MATERNAL BODY WEIGHT (G)						
DAY 0	MEAN±S.D.	270.2 ± 18.9	271.0 ± 15.9	272.7 ± 20.0	262.0 ± 21.0	240.0 ± 17.6**
DAY 1	MEAN±S.D.	277.1 ± 18.2	276.5 ± 15.4	278.2 ± 20.4	268.8 ± 21.5	244.2 ± 16.0**
DAY 2	MEAN±S.D.	281.8 ± 17.2	281.4 ± 14.6	282.6 ± 20.3	272.2 ± 21.0	245.3 ± 17.8**
DAY 3	MEAN±S.D.	284.3 ± 18.3	285.0 ± 16.0	286.2 ± 20.9	273.0 ± 20.1*	246.0 ± 18.3**
DAY 4	MEAN±S.D.	287.9 ± 18.1	288.1 ± 15.9	286.8 ± 21.4	276.3 ± 21.3*	247.2 ± 18.8**
DAY 5	MEAN±S.D.	290.4 ± 19.1	289.5 ± 15.8	290.6 ± 20.9	277.8 ± 22.4*	250.1 ± 18.5**
DAY 6	MEAN±S.D.	293.5 ± 18.5	293.1 ± 16.5	293.1 ± 20.4	280.9 ± 21.9*	253.2 ± 19.4**
DAY 7	MEAN±S.D.	295.5 ± 19.2	293.7 ± 16.7	294.3 ± 20.4	282.5 ± 23.0*	254.6 ± 19.3**
DAY 8	MEAN±S.D.	299.3 ± 19.7	296.1 ± 17.0	297.5 ± 21.0	285.4 ± 22.2*	255.8 ± 19.4**
DAY 9	MEAN±S.D.	302.7 ± 19.7	300.3 ± 17.1	301.2 ± 20.9	288.0 ± 22.5**	258.3 ± 18.9**
DAY 10	MEAN±S.D.	307.8 ± 19.3	305.8 ± 18.0	306.2 ± 20.0	293.5 ± 22.7*	264.3 ± 18.9**

DAY = DAY OF GESTATION
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for rats that did not have a confirmed mating date.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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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 C4 (PAGE 2): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	35	35	35	34a	35
PREGNANT	N	33	32	28	29	30
INCLUDED IN ANALYSES	N	22b,c	25c	22c	17b,c	21c
MATERNAL BODY WEIGHT (G)						
DAY 11	MEAN±S.D.	312.5 ± 19.2	313.5 ± 15.9	313.4 ± 19.5	298.5 ± 21.4*	266.0 ± 20.9**
DAY 12	MEAN±S.D.	317.0 ± 20.0	317.2 ± 16.5	316.7 ± 20.6	303.6 ± 22.6	268.1 ± 20.8**
DAY 13	MEAN±S.D.	320.2 ± 19.8	320.4 ± 16.7	320.6 ± 19.4	306.9 ± 21.3	271.9 ± 21.7**
DAY 14	MEAN±S.D.	325.0 ± 20.0	325.4 ± 17.0	326.2 ± 19.7	311.2 ± 22.1	276.7 ± 23.5**
DAY 15	MEAN±S.D.	332.2 ± 20.1	332.7 ± 17.9	333.1 ± 19.3	320.2 ± 22.2	283.4 ± 23.1**
DAY 16	MEAN±S.D.	342.0 ± 21.9	342.7 ± 17.6	343.9 ± 19.0	330.6 ± 22.8	294.1 ± 21.8**
DAY 17	MEAN±S.D.	356.3 ± 23.5	357.6 ± 18.2	358.4 ± 18.5	345.4 ± 24.9	308.6 ± 21.9**
DAY 18	MEAN±S.D.	370.9 ± 25.9	371.0 ± 19.9	370.6 ± 19.7	359.7 ± 26.4	325.0 ± 22.0**
DAY 19	MEAN±S.D.	383.5 ± 26.0	383.1 ± 18.6	384.5 ± 19.7	373.2 ± 28.2	339.6 ± 22.4**
DAY 20	MEAN±S.D.	396.4 ± 27.3	397.5 ± 19.8	397.8 ± 21.7	386.1 ± 29.6	348.8 ± 23.3**

DAY = DAY OF GESTATION
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for rats that did not have a confirmed mating date.
c. Excludes values for rats that were assigned to Caesarean-sectioning on day 10 of gestation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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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 C5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS TESTED	N	35	35	35	34a	35
PREGNANT	N	33	32	28	29	30
INCLUDED IN ANALYSES	N	32b	32	28	26b	30
MATERNAL BODY WEIGHT CHANGE (G)						
DAYS 0 - 7	MEAN±S.D.	+25.3 ± 7.5	+22.7 ± 7.4	+21.6 ± 6.5	+20.5 ± 7.1*	+14.6 ± 6.5**
DAYS 7 - 10	MEAN±S.D.	+12.2 ± 4.4	+12.0 ± 5.2	+12.0 ± 5.6	+10.9 ± 6.0	+9.7 ± 7.6
DAYS 10 - 12	MEAN±S.D.	+10.2 ± 3.4 [22]c	+10.3 ± 4.6 [25]c	+8.8 ± 4.1 [22]c	+10.5 ± 5.5 [17]c	+5.3 ± 6.3** [21]c
DAYS 12 - 15	MEAN±S.D.	+15.3 ± 4.2 [22]c	+15.5 ± 4.7 [25]c	+16.4 ± 5.5 [22]c	+16.6 ± 4.8 [17]c	+15.3 ± 6.7 [21]c
DAYS 15 - 18	MEAN±S.D.	+38.7 ± 7.8 [22]c	+38.2 ± 7.4 [25]c	+37.4 ± 8.8 [22]c	+39.5 ± 8.0 [17]c	+41.5 ± 6.2 [21]c
DAYS 18 - 20	MEAN±S.D.	+25.5 ± 6.8 [22]c	+26.5 ± 5.5 [25]c	+27.2 ± 7.4 [22]c	+26.4 ± 7.4 [17]c	+23.8 ± 4.2 [21]c
DAYS 0 - 20	MEAN±S.D.	+125.1 ± 15.9 [22]c	+123.8 ± 13.3 [25]c	+121.9 ± 20.2 [22]c	+123.1 ± 18.3 [17]c	+108.0 ± 10.6** [21]c

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for rats that did not have a confirmed mating date.
c. Excludes values for rats that were assigned to Caesarean-sectioning on day 10 of gestation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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TABLE C6 (PAGE 1): MATERNAL BODY WEIGHTS - LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	24a	25
PREGNANT	N	23	25	22	20	21
DELIVERED LITTERS	N	23	25	22	20	21
MATERNAL BODY WEIGHT (G)						
DAY 1	MEAN±S.D.	298.3 ± 19.4	299.0 ± 18.0 [24]b	298.0 ± 23.2	297.8 ± 21.6	273.3 ± 19.7** [19]c
DAY 4	MEAN±S.D.	303.8 ± 24.0	304.0 ± 17.9	299.4 ± 21.3	290.7 ± 22.5 [18]d	d
DAY 7	MEAN±S.D.	311.8 ± 21.3	306.0 ± 14.9	305.5 ± 18.9	294.7 ± 22.7 [18]d	
DAY 10	MEAN±S.D.	325.1 ± 20.7	318.0 ± 15.3	320.3 ± 16.6	306.4 ± 20.2** [18]d	
DAY 14	MEAN±S.D.	327.0 ± 19.6	326.5 ± 19.5	324.0 ± 22.4	321.4 ± 22.2 [18]d	
DAY 21	MEAN±S.D.	331.2 ± 21.3	326.4 ± 14.9	331.8 ± 16.1	329.4 ± 20.4 [18]d	

DAY = DAY OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for dam 8842, which delivered one additional pup on day 2 of lactation.
c. Excludes values that were not recorded.
d. Excludes values for dams that had no surviving pups.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

000596

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 C7 (PAGE 1): MATERNAL BODY WEIGHT CHANGES LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	24a	25
PREGNANT	N	23	25	22	20	21
DELIVERED LITTERS	N	23	25	22	20	21
INCLUDED IN ANALYSES	N	23	25	22	18b	0b
MATERNAL BODY WEIGHT CHANGE (G)						
DAYS 1 - 4	MEAN±S.D.	+5.5 ± 11.0	+5.3 ± 8.4 [24]c	+1.4 ± 11.1	-6.7 ± 11.0**	
DAYS 4 - 7	MEAN±S.D.	+8.0 ± 10.8	+2.0 ± 7.6	+6.1 ± 11.5	+4.0 ± 7.9	
DAYS 7 - 10	MEAN±S.D.	+13.3 ± 17.4	+12.0 ± 6.0	+14.8 ± 5.9	+11.7 ± 6.4	
DAYS 10 - 14	MEAN±S.D.	+1.9 ± 18.4	+8.5 ± 10.7	+3.6 ± 15.2	+15.0 ± 6.4*	
DAYS 14 - 21	MEAN±S.D.	+4.1 ± 19.4	-0.1 ± 10.0	+7.8 ± 15.2	+8.0 ± 9.3	
DAYS 1 - 21	MEAN±S.D.	+32.8 ± 19.7	+27.8 ± 12.3 [24]c	+33.8 ± 17.8	+32.0 ± 14.6	

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for dams that had no surviving pups.
c. Excludes values for dam 8842, which delivered one additional pup on day 2 of lactation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

000597

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 C8 (PAGE 1): ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - PRECOHABITATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	35	35
FEED CONSUMPTION (G/DAY)						
DAYS 1 - 8	MEAN±S.D.	19.2 ± 2.2 [34]a	19.6 ± 3.0	19.3 ± 2.6	19.7 ± 3.0	17.8 ± 1.9*
DAYS 8 - 15	MEAN±S.D.	19.8 ± 2.2	19.9 ± 2.1 [31]a	18.8 ± 2.4 [32]a	19.3 ± 2.3 [34]a	17.2 ± 2.8**
DAYS 15 - 22	MEAN±S.D.	19.3 ± 2.6	19.7 ± 1.8	19.4 ± 2.0	18.4 ± 1.8 [34]a	16.0 ± 2.0**
DAYS 22 - 29	MEAN±S.D.	20.0 ± 2.5	19.5 ± 2.0	19.3 ± 2.0 [34]a	18.5 ± 2.2**	16.2 ± 1.8**
DAYS 29 - 36	MEAN±S.D.	19.2 ± 2.2 [34]a	19.1 ± 2.0 [34]a	18.6 ± 2.0	18.0 ± 2.7*	14.7 ± 1.7**
DAYS 36 - 42b	MEAN±S.D.	19.3 ± 2.0	19.4 ± 2.0 [34]a	18.9 ± 1.8	18.7 ± 2.5 [34]c	16.3 ± 2.7**
DAYS 1 - 42b	MEAN±S.D.	19.5 ± 1.9	19.6 ± 1.5 [34]a	19.1 ± 1.7	18.9 ± 2.1 [34]c	16.4 ± 1.6**

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.
c. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

000598

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 C9 (PAGE 1): RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - PRECOHABITATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	35	35
FEED CONSUMPTION (G/KG/DAY)						
DAYS 1 - 8	MEAN±S.D.	79.7 ± 7.2 [34]a	81.5 ± 11.5	80.0 ± 9.2	81.6 ± 11.8	74.6 ± 6.2*
DAYS 8 - 15	MEAN±S.D.	79.8 ± 7.8	79.8 ± 8.5 [31]a	75.5 ± 8.0 [32]a	78.1 ± 8.1 [34]a	71.6 ± 10.2**
DAYS 15 - 22	MEAN±S.D.	75.7 ± 7.6	77.6 ± 6.9	76.3 ± 6.1	73.3 ± 5.8 [34]a	66.8 ± 6.0**
DAYS 22 - 29	MEAN±S.D.	76.6 ± 7.1	75.4 ± 5.7	73.8 ± 5.9 [34]a	72.4 ± 6.3**	67.8 ± 5.5**
DAYS 29 - 36	MEAN±S.D.	71.5 ± 5.2 [34]a	72.0 ± 6.9 [34]a	69.6 ± 4.7	69.0 ± 7.5	61.6 ± 5.2**
DAYS 36 - 42b	MEAN±S.D.	71.0 ± 5.9	71.9 ± 5.8 [34]a	69.9 ± 4.7	71.2 ± 7.4 [34]c	68.2 ± 10.4
DAYS 1 - 42b	MEAN±S.D.	75.7 ± 4.5	76.5 ± 4.4 [34]a	74.3 ± 4.3	74.5 ± 6.0 [34]c	68.5 ± 4.3**

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.
c. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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

TABLE C10 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	34a	35
PREGNANT	N	33	32	28	29	30
INCLUDED IN ANALYSES	N	32b	32	28	26b	30
MATERNAL FEED CONSUMPTION (G/DAY)						
DAYS 0 - 7	MEAN±S.D.	21.8 ± 2.1	21.6 ± 2.1	21.0 ± 2.1	20.4 ± 2.2*	17.0 ± 2.1**
DAYS 7 - 10	MEAN±S.D.	23.4 ± 2.2	23.6 ± 2.4	22.6 ± 3.0	21.0 ± 2.8**	17.7 ± 2.6**
DAYS 10 - 12	MEAN±S.D.	24.2 ± 3.0 [22]c	24.3 ± 2.5 [25]c	22.9 ± 3.2 [22]c	23.6 ± 3.4 [17]c	18.6 ± 3.3** [21]c
DAYS 12 - 15	MEAN±S.D.	23.8 ± 2.4 [22]c	23.6 ± 2.1 [25]c	22.8 ± 2.5 [22]c	23.4 ± 2.8 [17]c	19.6 ± 2.7** [21]c
DAYS 15 - 18	MEAN±S.D.	24.8 ± 2.8 [22]c	24.4 ± 2.3 [25]c	24.2 ± 1.9 [22]c	26.6 ± 2.9* [17]c	23.3 ± 2.7 [20]c,d
DAYS 18 - 20	MEAN±S.D.	22.2 ± 2.6 [22]c	22.4 ± 2.3 [24]c,d	22.9 ± 2.5 [22]c	23.6 ± 3.3 [17]c	21.2 ± 2.5 [21]c
DAYS 0 - 20	MEAN±S.D.	23.0 ± 1.9 [22]c	22.9 ± 1.2 [24]c,d	22.4 ± 2.0 [22]c	22.8 ± 2.2 [17]c	19.1 ± 1.8** [21]c

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for rats that did not have a confirmed mating date.
c. Excludes values for rats that were assigned to Caesarean-sectioning on day 10 of gestation.
d. Excludes values that were associated with spillage or wet feed.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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

TABLE C11 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS TESTED	N	35	35	35	34a	35
PREGNANT	N	33	32	28	29	30
INCLUDED IN ANALYSES	N	32b	32	28	26b	30
MATERNAL FEED CONSUMPTION (G/KG/DAY)						
DAYS 0 - 7	MEAN±S.D.	76.7 ± 6.6	75.9 ± 5.6	73.7 ± 6.4	74.6 ± 5.9	68.8 ± 6.0**
DAYS 7 - 10	MEAN±S.D.	77.7 ± 5.4	78.8 ± 5.4	75.3 ± 9.2	72.8 ± 7.4*	68.7 ± 8.4**
DAYS 10 - 12	MEAN±S.D.	77.2 ± 6.3 [22]c	77.8 ± 7.3 [25]c	73.2 ± 9.7 [22]c	78.7 ± 7.4 [17]c	70.1 ± 12.1 [21]c
DAYS 12 - 15	MEAN±S.D.	73.5 ± 5.6 [22]c	72.8 ± 5.4 [25]c	70.4 ± 6.6 [22]c	75.4 ± 6.2 [17]c	71.3 ± 8.5 [21]c
DAYS 15 - 18	MEAN±S.D.	70.7 ± 6.1 [22]c	69.4 ± 6.3 [25]c	69.0 ± 5.7 [22]c	78.5 ± 5.8** [17]c	77.4 ± 7.4** [20]c,d
DAYS 18 - 20	MEAN±S.D.	58.1 ± 6.1 [22]c	58.7 ± 6.1 [24]c,d	59.7 ± 6.7 [22]c	63.4 ± 8.0* [17]c	62.8 ± 6.9* [21]c
DAYS 0 - 20	MEAN±S.D.	72.7 ± 4.3 [22]c	72.4 ± 2.9 [24]c,d	70.5 ± 5.3 [22]c	74.8 ± 4.4 [17]c	70.0 ± 4.7 [21]c

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for rats that did not have a confirmed mating date.
c. Excludes values for rats that were assigned to Caesarean-sectioning on day 10 of gestation.
d. Excludes values that were associated with spillage or wet feed.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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

TABLE C12 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	24a	25
PREGNANT	N	23	25	22	20	21
DELIVERED LITTERS	N	23	25	22	20	21
INCLUDED IN ANALYSES	N	23	25	22	18b	0b
MATERNAL FEED CONSUMPTION (G/DAY)						
DAYS 1 - 4	MEAN±S.D.	25.4 ± 6.9 [22]c	24.0 ± 4.4 [23]c,d	22.0 ± 4.5 [21]c	16.7 ± 4.0**	
DAYS 4 - 7	MEAN±S.D.	37.5 ± 7.5	37.6 ± 5.4	36.3 ± 8.7 [20]c	28.9 ± 6.6**	
DAYS 7 - 10	MEAN±S.D.	47.2 ± 5.2 [22]c	44.8 ± 4.4	45.3 ± 5.1	36.6 ± 7.6**	
DAYS 10 - 14e	MEAN±S.D.	56.9 ± 5.1 [22]c	56.7 ± 5.8	53.6 ± 9.2	46.8 ± 9.2**	
DAYS 1 - 14e	MEAN±S.D.	43.4 ± 5.4 [22]c	42.5 ± 4.5 [24]d	41.0 ± 6.9	33.4 ± 6.5**	

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for dams that had no surviving pups.
c. Excludes values that were not recorded, as well as those associated with spillage or wet feed.
d. Excludes values for dam 8842, which delivered one additional pup on day 2 of lactation.
e. 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 21 of lactation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

000602

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 C13 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - LACTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	24a	25
PREGNANT	N	23	25	22	20	21
DELIVERED LITTERS	N	23	25	22	20	21
INCLUDED IN ANALYSES	N	23	25	22.	18b	0b
MATERNAL FEED CONSUMPTION (G/KG/DAY)						
DAYS 1 - 4	MEAN±S.D.	84.6 ± 22.2 [22]c	79.9 ± 14.1 [23]c,d	74.4 ± 17.0 [21]c	56.9 ± 13.8**	
DAYS 4 - 7	MEAN±S.D.	122.2 ± 24.7	123.6 ± 19.8	120.0 ± 28.3 [20]c	98.6 ± 20.0**	
DAYS 7 - 10	MEAN±S.D.	147.9 ± 15.6 [22]c	144.0 ± 15.4	145.4 ± 18.6	121.7 ± 23.5**	
DAYS 10 - 14e	MEAN±S.D.	174.0 ± 14.1 [22]c	175.9 ± 17.7	166.7 ± 29.0	148.9 ± 25.9**	
DAYS 1 - 14e	MEAN±S.D.	137.8 ± 16.2 [22]c	136.9 ± 15.5 [24]d	133.0 ± 23.8	110.4 ± 19.6**	

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 8938, which was moribund sacrificed on day 36 of study.
b. Excludes values for dams that had no surviving pups.
c. Excludes values that were not recorded, as well as those associated with spillage or wet feed.
d. Excludes values for dam 8842, which delivered one additional pup on day 2 of lactation.
e. 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 21 of lactation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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 C14 (PAGE 1): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
ESTROUS CYCLING						
RATS EVALUATED	N	15	15	15	15	15
ESTROUS STAGES/ 28 DAYS	MEAN±S.D.	6.5 ± 0.7	6.5 ± 0.8	5.7 ± 1.3	6.1 ± 0.8	6.7 ± 0.7
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	3	4	5	2	0
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	1	0	1	0	0

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 C14 (PAGE 2): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
MATING AND FERTILITY						
RATS IN COHABITATION	N	35	35	35	34a	35
DAYS IN COHABITATION b	MEAN±S.D.	2.7 ± 1.8 [34]	2.6 ± 1.3	2.9 ± 3.1	2.1 ± 1.2 [31]	3.2 ± 3.2
RATS THAT MATED	N(%)	35(100.0)	35(100.0)	34(97.1)	34(100.0)	35(100.0)
FERTILITY INDEX c	N/N (%)	33/35 (94.3)	32/35 (91.4)	28/34 (82.4)	29/34 (85.3)	30/35 (85.7)
RATS WITH CONFIRMED MATING DATES	N	34	33	34	31	35
MATED BY FIRST MALE d DAYS 1-7	N(%)	34(100.0)	33(100.0)	33(97.0)	31(100.0)	32(91.4)
MATED BY SECOND MALE d DAYS 8-14	N(%)	0(0.0)	0(0.0)	1(2.9)	0(0.0)	3(8.6)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	33/35 (94.3)	32/35 (91.4)	28/35 (80.0)	29/34 (85.3)	30/35 (85.7)

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for female rat 8938, which was moribund sacrificed on day 36 of study.
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Number of pregnancies/number of rats that mated.
d. Restricted to rats with a confirmed mating date.

000605

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 C15 (PAGE 1): NECROPSY OBSERVATIONS SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS EXAMINED a	N	35	35	35	35	35
MORIBUND SACRIFICED	N	0	0	0	1b	0
APPEARED NORMAL	N	35	35	34	34b	35
SPLEEN: ADHERED TO ABDOMINAL ADIPOSE TISSUE	N	0	0	0	1	0
KIDNEYS: RIGHT, PELVIS, MODERATE DILATION	N	0	0	1	0	0

a. Refer to the individual clinical observations table (Table C22) for external observations confirmed at necropsy.
b. Rat 8938 was moribund sacrificed on day 36 of study.

000606

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 C16 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
RATS ASSIGNED TO CAESAREAN-SECTIONING	N	10	10	10	10	10
PREGNANT	N(%)	10(100.0)	7(70.0)	6(60.0)	9(90.0)	9(90.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 10 OF GESTATION	N	10	7	6	9	9
CORPORA LUTEA	MEAN±S.D.	16.6 ± 3.6	14.6 ± 6.6	14.8 ± 4.3	15.7 ± 4.6	16.2 ± 3.2
IMPLANTATIONS	MEAN±S.D.	13.5 ± 4.0	11.8 ± 5.6	12.2 ± 5.6	12.9 ± 4.2	13.1 ± 4.0
VIABLE EMBRYOS	N	133	83	73	115	118
	MEAN±S.D.	13.3 ± 4.1	11.8 ± 5.6	12.2 ± 5.6	12.8 ± 4.1	13.1 ± 4.0
NONVIABLE EMBRYOS	N	2	0	0	1	0
	MEAN±S.D.	0.2 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	1(10.0)	0(0.0)	0(0.0)	1(11.1)	0(0.0)
DAMS WITH ALL NONVIABLE EMBRYOS	N	0	0	0	0	0
DAMS WITH VIABLE EMBRYOS	N(%)	10(100.0)	7(100.0)	6(100.0)	9(100.0)	9(100.0)
% NONVIABLE EMBRYOS/LITTER	MEAN±S.D.	1.7 ± 5.3	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 1.8	0.0 ± 0.0

000607

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 C17 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	24a	25
PREGNANT	N(%)	23(92.0)	25(100.0)	22(88.0)	20(83.3)	21(84.0)
DELIVERED LITTERS	N(%)	23(100.0)	25(100.0)	22(100.0)	20(100.0)	21(100.0)
DURATION OF GESTATION b	MEAN±S.D.	22.7 ± 0.4 [22]	22.8 ± 0.4 [24]	22.5 ± 0.7 [22]	22.4 ± 0.5 [17]	22.2 ± 0.4** [21]
IMPLANTATION SITES	N	342	384	323	295	262
PER DELIVERED LITTER	MEAN±S.D.	14.9 ± 1.9	15.4 ± 2.2	14.7 ± 3.5	14.8 ± 1.7	12.5 ± 1.4**
DAMS WITH STILLBORN PUPS	N(%)	5(21.7)	2(8.0)	5(22.7)	4(20.0)	15(71.4)**
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
GESTATION INDEX c	%	100.0	100.0	100.0	100.0	95.2
	N/N	23/ 23	25/ 25	22/ 22	20/ 20	20/ 21
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	2(10.0)	20(100.0)**
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N	0	0	0	0	0

a. Rat 8938 was moribund sacrificed on day 36 of study.
b. 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.
c. Number of rats with live offspring/number of pregnant rats.
** Significantly different from the carrier group value ($p \leq 0.01$).

000608

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 C18 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	23	25	22	20	20
PUPS DELIVERED (TOTAL)	N	323	350a	303	260	200
	MEAN±S.D.	14.0 ± 2.1	14.6 ± 2.3a	13.8 ± 3.4	13.0 ± 2.5	10.0 ± 3.4**
LIVEBORN	MEAN±S.D.	13.6 ± 2.3	14.4 ± 2.5a	13.5 ± 3.3	12.7 ± 2.6	7.8 ± 4.0**
	N(%)	313(96.9)	346(98.9)a	297(98.0)	254(97.7)	156(78.0)**
STILLBORN	MEAN±S.D.	0.3 ± 0.7	0.1 ± 0.3a	0.3 ± 0.6	0.3 ± 0.6	2.2 ± 2.3**
	N(%)	7(2.2)	2(0.6)a	6(2.0)	6(2.3)	44(22.0)**
UNKNOWN VITAL STATUS	N	3	2a	0	0	0
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED						
DAY 1	N/N(%)	2/313(0.6)	3/346(0.9)a	1/297(0.3)	27/254(10.6)	71/156(45.5)**
DAYS 2- 4	N/N(%)	2/311(0.6)	3/358(0.8)	4/296(1.4)	59/227(26.0)*	85/ 85(100.0)**
DAYS 5- 7	N/N(%)	0/184(0.0)	0/200(0.0)	0/169(0.0)	6/131(4.6)**	
DAYS 8-14	N/N(%)	0/184(0.0)	0/200(0.0)	1/169(0.6)	1/125(0.8)	
DAYS 15-21	N/N(%)	0/184(0.0)	0/200(0.0)	2/168(1.2)	0/124(0.0)	
VIABILITY INDEX b	%	98.7	98.3	98.3	66.1*	0.0**
	N/N	309/313	340/346a	292/297	168/254	0/156
LACTATION INDEX c	%	100.0	100.0	98.2	94.6**	
	N/N	184/184	200/200	166/169	124/131	

DAY(S) = DAY(S) POSTPARTUM

a. Excludes values for litter 8842; the dam delivered one additional pup on day 2 of lactation.
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.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

000609

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 C18 (PAGE 2): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	23	25	22	20	20
SURVIVING PUPS/LITTER a						
DAY 1 b	MEAN±S.D.	13.6 ± 2.3	14.4 ± 2.4 [24]c	13.5 ± 3.3	12.7 ± 2.6	7.8 ± 4.0**
DAY 4 PRECULLING	MEAN±S.D.	13.4 ± 2.1	14.2 ± 2.4	13.3 ± 3.3	8.4 ± 4.0	0.0 ± 0.0**
DAY 4 POSTCULLING	MEAN±S.D.	8.0 ± 0.0	8.0 ± 0.0	7.7 ± 1.1	6.6 ± 2.6*	0.0 ± 0.0
DAY 7	MEAN±S.D.	8.0 ± 0.0	8.0 ± 0.0	7.7 ± 1.1	6.2 ± 2.8**	0.0 ± 0.0
DAY 14	MEAN±S.D.	8.0 ± 0.0	8.0 ± 0.0	7.6 ± 1.1	6.2 ± 2.8**	0.0 ± 0.0
DAY 21	MEAN±S.D.	8.0 ± 0.0	8.0 ± 0.0	7.5 ± 1.2	6.2 ± 2.8**	0.0 ± 0.0
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED						
DAY 1 b	MEAN±S.D.	46.3 ± 17.0	53.9 ± 14.4 [24]c	52.8 ± 11.9	55.1 ± 17.8	46.7 ± 22.0
DAY 4 PRECULLING	MEAN±S.D.	46.8 ± 16.9	53.2 ± 15.0	53.0 ± 11.6	57.0 ± 21.4 [18]d	d
DAY 4 POSTCULLING	MEAN±S.D.	48.9 ± 11.2	49.0 ± 6.2	50.8 ± 5.5 [20]e	53.8 ± 19.7 [18]d	
DAY 7	MEAN±S.D.	48.9 ± 11.2	49.0 ± 6.2	51.9 ± 6.3	51.7 ± 23.2 [18]d	
DAY 14	MEAN±S.D.	48.9 ± 11.2	49.0 ± 6.2	51.6 ± 5.9	51.8 ± 23.0 [18]d	
DAY 21	MEAN±S.D.	48.9 ± 11.2	49.0 ± 6.2	52.4 ± 6.7	51.8 ± 23.0 [18]d	

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 8842; the dam delivered one additional pup on day 2 of lactation.
d. Excludes values for litters that had no surviving pups.
e. Excludes values for litters 8874 and 8899; a sexing error was found prior to weighing on day 1 postpartum.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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

TABLE C18 (PAGE 3): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4	IV 1.6	V 3.2
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	23	25	22	20	20
INCLUDED IN ANALYSES	N	23	25	22	20	11a
LIVE LITTER SIZE AT WEIGHING						
DAY 1	MEAN±S.D.	13.6 ± 2.2	14.3 ± 2.4 [24]b	13.4 ± 3.4	11.6 ± 3.5	9.1 ± 3.7**
DAY 4 PRECULLING	MEAN±S.D.	13.4 ± 2.1	14.2 ± 2.4	13.3 ± 3.3	9.3 ± 3.0** [18]a	a
DAY 4 POSTCULLING	MEAN±S.D.	8.0 ± 0.0	8.0 ± 0.0	7.7 ± 1.1	7.3 ± 1.4 [18]a	
DAY 7	MEAN±S.D.	8.0 ± 0.0	8.0 ± 0.0	7.7 ± 1.1	6.9 ± 1.9* [18]a	
DAY 14	MEAN±S.D.	8.0 ± 0.0	8.0 ± 0.0	7.6 ± 1.1	6.9 ± 1.9** [18]a	
DAY 21	MEAN±S.D.	8.0 ± 0.0	8.0 ± 0.0	7.5 ± 1.2	6.9 ± 1.9** [18]a	
PUP WEIGHT/LITTER (GRAMS)						
DAY 1	MEAN±S.D.	6.6 ± 0.6	6.6 ± 0.5 [24]b	6.4 ± 0.7	5.7 ± 0.5**	5.3 ± 0.4** [10]a
DAY 4 PRECULLING	MEAN±S.D.	9.2 ± 1.2	8.9 ± 0.8	8.9 ± 1.3	7.3 ± 1.1** [18]a	a
DAY 4 POSTCULLING	MEAN±S.D.	9.4 ± 1.2	9.0 ± 0.7	9.0 ± 1.3	7.5 ± 1.2** [18]a	
DAY 7	MEAN±S.D.	14.8 ± 1.9	14.3 ± 1.2	14.0 ± 1.4	11.0 ± 1.8** [18]a	
DAY 14	MEAN±S.D.	31.1 ± 3.3	30.5 ± 2.3	29.9 ± 2.3	24.4 ± 3.3** [18]a	
DAY 21	MEAN±S.D.	49.8 ± 5.1	48.6 ± 3.6	48.2 ± 4.5	40.3 ± 4.3** [18]a	

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for litters that had no surviving pups.
b. Excludes values for litter 8842; the dam delivered one additional pup on day 2 of lactation.
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

000611

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 C19 (PAGE 1): CLINICAL OBSERVATIONS FROM BIRTH TO DAY 21 POSTPARTUM - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
LITTERS EXAMINED (N)		23	25	22	20	20
TRANSIENT CLINICAL OBSERVATIONS: a		TOTAL FREQUENCY (DAYS x PUPS)/LITTERS WITH OBSERVATIONS				
NOT NURSING	N/N	0/ 0	0/ 0	0/ 0	0/ 0	24/ 3**
PLACENTAS NOT REMOVED	N/N	0/ 0	0/ 0	0/ 0	0/ 0	22/ 2
NOT NESTING	N/N	0/ 0	0/ 0	0/ 0	0/ 0	2/ 1
PERSISTENT CLINICAL OBSERVATIONS: a		TOTAL FREQUENCY (DAYS x PUPS)/LITTERS WITH OBSERVATIONS				
TAIL: MISSING	N/N	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
LEFT FORELIMB: CANNIBALIZED	N/N	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
HEAD: DOMED	N/N	0/ 0	0/ 0	17/ 1	7/ 1	0/ 0
PORTION OF TAIL MISSING	N/N	0/ 0	0/ 0	0/ 0	20/ 1	0/ 0
UMBILICAL HERNIA	N/N	0/ 0	15/ 1	0/ 0	0/ 0	0/ 0
LEFT HINDPAW: DIGITS, MISSING	N/N	21/ 1	0/ 0	0/ 0	0/ 0	0/ 0
TAIL: BENT	N/N	8/ 1	0/ 0	0/ 0	0/ 0	0/ 0

STATISTICAL ANALYSES WERE RESTRICTED TO THE NUMBER OF LITTERS WITH OBSERVATIONS.
N/N = TOTAL FREQUENCY (DAYS x PUPS)/LITTERS WITH OBSERVATIONS
a. Tabulation restricted to adverse observations; all other pups appeared normal.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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 C20 (PAGE 1): REFLEX AND PHYSICAL DEVELOPMENT - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP			I	II	III	IV	V
MATERNAL DOSAGE (MG/KG/DAY)			0 (VEHICLE)	0.1	0.4	1.6	3.2
SURFACE RIGHTING a		N	23	25	22	20	10b
DAY	1	MEAN±S.D.	46.0 ± 20.0	51.6 ± 24.3 [24]c	37.0 ± 22.0	29.4 ± 23.5	3.8 ± 8.1**
DAY	2	MEAN±S.D.	38.7 ± 24.7	34.0 ± 24.8	32.7 ± 17.9	20.4 ± 16.1 [19]b	b
DAY	3	MEAN±S.D.	47.8 ± 27.6	43.4 ± 27.4	39.4 ± 28.6	11.9 ± 10.7** [19]b	
DAY	4	MEAN±S.D.	71.0 ± 31.4	74.4 ± 22.7	61.5 ± 28.1	32.4 ± 21.5** [18]b	
DAY	5	MEAN±S.D.	85.9 ± 21.8	91.0 ± 20.6	84.1 ± 19.0	41.2 ± 32.9** [18]b	
DAY	6	MEAN±S.D.	90.8 ± 21.7	95.5 ± 12.4	90.9 ± 17.3	47.1 ± 30.1** [18]b	
DAY	7	MEAN±S.D.	94.6 ± 13.0	93.5 ± 16.2	93.8 ± 13.2	60.2 ± 33.6** [18]b	
DAY	8	MEAN±S.D.	96.7 ± 8.6	95.5 ± 10.8	98.3 ± 5.8	77.6 ± 29.3** [18]b	
DAY	9	MEAN±S.D.	97.8 ± 6.1	98.5 ± 5.5	99.4 ± 2.7	85.4 ± 27.9 [18]b	
DAY	10	MEAN±S.D.	99.4 ± 2.6	99.5 ± 2.5	100.0 ± 0.0	89.6 ± 28.5* [18]b	
DAY	11	MEAN±S.D.	100.0 ± 0.0	99.5 ± 2.5	100.0 ± 0.0	94.4 ± 16.2 [18]b	
DAY	12	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.0 ± 24.0 [18]b	
DAY	13	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.4 ± 23.6 [18]b	
DAY	14	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.4 ± 23.6 [18]b	

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. The average percentage of pups meeting the criterion for the developmental landmark tested on each day postpartum.
b. Excludes values for dams that had no surviving pups.
c. Excludes values for dam 8842, which delivered one additional pup on day 2 of lactation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

000613

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 C20 (PAGE 2): REFLEX AND PHYSICAL DEVELOPMENT - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
SURFACE RIGHTING (Cont.)a	N	23	25	22	20	21
INCLUDED IN ANALYSES	N	23	25	22	18b	0b
DAY 15	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	97.2 ± 11.8	
DAY 16	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	97.2 ± 11.8	
DAY 17	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	
	MEAN%±S.D.c	2.4 ± 1.8	2.0 ± 1.2	2.7 ± 1.6	4.6 ± 2.6*	
PINNA UNFOLDING a	N	23	25	22	19b	0b
DAY 2	MEAN±S.D.	16.3 ± 36.6	3.2 ± 12.6	6.0 ± 22.1	1.6 ± 7.1	
DAY 3	MEAN±S.D.	73.1 ± 33.5	50.8 ± 35.4*	43.9 ± 38.8*	8.4 ± 19.8**	
DAY 4	MEAN±S.D.	99.2 ± 3.7	97.6 ± 8.5	98.3 ± 6.3	47.7 ± 47.6** [18]b	
DAY 5	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	81.9 ± 32.1** [18]b	
DAY 6	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.0 ± 20.7 [18]b	
DAY 7	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0 [18]b	
	MEAN%±S.D.c	3.1 ± 0.7	3.4 ± 0.6	3.5 ± 0.7	4.7 ± 1.0** [18]b	

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. The average percentage of pups meeting the criterion for the developmental landmark tested on each day postpartum.
b. Excludes values for dams that had no surviving pups.
c. The average day postpartum that at least 50% of the pups had the developmental measure present.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

000614

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 C20 (PAGE 3): REFLEX AND PHYSICAL DEVELOPMENT - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
EYE OPENING a	N	23	25	22	18b	0b
DAY 12	MEAN±S.D.	0.0 ± 0.0	0.5 ± 2.5	0.6 ± 2.7	3.5 ± 12.0	
DAY 13	MEAN±S.D.	7.1 ± 14.0	3.5 ± 11.1	3.9 ± 9.2	4.2 ± 12.9	
DAY 14	MEAN±S.D.	55.4 ± 32.6	34.0 ± 31.1*	32.0 ± 30.6*	13.9 ± 21.4**	
DAY 15	MEAN±S.D.	93.5 ± 9.9	86.0 ± 25.6	83.5 ± 24.2	37.9 ± 38.1**	
DAY 16	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	99.4 ± 3.0	74.8 ± 33.9**	
DAY 17	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	98.6 ± 5.9	
DAY 18	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	
	MEAN±S.D.c	14.3 ± 0.6	14.8 ± 0.7	14.9 ± 0.5**	15.7 ± 1.3**	

DAY = DAY POSTPARTUM
a. The average percentage of pups meeting the criterion for the developmental landmark tested on each day postpartum.
b. Excludes values for dams that had no surviving pups.
c. The average day postpartum that at least 50% of the pups had the developmental measure present.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

000615

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 C20 (PAGE 4): REFLEX AND PHYSICAL DEVELOPMENT - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP			I	II	III	IV	V
MATERNAL DOSAGE (MG/KG/DAY)			0 (VEHICLE)	0.1	0.4	1.6	3.2
ACOUSTIC STARTLE a		N	23	25	22	18b	0b
DAY	13	MEAN±S.D.	75.5 ± 31.9	83.0 ± 20.4	69.7 ± 21.0	39.3 ± 27.4**	
DAY	14	MEAN±S.D.	84.2 ± 25.1	87.0 ± 18.2	78.6 ± 23.7	52.3 ± 40.8*	
DAY	15	MEAN±S.D.	88.6 ± 21.9	78.5 ± 34.1	77.1 ± 27.7	68.7 ± 29.6	
DAY	16	MEAN±S.D.	95.1 ± 14.5	81.5 ± 32.9	80.9 ± 29.7	67.3 ± 36.0**	
DAY	17	MEAN±S.D.	98.9 ± 5.2	91.5 ± 20.6	89.2 ± 22.9	76.6 ± 35.1*	
DAY	18	MEAN±S.D.	100.0 ± 0.0	95.5 ± 14.4	96.0 ± 11.8	81.9 ± 36.2**	
DAY	19	MEAN±S.D.	100.0 ± 0.0	99.0 ± 5.0	98.3 ± 8.0	84.5 ± 32.1**	
DAY	20	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.4 ± 16.2*	
DAY	21	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	97.2 ± 11.8	
		MEAN±S.D.c	13.3 ± 0.6	13.1 ± 0.3	13.0 ± 0.2	14.0 ± 1.4	

DAY = DAY POSTPARTUM

a. The average percentage of pups meeting the criterion for the developmental landmark tested on each day postpartum.

b. Excludes values for dams that had no surviving pups.

c. The average day postpartum that at least 50% of the pups had the developmental measure present.

* Significantly different from the vehicle control group value ($p \leq 0.05$).

** Significantly different from the vehicle control group value ($p \leq 0.01$).

000616

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 C20 (PAGE 5): REFLEX AND PHYSICAL DEVELOPMENT - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
AIR Rrighting a	N	23	25	22	18b	0b
DAY 14	MEAN±S.D.	23.9 ± 27.7	13.5 ± 14.4	13.0 ± 15.8	3.5 ± 8.4**	
DAY 15	MEAN±S.D.	48.4 ± 28.8	33.5 ± 22.2	35.5 ± 23.0	3.5 ± 5.8**	
DAY 16	MEAN±S.D.	64.7 ± 26.8	66.0 ± 21.5	46.8 ± 26.3*	21.3 ± 25.0**	
DAY 17	MEAN±S.D.	79.3 ± 25.7	77.0 ± 19.0	73.0 ± 21.9	40.8 ± 31.5**	
DAY 18	MEAN±S.D.	97.8 ± 8.1	90.5 ± 15.0	93.9 ± 13.3	60.6 ± 22.5**	
DAY 19	MEAN±S.D.	100.0 ± 0.0	99.0 ± 3.5	98.3 ± 8.0	75.2 ± 30.1**	
DAY 20	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.8 ± 16.2**	
DAY 21	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.8 ± 16.2**	
	MEAN±S.D.c	15.5 ± 1.2	15.9 ± 0.8	16.1 ± 1.1	17.5 ± 0.8**	
PUPIL CONSTRICTION a	N	23	25	22	18b	0b
DAY 21	MEAN±S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. The average percentage of pups meeting the criterion for the developmental landmark tested on each day postpartum.
b. Excludes values for dams that had no surviving pups.
c. The average day postpartum that at least 50% of the pups had the developmental measure present.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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TABLE C21 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4	1.6	3.2
LITTERS EXAMINED (N)		23	25	22	20	21
TOTAL PUPS STILLBORN OR FOUND DEAD a	N	8	6	8	71	172
STILLBORN	N	5	1	4	6	41
FOUND DEAD	N	3	5	4	65	131
NO MILK IN STOMACH b	N(%)	2(66.7)	0(0.0)	3(75.0)	49(75.4)	102(77.9)
HINDLIMBS AND/OR PORTION OF TAIL: MISSING c	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	9(5.2)
WHOLE BODY EDEMA	N(%)	0(0.0)	0(0.0)	0(0.0)	1(1.4)	0(0.0)
PUPS SACRIFICED AND NECROPSIED ON DAYS 4 OR 21 POSTPARTUM a						
LITTERS EVALUATED	N	23	25	22	18d	0d
PUPS EVALUATED	N	259	305	209	161	0
APPEARED NORMAL						
LITTER INCIDENCE	N(%)	23(100.0)	25(100.0)	20(90.9)	18(100.0)	
PUP INCIDENCE	N(%)	259(100.0)	305(100.0)	206(98.6)	161(100.0)	
BRAIN: LATERAL VENTRICLES, MODERATE TO SEVERE DILATION						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)	
PUP INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.0)	0(0.0)	
KIDNEYS: RIGHT, PELVIS, SLIGHT DILATION						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)	
PUP INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	

a. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation.
b. Analysis restricted to pups found dead or moribund sacrificed and necropsied.
c. Presumed cannibalized by dam.
d. Excludes values for litters that had no surviving pups.

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APPENDIX D

REPORT TABLES - F1 GENERATION MALE RATS

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 D1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0 (VEHICLE)	II 0.1	III 0.4
MAXIMUM POSSIBLE INCIDENCE	2207/ 25	2196/ 25	2215/ 25
FOUND DEAD	2a,b	1c	0
INCISORS: TOTAL	96/ 3	152/ 8	97/ 4
MISALIGNED	87/ 2	108/ 4	94/ 3
MISSING/BROKEN	13/ 2	76/ 7	17/ 2
CHROMORHINORRHEA	7/ 1b	9/ 4	6/ 4
ABRASION d	0/ 0	61/ 4	32/ 2
CHROMODACRYORRHEA	58/ 1	50/ 4	38/ 1
BASE OF TAIL: LACERATION	0/ 0	0/ 0	11/ 1
SNOUT: SWOLLEN	0/ 0	8/ 2	3/ 1
RED PERIORAL SUBSTANCE	0/ 0	1/ 1	1/ 1
PENIS: RED SUBSTANCE	0/ 0	0/ 0	2/ 1
LOCALIZED ALOPECIA: TOTAL	6/ 1	66/ 4	0/ 0
BACK	0/ 0	16/ 3**	0/ 0
LIMBS	6/ 1	29/ 1	0/ 0
HEAD	0/ 0	24/ 1	0/ 0
BACK: SCAB	14/ 1	8/ 2	0/ 0
URINE-STAINED ABDOMINAL FUR	0/ 0	4/ 1	0/ 0
SOFT OR LIQUID FECES	0/ 0	1/ 1	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Rat 13105 was found dead on day 87 postweaning.
b. Rat 13116 was found dead on day 86 postweaning.
c. Rat 13138 was found dead on day 66 postweaning.
d. Located on the head, corner of left eye, left forelimb or back.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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 D1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0 (VEHICLE)	II 0.1	III 0.4
MAXIMUM POSSIBLE INCIDENCE	2207/ 25	2196/ 25	2215/ 25
FOUND DEAD	2a,b	1c	0
EARS: SWOLLEN AND PURPLE	9/ 1	0/ 0	0/ 0
RALES	2/ 1	0/ 0	0/ 0
GASPING	1/ 1	0/ 0	0/ 0
EXCESS SALIVATION	1/ 1	0/ 0	0/ 0
EYES: DILATED PUPIL	1/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Rat 13105 was found dead on day 87 postweaning.
b. Rat 13116 was found dead on day 86 postweaning.
c. Rat 13138 was found dead on day 66 postweaning.

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 D2 (PAGE 1): BODY WEIGHTS - SUMMARY F1 GENERATION MALE RATS

DOSAGE GROUP			I	II	III
DOSAGE (MG/KG/DAY)			0 (VEHICLE)	0.1	0.4
RATS TESTED		N	25	25	25
BODY WEIGHT (G)					
DAY 1		MEAN±S.D.	54.0 ± 5.8	52.1 ± 5.3	50.8 ± 4.9 [23]a
DAY 8		MEAN±S.D.	101.6 ± 10.3	98.2 ± 8.1	96.4 ± 8.5
DAY 15		MEAN±S.D.	161.8 ± 13.6	156.2 ± 11.6	154.5 ± 11.8
DAY 22		MEAN±S.D.	223.7 ± 17.1	219.6 ± 14.0	216.6 ± 15.4
DAY 29		MEAN±S.D.	285.0 ± 21.1	279.4 ± 17.2	277.2 ± 21.0
DAY 36		MEAN±S.D.	338.2 ± 25.6	334.7 ± 20.9	332.4 ± 22.8
DAY 43		MEAN±S.D.	381.1 ± 30.3	377.9 ± 21.6	373.3 ± 26.2
DAY 50		MEAN±S.D.	420.5 ± 33.4	414.0 ± 25.3	410.6 ± 32.9
DAY 57		MEAN±S.D.	448.4 ± 34.2	437.0 ± 29.5	436.8 ± 37.4
DAY 64		MEAN±S.D.	472.2 ± 36.9	465.1 ± 34.3	462.6 ± 44.0
PRECOHABITATION b		MEAN±S.D.	508.6 ± 42.8	499.2 ± 39.0 [24]c	494.8 ± 51.1
TERMINAL BODY WEIGHT d		MEAN±S.D.	542.0 ± 50.9 [23]c	533.0 ± 43.6 [24]c	522.3 ± 54.6

DAY = DAY POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values that were not recorded.
b. Precohabitation body weights were recorded on the day that cohabitation began for the F1 generation rats; at that time these rats were 85 to 97 days of age.
c. Excludes values for rats that were found dead.
d. Terminal body weights were recorded when the rats were 100 to 112 days of age.

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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 D3 (PAGE 1): BODY WEIGHT CHANGES - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
BODY WEIGHT CHANGE (G)				
DAYS 1 - 8	MEAN±S.D.	+47.7 ± 5.5	+46.1 ± 3.9	+46.5 ± 4.8 [23]a
DAYS 8 - 15	MEAN±S.D.	+60.2 ± 6.8	+58.0 ± 5.3	+58.1 ± 4.9
DAYS 15 - 22	MEAN±S.D.	+61.8 ± 6.2	+63.5 ± 4.8	+62.2 ± 5.7
DAYS 22 - 29	MEAN±S.D.	+61.4 ± 6.5	+59.7 ± 4.7	+60.6 ± 8.0
DAYS 29 - 36	MEAN±S.D.	+53.2 ± 10.8	+55.3 ± 9.2	+55.2 ± 5.0
DAYS 36 - 43	MEAN±S.D.	+42.9 ± 8.5	+43.2 ± 7.3	+40.9 ± 8.9
DAYS 43 - 50	MEAN±S.D.	+39.4 ± 6.5	+36.1 ± 10.9	+37.3 ± 10.2
DAYS 50 - 57	MEAN±S.D.	+27.8 ± 8.7	+23.0 ± 22.9	+26.3 ± 7.2
DAYS 57 - 64	MEAN±S.D.	+23.9 ± 8.6	+28.2 ± 24.9	+25.8 ± 13.9
DAY 1 - PRECOHABITATION b	MEAN±S.D.	+454.6 ± 40.7	+447.1 ± 36.5 [24]c	+443.5 ± 51.4 [23]a
DAY 1 - TERMINATION d	MEAN±S.D.	+488.0 ± 48.6 [23]c	+480.9 ± 41.2 [24]c	+471.2 ± 55.3 [23]a

DAY(S) = DAY(S) POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values that were not recorded.
b. Precohabitation body weights were recorded on the day that cohabitation began for the F1 generation rats; at that time these rats were 85 to 97 days of age.
c. Excludes values for rats that were found dead.
d. Terminal body weights were recorded when the rats were 79 to 91 days of age.

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 D4 (PAGE 1): ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS TESTED	N	25	25	25
FEED CONSUMPTION (G/DAY)				
DAYS 1 - 8	MEAN±S.D.	14.7 ± 1.5	13.8 ± 1.3*	13.4 ± 2.9**
DAYS 8 - 15	MEAN±S.D.	21.7 ± 1.5	22.1 ± 2.3	20.8 ± 3.2
DAYS 15 - 22	MEAN±S.D.	26.0 ± 2.2 [24]a	25.8 ± 1.4	25.4 ± 1.7
DAYS 22 - 29	MEAN±S.D.	28.8 ± 2.0	29.2 ± 1.6	28.5 ± 2.3
DAYS 29 - 36	MEAN±S.D.	30.3 ± 3.5	30.0 ± 3.1	29.9 ± 2.3
DAYS 36 - 43	MEAN±S.D.	32.2 ± 2.8	32.6 ± 2.9	31.7 ± 2.6
DAYS 43 - 50	MEAN±S.D.	32.4 ± 3.4 [24]a	33.0 ± 3.3 [24]a	31.7 ± 3.1
DAYS 50 - 57	MEAN±S.D.	32.3 ± 2.8	31.7 ± 2.2	31.5 ± 3.1
DAYS 57 - 64b	MEAN±S.D.	30.5 ± 2.7 [22]a	30.2 ± 4.1	30.9 ± 3.2
DAYS 1 - 64b	MEAN±S.D.	27.3 ± 1.7 [22]a	27.6 ± 1.5	27.1 ± 1.9

DAYS = DAYS POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Spilled feed precluded the calculation of this value.
b. Last value recorded before cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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

TABLE D5 (PAGE 1): RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS TESTED	N	25	25	25
FEED CONSUMPTION (G/KG/DAY)				
DAYS 1 - 8	MEAN±S.D.	189.9 ± 17.6	184.9 ± 17.8	183.6 ± 38.1 [23]a
DAYS 8 - 15	MEAN±S.D.	165.5 ± 11.0	174.5 ± 16.3	166.4 ± 23.9
DAYS 15 - 22	MEAN±S.D.	135.6 ± 7.2 [24]b	137.6 ± 7.1	137.3 ± 7.6
DAYS 22 - 29	MEAN±S.D.	113.5 ± 5.4	117.1 ± 4.6*	115.3 ± 4.8
DAYS 29 - 36	MEAN±S.D.	97.3 ± 8.9	97.7 ± 8.1	98.1 ± 4.2
DAYS 36 - 43	MEAN±S.D.	89.6 ± 6.2	91.4 ± 6.9	90.0 ± 6.6
DAYS 43 - 50	MEAN±S.D.	80.7 ± 5.5 [24]b	83.4 ± 7.6 [24]b	80.9 ± 6.0
DAYS 50 - 57	MEAN±S.D.	74.5 ± 4.6	74.7 ± 4.1	74.2 ± 3.8
DAYS 57 - 64c	MEAN±S.D.	67.2 ± 4.7 [22]b	67.1 ± 8.4	68.6 ± 3.8
DAYS 1 - 64c	MEAN±S.D.	96.2 ± 4.1 [22]b	97.4 ± 3.8	96.6 ± 3.6 [23]b

DAYS = DAYS POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values that were not recorded.
b. Spilled feed precluded the calculation of this value.
c. Last value recorded before cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

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TABLE D6 (PAGE 1): SEXUAL MATURATION - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
MALE RATS	N	25	25	25
PREPUTIAL SEPARATION a	MEAN±S.D.	45.0 ± 2.1	45.7 ± 2.3	45.1 ± 1.8

a. Average day postpartum that the prepuce was observed to be separated.

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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 D7 (PAGE 1): PASSIVE AVOIDANCE PERFORMANCE SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
MALE RATS				
SESSION 1a	N	23	25	22
TRIALS TO CRITERION	MEAN±S.D.	4.8 ± 1.0	6.1 ± 2.9	4.8 ± 1.1
LATENCY TRIAL 1b	MEAN±S.D.	8.9 ± 5.0	7.8 ± 4.8	10.5 ± 6.0
LATENCY TRIAL 2b	MEAN±S.D.	21.4 ± 15.8	20.1 ± 19.7	23.9 ± 20.7
FAILED TO LEARN c	N(%)	0(0.0)	1(4.0)	0(0.0)
SESSION 2a	N	23	24	22
TRIALS TO CRITERION	MEAN±S.D.	3.3 ± 2.6	3.6 ± 2.2	3.5 ± 2.7
LATENCY TRIAL 1b	MEAN±S.D.	32.8 ± 22.2	28.2 ± 23.5	33.1 ± 22.4

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase).

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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 D8 (PAGE 1): WATERMAZE PERFORMANCE - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
MALE RATS				
SESSION 1a	N	23	25	21
TRIALS TO CRITERION	MEAN±S.D.	9.1 ± 3.1	9.4 ± 3.0	9.0 ± 3.0
ERRORS PER TRIAL	MEAN±S.D.	0.37 ± 0.17	0.40 ± 0.24	0.43 ± 0.22
LATENCY TRIAL 2b	MEAN±S.D.	11.7 ± 7.4	13.7 ± 13.9	10.6 ± 6.0
FAILED TO LEARN c	N(%)	0(0.0)	2(8.0)	0(0.0)
SESSION 2a	N	23	23	21
TRIALS TO CRITERION	MEAN±S.D.	6.4 ± 2.6	6.3 ± 1.9	5.5 ± 0.9
ERRORS PER TRIAL	MEAN±S.D.	0.12 ± 0.20	0.08 ± 0.11	0.09 ± 0.15
LATENCY TRIAL 1b	MEAN±S.D.	10.3 ± 8.3	9.1 ± 6.5	10.7 ± 6.7

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from group averages and statistical analyses.

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 D9 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS IN COHABITATION	N	23a	24b	25
DAYS IN COHABITATION c	MEAN±S.D	3.2 ± 2.1	3.4 ± 1.9	2.6 ± 1.5
RATS THAT MATED d	N(%)	20(87.0)	22(91.7)	25(100.0)
FERTILITY INDEX e,f	N/N	19/20	20/22	24/25
	(%)	(95.0)	(90.9)	(96.0)
RATS WITH CONFIRMED MATING DATES g	N	20	22	25
RATS MATING h DAYS 1-7	N(%)	20(100.0)	22(92.0)	25(100.0)
RATS PREGNANT/RATS IN COHABITATION g	N/N	19/23	20/24	24/25
	(%)	(82.6)	(83.3)	(96.0)

a. Excludes values for rats that were not assigned to cohabitation because there were no available female rats.
b. Excludes values for rat 13138, which was found dead day 66 postweaning.
c. Restricted to rats with a confirmed mating date on days 1 to 7 of cohabitation and rats that did not mate.
d. Includes only one mating for each male rat.
e. Number of pregnancies/number of rats that mated.
f. Includes only one pregnancy for each rat that impregnated more than one female rat.
g. Includes only one confirmed mating for each male rat.
h. Restricted to rats with a confirmed mating date during days 1 to 7 of cohabitation.

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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 D10 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4
RATS EXAMINED a	N	25	25	25
FOUND DEAD	N	2b,c	1d	0
APPEARED NORMAL	N	23	21d	23
ABDOMINAL ADIPOSE TISSUE:				
RED WITH FIRM AREAS	N	0	0	1
KIDNEYS:				
BILATERAL, LARGE	N	0	0	1
PARENCHYMA, NUMEROUS FLUID-FILLED CYSTS	N	0	0	1
RIGHT OR LEFT, PELVIS, SLIGHT DILATION	N	0	2	0
LEFT, PELVIS, SIX CALCULI	N	0	1	0
TESTES:				
BILATERAL OR RIGHT, PURPLE AND/OR FLACCID	N	0	3**	0
EPIDIDYMIDES:				
BILATERAL OR RIGHT, FLACCID	N	0	3**	0
LEFT OR RIGHT, SMALL	N	0	2	0
URETERS:				
LEFT, DILATED	N	0	1	0

a. Refer to the individual clinical observations table (Table D13) for external observations confirmed at necropsy.
b. Rat 13105 was found dead on day 87 postweaning.
c. Rat 13116 was found dead on day 86 postweaning.
d. Rat 13138 was found dead on day 66 postweaning.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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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 D10 (PAGE 2): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS EXAMINED a	N	25	25	25
FOUND DEAD	N	2b,c	1d	0
URINARY BLADDER:				
WALLS, THICKENED AND NUMEROUS CALCULI	N	0	1	0
THORACIC CAVITY:				
DARK RED CLOTTED MATERIAL	N	1c	0	0
LUNGS:				
ALL LOBES, DARK RED	N	1b	0	0
EXTERNAL OBSERVATIONS:				
RED PERINASAL SUBSTANCE	N	1c	0	0

a. Refer to the individual clinical observations table (Table D13) for external observations confirmed at necropsy.
b. Rat 13105 was found dead on day 87 postweaning.
c. Rat 13116 was found dead on day 86 postweaning.
d. Rat 13138 was found dead on day 66 postweaning.

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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 D11 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a	24a	25
TERMINAL BODY WEIGHT	MEAN±S.D.	542.0 ± 50.9	533.0 ± 43.6	522.3 ± 54.6
EPIDIDYMIS LEFT	MEAN±S.D.	0.67 ± 0.07	0.65 ± 0.06 [23]b	0.63 ± 0.04
TESTIS LEFT	MEAN±S.D.	1.77 ± 0.16	1.80 ± 0.16 [23]b	1.79 ± 0.12
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	1.75 ± 0.33	1.72 ± 0.24 [22]b	1.62 ± 0.20 [24]b
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	1.04 ± 0.17	1.05 ± 0.19	0.96 ± 0.15
EPIDIDYMIS RIGHT	MEAN±S.D.	0.67 ± 0.06	0.66 ± 0.05 [21]b	0.64 ± 0.04
TESTIS RIGHT	MEAN±S.D.	1.78 ± 0.18	1.79 ± 0.15 [21]b	1.82 ± 0.12
PROSTATE	MEAN±S.D.	0.93 ± 0.19	0.91 ± 0.22	0.89 ± 0.19

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead.
b. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).

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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 D12 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a	24a	25
TERMINAL BODY WEIGHT	MEAN±S.D.	542.0 ± 50.9	533.0 ± 43.6	522.3 ± 54.6
EPIDIDYMIS LEFT	MEAN±S.D.	0.124 ± 0.017	0.122 ± 0.014 [23]b	0.121 ± 0.014
TESTIS LEFT	MEAN±S.D.	0.329 ± 0.041	0.332 ± 0.043 [23]b	0.346 ± 0.037
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.325 ± 0.068	0.325 ± 0.055 [22]b	0.316 ± 0.055 [24]b
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.193 ± 0.037	0.198 ± 0.037	0.184 ± 0.032
EPIDIDYMIS RIGHT	MEAN±S.D.	0.124 ± 0.016	0.125 ± 0.012 [21]b	0.122 ± 0.015
TESTIS RIGHT	MEAN±S.D.	0.331 ± 0.043	0.339 ± 0.042 [21]b	0.351 ± 0.037
PROSTATE	MEAN±S.D.	0.172 ± 0.035	0.171 ± 0.043	0.170 ± 0.035

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) x 100.
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead.
b. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).

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APPENDIX E

REPORT TABLES – F1 GENERATION FEMALE RATS

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 E1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0 (VEHICLE)	II 0.1	III 0.4
PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION):			
MAXIMUM POSSIBLE INCIDENCE	1721/ 25	1844/ 25	1840/ 25
SACRIFICED	1a	0	0
FOUND DEAD	1b	0	0
INCISORS: TOTAL	29/ 3	32/ 4	77/ 6
MISALIGNED	29/ 3	9/ 1	52/ 4
MISSING/BROKEN	14/ 2	23/ 3	25/ 2
CHROMODACRYORRHEA	5/ 1	9/ 1	19/ 3
EARS: SWOLLEN AND PURPLE	8/ 2	4/ 2	0/ 0
LOCALIZED ALOPECIA: TOTAL	13/ 2	24/ 2	0/ 0
BACK	0/ 0	22/ 2	0/ 0
HEAD	0/ 0	2/ 1	0/ 0
LIMBS	13/ 2	0/ 0	0/ 0
BACK: ABRASION	0/ 0	10/ 1	0/ 0
BACK: SCAB	0/ 0	1/ 1	0/ 0
URINE-STAINED ABDOMINAL FUR	10/ 2	0/ 0	0/ 0
LEFT HINDPAW: THIRD AND FOURTH DIGITS MISSING	74/ 1	0/ 0	0/ 0
PERIVAGINAL SWELLING	6/ 1	0/ 0	0/ 0
LEFT FOREPAW: SWOLLEN	1/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Rat 13205 was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Rat 13221 was found dead on day 28 postweaning.

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 E1 (PAGE 2): CLINICAL OBSERVATIONS SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0 (VEHICLE)	II 0.1	III 0.4
PRESUMED GESTATION: a			
MAXIMUM POSSIBLE INCIDENCE	509/ 23	539/ 24	548/ 25
MORTALITY	0	0	0
INCISORS: TOTAL	66/ 4	90/ 5	142/ 8
MISALIGNED	62/ 4	77/ 5	123/ 6
MISSING/BROKEN	21/ 2	41/ 3	22/ 3
CHROMODACRYORRHEA	10/ 2	26/ 1	40/ 3
EARS: SWOLLEN AND PURPLE	44/ 2	75/ 4	23/ 2
RED PERIVAGINAL SUBSTANCE	0/ 0	1/ 1	2/ 2
LOCALIZED ALOPECIA: TOTAL	61/ 3	32/ 3	21/ 2
LIMBS	61/ 3	27/ 2	15/ 1
HEAD	0/ 0	5/ 1	6/ 1
UNDERSIDE	15/ 1	0/ 0	0/ 0
URINE-STAINED ABDOMINAL FUR	0/ 0	0/ 0	1/ 1
CHROMORHINORRHEA	14/ 2	1/ 1	0/ 0
SOFT OR LIQUID FECES	0/ 0	1/ 1	0/ 0
LEFT HINDPAW: THIRD AND FOURTH DIGITS MISSING	23/ 1	0/ 0	0/ 0
TAIL: SHEATH MISSING	12/ 1	0/ 0	0/ 0
TIP OF TAIL BLACK	12/ 1	0/ 0	0/ 0
TIP OF TAIL MISSING	10/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Restricted to rats with a confirmed mating date.

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 E1 (PAGE 3): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0 (VEHICLE)	II 0.1	III 0.4
LACTATION:			
MAXIMUM POSSIBLE INCIDENCE	462/ 22	441/ 21	493/ 24
FOUND DEAD	0	0	1a
INCISORS: TOTAL	65/ 4	43/ 3	116/ 6
MISALIGNED	44/ 3	42/ 2	116/ 6
MISSING/BROKEN	42/ 2	34/ 3	18/ 3
RED PERIVAGINAL SUBSTANCE	0/ 0	0/ 0	5/ 5**
EARS: SWOLLEN AND PURPLE	45/ 3	84/ 4	42/ 2
CHROMODACRYORRHEA	21/ 1	7/ 1	37/ 2
LOCALIZED ALOPECIA: TOTAL	63/ 3	42/ 2	21/ 1
LIMBS	63/ 3	42/ 2	21/ 1
UNDERSIDE	21/ 1	0/ 0	13/ 1
CHROMORHINORRHEA	6/ 1	2/ 1	0/ 0
CHEST: ABRASION	0/ 0	9/ 1	0/ 0
TIP OF TAIL MISSING	21/ 1	0/ 0	0/ 0
LEFT HINDPAW: THIRD AND FOURTH DIGITS MISSING	21/ 1	0/ 0	0/ 0
URINE-STAINED ABDOMINAL FUR	1/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
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 13257 was found dead on day 10 of lactation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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TABLE E2 (PAGE 1): BODY WEIGHTS PRECOHABITATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	24a	25	25
BODY WEIGHT (G)				
DAY 1	MEAN±S.D.	51.1 ± 6.1	50.5 ± 4.2	48.7 ± 5.1
DAY 8	MEAN±S.D.	91.2 ± 9.3	91.8 ± 5.2	91.2 ± 7.6
DAY 15	MEAN±S.D.	134.5 ± 12.5	138.0 ± 7.6	134.5 ± 9.9
DAY 22	MEAN±S.D.	168.2 ± 16.0	173.4 ± 9.7	168.5 ± 12.4
DAY 29	MEAN±S.D.	194.0 ± 21.7 [23]b	200.5 ± 13.1	193.9 ± 16.6
DAY 36	MEAN±S.D.	217.4 ± 24.9 [23]b	226.8 ± 15.2	217.8 ± 17.4
DAY 43	MEAN±S.D.	234.9 ± 25.2 [23]b	245.1 ± 14.6	237.9 ± 20.0
DAY 50	MEAN±S.D.	251.1 ± 27.4 [23]b	261.4 ± 20.0	251.2 ± 20.9
DAY 57	MEAN±S.D.	259.6 ± 29.0 [23]b	272.7 ± 20.7	262.7 ± 24.4
DAY 64	MEAN±S.D.	273.2 ± 31.4 [23]b	282.7 ± 19.6	272.3 ± 25.8
PRECOHABITATION c	MEAN±S.D.	282.8 ± 33.6 [23]b	294.0 ± 16.3	280.3 ± 26.9

DAY = DAY POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead day 28 postweaning.
c. Precohabitation body weights were recorded on the day that cohabitation began for the F1 generation rats; at that time these rats were 85 to 97 days of age.

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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 E3 (PAGE 1): BODY WEIGHT CHANGES - PRECOHABITATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	24a	25	25
BODY WEIGHT CHANGE (G)				
DAYS 1 - 8	MEAN±S.D.	+40.0 ± 4.2	+41.4 ± 2.9	+42.5 ± 5.0
DAYS 8 - 15	MEAN±S.D.	+43.3 ± 6.3	+46.1 ± 4.2	+43.3 ± 4.5
DAYS 15 - 22	MEAN±S.D.	+33.8 ± 7.0	+35.5 ± 5.2	+34.0 ± 6.8
DAYS 22 - 29	MEAN±S.D.	+25.8 ± 12.8 [23]a,b	+27.0 ± 5.8	+25.4 ± 8.7
DAYS 29 - 36	MEAN±S.D.	+23.4 ± 10.4 [23]a,b	+26.3 ± 5.8	+23.8 ± 5.7
DAYS 36 - 43	MEAN±S.D.	+17.5 ± 9.2 [23]a,b	+18.3 ± 5.1	+20.1 ± 7.4
DAYS 43 - 50	MEAN±S.D.	+16.2 ± 6.6 [23]a,b	+16.3 ± 8.0	+13.3 ± 5.9
DAYS 50 - 57	MEAN±S.D.	+8.5 ± 6.8 [23]a,b	+11.3 ± 9.3	+11.6 ± 6.9
DAYS 57 - 64	MEAN±S.D.	+13.6 ± 7.6 [23]a,b	+10.0 ± 9.4	+9.6 ± 7.8
DAYS 1 - 64	MEAN±S.D.	+222.3 ± 28.9 [23]a,b	+232.2 ± 20.3	+223.6 ± 24.0
DAY 1 - PRECOHABITATION c	MEAN±S.D.	+231.9 ± 30.7 [23]a,b	+243.6 ± 17.3	+231.6 ± 25.1

DAY(S) = DAY(S) POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead day 28 postweaning.
c. Precohabitation body weights were recorded on the day that cohabitation began for the F1 generation rats; at that time these rats were 85 to 97 days of age.

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 E4 (PAGE 1): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP			I	II	III
DOSAGE (MG/KG/DAY)			0 (VEHICLE)	0.1	0.4
RATS TESTED		N	25	25	25
INCLUDED IN ANALYSES		N	23a,b	25	25
PREGNANT		N	22	21	24
MATERNAL BODY WEIGHT (G)					
DAY 0		MEAN±S.D.	284.4 ± 30.8	295.3 ± 17.3	279.9 ± 24.8
DAY 1		MEAN±S.D.	291.4 ± 31.1	302.8 ± 18.9	288.1 ± 25.4
DAY 2		MEAN±S.D.	298.1 ± 30.5	308.1 ± 19.3	294.5 ± 24.8
DAY 3		MEAN±S.D.	303.1 ± 31.0	312.8 ± 19.3	297.5 ± 25.9
DAY 4		MEAN±S.D.	305.5 ± 31.1	315.3 ± 19.1	300.2 ± 25.3
DAY 5		MEAN±S.D.	309.2 ± 31.2	318.7 ± 19.2	304.4 ± 26.5
DAY 6		MEAN±S.D.	313.2 ± 32.7	321.6 ± 18.7	306.9 ± 25.1
DAY 7		MEAN±S.D.	317.4 ± 33.2	322.8 ± 19.9	309.0 ± 26.0
DAY 8		MEAN±S.D.	321.6 ± 32.8	328.0 ± 19.9	313.2 ± 27.4
DAY 9		MEAN±S.D.	326.2 ± 33.0	331.5 ± 19.5	317.7 ± 26.1
DAY 10		MEAN±S.D.	331.7 ± 33.6	336.5 ± 20.1	322.3 ± 28.2

DAY = DAY OF GESTATION
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 postweaning.

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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 E4 (PAGE 2): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a,b	25	25
PREGNANT	N	22	21	24
MATERNAL BODY WEIGHT (G)				
DAY 11	MEAN±S.D.	337.0 ± 32.3	341.0 ± 21.5	329.0 ± 27.1
DAY 12	MEAN±S.D.	342.4 ± 34.7	347.2 ± 22.9	331.8 ± 26.9
DAY 13	MEAN±S.D.	345.6 ± 33.8	350.3 ± 21.0	336.4 ± 28.7
DAY 14	MEAN±S.D.	351.9 ± 34.9	354.7 ± 21.1	342.3 ± 27.3
DAY 15	MEAN±S.D.	358.9 ± 35.0	364.0 ± 22.3	347.9 ± 29.5
DAY 16	MEAN±S.D.	366.2 ± 35.0	373.5 ± 22.4	358.6 ± 29.6
DAY 17	MEAN±S.D.	379.4 ± 34.4	385.8 ± 25.3	372.3 ± 30.2
DAY 18	MEAN±S.D.	394.6 ± 32.8	401.6 ± 27.4	388.1 ± 32.1
DAY 19	MEAN±S.D.	408.3 ± 35.6	414.7 ± 27.7	402.8 ± 32.0
DAY 20	MEAN±S.D.	420.8 ± 34.4	428.0 ± 30.3	417.7 ± 34.4

DAY = DAY OF GESTATION
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 postweaning.

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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 E5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a,b	25	25
PREGNANT	N	22	21	24
MATERNAL BODY WEIGHT CHANGE (G)				
DAYS 0 - 7	MEAN±S.D.	+33.0 ± 6.9	+27.4 ± 9.0	+29.2 ± 7.1
DAYS 7 - 10	MEAN±S.D.	+14.3 ± 5.0	+13.8 ± 6.9	+13.2 ± 5.2
DAYS 10 - 14	MEAN±S.D.	+20.2 ± 5.9	+18.1 ± 9.9	+20.0 ± 6.2
DAYS 14 - 17	MEAN±S.D.	+27.5 ± 13.7	+31.2 ± 9.4	+30.0 ± 8.6
DAYS 17 - 20	MEAN±S.D.	+41.4 ± 11.0	+42.2 ± 9.3	+45.4 ± 9.0
DAYS 0 - 20	MEAN±S.D.	+136.4 ± 16.0	+132.7 ± 22.7	+137.8 ± 16.4

DAYS = DAYS OF GESTATION

a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.

b. Excludes values for rat 13221, which was found dead on day 28 postweaning.

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 E6 (PAGE 1): MATERNAL BODY WEIGHTS - LACTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a,b	25	25
PREGNANT	N	22	21	24
DELIVERED LITTERS	N	22	21	24
MATERNAL BODY WEIGHT (G)				
DAY 1	MEAN±S.D.	325.7 ± 35.8	334.7 ± 16.9	323.4 ± 28.4
DAY 4	MEAN±S.D.	328.0 ± 33.0	337.5 ± 20.9	318.8 ± 25.8
DAY 7	MEAN±S.D.	331.5 ± 31.2	340.2 ± 19.1	324.2 ± 23.2
DAY 14	MEAN±S.D.	358.9 ± 29.2	368.3 ± 21.8	352.4 ± 23.6 [23]c
DAY 21	MEAN±S.D.	338.0 ± 31.0	356.0 ± 18.4	341.3 ± 17.8 [23]c

DAY = DAY OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 postweaning.
c. Excludes values for dam 13257, which was found dead on day 10 of lactation.

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 E7 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - LACTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.1	III 0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a,b	25	25
PREGNANT	N	22	21	24
DELIVERED LITTERS	N	22	21	24
MATERNAL BODY WEIGHT CHANGE (G)				
DAYS 1 - 4	MEAN±S.D.	+2.3 ± 13.6	+2.8 ± 10.7	-4.6 ± 8.4*
DAYS 4 - 7	MEAN±S.D.	+3.6 ± 11.8	+2.7 ± 10.7	+5.4 ± 8.2
DAYS 7 - 14	MEAN±S.D.	+27.3 ± 12.8	+28.0 ± 15.6	+30.1 ± 10.4 [23]c
DAYS 14 - 21	MEAN±S.D.	-20.9 ± 17.1	-12.3 ± 12.6	-11.0 ± 17.0 [23]c
DAYS 1 - 21	MEAN±S.D.	+12.3 ± 19.1	+21.3 ± 15.3	+21.2 ± 18.7 [23]c

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 postweaning.
c. Excludes values for dam 13257, which was found dead on day 10 of lactation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

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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 E8 (PAGE 1): ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - PRECOHABITATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	24a	25	25
FEED CONSUMPTION (G/DAY)				
DAYS 1 - 8	MEAN±S.D.	13.6 ± 1.3	13.7 ± 1.2	12.6 ± 1.7*
DAYS 8 - 15	MEAN±S.D.	18.9 ± 1.4 [23]b	19.1 ± 1.5	18.9 ± 2.4
DAYS 15 - 22	MEAN±S.D.	20.4 ± 2.3 [23]b	21.2 ± 1.6	20.8 ± 1.9 [24]b
DAYS 22 - 29	MEAN±S.D.	20.5 ± 2.8 [23]c	21.4 ± 2.1	20.8 ± 2.0
DAYS 29 - 36	MEAN±S.D.	20.5 ± 2.7 [23]c	21.4 ± 2.4	20.7 ± 2.1 [24]b
DAYS 36 - 43	MEAN±S.D.	22.4 ± 3.8 [22]b,c	24.4 ± 4.0	23.6 ± 3.9
DAYS 43 - 50	MEAN±S.D.	22.9 ± 3.2 [22]b,c	23.8 ± 3.5 [24]b	23.6 ± 3.9 [24]b
DAYS 50 - 57	MEAN±S.D.	23.2 ± 3.7 [23]c	23.5 ± 3.0	22.3 ± 2.9
DAYS 57 - 64d	MEAN±S.D.	21.5 ± 2.3 [23]c	21.9 ± 2.0 [24]b	21.5 ± 3.0 [24]b
DAYS 1 - 64d	MEAN±S.D.	20.4 ± 1.8 [23]c	21.2 ± 1.7 [24]b	20.6 ± 1.9 [24]b

DAYS = DAYS POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values that were associated with spillage or wet feed.
c. Excludes values for rat 13221, which was found dead day 28 postweaning.
d. Last value recorded before cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

000645

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 E9 (PAGE 1): RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - PRECOHABITATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	24a	25	25
FEED CONSUMPTION (G/KG/DAY)				
DAYS 1 - 8	MEAN±S.D.	191.9 ± 18.7	192.8 ± 14.1	180.9 ± 23.4
DAYS 8 - 15	MEAN±S.D.	167.9 ± 15.3 [23]b	166.7 ± 11.4	167.7 ± 18.5
DAYS 15 - 22	MEAN±S.D.	135.9 ± 11.0 [23]b	136.4 ± 7.6	137.8 ± 11.9 [24]b
DAYS 22 - 29	MEAN±S.D.	113.2 ± 12.9 [23]c	114.2 ± 6.8	114.6 ± 6.8
DAYS 29 - 36	MEAN±S.D.	99.9 ± 9.6 [23]c	100.3 ± 7.8	100.9 ± 7.8 [24]b
DAYS 36 - 43	MEAN±S.D.	100.2 ± 18.4 [22]b,c	103.4 ± 16.7	104.1 ± 18.9
DAYS 43 - 50	MEAN±S.D.	95.3 ± 13.6 [22]b,c	93.9 ± 11.6 [24]b	96.8 ± 17.6 [24]b
DAYS 50 - 57	MEAN±S.D.	91.5 ± 14.7 [23]c	88.4 ± 11.3	86.9 ± 8.1
DAYS 57 - 64d	MEAN±S.D.	80.9 ± 6.4 [23]c	79.0 ± 6.7 [24]b	80.3 ± 9.2 [24]b
DAYS 1 - 64d	MEAN±S.D.	109.6 ± 8.8 [23]c	109.2 ± 6.6 [24]b	109.5 ± 9.1 [24]b

DAYS = DAYS POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values that were associated with spillage or wet feed.
c. Excludes values for rat 13221, which was found dead day 28 postweaning.
d. Last value recorded before cohabitation.

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 E10 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - GESTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a,b	25	25
PREGNANT	N	22	21	24
MATERNAL FEED CONSUMPTION (G/DAY)				
DAYS 0 - 7	MEAN±S.D.	25.2 ± 2.2	25.0 ± 2.6 [20]c	24.0 ± 2.4
DAYS 7 - 10	MEAN±S.D.	27.2 ± 3.0	26.2 ± 4.2	25.4 ± 3.2
DAYS 10 - 14	MEAN±S.D.	26.8 ± 3.2	25.6 ± 3.1	25.0 ± 2.6 [23]c
DAYS 14 - 17	MEAN±S.D.	27.8 ± 3.9	26.0 ± 4.1	26.9 ± 3.3
DAYS 17 - 20	MEAN±S.D.	25.2 ± 3.6	25.0 ± 3.6	25.5 ± 4.0
DAYS 0 - 20	MEAN±S.D.	26.2 ± 2.4	25.5 ± 2.5	25.1 ± 2.4

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 postweaning.
c. Excludes values that were associated with spillage.

000647

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 E11 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - GESTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a,b	25	25
PREGNANT	N	22	21	24
MATERNAL FEED CONSUMPTION (G/KG/DAY)				
DAYS 0 - 7	MEAN±S.D.	83.6 ± 5.8	80.0 ± 5.4 [20]c	80.9 ± 7.4
DAYS 7 - 10	MEAN±S.D.	84.1 ± 6.9	79.3 ± 10.0	80.4 ± 7.6
DAYS 10 - 14	MEAN±S.D.	78.8 ± 7.8	74.0 ± 7.6	75.8 ± 7.1 [23]c
DAYS 14 - 17	MEAN±S.D.	76.7 ± 10.2	70.3 ± 10.0	75.7 ± 6.8
DAYS 17 - 20	MEAN±S.D.	63.1 ± 8.2	61.3 ± 7.6	64.5 ± 8.8
DAYS 0 - 20	MEAN±S.D.	77.8 ± 5.6	73.8 ± 5.0	75.9 ± 5.2

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 postweaning.
c. Excludes values that were associated with spillage.

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 E12 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - LACTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS TESTED	N	25	25	25
INCLUDED IN ANALYSES	N	23a,b	25	25
PREGNANT	N	22	21	24
DELIVERED LITTERS	N	22	21	24
MATERNAL FEED CONSUMPTION (G/DAY)				
DAYS 1 - 4	MEAN±S.D.	29.2 ± 6.9	30.1 ± 5.7 [19]c	25.2 ± 4.7*
DAYS 4 - 7	MEAN±S.D.	36.9 ± 4.8	39.6 ± 7.8	35.9 ± 6.4
DAYS 7 - 14d	MEAN±S.D.	54.0 ± 6.1	57.4 ± 7.1	52.0 ± 7.3 [23]e
DAYS 1 - 14d	MEAN±S.D.	44.4 ± 4.6	47.1 ± 6.2	42.2 ± 5.5 [23]e

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 postweaning.
c. Excludes values that were associated with spillage.
d. 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 21 of lactation.
e. Excludes values for dam 13257, which was found dead on day 10 of lactation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

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 E13 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - LACTATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP			I	II	III
DOSAGE (MG/KG/DAY)			0 (VEHICLE)	0.1	0.4
RATS TESTED		N	25	25	25
INCLUDED IN ANALYSES		N	23a,b	25	25
PREGNANT		N	22	21	24
DELIVERED LITTERS		N	22	21	24
MATERNAL FEED CONSUMPTION (G/KG/DAY)					
DAYS 1 - 4		MEAN±S.D.	90.6 ± 24.7	89.0 ± 15.0 [19]c	78.9 ± 16.0
DAYS 4 - 7		MEAN±S.D.	113.2 ± 20.8	117.1 ± 22.9	111.9 ± 20.2
DAYS 7 - 14d		MEAN±S.D.	157.6 ± 21.9	162.3 ± 20.2	154.1 ± 17.7 [23]e
DAYS 1 - 14d		MEAN±S.D.	133.4 ± 19.9	136.6 ± 17.6	128.8 ± 15.1 [23]e

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 postweaning.
c. Excludes values that were associated with spillage.
d. 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 21 of lactation.
e. Excludes values for dam 13257, which was found dead on day 10 of lactation.

000650

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 E14 (PAGE 1): SEXUAL MATURATION - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
FEMALE RATS	N	25	25	25
INCLUDED IN ANALYSES	N	24a	25	25
VAGINA PATENT b	MEAN±S.D.	31.1 ± 1.8	31.1 ± 2.0	30.5 ± 1.4

a. Excludes values for female rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Average day postpartum that the vagina was patent.

000651

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 E15 (PAGE 1): PASSIVE AVOIDANCE PERFORMANCE - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
FEMALE RATS				
SESSION 1a	N	22b	25	22
TRIALS TO CRITERION	MEAN±S.D.	6.4 ± 3.1	5.2 ± 2.0	5.0 ± 1.0
LATENCY TRIAL 1c	MEAN±S.D.	8.0 ± 5.0	7.1 ± 3.5	11.3 ± 12.5
LATENCY TRIAL 2c	MEAN±S.D.	23.2 ± 18.1	25.6 ± 19.6	24.1 ± 19.1
FAILED TO LEARN d	N(%)	1(4.5)	0(0.0)	0(0.0)
SESSION 2a	N	21	25	22
TRIALS TO CRITERION	MEAN±S.D.	4.0 ± 2.9	3.3 ± 2.7	2.8 ± 1.2*
LATENCY TRIAL 1c	MEAN±S.D.	26.0 ± 21.3	38.4 ± 23.7	40.3 ± 24.6

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
c. The latency was recorded in seconds.
d. Number of rats that did not meet the criterion in Session 1 (Learning Phase).
* Significantly different from the vehicle control group value ($p \leq 0.05$).

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 E16 (PAGE 1): WATERMAZE PERFORMANCE - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
FEMALE RATS				
SESSION 1a	N	21	25	21
TRIALS TO CRITERION	MEAN±S.D.	11.2 ± 2.7	9.8 ± 2.8	11.8 ± 2.5
ERRORS PER TRIAL	MEAN±S.D.	0.51 ± 0.28	0.40 ± 0.18	0.51 ± 0.27
LATENCY TRIAL 2b	MEAN±S.D.	12.2 ± 7.4	13.0 ± 7.5	14.5 ± 8.2
FAILED TO LEARN c	N(%)	3(14.3)	3(12.0)	2(9.5)
SESSION 2a	N	18	22	18d
TRIALS TO CRITERION	MEAN±S.D.	7.3 ± 3.2	7.5 ± 3.0	6.3 ± 1.8
ERRORS PER TRIAL	MEAN±S.D.	0.16 ± 0.18	0.15 ± 0.14	0.17 ± 0.20
LATENCY TRIAL 1b	MEAN±S.D.	14.4 ± 12.1	10.4 ± 5.6	10.7 ± 3.5

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from group averages and statistical analyses.
d. Excludes values for rat 13264, which was not tested during Session 2 (Retention Phase).

000653

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 E17 (PAGE 1): MATING AND FERTILITY SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS IN COHABITATION	N	23a,b	25	25
DAYS IN COHABITATION c	MEAN±S.D	3.6 ± 3.2	3.9 ± 2.8	2.6 ± 1.5
RATS THAT MATED	N(%)	23(100.0)	24(96.0)	25(100.0)
FERTILITY INDEX d	N/N	22/23	21/24	24/25
	(%)	(95.6)	(87.5)	(96.0)
RATS WITH CONFIRMED MATING DATES	N	23	24	25
RATS MATING e				
DAYS 1- 7	N(%)	20(87.0)	22(91.7)	25(100.0)
DAYS 8-14	N(%)	3(13.0)f	2(8.3)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N	22/23	21/25	24/25
	(%)	(95.6)	(84.0)	(96.0)

a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13121, which was found dead day 28 postweaning.
c. Restricted to rats with a confirmed mating date and rats that did not mate.
d. Number of pregnancies/number of rats that mated.
e. Restricted to rats with a confirmed mating date.
f. Includes data for rat 13228; rat was only in cohabitation six days with the first male because there were no available male rats on day 1 of cohabitation.

000654

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 E18 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS EXAMINED a	N	25	25	25
FOUND DEAD	N	1b	0	1c
SACRIFICED	N	1d	0	0
APPEARED NORMAL	N	23b,d	25	24
THORACIC CAVITY:				
LIGHT ORANGE FLUID	N	0	0	1c
TRACHEA:				
WHITE FOAM PRESENT	N	0	0	1c
LUNGS:				
DARK RED	N	0	0	1c
URINARY BLADDER:				
ONE CALCULUS	N	2	0	0
WALLS THICKENED	N	1	0	0
KIDNEYS:				
BILATERAL OR LEFT, CORTEX PITTED AND/OR ROUGH	N	2	0	0
RIGHT, PELVIS, ONE CALCULUS	N	1	0	0
RIGHT, PELVIS, TAN GRANULAR MATERIAL	N	1	0	0

a. Refer to the individual clinical observations table (Table E23) for external observations confirmed at necropsy.
b. Rat 13221 was found dead on day 28 postweaning.
c. Dam 13257 was found dead on day 10 of lactation.
d. Rat 13205 was excluded from study on day 7 postweaning; the sex was incorrectly identified.

000655

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 E19 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
RATS ASSIGNED TO NATURAL DELIVERY	N	23a,b	25	25
PREGNANT	N(%)	22(95.6)	21(84.0)	24(96.0)
DELIVERED LITTERS	N(%)	22(100.0)	21(100.0)	24(100.0)
DURATION OF GESTATION c	MEAN±S.D.	22.9 ± 0.5	22.9 ± 0.4	22.8 ± 0.4
IMPLANTATION SITES	N	338	352	369
PER DELIVERED LITTER	MEAN±S.D.	15.4 ± 2.9	16.8 ± 2.2	15.4 ± 2.2
DAMS WITH STILLBORN PUPS	N(%)	4(18.2)	3(14.3)	9(37.5)
DAMS WITH NO LIVEBORN PUPS	N	0	0	0
GESTATION INDEX d	%	100.0	100.0	100.0
	N/N	22/ 22	21/ 21	24/ 24
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

a. Excludes values for rat 13205, which was excluded from study on day 7 postweaning; the sex was incorrectly identified.
b. Excludes values for rat 13221, which was found dead on day 28 of study.
c. 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.
d. Number of rats with live offspring/number of pregnant rats.

000656

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.

000657

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.

000658

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 ($p \leq 0.01$).

000659

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 E21 (PAGE 1): CLINICAL OBSERVATIONS FROM BIRTH TO DAY 21 POSTPARTUM - SUMMARY - F2 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
LITTERS EXAMINED (N)		22	21	24
TRANSIENT CLINICAL OBSERVATIONS: a		TOTAL FREQUENCY (DAYS x PUPS)/LITTERS WITH OBSERVATIONS		
NOT NURSING OR NESTING	N/N	1/ 1	0/ 0	0/ 0
DECREASED MOTOR ACTIVITY	N/N	1/ 1	0/ 0	0/ 0
COLD TO TOUCH	N/N	1/ 1	0/ 0	0/ 0
PERSISTENT CLINICAL OBSERVATIONS: a		TOTAL FREQUENCY (DAYS x PUPS)/LITTERS WITH OBSERVATIONS		
TIP OF TAIL MISSING	N/N	21/ 1	0/ 0	1/ 1
UMBILICAL HERNIA	N/N	0/ 0	0/ 0	15/ 1

STATISTICAL ANALYSES WERE RESTRICTED TO THE NUMBER OF LITTERS WITH OBSERVATIONS.
N/N = TOTAL FREQUENCY (DAYS x PUPS)/LITTERS WITH OBSERVATIONS
a. Tabulation restricted to adverse observations; all other pups appeared normal.

000660

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 E22 (PAGE 1): NECROPSY OBSERVATIONS SUMMARY - F2 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.4
LITTERS EXAMINED (N)		22	21	24
TOTAL PUPS STILLBORN OR FOUND DEAD a	N	9	12	19
STILLBORN	N	4	7	12
FOUND DEAD	N	5	5	7
NO MILK IN STOMACH b	N(%)	2(40.0)	4(80.0)	4(57.1)
PUPS SACRIFICED AND NECROPSIED ON DAYS 4, 10 OR 21 POSTPARTUM a				
LITTERS EVALUATED	N	22	21	24
PUPS EVALUATED	N	301	311	325
APPEARED NORMAL				
LITTER INCIDENCE	N(%)	21(95.4)	20(95.2)	24(100.0)
PUP INCIDENCE	N(%)	300(99.7)	310(99.7)	325(100.0)
LIVER: MEDIAN AND LEFT LATERAL LOBES, TAN RAISED AREA				
LITTER INCIDENCE	N(%)	0(0.0)	1(4.8)	0(0.0)
PUP INCIDENCE	N(%)	0(0.0)	1(0.3)	0(0.0)
BRAIN: HYDROCEPHALY, EXTREME DILATION OF ALL VENTRICLES				
LITTER INCIDENCE	N(%)	1(4.5)	0(0.0)	0(0.0)
PUP INCIDENCE	N(%)	1(0.3)	0(0.0)	0(0.0)

a. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation.
b. Analysis restricted to pups found dead and necropsied.

000661

APPENDIX F

PROTOCOL AND AMENDMENTS

RESEARCH Argus LABORATORIES

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044
T: (215) 443-8710 F: (215) 443-8587

PROTOCOL 418-008

SPONSOR'S STUDY NUMBER: 6295.9

STUDY TITLE: Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats.

PURPOSE: The purpose of this study is to test for toxic effects/ disturbances resulting from PFOS treatment of Crl:CD®BR VAF/Plus® male and female rats before cohabitation through mating, gestation and lactation. This study evaluates ICH Harmonised Tripartite Guideline stages A through F of the reproductive process and should detect effects on the estrous cycle, tubal transport, implantation, gestation, parturition, lactation and maternal behavior in female rats, on the development of the offspring of the treated male and female rats, and permit detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be detected by histological examinations of male rat reproductive organs. Because manifestations of effects induced during this period may be delayed in the offspring, observations will be continued through production of F2 generation litters.

TESTING FACILITY: Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1297
Telephone: (215) 443-8710
Telefax: (215) 443-8587

STUDY DIRECTOR: Raymond G. York, Ph.D., DABT
Associate Director of Research

SPONSOR: 3M Toxicology Services
3M Center, Building 220-2E-02
St. Paul, Minnesota 55144-1000

STUDY MONITOR: Marvin T. Case, D.V.M., Ph.D.
Telephone: (612) 733-5180
Telefax: (612) 733-1773

ALTERNATE STUDY MONITOR: Andrew M. Seacat, Ph.D.
Telephone: (612) 575-3161
Telefax: (612) 733-1773

REGULATORY CITATIONS:

Study Design as Modification of: 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.

STUDY SCHEDULE:

See ATTACHMENT 1 to the protocol.

TEST ARTICLE AND VEHICLE:

Identification:

Test Article:

Name:	PFOS.
Physical Description:	Light-colored powder.
Lot/Batch Number:	217.
Specific Gravity:	~0.6.
Purity:	98.9%.
Expiration Date:	May, 2000.

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

Vehicle:

0.5% Tween 80 in Reversed Osmosis Membrane Processed Deionized Water (R.O. Deionized Water). Supplier and lot identification of Tween 80 to be documented in the raw data.

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the vehicle that would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

Safety Precautions:

Gloves, mask, appropriate eye protection and a uniform/lab coat are to be worn during formulation preparation and dosage administration. The Material Safety Data Sheet (MSDS) is attached to the protocol (ATTACHMENT 2).

Storage:

Bulk Test Article:	Room temperature.
Vehicle Components:	Room temperature.
Prepared Vehicle:	Room temperature.
Prepared Formulations:	Frozen (-20°C).

All test article shipments to the Testing Facility should be addressed to the attention of Julian Gulbinski, Manager of Formulations, at the previously cited address and telephone number.

Shipments should include information concerning storage conditions and shipping cartons should be labeled appropriately. The recipient should be notified in advance of shipment.

FORMULATION:

Frequency of Preparation:

Formulations (suspensions) will be prepared daily at the Testing Facility.

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:

Stability:

Stability data for prepared formulations bracketing the range of concentrations and conditions of this study are on file with the Sponsor and will not be determined during the conduct of this study. Suspensions will be prepared daily at the Testing Facility.

Homogeneity Analyses:

Homogeneity of the test article in prepared suspensions will be verified during the course of this study. A syringe will be used to withdraw samples (5 mL each) from the top, middle and bottom of the highest concentration on the first day of preparation. Each sample (5 mL) will be divided into two aliquots, one of 2 mL and one of 3 mL. One aliquot (2 mL) will be shipped for analysis; the other aliquot (3 mL) will be retained at the Testing Facility as a backup sample. Backup samples will be stored under the previously cited conditions and discarded at the Testing Facility upon the request of the Sponsor.

Concentration Analyses:

Concentration of the prepared test article suspensions will be verified during the course of this study. A syringe will be used to withdraw samples (5 mL each) from each concentration during the first and sixth week of dosage administration. Each sample (5 mL each) will be divided into two aliquots, one of 2 mL and one of 3 mL. One aliquot (2 mL) will be shipped for analysis; the other aliquot (3 mL) will be retained at the Testing Facility as a backup sample. Backup samples will be stored under the previously cited conditions and discarded at the Testing Facility upon the 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

Both the recipient and the Study Monitor 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.

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 has been demonstrated to be sensitive to reproductive and developmental toxins and has been widely used throughout industry for reproductive and developmental toxicity evaluations; 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:	195 virgin male and 205 virgin female rats.
Population selected for study:	175 male rats (35 per dosage group) and 175 female rats (35 per dosage group).

Ten mated female rats will be assigned to Caesarean-sectioning on day 10 of presumed gestation; the remaining female rats will be permitted to deliver litters.

250 F1 generation pups (25 per sex per dosage group) will be selected at weaning on day 21 postpartum for continued postnatal observation.

Body Weight and Age:

Male rats will be ordered to weigh from 300 g to 325 g each at receipt, at which time they will be expected to be at least 60 days of age. Female rats will be ordered to weigh from 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 ranges will be included in the final report.

Sex:

Both Fo and F1 generation male and female rats will be evaluated. Only Fo generation male and female rats will be given the test article.

Source:

Charles River Laboratories, Inc., Raleigh, North Carolina.

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 and female rats are assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study before administration of the first dosage of the test article.

F1/F2 Generations:

Pups will not be individually identified during lactation; all parameters will be evaluated in terms of the litter. At weaning, each rat selected for continued observation will be identified with a Monel® self-piercing ear tag.

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 assigned to natural delivery will be individually housed in nesting boxes. Each dam and delivered litter will be housed in a common nesting box during the postpartum period.

F1 Generation Rats/F2 Generation Litters:

After weaning, the F1 generation rats will be individually housed before cohabitation, housed in pairs (one male rat per female rat) during cohabitation, and individually housed after cohabitation. The same type of caging will be used as described for the Fo generation rats. Beginning no later than day 20 of presumed gestation, F1 generation female rats will be individually housed in nesting boxes. Each dam and delivered litter will be housed in a common nesting box during the postpartum period.

Nesting Material:

Bedding material (bed-o'cobs®) will be supplied to female rats assigned to natural delivery.

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:

Fo Generation:

Upon arrival, rats will be assigned to individual housing on the basis of computer-generated random units. After acclimation, male and female rats will be selected for study on the basis of physical appearance and body weights recorded during acclimation. The 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 rats to cohabitation, one male rat per female rat. The cohabitation period will consist of a maximum of 14 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. Female rats not mated within the first 7 days of cohabitation will be assigned alternate male rats that have mated (same dosage group) and will remain in cohabitation for a maximum of seven additional days.

The first ten female rats per dosage group with a confirmed date of mating will be assigned to Caesarean-sectioning on day 10 of presumed gestation. The remaining female rats will be permitted to naturally deliver litters.

A table of random units will be used to assign five rats per group to a pharmacokinetic sample collection at scheduled sacrifice after completion of the cohabitation period (male rats siring litters with dams allowed to naturally deliver a litter) or on day 21 postpartum (female rats allowed to naturally deliver litters).

F1/F2 Generation Pups:

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, a table of random units will be used to select pups to be culled, and litters will be reduced to eight pups each. Whenever possible, the same number of male and female pups per litter will be continued on study.

At weaning of the F1 generation pups on day 21 postpartum, a table of random units will be used to select 25 male and 25 female pups per group, resulting in a total of 250 F1 generation rats (125 per sex) chosen for continued evaluation. At least one male pup and one female pup per litter, when possible, will be selected.

ADMINISTRATION:

Route and Reason for Choice:

The oral (gavage) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one of the possible routes of human exposure.

Method and Frequency:

Dosages will be adjusted for the most recently recorded body weight and given at approximately the same time each day.

Fo Generation Male Rats:

Male rats will be given the test article once daily beginning 28 days before cohabitation (maximum 14 days) and continuing through the day before sacrifice. Male rats will be sacrificed after completion of the cohabitation period.

Fo Generation Female Rats:

Female rats will be given the test article once daily beginning 28 days before cohabitation (maximum of 14 days) and continuing through day 9 of presumed gestation (rats assigned to Caesarean-sectioning), day 24 of presumed gestation (rats assigned to natural delivery that do not deliver a litter) or day 20 postpartum (rats that deliver a litter).

F1 Generation:

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 will be selected by the Sponsor on the basis of previous studies conducted with the test article.

Dosage Levels, Concentrations and Volumes:

Dosage Group	Number of Rats Per Sex	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Argus Batch Number
I	35	0 (Vehicle)	0	5	B-418-008-A(Day.Month.Year)
II	35	0.1	0.02	5	B-418-008-B(Day.Month.Year)
III	35	0.4	0.08	5	B-418-008-C(Day.Month.Year)
IV	35	1.6	0.32	5	B-418-008-D(Day.Month.Year)
V	35	3.2	0.64	5	B-418-008-E(Day.Month.Year)

The test article will be considered 100% pure for the purpose of dosage calculations.

TESTS, ANALYSES AND MEASUREMENTS - Fo GENERATION:

Viability - Male and Female Rats:

All Periods: At least twice daily.

Clinical Observations and/or General Appearance - Male and Female Rats:

Acclimation Period: At least once.

Dosage Period: Twice daily. Prior to dosage administration and once approximately one hour postdosage.

Maternal Behavior: Days 1, 4, 7, 14 and 21 postpartum. Any 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 - Male Rats:

Acclimation Period: At least once.

Dosage Period: Weekly.

Sacrifice: Terminal weight.

Body Weights - Female Rats:

Acclimation Period:	At least once.
Dosage Period:	Weekly to cohabitation. Daily during presumed gestation and on Days 1, 4, 7 and 14 postpartum (rats assigned to natural delivery).
Sacrifice:	Terminal weight.

Feed Consumption Values - Male Rats (recorded and tabulated):

Dosage Period:	Weekly.

Feed Consumption Values - Female Rats (recorded and tabulated):

Dosage Period:	Weekly to cohabitation. Daily during presumed gestation. Days 1, 4, 7 and 14 postpartum (rats assigned to natural delivery).

Feed consumption not tabulated after day 14 postpartum, when it is expected that pups will begin to consume maternal feed.

Feed Consumption Values - Male and Female Rats:

Feed consumption values may be recorded more frequently than cited above if it is necessary to replenish the feed. During cohabitation, when two rats occupy the same cage with one feed jar, replenishment of the feed jars will be documented. Individual values will not be recorded or tabulated.

Estrous Cycling and Mating:

A table of random units will be used to select 15 female rats per group for evaluation of estrous cycling by examination of vaginal cytology for 14 days before the start of the cohabitation period.

During cohabitation, all female rats will be evaluated daily until spermatozoa are observed in a smear of the vaginal contents and/or a copulatory plug is observed *in situ*.

Duration of Gestation:

The duration of gestation is calculated from day 0 of presumed gestation to the day the first pup is observed.

Fertility Parameters:

Fertility Index (percentage of matings that result in pregnancies).

Gestation Index (percentage of pregnancies that result in birth of live litters).

Number of offspring per litter (live and dead pups).

Number of implantation sites.

General condition of dam and litter during the postpartum period.

Viability Indices (percentage of pups born that survive 4 and 7 days).

Lactation Index (percentage of pups born that survive 21 days).

Caesarean-Sectioning Observations:

Rats will be Caesarean-sectioned on day 10 of presumed gestation. Placentae that appear abnormal (size, color or shape) will be noted in the raw data. The rats 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.)

Natural Delivery:

Female rats will be evaluated for:

Clinical Observations During Parturition.

Duration of Gestation (day 0 of presumed gestation to the time the first pup is observed).

Length of Parturition (time of delivery of last pup minus the time of delivery of the first pup divided by N-1 pups in each litter).

Litter Size (defined as all pups delivered).

Pup Viability at Birth.

METHOD OF SACRIFICE - Fo GENERATION:

Rats will be sacrificed by carbon dioxide asphyxiation. Embryos will be discarded after examination.

NECROPSY - Fo GENERATION:

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.

Male and Female Rats Assigned to Pharmacokinetic Sample Collection:

At scheduled sacrifice after completion of the cohabitation period (male rats siring litters with dams allowed to naturally deliver a litter) and on day 21 postpartum (female rats allowed to naturally deliver a litter), five rats per group will be assigned to a pharmacokinetic sample collection. In addition to the appropriate evaluations described below, blood samples (approximately 4 mL per rat) will be collected from the inferior vena cava into serum separator tubes and centrifuged. The resulting serum (approximately 2 mL) will be immediately frozen on dry ice and maintained frozen (-70°C) until shipment to the Sponsor for analysis. The liver will be excised, weighed, and a sample section (lateral lobe) will be frozen and retained at -70°C until shipment to the Sponsor for analysis.

After completion of sample collection, serum and liver section (lateral lobe) samples will be shipped (frozen on dry ice) to Kris J. Hansen, Ph.D., at the previously cited address for analysis. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

Scheduled Sacrifice of Male Rats:

After completion of the cohabitation period, male rats will be sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. The following organs will be excised and weighed and retained for possible histologic evaluation: testes, epididymides, prostate and seminal vesicles (weighed with and without fluid). The testes will be fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin for possible histopathological evaluation. The remaining organs will be retained in neutral buffered 10% formalin.

Scheduled Sacrifice - Female Rats Assigned to Caesarean-Sectioning:

On day 10 of presumed gestation, female rats will be sacrificed, Caesarean-sectioned, and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. Uteri of apparently nonpregnant rats will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5]. Uteri of nonpregnant rats and all ovaries will be retained in neutral buffered 10% formalin for possible future evaluation.

Scheduled Sacrifice - Female Rats Assigned to Natural Delivery:

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].

After completion of the 21-day postpartum period, 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.

Dams with No Surviving Pups:

Dams with no surviving pups will be sacrificed after the last pup is found dead, missing or presumed cannibalized. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. 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 or abortion 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. Testes, epididymides, prostate and seminal vesicles of male rats will be excised and individual organ weights will be recorded (seminal vesicles weighed with and without fluid). The testes will be fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin. The remaining organs will be retained in neutral buffered 10% formalin. Pregnancy status and uterine contents of female rats will be recorded. Aborted fetuses and/or delivered pups will be examined to the extent possible. Ovaries will be retained in neutral buffered 10% formalin. Uteri of apparently nonpregnant rats will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5].

TESTS, ANALYSES AND MEASUREMENTS - F1 GENERATION:

Viability:

Preweaning Period:	Litters will be observed for dead pups at least twice daily. The pups in each litter will be counted once daily.
Postweaning Period:	Twice daily.

Clinical Observations and/or General Appearance:

Preweaning Period:	Once daily.
Postweaning Period:	Once weekly.
Maternal Behavior:	Days 1, 4, 7, 14 and 21 postpartum. Any 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 the Study Monitor.

Body Weights:

Preweaning Period:	Days 1 (birth), 4, 7, 14 and 21 postpartum.
Postweaning Period:	Weekly.
Presumed Gestation Period:	Days 0, 7, 10, 14, 17 and 20 (female rats only).
Lactation Period:	Days 1, 4, 7, 10 and 14 (female rats only).
Sacrifice:	Terminal weight.

Feed Consumption Values (recorded and tabulated):

Preweaning Period:	Not recorded.
Postweaning Period:	Weekly except during cohabitation.
Presumed Gestation Period:	Days 0, 7, 10, 14, 17 and 20 (female rats only).
Lactation Period:	Days 1, 4, 7, 10 and 14 (female rats only).

Feed consumption values may be recorded more frequently if it is necessary to replenish the feed. During cohabitation, when two rats occupy the same cage with one feed jar, values will be documented when feed jars are filled. These intervals will not be tabulated.

Preweaning Developmental Observations:

The number of pups meeting the criterion is recorded on each day of testing. Testing continues until the day the criterion is attained by all pups in the litter.

Surface Righting Reflex (ability to right in 5 seconds): From day 1 postpartum.

Pinna Unfolding: From day 2 postpartum.

Eye Opening: From day 12 postpartum.

Acoustic Startle Response: From day 13 postpartum.

Air Righting Reflex: From day 14 postpartum.

Pupil constriction is evaluated once, on day 21 postpartum.

Postweaning Developmental Observations:

Sexual Maturation:

Female rats will be evaluated for the age of vaginal patency, beginning on day 28 postpartum. Male rats will be evaluated for the age of preputial separation, beginning on day 39 postpartum.

Passive Avoidance Testing:

Beginning at 24 ± 1 day postpartum, one male rat and one female rat from each litter, where possible, will be evaluated in a passive avoidance test for learning, short-term retention and long-term retention.

The passive avoidance apparatus consists of a two-compartment chamber with hinged Plexiglas® lids. One compartment is fitted with a bright light and Plexiglas® floor. The other compartment is fitted with a grid floor to which a brief (1 sec) pulse of mild electric current (1 mA) can be delivered. The two compartments are separated by a sliding door. On each test trial, the rat is placed into the "bright" compartment, the sliding door is opened and the light is turned on. The rat is allowed to explore the apparatus until it enters the "dark" compartment. The sliding door is then immediately closed, the light is turned off and the brief pulse of current is delivered to the grid floor. The rat is then removed from the apparatus and placed into a holding cage for 30 seconds before the start of the next trial. Trials are repeated until the rat remains in the "bright"

compartment for 60 seconds on two consecutive trials (the criterion for learning) or until 15 trials have been completed. The latency to enter the dark compartment or the maximum 60-second interval is recorded for each trial.

Each rat is tested twice. The test sessions are separated by a one-week interval, and the criterion is the same for both days of testing.

Dosage groups are compared for the following dependent measures:

The number of trials to the criterion in the first session--this measure will be used to compare groups for overall learning performance.

The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the first test session--this measure will be used to compare groups for activity levels and exploratory tendencies in a novel environment.

The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session--this measure will be used to compare groups for short-term retention.

The number of trials to the criterion in the second test session--this measure will be used to compare groups for long-term retention.

The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the second session--this value is another indication of long-term retention.

Watermaze Testing:

Beginning at approximately 70 days postpartum, one male rat and one female rat from each litter will be evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory.

Each rat is tested in a watertight 16-gauge stainless steel modified M-maze. The maze is filled with water to a depth of approximately nine inches, and the water is monitored for temperature (range of 21°C ± 1°C).

On each test trial, the rat will be placed into the starting position (base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals of the M-maze, in order to be removed from the water. On the first trial, the rat is required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial 1 is designated the incorrect goal during the remaining trials. Rats that fail to make a correct goal choice within 60 seconds in any given trial are guided to the correct goal and are then removed from the water. A 15-second intertrial interval will separate each trial. Each rat is required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test

session is 15. Latency (measured in seconds) to choose the correct goal or the maximum 60-second interval is recorded for each trial, as is the number of errors (incorrect turns in the maze) during each trial.

Each rat is tested twice. The test sessions are separated by a one-week interval, and the correct goal and the criterion are the same for both test sessions.

Dosage groups are compared for the following dependent measures:

The number of trials to criterion on the first day of testing--this measure will be used to compare groups for overall learning performance.

The average number of errors (incorrect turns in the maze) for each trial on the first day of testing--this measure will also be used to compare groups for overall learning performance.

The latency (in seconds) to reach the correct goal on trial 2 of the first day of testing--this measure will be used to compare groups for short-term retention.

The number of trials to criterion on the second day of testing--this measure will be used to compare groups for long-term retention.

The average number of errors for each trial on the second day of testing--this measure will also be used to compare groups for long-term retention.

The latency (in seconds) to reach the correct goal on trial 1 of day 2 of testing--this is another indicator of long-term retention.

Reproductive Capacity:

At approximately 90 days of age, the F1 generation rats within each dosage group will be assigned to cohabitation, one male rat per female rat, based on computer-generated random units or random unit tables, with the exclusion of sibling matings. The cohabitation period will consist of a maximum of 14 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. Female rats that do not mate within the first 7 days of cohabitation will be assigned alternate male rats from the same dosage group that have mated. Female rats will be allowed to naturally deliver and maintain litters through a 21-day postpartum period.

Mating Performance:

As cited above for Fo generation rats.

Duration of Gestation:

As cited above for Fo generation rats.

Fertility Parameters:

As cited above for Fo generation rats.

F2 Generation Litter Data:

Viability, clinical observations and body weights for F2 generation pups will be recorded as cited above for F1 generation litters.

METHOD OF SACRIFICE - F1 GENERATION RATS/F2 GENERATION PUPS:

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.

Scheduled Sacrifice - F1 Generation Male Rats:

Rats will be sacrificed after completion of the 14-day cohabitation period. A gross necropsy of the thoracic, abdominal and pelvic visceral will be performed. Testes and epididymides of male rats will be excised and individual organ weights will be recorded. The epididymides will be retained in neutral buffered 10% formalin. The testes will be fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin.

Scheduled Sacrifice - F1 Generation Female Rats:

Female rats will be sacrificed after completion of the 21-day postpartum period. The number and distribution of implantation sites will be recorded. Rats that do not deliver a litter will be sacrificed on day 25 of presumed gestation and uteri will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5]. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. Female rats without a confirmed mating date that do not deliver a litter will be sacrificed on an estimated day 25 of presumed gestation.

F1 Generation Rats Found Dead or Moribund:

Rats that die or are sacrificed because of moribund condition or abortion 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. Testes, epididymides, prostate and seminal vesicles of male rats will be excised and individual organ weights will be recorded (seminal vesicles weighed with and without fluid). The testes will be fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin. The remaining organs will be retained in neutral buffered 10% formalin. Pregnancy status and uterine contents of female rats will be recorded. Aborted fetuses and/or delivered pups will be examined to the extent possible. Ovaries will be retained in neutral buffered 10% formalin. Uteri of apparently nonpregnant rats will be stained with 10% ammonium sulfide to confirm the absence of implantation sites[5].

F1 Generation Dams with No Surviving Pups:

Dams with no surviving pups will be sacrificed after the last pup is found dead, missing or presumed cannibalized. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. Postpartum data for these dams will be excluded from summary tables.

F1/F2 Generation 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. Should postmortem autolysis preclude these evaluations, it will be noted in the necropsy data.

F1/F2 Generation Pups Found Dead or Moribund on Days 2 to 21 Postpartum:

Pups found dead or sacrificed due to moribund condition will be examined for gross lesions and for the cause of the moribund condition or death. 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. Should postmortem autolysis preclude these evaluations it will be noted in the necropsy data.

F1/F2 Generation Pups Not Selected for Continued Observation:

F1 and F2 generation pups culled on day 4 postpartum will be sacrificed and examined for gross lesions; pups with gross lesions will be preserved in Bouin's solution. Necropsy will include a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly.

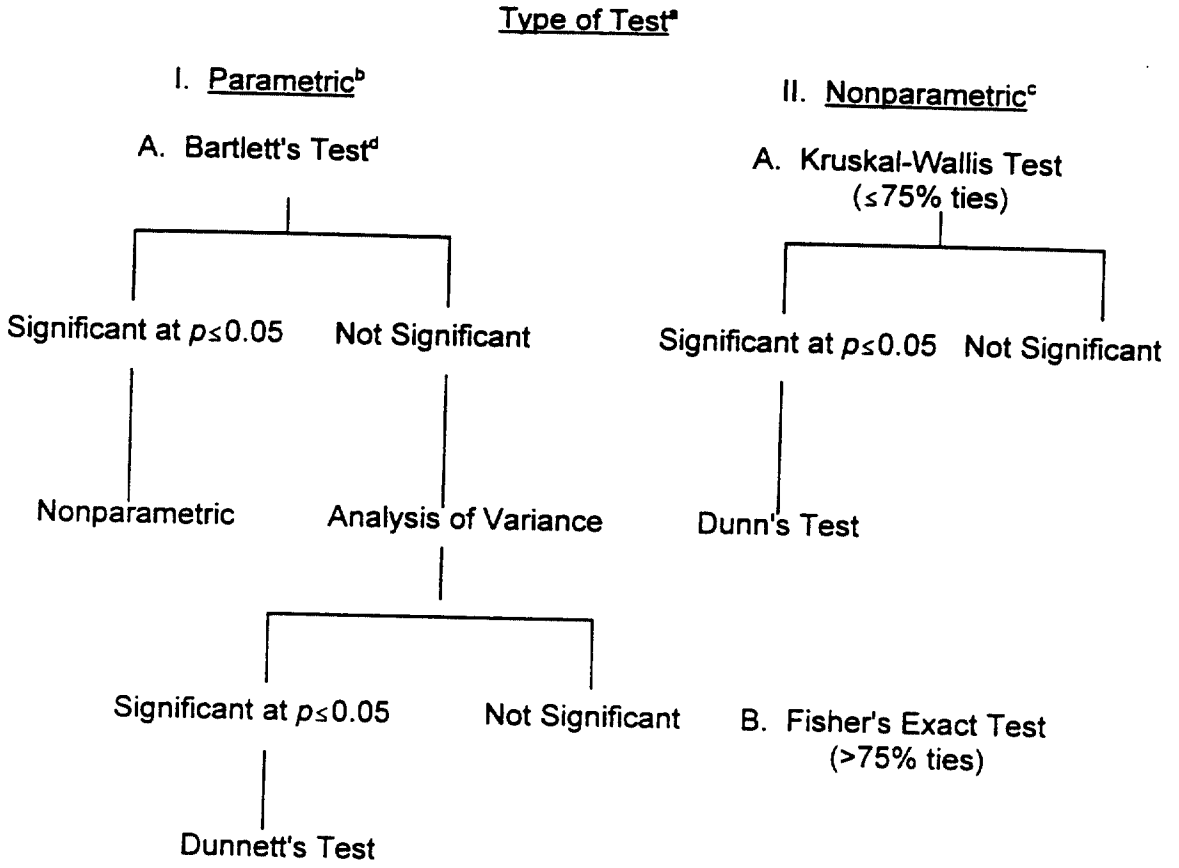
All F1 generation pups culled on day 21 postpartum will be sacrificed and examined for gross lesions; gross lesions will be preserved in neutral buffered 10% formalin. Necropsy will include a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly.

Scheduled Sacrifice - F2 Generation Pups:

On day 21 postpartum, pups will be sacrificed and examined for gross lesions. Necropsy will include a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly.

PROPOSED STATISTICAL METHODS[6-12]:

Averages and percentages will be calculated. Litter values will be used where appropriate. Additional procedures and/or analyses may be performed, if appropriate.



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a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
b. Used only to analyze data with homogeneity of variance.
c. Proportion data are not included in this category.
d. Test for homogeneity of variance.

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.
Blood Sample Collection, Processing and Shipment.
Body Weights.
Feed Consumption Values.
Caesarean-Sectioning Observations.
Natural Delivery Observations.
Litter Observations.
Reflex and Physical Development and Behavioral Observations - F1 Generation Pups.
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., ATS
Director of Research: Alan M. Hoberman, Ph.D., DABT
Associate Director of Research and Study Director: Raymond G. York, Ph.D., DABT
Director of Laboratory Operations: John F. Barnett, B.S.
Manager of Study Coordination: Valerie A. Sharper, M.S.
Manager of Animal Operations and Member, Institutional Animal Care and Use Committee: Dena C. Lebo, V.M.D.
Manager of Regulatory Compliance: Kathleen A. Moran, M.S.
Consultant, Veterinary Pathology: W. Ray Brown, D.V.M., Ph.D., ACVP

FINAL REPORT:

A comprehensive draft final report will be prepared on completion of the study and will be finalized following consultation with the Sponsor. The report will include the following:

Summary and Conclusion.
Experimental Design and Method.
Evaluation of Test Results.
Appendices: Figures, Summary and Individual Tables Summarizing the Above Data, Protocol and Associated Amendments and Deviations, Study Director's GLP Compliance Statement, Reports of Supporting Data (if appropriate) and QAU Statement.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE STATEMENT:

The procedures described in this protocol have been reviewed by the Testing Facility's Institutional Animal Care and Use Committee. All procedures described in this protocol that involve study animals will be conducted in a manner to avoid or minimize discomfort, distress or pain to the animals.

The Sponsor's signature below documents the fact that information concerning the necessity for conducting this study and the fact that this is not an unnecessarily duplicative study may be obtained from the Sponsor. No alternative (*in vitro*) procedures were available for meeting the stated purposes of the study.

REFERENCES:

1. Christian, M.S. 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.

6. Snedecor, G.W. and Cochran, W.G. (1967). Variance test for homogeneity of the binomial distribution. *Statistical Methods*, 6th Edition, Iowa State University Press, Ames, pp. 240-241.

7. Sokal, R.R. and Rohlf, F.J. (1969). Bartlett's test of homogeneity of variances. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 370-371.

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9. Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1129.

10. Sokal, R.R. and Rohlf, F.J. (1969). Kruskal-Wallis Test. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 388-389.

11. Dunn, O.J. (1964). Multiple comparisons using rank sums. Technometrics 6(3):241-252.

12. Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*, McGraw-Hill, New York, pp. 96-104.

PROTOCOL APPROVAL:

FOR THE TESTING FACILITY

George E. Dearlove, Ph.D., DABT
Associate Director of Research

21-May-98
Date

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

21-May-98
Date

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

21-May-98
Date

FOR THE SPONSOR

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

22 May 1998
Date

ATTACHMENT 1

STUDY SCHEDULE

ATTACHMENT 1

SCHEDULE[a]

12 MAY 98	Animal Receipt - Acclimation Begins (Fo generation rats).
26 MAY 98	Start of Dosage Period - Fo Generation Male Rats (28 days before cohabitation and continuing through a 14-day cohabitation period until sacrifice after successful mating has been determined).
26 MAY 98 - 15 JUL 98	Dosage Period - Female Rats Assigned to Caesarean-Sectioning (28 days before cohabitation and continuing through day 09 of presumed gestation).
26 MAY 98 - 19 AUG 98	Dosage Period - Female Rats Assigned to Natural Delivery [28 days before cohabitation through day 24 of presumed gestation (rats that do not deliver a litter) or day 20 postpartum (rats that deliver a litter)].
09 JUN 98 - 22 JUN 98	Dosage Period Estrous Cycle Evaluation.
22 JUN 98 PM - 29 JUN 98 AM 29 JUN 98 PM - 06 JUL 98 AM	Cohabitation Period (Maximum of 14 days). Male 1 (07 days) Male 2 (07 days)
23 JUN 98 06 JUL 98	First Possible Day 0 of Presumed Gestation. Last Possible Day 0 of Presumed Gestation.
20 JUL 98	Fo Generation Male Rats Sacrificed after Completion of the Cohabitation Period (Earliest possible date).

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a. The study initiation date is the day the Study Director signs the protocol.

ATTACHMENT 1

03 JUL 98	First Possible Day 10 of Presumed Gestation Caesarean-sectioning.
16 JUL 98	Last Possible Day 10 of Presumed Gestation Caesarean-sectioning.
14 JUL 98	First Possible Delivery (Day 21 of presumed gestation).
31 JUL 98	Last Possible Delivery (Day 25 of presumed gestation).
18 JUL 98	First Possible Day 25 of Presumed Gestation Female Sacrifice.
31 JUL 98	Last Possible Day 25 of Presumed Gestation Female Sacrifice.
03 AUG 98	First Possible Day 21 Weaning (Dams and F1 generation pups not selected for continued observation sacrificed).
20 AUG 98	Last Possible Day 21 Weaning.
04 AUG 98	F1 Generation Postweaning Observations Begin (Details of tests cited in protocol).
19 OCT 98 - 02 NOV 98	F1 Generation Cohabitation Period (Initiated when rats are approximately 90 days of age - approximate initial date).
16 NOV 98	F1 Generation Male Rats Sacrificed after Completion of Cohabitation Period - Approximate Earliest Possible Date.
10 NOV 98 - 27 NOV 98	Delivery Period - F1 Generation Dams/F2 Generation Litters (Approximate dates).
30 NOV 98 - 17 DEC 98	Sacrifice of F1 Generation Dams and F2 Generation Litters on Day 21 Postpartum (Approximate dates).
06 APR 99	Draft Final Report.

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

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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

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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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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.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

ATTACHMENT 3

TEST ARTICLE 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 control article for oral administration to rats on Argus Study 418-008.

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.

2. Suspensions will be prepared:
   _X_ Daily ___ 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 solutions adjusted for Free base and % Purity.
   ___ Yes _X_ No (Calculations based on 100%)
   ___ Free Base ___ Purity

6. Sampling requirements: Cited in protocol.

7. Storage: Cited in protocol.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

NOTE: Test article will be prepared as a serial dilution from the high dosage to the low dosage. Stir bars are to be added to the containers; mixing should occur during sampling and/or dosage administration.

C. Preparation of the Control Group:

1. Add the required amount of vehicle to an appropriate vessel. (See TEST ARTICLE CALCULATIONS for exact quantities.)

D. Test Article Solution Preparation:

1. To prepare the 0.64-mg/mL, (group V) solution, add 160 mg of test article into an appropriately sized, labeled container.

2. Q. S. to 250 mL with the vehicle and mix by inversion.

3. Add a stir bar and heat the preparation to 80 °C in a water bath for approximately 30 minutes (or until the test article has dissolved).

4. Remove the solution from the water bath, and slowly spin over night while the solution equilibrates to room temperature.

5. To prepare group IV, (the 0.32-mg/mL solution), add 100 mL of group V solution to an appropriately labeled container, then q.s. to 200 mL with vehicle. Add a stir bar and mix until uniform.

6. To prepare group III, (the 0.08-mg/mL solution), add 50 mL of group IV solution to an appropriately labeled container, then q.s. to 200 mL with vehicle. Add a stir bar and mix until uniform.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

7. To prepare group II, (the 0.02-mg/mL solution), add 50 mL of group III solution to an appropriately labeled container, then q.s. to 200 mL with vehicle. Add a stir bar and mix until uniform.

Written by: _____

Approved by: _____ Date: 21-May-98

@MC 1-11-99

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

Initial/Date : MC 1-11-99

ARGUS

TEST ARTICLE/SUBSTANCE PREPARATION PROCEDURE CLARIFICATION

Protocol: 418-008 **Version:** 418-008 (21 MAY 95)

Date of Clarification	Preparation Step #	Clarification
5/25/98 ① +Ⓕ Ⓐ CR 7/15/98	C. 1	200 mL of Vehicle is added to prepare Control Group CR 7/15/98

Raymond H. Hart 15-JUL-98
Study Director Date

Study Director Date

Study Director Date

Study Director Date

Reviewed by: MC Date: 7-15-98

08.15.97 TASPPM-01-02

① Ⓕ CR 7/15/98

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PROTOCOL 418-008

COMBINED ORAL(GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: 6295.9

Amendment 1 - June 2, 1998

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1. Storage (page 3 of the protocol):

   [Effective Date: May 26, 1998] Prepared formulations will be stored at room temperature overnight, rather than frozen.

   Reason for Change:

   This change was made to clarify the protocol and facilitate dose formulation preparation.

2. Frequency of Preparation (page 4 of the protocol):

   [Effective Date: May 26, 1998] The vehicle (0.5% Tween 80® in R.O. Deionized Water) will be prepared weekly by adding 15 mLs of Tween 80® to 2985 mL of R.O. Deionized Water.

   Reason for Change:

   This change was made to clarify the protocol and facilitate dose formulation preparation.

3. Stability (page 4 of the protocol):

   [Effective Date: May 27, 1998] The Sponsor has confirmed a 48-hour stability on the test article in 0.5% Tween 80® solutions. This allows for preparations to be made one day prior to the day of dose administration. Due to the lengthy

preparation procedure, dose solutions may be made one day prior to the day of dosing.

Reason for Change:

This change was made to clarify the protocol and facilitate dose formulation preparation.

02 Jun-98
Alan M. Hoberman, Ph.D., DABT Date
Director of Research

02-Jun-98
Raymond G. York, Ph.D., DABT Date
Associate Director of Research
and Study Director

02 June 98
Dena C. Lebo, V.M.D. Date
Member, Institutional Animal Care
and Use Committee

8 June 1998
Marvin T. Case, D.V.M., Ph.D. Date
Study Monitor

RESEARCH Argus LABORATORIES

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PROTOCOL 418-008

COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: 6295.9

Amendment 2 - June 11, 1998

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1. Body Weights - Female Rats (page 12 of the protocol):

   [Effective Date: May 26, 1998] Body weights and feed consumption values will also be collected on Fo generation females on postpartum day 10.

   Reason for Change:

   This information was added to the protocol to match data collection on F1 generation females.

2. Scheduled Sacrifice of Male Rats (page 14 of the protocol):

   [Effective Date: May 26, 1998] At scheduled sacrifice of Fo male rats, all organs will be weighed individually.

   Reason for Change:

   This change clarifies the protocol.

3. Scheduled Sacrifice - Female Rats Assigned to Caesarean-Sectioning (page 15 of the protocol):

   [Effective Date: May 26, 1998] Uteri of non-pregnant rats will not be retained.

Reason for Change:

This change was made at the request of the Sponsor.

4. Scheduled Sacrifice - F1 Generation Male Rats (page 20 of the protocol):

[Effective Date: May 26, 1998] Seminal vesicles (with and without fluids) and prostates, will be weighed at scheduled sacrifice of all F1 generation male rats.

Reason for Change:

This information was added to the protocol to match data collection on Fo generation males.

A. Hoberman 11-JUN-98
Alan M. Hoberman, Ph.D., DABT Date
Director of Research

Raymond G. York 11-JUN-98
Raymond G. York, Ph.D., DABT Date
Associate Director of Research
and Study Director

Dena C. Lebo 11 Jun 98
Dena C. Lebo, V.M.D. Date
Member, Institutional Animal Care
and Use Committee

Marvin T. Case 12 Jun 1998
Marvin T. Case, D.V.M., Ph.D. Date
Study Monitor

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COMBINED ORAL(GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: 6295.9

Amendment 3 - June 25, 1998

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1. Administration, Method and Frequency (Page 10 of the protocol):

[Effective Date: June 19, 1998] Due to a programming error in dosage calculation the dosage period will be extended for an additional 2 weeks and the cohabitation period will occur two weeks later than originally scheduled. The error in dosage calculations caused the male rats to receive approximately 89% to 90% of the targeted dosage and female rats to receive approximately 95% to 99% of the targeted dosage. This occurred for six of the seven days in the second and third weeks and two days of the fourth week of the dosage period.

Reason for Change:

This change was made to ensure that all rats receive the correct dosage before cohabitation.

2. Schedule (Attachment 1 of the protocol):

SCHEDULE

12 MAY 98	Animal Receipt - Acclimation Begins (Fo generation rats).
26 MAY 98	Start of Dosage Period - Fo Generation Male Rats (42 days before cohabitation and continuing through a 14-day cohabitation period until sacrifice after successful mating has been determined).
26 MAY 98 - 29 JUL 98	Dosage Period - Female Rats Assigned to Caesarean-Sectioning (42 days before cohabitation and continuing through day 09 of presumed gestation).
26 MAY 98 - 02 SEP 98	Dosage Period - Female Rats Assigned to Natural Delivery [42 days before cohabitation through day 24 of presumed gestation (rats that do not deliver a litter) or day 20 postpartum (rats that deliver a litter)].
09 JUN 98 - 06 JUL 98	Dosage Period Estrous Cycle Evaluation.
06 JUL 98 PM - 13 JUL 98 AM 13 JUL 98 PM - 20 JUL 98 AM	Cohabitation Period (Maximum of 14 days). Male 1 (07 days) Male 2 (07 days)
07 JUL 98 20 JUL 98	First Possible Day 0 of Presumed Gestation. Last Possible Day 0 of Presumed Gestation.
03 AUG 98	Fo Generation Male Rats Sacrificed after Completion of the Cohabitation Period (Earliest possible date).
17 JUL 98	First Possible Day 10 of Presumed Gestation Caesarean-sectioning.
30 JUL 98	Last Possible Day 10 of Presumed Gestation Caesarean-sectioning.

28 JUL 98	First Possible Delivery (Day 21 of presumed gestation).
14 AUG 98	Last Possible Delivery (Day 25 of presumed gestation).
01 AUG 98	First Possible Day 25 of Presumed Gestation Female Sacrifice.
14 AUG 98	Last Possible Day 25 of Presumed Gestation Female Sacrifice.
17 AUG 98	First Possible Day 21 Weaning (Dams and F1 generation pups not selected for continued observation sacrificed).
03 SEP 98	Last Possible Day 21 Weaning.
18 AUG 98	F1 Generation Postweaning Observations Begin (Details of tests cited in protocol).
02 NOV 98 - 16 NOV 98	F1 Generation Cohabitation Period (Initiated when rats are approximately 90 days of age - approximate initial date).
30 NOV 98	F1 Generation Male Rats Sacrificed after Completion of Cohabitation Period - Approximate Earliest Possible Date.
24 NOV 98 - 11 DEC 98	Delivery Period - F1 Generation Dams/F2 Generation Litters (Approximate dates).
14 DEC 98 - 31 DEC 98	Sacrifice of F1 Generation Dams and F2 Generation Litters on Day 21 Postpartum (Approximate dates).
06 APR 99	Draft Final

Reason for Change:

This schedule was changed due to the extension of the dosing period by two weeks and the cohabitation period occurring two weeks later than scheduled.

25 JUN 98

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

25-JUN 98

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

25 Jun 98

Dena C. Lebo, V.M.D. Date
Member, Institutional Animal Care
and Use Committee

1 July 98

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

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PROTOCOL 418-008

COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS.

SPONSOR'S STUDY NUMBER: 6295.9

Amendment 4 - July 14, 1998

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1. Test Article Preparation Procedure (Version 29 JUN 98 of Attachment 3 to the protocol):

   [Effective Date: June 29, 1998] The volume of the test article solutions prepared each day will be increased. The new Test Article Preparation Procedure is attached to this amendment.

   Reason for Change:

   It is necessary to increase the volume of the test article solutions prepared each day because of the increasing body weights of the rats.

for George E. Dearlove, Ph.D., DABT Date
Associate Director of Research

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

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

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

ATTACHMENT 3

TEST ARTICLE 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 control article for oral administration to rats on Argus Study 418-008.

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.

2. Suspensions will be prepared:
   _X_ Daily ___ 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 solutions adjusted for Free base and % Purity.
   __ Yes _X_ No (Calculations based on 100%)
   ___ Free Base ___ Purity

6. Sampling requirements: Cited in protocol.

7. Storage: Cited in protocol.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

NOTE: Test article will be prepared as a serial dilution from the high dosage to the low dosage. Stir bars are to be added to the containers; mixing should occur during sampling and/or dosage administration.

C. Preparation of the Control Group:

1. Add the required amount of vehicle to an appropriate vessel. (See TEST ARTICLE CALCULATIONS for exact quantities.)

D. Test Article Solution Preparation:

1. To prepare the 0.64-mg/mL, (group V) solution, add 224 mg of test article into an appropriately sized, labeled container.

2. Q. S. to 350 mL with the vehicle.

3. Add a stir bar and heat the preparation to 80 °C in a water bath for approximately 30 minutes (or until the test article has dissolved).

4. Remove the solution from the water bath, and spin while the solution equilibrates to room temperature.

5. To prepare group IV, (the 0.32-mg/mL solution), add 150 mL of group V solution to an appropriately labeled container, then q.s. to 300 mL with vehicle. Add a stir bar and mix until uniform.

6. To prepare group III, (the 0.08-mg/mL solution), add 65 mL of group IV solution to an appropriately labeled container, then q.s. to 260 mL with vehicle. Add a stir bar and mix until uniform.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

7. To prepare group II, (the 0.02-mg/mL solution), add 50 mL of group III solution to an appropriately labeled container, then q.s. to 200 mL with vehicle. Add a stir bar and mix until uniform.

Written by: _____

Approved by: _____ Date: 13-JUL-98

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

Initial/Date : _____ 11-JAN 99

PRIMEDICA

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PROTOCOL 418-008

COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS.

SPONSOR'S STUDY NUMBER: 6295.9

Amendment 5 - July 17, 1998

1. Concentration Analyses (page 5 of the protocol):

   Samples (5 mL each) from each concentration will be taken during the first and last week of the F1 dosage administration to verify concentrations of the prepared test article formulations.

   Reason for Change:

   This change was made because dosage administration was extended to include the F1 generation male and female rats.

2. Sex (page 6 of the protocol):

   The F1 generation male and female pups will be given the test article or vehicle.

   Reason for Change:

   These changes were made at the request of the Sponsor in order to provide information about the effects of the test article on the second generation.

3. Method and Frequency (page 10 of the protocol):

   The F1 generation male and female pups will be administered the test article orally (gavage) on day 1 postweaning through the day before sacrifice. F2 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.

Reason for Change:

These changes were made at the request of the Sponsor and follow the method of exposure to Fo generation.

4. Dosage Levels, Concentrations and Volumes (page 11 of the protocol):

Dosage Group	Number of Fo Generation Rats Per Sex	Number of F1 Generation Rats Per Sex	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Argus Batch Number
I	35	25	0 (Vehicle)	0	5	B-418-008-A(Day.Month.Year)
II	35	25	0.1	0.02	5	B-418-008-B(Day.Month.Year)
III	35	25	0.4	0.08	5	B-418-008-C(Day.Month.Year)
IV	35	25	1.6	0.32	5	B-418-008-D(Day.Month.Year)
V	35	25	3.2	0.64	5	B-418-008-E(Day.Month.Year)

Reason for Change:

This change was made because dosage administration was extended to include the F1 generation male and female rats.

5. Tests, Analyses and Measurements - F1 Generation (page 16 of the protocol):

Clinical Observations and/or General Appearance:

Preweaning Period:	Once daily.
Dosage Period:	Twice daily. Prior to dosage administration and once approximately one hour postdosage.
Maternal Behavior:	Days 1, 4, 7 , 14 and 21 postpartum. Any observed abnormal behavior will be recorded daily

Body Weights - Male:

Preweaning Period:	Days 1 (birth), 4, 7, 14 and 21 postpartum.
Dosage Period:	Weekly.
Sacrifice:	Terminal weight.

Body Weights - Female:

Preweaning Period:	Days 1 (birth), 4, 7, 14 and 21 postpartum.
Dosage Period:	Weekly to cohabitation. Daily during presumed gestation and on Days 1, 4, 7 and 14 postpartum (rats assigned to natural delivery).
Sacrifice:	Terminal weight.

Reason for Change:

These changes were made because dosage administration was extended to include the F1 generation male and female rats.

6. F1/F2 Generation Pups Not Selected for Continued Observation (page 22 of the protocol):

On day 4 postpartum, the stomach contents (milk curd) will be collected from culled pups from Groups I, II and V (or highest dosage group available). Samples will be collected from all pups from four to five of the largest litters in these three dosage groups. Individual pup samples will be combined by litter into polypropylene tubes and frozen at -20°C. After completion of sample collection, samples will be shipped (frozen on dry ice) to Kris J. Hansen, Ph.D. at 3M Environmental Technology and Safety Services, 935 Bush Avenue, Building 2-3E-09,, St. Paul, Minnesota 55133-3331 for analysis. Both the recipient and the Study Monitor will be notified in advance of sample shipment.

Reason for Change:

Collection of milk samples were requested by the Sponsor to determine if the test article is reaching the pups via lactation.

for George E. Dearlove, Ph.D., DABT Date
Associate Director of Research

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

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

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

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PROTOCOL 418-008

COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: 6295.9

Amendment 6 - 13 August 1998

1. Necropsy (page 20 of the protocol):

   On postpartum day 21, the 1.6 mg/kg/day dosage group (Group IV) dams and litters will be sacrificed.

   Reason for Change:

   Group IV was terminated at weaning because of the severe pup toxicity during lactation (mortality and reduced body weights and delayed development).

2. Male and Female Rats Assigned to Pharmacokinetic Sample Collection (page 14 of the protocol):

   On postpartum Day 21, the livers of the pups from five litters in each of the remaining groups will be excised, pooled per litter, frozen and retained at -70°C until shipment to the Sponsor.

   Reason for Change:

   This change was made at the request of the Sponsor for possible analysis.

3. Scheduled Sacrifice - Female Rats Assigned to Natural Delivery and Dams with No Surviving Pups (page 15 of the protocol):

   Ovaries will be retained in neutral buffered 10% formalin.

Reason for Change:

This change was made to clarify the protocol.

George E. Dearlove 13-AUG-98
George E. Dearlove, Ph.D., DABT Date
Associate Director of Research

Raymond G. York 13-AUG-98
Raymond G. York, Ph.D., DABT Date
Associate Director of Research
Study Director

Barbara G. Patterson 13-Aug-1998
for Barbara J. Patterson, B.A. Date
Chairperson, Institutional Animal Care
and Use Committee

Marvin T Case 27 Aug 98
Marvin T. Case, D.V.M., Ph.D. Date
Study Monitor

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COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: 6295.9

Amendment 7 - 24 November 1998

1. Natural Delivery (page 13 of the protocol):

The Length of Parturition (time of delivery of last pup minus the time of delivery of the first pup divided by N-1 pups in each litter) will not be calculated.

Reason for Change:

A litter watch was not required by the Sponsor.

George E. Dearlove, Ph.D., DABT Date
Associate Director of Research

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

Dena C. Lebo, V.M.D. Date
Chairperson, Institutional Animal Care and Use Committee

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

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PROTOCOL 418-008

COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS

SPONSOR'S STUDY NUMBER: 6295.9

Amendment 8 - 18 May 1999

1. Concentration Analyses (page 5 of the protocol):

   The concentration samples were sent to the Sponsor. Sample analyses will be conducted at the discretion of the Sponsor and no report will be sent to the Testing Facility.

   Reason for Change:

   This change was made at the request of the Sponsor to clarify the protocol.

2. Male and Female Rats Assigned to Pharmacokinetic Sample Collection (page 14 of the protocol):

   The liver and serum samples were sent to the Sponsor. Samples will be analyzed at the discretion of the Sponsor.

   Reason for Change:

   This change was made at the request of the Sponsor to clarify the protocol.

3. F1/F2 Generation Pups Not Selected for Continued Observation (page 22 and Amendment 5 of the protocol):

   The stomach contents of these pups were sent to the Sponsor for analysis. Stomach contents will be analyzed at the discretion of the Sponsor.

Reason for Change:

This change was made at the request of the Sponsor to clarify the protocol.

George E. Dearlove 18 MAY 99

George E. Dearlove, Ph.D., DABT Date
Associate Director of Research

Raymond G. York 18-MAY-99

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

Theresa A. Worland 18 MAY 99
for

Dena C. Lebo, V.M.D. Date
Chairperson, Institutional Animal Care
and Use Committee

Marvin T Case 19 May 99

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

APPENDIX G

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

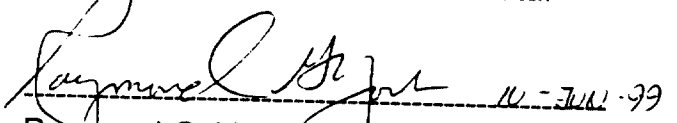
DEVIATIONS FROM THE PROTOCOL AND THE STANDARD OPERATING PROCEDURES OF THE TESTING FACILITY

1. On 8 August 1998, the 5 mL concentration sample was not taken during the first week of dosage administration for the F1 generation rats. This deviation did not adversely affect the outcome or interpretation of the study because sufficient data were collected to accurately evaluate this parameter.

2. On 3 to 8 June 1998 [study days (DSs) 9 to 14], 10 to 15 June 1998 (DSs 16 to 21), 17 June 1998 (DS 23) and 18 June 1998 (DS 24), all Fo generation male and female rats were dosed with the dosage volumes based on body weights recorded on DS 1 (26 May 1998), rather than on weekly body weights. As a result of this error, male rats were administered approximately 89% to 90% of the targeted dosages and female rats were administered approximately 95% to 99% of the targeted dosages. This deviation did not adversely affect the outcome or interpretation of the study because the precohabitation dosage period was extended for an additional two weeks to ensure that all rats were administered the correct dosages before cohabitation.

3. On 21 August 1998 (postweaning day 1), F1 generation male rats 13158 and 13159 in the 0.4 mg/kg/day dosage group were neither weighed nor dosed. The dosage volume for the rest of the week was based on the postweaning day 2 body weight. This deviation did not adversely affect the outcome or interpretation of the study because a sufficient number of postweaning day 1 body weights were collected to evaluate this parameter, and the dosage volumes were based on the day 2 postweaning weight were presumed to be very similar to dosage volumes based on the day 1 postweaning weight.

All deviations are documented in the raw data.



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

APPENDIX H

TEMPERATURE AND RELATIVE HUMIDITY REPORTS

ARGUS

Temperature and Relative Humidity Report
Location: Room 17

Protocol Number: 418-008

Range of Dates: 12-May-1998 12:00 to 12-Jun-1998 09:46

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: Rat				
Total Number of Days:	32		32	
Total Number of Hours:	741.5		741.5	
Total Number of Data Points:	742		742	
Mean (± SD):	69.6	(± 1.1)	46.2	(± 3.9)
Maximum:	71.9		61.2	
Median:	69.7		46.5	
Minimum:	66.4		37.9	
Number of Points in Range (%):	742	(100.0)	742	(100.0)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 04-Sep-1998 at 13:18

COMMENTS: _____

_____

_____

_____

REVIEWED BY: _____ **DATE:** 9/4/98

729

GOO744

ARGUS

Temperature and Relative Humidity Report
Location: Room 03

Protocol Number: 418-008

Range of Dates: 12-Jun-1998 09:46 to 21-Sep-1998 13:40

	Temperature 64°F to 79°F		Relative Humidity 30% to 70%	
Target Range:				
Species: Rat				
Total Number of Days:	102		102	
Total Number of Hours:	2427.74		2427.74	
Total Number of Data Points:	2423		2423	
Mean (± SD):	69.0	(± 2.0)	61.6	(± 4.8)
Maximum:	75.3		72.3	
Median:	69.1		63.1	
Minimum:	62.0		45.7	
Number of Points in Range (%):	2405	(99.3)	2404	(99.2)
Number of Points High (%):	0	(0.0)	19	(0.8)
Number of Points Low (%):	18	(0.7)	0	(0.0)

Report Generated: 31-Mar-1999 at 09:02

COMMENTS: _____

_____

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REVIEWED BY: _____ **DATE:** 3-31-99

730

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ARGUS

Temperature and Relative Humidity Report
Location: Room 05

Protocol Number: 418-008

Range of Dates: 27-Jul-1998 12:50 to 28-Dec-1998 08:29

	Temperature 64°F to 79°F		Relative Humidity 30% to 70%	
Target Range:				
Species: Rat				
Total Number of Days:	155		155	
Total Number of Hours:	3691.26		3691.26	
Total Number of Data Points:	3677		3677	
Mean (± SD):	69.3	(± 1.8)	56.3	(± 6.7)
Maximum:	82.7		71.3	
Median:	69.7		58.1	
Minimum:	64.6		34.3	
Number of Points in Range (%):	3661	(99.6)	3667	(99.7)
Number of Points High (%):	16	(0.4)	10	(0.3)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 31-Mar-1999 at 09:22

COMMENTS: _____

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REVIEWED BY: _____ **DATE:** 3-31-99

731

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ARGUS

Temperature and Relative Humidity Report
Location: Room 35-37

Protocol Number: 418-008

Range of Dates: 21-Sep-1998 13:40 to 28-Dec-1998 08:29

	Temperature 64°F to 79°F		Relative Humidity 30% to 70%	
Target Range:				
Species: Rat				
Total Number of Days:	99		99	
Total Number of Hours:	2346.52		2346.52	
Total Number of Data Points:	2336		2336	
Mean (± SD):	69.7	(± 0.7)	52.5	(± 4.0)
Maximum:	73.0		69.5	
Median:	69.7		51.7	
Minimum:	67.8		23.6	
Number of Points in Range (%):	2336	(100.0)	2332	(99.8)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	4	(0.2)

Report Generated: 31-Mar-1999 at 09:52

COMMENTS: _____

REVIEWED BY: John T. Barnett Jr **DATE:** 3/31/99

732

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ARGUS

Temperature Deviations Report
Location: Room 03

Protocol Number: 418-008

Range of Dates: 12-Jun-1998 09:46 to 21-Sep-1998 13:40

Temperature Target Range: 64°F to 79°F
Species: Rat

Date	Time	Temp.
17-Jun-1998	03:00	63.9 L
17-Jun-1998	04:00	63.5 L
17-Jun-1998	05:00	63.2 L
17-Jun-1998	06:00	62.9 L
17-Jun-1998	07:00	62.7 L
28-Jun-1998	06:00	63.9 L
28-Jun-1998	07:00	63.7 L
08-Jul-1998	21:00	63.6 L
08-Jul-1998	22:00	63.2 L
08-Jul-1998	23:00	62.8 L
09-Jul-1998	00:00	62.6 L
09-Jul-1998	01:00	62.5 L
09-Jul-1998	02:00	62.3 L
09-Jul-1998	03:00	62.2 L
09-Jul-1998	04:00	62.1 L
09-Jul-1998	05:00	62.0 L
09-Jul-1998	06:00	62.0 L
09-Jul-1998	07:00	62.4 L

H = Value out of range - High L = Value out of range - Low
Temp. = Temperature °F

Report Generated: 31-Mar-1999 at 09:09

✓ 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: 31-Mar-99

733

ARGUS

Relative Humidity Deviations Report
Location: Room 03

Protocol Number: 418-008

Range of Dates: 12-Jun-1998 09:46 to 21-Sep-1998 13:40

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

Date	Time	R.H.	Date	Time	R.H.
26-Jun-1998	11:00	70.7 H			
26-Jun-1998	12:00	71.0 H			
09-Jul-1998	08:00	70.1 H			
09-Jul-1998	11:00	70.2 H			
09-Jul-1998	12:00	70.6 H			
21-Jul-1998	12:00	70.2 H			
24-Jul-1998	10:00	70.1 H			
16-Aug-1998	17:00	72.0 H			
16-Aug-1998	19:00	71.0 H			
18-Aug-1998	01:00	70.2 H			
24-Aug-1998	15:00	70.3 H			
25-Aug-1998	17:00	70.6 H			
27-Aug-1998	15:00	72.3 H			
30-Aug-1998	13:00	70.6 H			
31-Aug-1998	11:00	71.2 H			
15-Sep-1998	21:00	70.2 H			
16-Sep-1998	14:00	72.1 H			

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

Report Generated: 31-Mar-1999 at 09:15

✓ 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:

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Study Director: [signature] Date: 31-MAR-99

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ARGUS

Temperature Deviations Report
Location: Room 05

Protocol Number: 418-008

Range of Dates: 27-Jul-1998 12:50 to 28-Dec-1998 08:29

Temperature Target Range: 64°F to 79°F
Species: Rat

Date	Time	Temp.	Date	Time	Temp.
19-Sep-1998	16:00	80.8 H			
19-Sep-1998	17:00	81.7 H			
19-Sep-1998	18:00	82.4 H			
19-Sep-1998	19:00	82.7 H			
19-Sep-1998	20:00	82.4 H			
19-Sep-1998	21:00	82.1 H			
19-Sep-1998	22:00	81.9 H			
19-Sep-1998	23:00	81.8 H			
20-Sep-1998	00:00	81.6 H			
20-Sep-1998	01:00	81.5 H			
20-Sep-1998	02:00	81.3 H			
20-Sep-1998	03:00	81.2 H			
20-Sep-1998	04:00	81.1 H			
20-Sep-1998	05:00	81.0 H			
20-Sep-1998	06:00	81.1 H			
20-Sep-1998	07:00	81.1 H			

H = Value out of range - High L = Value out of range - Low
Temp. = Temperature °F

Report Generated: 31-Mar-1999 at 09:36

✓ 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: 31-MAR-99

735

ARGUS

Relative Humidity Deviations Report
Location: Room 05

Protocol Number: 418-008

Range of Dates: 27-Jul-1998 12:50 to 28-Dec-1998 08:29

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

Date	Time	R.H.	Date	Time	R.H.
26-Sep-1998	14:00	70.7 H			
26-Sep-1998	16:00	70.1 H			
26-Sep-1998	19:00	70.1 H			
30-Sep-1998	17:00	70.1 H			
09-Oct-1998	02:00	70.5 H			
14-Oct-1998	07:00	70.4 H			
14-Oct-1998	11:00	70.1 H			
03-Nov-1998	16:00	70.3 H			
07-Dec-1998	08:00	71.3 H			

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

Report Generated: 31-Mar-1999 at 09:45

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

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

Study Director: _____ Date: 31-MAR-99

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ARGUS

Relative Humidity Deviations Report
Location: Room 35-37

Protocol Number: 418-008

Range of Dates: 21-Sep-1998 13:40 to 28-Dec-1998 08:29

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

Date	Time	R.H.	Date	Time	R.H.
23-Sep-1998	10:00	29.3 L			
23-Sep-1998	11:00	27.3 L			
23-Sep-1998	12:00	28.2 L			
23-Sep-1998	13:00	23.6 L			

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

Report Generated: 31-Mar-1999 at 10:12

✓ 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: Raymond Hagan Date: 31-MAR-99

737

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APPENDIX I

STATEMENT OF THE STUDY DIRECTOR

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044
T: (215) 443-8710 F: (215) 443-8587

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

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, Ph.D., DABT Date
Associate Director of Research
and Study Director
Argus Research Laboratories, Inc.

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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 (1997). *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 J

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 418-008: Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats
Sponsor's Study Number: 6295.9

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 13 MAY 98.

Critical phases of this study were inspected 24 times; study information and raw data were audited six times (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 05 DEC 98 and 29 APR 99, and for revisions requested by the Sponsor 18 MAY 99, 7 JUN 99, 9 JUN 99, and for finalization on 10 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 10Jun99 Heather L. Rabuttino 10 JUN 99

Nancy J. Gongliewski Date
Quality Assurance Manager

Heather L. Rabuttino, M.S. Date
Quality Assurance Supervisor
and Principal Auditor

TABLE 1

CRITICAL PHASES INSPECTED

Test Article Administration - Gavage

Dates of inspection: 28 MAY 98, 02 JUN 98, 03 SEP 98

Dates results reported to the Study Director and Management:
15 JUN 98, 15 JUN 98, 18 SEP 98

Estrous Cycle Evaluation

Date of inspection: 11 JUN 98

Date results reported to the Study Director and Management: 11 JUN 98

Cohabitation

Dates of inspection: 08 JUL 98, 04 NOV 98

Dates results reported to the Study Director and Management:
14 JUL 98, 12 NOV 98

Scheduled Sacrifice - Day 10

Date of inspection: 17 JUL 98

Date results reported to the Study Director and Management: 03 AUG 98

Test Article Preparation

Dates of inspection: 29 JUL 98, 16 SEP 98

Dates results reported to the Study Director and Management:
14 AUG 98, 24 SEP 98

Natural Delivery

Dates of inspection: 31 JUL 98, 25 NOV 98

Dates results reported to the Study Director and Management:
14 AUG 98, 02 DEC 98

Blood Collection

Dates of inspection: 31 JUL 98, 18 AUG 98

Dates results reported to the Study Director and Management:
14 AUG 98, 21 AUG 98

Physical and Reflex Development - Surface Righting, Pinna Unfolding, Eye Opening, Auditory Startle, Air Righting, Pupil Constriction, Sexual Maturation

Dates of inspection: 31 JUL 98, 13 AUG 98, 18 AUG 98, 28 AUG 98, 11 SEP 98

Dates results reported to the Study Director and Management:
14 AUG 98, 03 SEP 98, 08 SEP 98, 11 SEP 98, 12 SEP 98

Male Necropsy

Date of inspection: 31 JUL 98

Date results reported to the Study Director and Management: 14 AUG 98

Necropsy - Dam and Litter Sacrifice

Dates of inspection: 18 AUG 98, 15 DEC 98

Dates results reported to the Study Director and Management:
21 AUG 98, 18 DEC 98

Day 21 Weaning

Date of inspection: 18 AUG 98

Date results reported to the Study Director and Management: 08 SEP 98

Behavioral Testing - Passive Avoidance, Watermaze,

Dates of inspection: 25 AUG 98, 08 OCT 98

Dates results reported to the Study Director and Management:
12 SEP 98, 12 OCT 98

TABLE 2

RAW DATA AUDIT(S)

The following study information and raw data were audited on 29 SEP 98, 01 OCT 98, 04 OCT 98 TO 05 OCT 98:

Protocol.
Protocol amendments.
List of personnel and computer operator codes.
Error codes and codes for clinical sigh observations.
Animal receipt, randomization, physical examination and acclimation.
In-life transaction record.
Feed consumption.
Necropsy.
Organ weights.
Tissue packing lists.
Edit requests.
Deviations.
Data review page/pages.
Blood collection data and packing lists.
Key for testing facility computer backup record abbreviations.

The results of this audit were reported to the Study Director and Management on 06 OCT 98.

The following study information and raw data were audited on 06 OCT 98, 12 OCT 98, 02 NOV 98 TO 06 NOV 98:

Animal receipt, randomization, physical examination and acclimation.
In-life transaction record.
Feed consumption.
Estrus cycle evaluation.
Cohabitation.
Caesarean-sectioning.
Maternal gross observations
Natural delivery observations.
Litter observations.
Pup body weights and status.
Table of random units.
Necropsy.
Tissue packing lists.
General comments.
Reflex and physical development.
Study maintenance records.
Temperature and relative humidity reports.
Feed, water and bedding analyses.
Edit requests.
Data review page.
Blood collection data and packing lists.
Pup stomach contents.

The results of this audit were reported to the Study Director and Management on 08 NOV 98.

The following study information and raw data were audited on 08 NOV 98 to 09 NOV 98:

Vehicle receipt, preparation and use.
Test article receipt, preparation and use.
Test article packing lists.

The results of this audit were reported to the Study Director and Management on 12 NOV 98.

The following study information and raw data were audited on 26 JAN 99 to 28 JAN 99:

In-life transaction record.
Feed consumption.
Passive avoidance.
Watermaze.
Genealogy chart.
Necropsy.
Organ weights.
Tissue packing lists.
Sexual Maturation.
Edit request.

The results of this audit were reported to the Study Director and Management on 28 JAN 99.

The following study information and raw data were audited on 28 JAN 99 to 01 FEB 99:

In-life transaction record.
Feed consumption.
Cohabitation.
Natural delivery observations.
Litter observations.
Pup body weights and status.
Passive avoidance.
Watermaze.
Table of random units.
Necropsy.
Tissue packing lists.
General comments.
Sexual maturation.
Study maintenance records.
Temperature and relative humidity reports.
Feed, water and bedding analyses.
Edit requests.
Dosage volumes.
Data review pages.

The results of this audit were reported to the Study Director and Management on 01 FEB 99.

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

Vehicle receipt, preparation and use.
Test article receipt, preparation and use.
Test article packing lists.

The results of this audit were reported to the Study Director and Management on 28 JAN 99.