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Draft Report

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

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VOLUME
I of II

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

UNAUDITED DRAFT
April 12, 2000

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

Author:

Peter J. Thomford, PhD

Study Completion Date:

To be determined

Testing Facility:

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Madison, Wisconsin 53704-2595

Laboratory Study Identification:

Covance 6329-223

Sponsor Study Identification:

3M Study No. T-6295.7

Volume I of II

COMPLIANCE STATEMENT

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

All aspects of this study were in accordance with the Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 792.

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

Date

Andrew M. Seacat, PhD
Study Monitor
3M

Date

STUDY IDENTIFICATION

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

Test Material	Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295)
Sponsor	3M Toxicology Services Building 220-2E-02, 3M Center St. Paul, Minnesota 55144-1000
Study Monitor	Andrew M. Seacat, PhD 3M 651.575.3161
Alternate Study Monitor	Marvin T. Case, DVM, PhD 3M Toxicology Services 651.733.5180
Study Location	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704-2595
Study Director	Peter J. Thomford, PhD Covance Laboratories Inc. PO Box 7545 Madison, Wisconsin 53707-7545 608.241.7207
Study Timetable	
Study Initiation Date	August 20, 1998
In-Life (Experimental) Start Date	August 26, 1998
In-Life Termination Date	March 7, 2000
Experimental Termination Date	To be determined

KEY PERSONNEL

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

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ABSTRACT

The purpose of this study was to assess the effect of the test material, Perfluorooctane Sulfonic Acid Potassium Salt [PFOS; T-6295 (hereafter referred to as PFOS)] on critical enzyme levels, hormones, and other selected biochemical parameters when administered daily by oral capsule to cynomolgus monkeys for at least 26 weeks. The treatment period was followed by an approximate 52-week recovery period.

Male and female cynomolgus monkeys were assigned to four groups (six animals/sex in Groups 1, 3, and 4; four animals/sex in Group 2). Each group received dose preparations containing the vehicle, lactose, or 0.03, 0.15, or 0.75 mg of PFOS/kg of body weight/day (mg/kg/day). Two animals/group in Groups 1, 3, and 4 were in a recovery period and were not treated for at least 52 weeks following the 26 week treatment period.

Food was provided *ad libitum*, except when animals were fasted. Water was provided *ad libitum*. The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. At least once daily, animals were examined for abnormalities and signs of toxicity, and food consumption was assessed qualitatively. Ophthalmic examinations were done before initiation of treatment and during Weeks 26 and 52. Body weight data were recorded weekly before initiation of treatment, on Days -1 and 1, and weekly thereafter. Blood and urine samples were collected for clinical hematology, clinical chemistry, and urinalysis tests before initiation of treatment and at specified intervals during treatment and recovery. Blood was also collected for blood hormone and PFOS level determinations before, during, and after treatment at specified intervals. Feces and liver samples were also collected at specified intervals. On Day 155 (Week 23), one male given 0.75 mg/kg/day died, and on Day 179 (Week 26), one male given 0.75 mg/kg/day was sacrificed due to poor health. On Days 184 and 185 (Week 27), four animals/sex/group (Groups 1 through 3) and four females and two males (Group 4) were anesthetized, weighed, exsanguinated, and necropsied. At necropsy at the scheduled and unscheduled sacrifices, a serum sample was collected, macroscopic observations were recorded, selected organs were weighed, and selected tissues were collected and preserved. Microscopic examinations were done on tissues from each animal in the control and high-dose groups and selected tissues from animals in the low- and mid-dose groups. Tissues were also collected for palmitoyl CoA oxidase determination, cell proliferation evaluation, PFOS determination, and electron microscopy. Additionally, the bile was collected from the gallbladder, and the gallbladder was preserved. At the recovery sacrifice on Day 549, the remaining Group 4

animals were anesthetized, weighed, exsanguinated, and necropsied. Macroscopic observations were recorded and specified tissues and serum were collected. Remaining animals in Groups 1 and 3 were donated or transferred to a follow-up study, Covance 6329-268.

At all dose levels, clinical observations, ophthalmic observations, and palmitoyl CoA oxidase determinations do not appear to be affected by treatment with PFOS.

Two males given 0.75 mg/kg/day died during treatment. These deaths were preceded by some adverse clinical observations (constricted pupil, pale gums, abnormal feces, excessive salivation, labored respiration, dehydrated appearance, hypoactive, ataxic, recumbent, low food consumption) and appeared to be related to the administration of PFOS. When compared with animals given the control material, covariate adjusted mean body weights (CAM) for males given 0.75 mg/kg/day were slightly lower beginning at Week 21, and for females given 0.75 mg/kg/day CAM body weights were, in general, significantly lower beginning at Week 11. Similar decreases were not seen in the other treated groups; therefore, this finding is likely test material-related. Test material-related effects on body weights were not apparent during recovery. Low food consumption was noted sporadically for animals in the groups given the control material and 0.03 mg/kg/day throughout treatment. The incidence of low food consumption was generally higher in the groups given 0.15 or 0.75 mg/kg/day as compared to animals given the control material and appeared to be test material-related. During recovery, effects on food consumption were reversed.

Estradiol values were generally lower on Days 62, 91, and 182 in males given 0.75 mg/kg/day, although because of the variation in the data only the Day 182 value was significant. Estrone values were generally higher in all of the treated females on Days 37, 62, and 91, although because of the variation in the data none of these values were significantly different, and this difference was not apparent on Day 182. Triiodothyronine values were notably lower in both males and females given 0.15 and 0.75 mg/kg/day on Days 91 and 182. With the single exception of males given 0.15 mg/kg/day on Day 91, all values were significantly lower. During recovery were occasional instances in which the hormone values in treated groups differed slightly from those of controls, but those differences were not consistent over time or between sexes, were not clearly dose-related, and did not appear to be clearly related to the administration of the test material. Apparent differences in the sexual maturity of both males and females used in this study complicates the interpretation of the hormone data.

The only clinical pathology parameters considered related to the test material were lower total cholesterol for animals given 0.75 mg/kg/day and lower high density lipoprotein cholesterol for animals given 0.15 or 0.75 mg/kg/day. These effects were reversed within the first 5 and 9 weeks of recovery, respectively.

At the terminal sacrifice, increased liver weights, macroscopic observations of mottled liver, hepatocellular hypertrophy, and hepatocellular vacuolation in animals given 0.75 mg/kg/day were considered related to PFOS treatment. However, the microscopic examination liver biopsies taken during recovery did not indicate any test material-related findings and none of the macroscopic observations made at the recovery sacrifice were considered test material-related. There were no microscopic findings in the liver from the animals in the high-dose recovery group. This indicates that the hepatic test material-related effects were reversible.

Treatment with PFOS by oral capsule for at least 26 weeks is generally well-tolerated in male and female cynomolgus monkeys at doses up to 0.15 mg/kg/day. Clinical and pathological findings considered to be associated with the treatment of PFOS after at least 26 weeks of treatment were found to be reversible during a 52-week recovery period. Based on the data presented in this report, the no-observable-effect level is 0.03 mg/kg/day. Dose analyses (provided by the Sponsor) and electron microscopy results (provided by PAI) are forthcoming.

PURPOSE

The purpose of this study was to assess the effect of the test material on critical enzyme levels, hormones, and other selected biochemical parameters when administered daily by capsule to cynomolgus monkeys for at least 26 weeks. The treatment period was followed by an approximate 52-week recovery period.

REGULATORY COMPLIANCE

All aspects of this study were done in accordance with the Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 792.

TEST MATERIAL, VEHICLE, AND SOLVENT

Test Material

The test material, Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295), Lot No. 217, is a white to off-white powder. It was received at Covance on September 4, 1997. The test material was stored at room temperature.

Information on synthesis methods, stability, purity, composition, or other characteristics that define the test material is on file with the Sponsor.

Vehicle

The vehicle was lactose (Spectrum, New Brunswick, New Jersey), Lot No. NN0192 (expiration date February 13, 1999). It was received at Covance on March 30, 1998.

The vehicle was stored at room temperature.

Information on synthesis methods, purity, stability, composition, or other characteristics that define the vehicle is on file with the manufacturer.

Solvent

The solvent was acetone (Spectrum, Gardena, California), Lot No. LH0253, (expiration date June 2000). It was received at Covance on June 23, 1997. The solvent was stored at room temperature.

Information on synthesis methods, composition, or other characteristics that define the solvent is on file with the manufacturer.

Gelatin Capsules

Gelatin capsules, Size Nos. 2 (Lot No. 122932, expiration date June 12, 2003) and 4 (Lot No. 544043, expiration date August 1, 2002) were manufactured by Torpac Inc., (Fairfield, New Jersey). Lot No. 122932 was received at Covance on June 12, 1998, and Lot No. 544043 was received on September 1 and November 11, 1998. The capsules were stored at room temperature. A copy of the Certificate of Analysis provided by the manufacturer is maintained in the data.

Reserve (Archive) Samples

A reserve sample (1 g) of each lot of the test material, vehicle, and each test material/lactose dilution was taken and stored at room temperature. These samples were transferred to the Sponsor after completion of the treatment phase (see Protocol Deviations).

Disposition

Remaining test material will be retained at Covance for use in possible future studies.

TEST ANIMALS AND HUSBANDRY

Animals

Young adult to adult cynomolgus monkeys were obtained from Covance Research Products Inc. (Denver, Pennsylvania) on June 30, 1998. The animals weighed 2.4 to 4.4 kg at initiation of treatment.

Identification
Each animal was assigned a permanent number upon arrival and identified with a collar tag before initiation of treatment. All data for an animal are recorded under this number.

Justification
PFOS is a known hepatic peroxisome proliferator (PP) in the rat. When exposed to a PP, nonhuman primates (such as the cynomolgus monkey) respond similarly to humans (i.e., low to no hepatic response) and therefore are an appropriate human surrogate species.

Husbandry
Animal Rooms 251 and 259 were used for this study. Environmental controls for the animal rooms were set to maintain 18 to 29°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. Variations from these conditions are documented in the data and are considered to have had no effect on the outcome of the study.

The animals were housed individually in suspended, stainless-steel cages.

Certified primate diet (#8726C, Harlan Teklad) was provided once or twice daily, unless otherwise specified. The lot numbers are recorded in the data. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file with Covance-Madison. Fruits or additional supplements were provided, but did not require analysis.

Water was provided *ad libitum*. Samples of the water are analyzed for specified microorganisms and environmental contaminants. The results are on file with Covance-Madison.

There were no known contaminants in the diet or water at levels that would have interfered with this study.

Acclimation

Twenty-four males and 24 females were received on June 30, 1998, and acclimated in Animal Room 251 for 57 days before initiation of treatment. In general, animals in this shipment appeared healthy. During acclimation, the animals were examined for abnormalities indicative of health problems. In addition, three tuberculosis tests, a physical examination, and a fecal flotation for parasites were performed on each animal.

PROCEDURES

This study was conducted in accordance with the Protocol dated August 20, 1998, and Protocol Amendment Nos. 1, 2, and 3. The protocol, protocol amendments, and protocol deviations are in Appendix 1.

Group Designations and Dosage Levels

Selection of animals for the study was based on data collected during acclimation. Animals were assigned to treatment groups using a computerized blocking procedure designed to achieve body weight balance with respect to treatment group.

Group	Dose Level (mg/kg/day)[a]	Total Material Dose Level (mg/kg/day)[b]	Number of Animals	
			Males	Females
1	0[a]	30[a]	6[d]	6[d]
2	0.03	15[b]	4	4
3	0.15	6[c]	6[d]	6[d]
4	0.75	30[c]	6[d]	6[d]

a The control group (Group 1) received the equivalent amount of lactose in gelatin capsules as the total material administered to Group 4.
b The low-dose (Group 2) received the test material diluted with lactose (1:499, w:w).
c The mid-dose (Group 3) and high-dose (Group 4) groups received the test material diluted with lactose (1:39, w:w).
d Two animals in Groups 1, 3, and 4 designated as recovery animals were treated for at least 26 weeks, then treatment was discontinued, and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 52 weeks posttreatment.

Dosing Procedures

Vehicle. Dose levels were based on the vehicle as supplied for Group 1. For Group 1 dose preparations, the specified amount of lactose was weighed, transferred into gelatin capsules, and the top and bottom halves of each capsule were joined. Capsules were prepared at least once weekly.

Test Material. The test material/lactose preparations for Groups 2 through 4 were diluted once before initiation of treatment; capsules were prepared at least once weekly.

A specified amount of test material was weighed, placed into a labeled mixing container, and the appropriate volume of acetone was added. After stirring manually until the test material was dissolved, the required amount of lactose was weighed and transferred to the container. The components were mixed thoroughly using a spatula. The prepared test material dilution was stirred periodically while allowed to stand exposed to the air until the acetone had evaporated. Preparations were diluted to facilitate capsule preparation.

Samples of the finished mixture for dose analyses were taken directly from the container.

The dose preparations were stored at room temperature between capsule preparations. The appropriate amount of prepared test material was weighed and transferred into Size 2 (Days 1 through 8) or 4 (Days 9 through 184) gelatin capsules and the top and bottom halves of each capsule were joined. Size 4 capsules were used instead of Size 2 to better facilitate dose administration. Individual daily doses were based on the most recently recorded body weight, with the exception of doses given on days when body weight measurements were performed; on those days, the previous body weight was used.

All capsule preparations were stored at room temperature until used for dosing.

Method of Administration. Gelatin capsules were used to facilitate comparison with data from previous toxicology studies that used the oral route. Also, oral is the most likely route of exposure in humans. Partial or intact capsules were noted in the vomitus of several animals on occasion; however, this is not considered to have adversely affected the results of the study.

The dose preparations were administered orally in gelatin capsules once daily 7 days/week for at least 26 weeks (see Protocol Deviations for exceptions).

Dose Analyses

Homogeneity and stability analyses were the responsibility of the Sponsor.

Samples (approximately 1 g each) were taken from the top, middle, and bottom of the test material/lactose preparations on Day -15 for homogeneity analysis. Samples collected from the middle of the preparations were also designated for prestudy stability analysis. A set of samples (approximately 1 g each) were taken from the low- and high-dose test material/lactose preparations at the end of the treatment phase for test material content analysis.

All samples were stored at room temperature until sent under ambient conditions to the Sponsor for analysis. Results of dose analyses will be provided for inclusion in the final report.

Observation of Animals

Clinical Observations. The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. Animals were also observed at least once daily (a.m.) for signs of poor health or abnormal behavior, and food consumption was assessed qualitatively; only abnormal findings were recorded. Once weekly and on the day of scheduled sacrifice, each animal was observed; abnormal findings or an indication of normal was recorded (see Protocol Deviations for exceptions). Additionally, postdose observations were recorded during treatment approximately 30 to 90 minutes after the last dose administration; only abnormal findings were recorded.

Ophthalmology. Ophthalmic examinations were done on each animal before initiation of treatment, before the scheduled terminal sacrifice during Week 26, and during Week 52 (see Protocol Deviations). The pupils were dilated with 1% Mydriacyl® and the anterior portion of the eye, optic media, and ocular fundus were examined with an indirect ophthalmoscope by a board-certified ophthalmologist.

Body Weights. Individual body weight data were recorded weekly before initiation of treatment, on Day -1, on the first day of treatment, and weekly thereafter. Body weights were also recorded for animals sacrificed at unscheduled intervals.

Clinical Pathology

Blood and urine samples were collected from each animal once before initiation of treatment (Day -27); on Days 37, 62, 91, 153, and 182 of treatment; and on Days 245, 271, 274, 322, 364, 456, and 546 during recovery (see Protocol Deviations). Animals were fasted overnight, and urine was collected overnight on wet ice before blood sampling; water was provided *ad libitum*. Blood was collected from the femoral vein. Potassium EDTA was the anticoagulant used for hematology tests; no anticoagulant was used for the chemistry tests. Blood samples were collected from the animal that was sacrificed at an unscheduled interval. Animals were bled in sequential order on Days 37, 62, and 91 and in random order at all other scheduled collections; this is not expected to have an impact on the clinical pathology results. The following were evaluated (see Protocol Deviations for exceptions).

Hematology

- red blood cell (erythrocyte) count
- hemoglobin
- hematocrit
- mean corpuscular volume
- mean corpuscular hemoglobin
- mean corpuscular hemoglobin concentration
- platelet count
- white blood cell (leukocyte) count
- differential blood cell count
  - segmented neutrophil count
  - lymphocyte count
  - monocyte count
  - eosinophil count
  - basophil count
- blood cell morphology
- reticulocyte count

Clinical Chemistry

- glucose
- urea nitrogen
- creatinine
- total protein
- albumin
- globulin
- total bilirubin
- cholesterol
- triglycerides
- alanine aminotransferase
- alkaline phosphatase
- aspartate aminotransferase
- gamma glutamyltransferase
- sorbitol dehydrogenase
- creatine kinase
- calcium
- inorganic phosphorus
- sodium
- potassium
- chloride
- bile acids
- amylase
- lipase
- pancreatic-specific amylase
- high density lipoprotein (HDL) (effective with collection on Day 153)

Urinalysis

volume (approximately 16 hours)	bilirubin
specific gravity	blood
pH	urobilinogen
protein	microscopic examination of sediment
glucose	appearance
ketones	

Blood Hormone Determination

Blood samples (approximately 5 mL) were collected from each animal three times before initiation of treatment (Days -50, -40, and -27); on Days 37, 62, 91, and 182 of treatment; and on Days 217, 245, 274, 322, 364, 458, and 549 during recovery. Animals were fasted overnight. Blood was collected from a femoral vein without using an anticoagulant. Samples were allowed to clot and centrifuged within 1 hour after collection; serum was harvested. The serum was divided into two approximately equal aliquots and stored in a freezer, set to maintain -60 to-80°C, until packed on dry ice and shipped to Ani Lytics Inc. for analysis of cortisol, testosterone, estradiol, estrone, estriol, thyroid stimulating hormone, total triiodothyronine, and total thyroxin. Beginning with the collection on Day 322 the samples were also analyzed for free triiodothyronine and free thyroxin.

Serum PFOS Level Determination

Blood samples (approximately 2 mL) were collected from each animal once before initiation of treatment (Day -27); during Weeks 1 (Day 7), 2, 4, 6, 8, 12, 16, 20, 24, and 26, and 27 (Day 183) of treatment; and during Weeks 27 (Days 184, 185, and 187), 28 (Day 190), 29 (Day 198), 30 (Day 204), 31 (Day 211), 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, and 79 (see Protocol Deviations). Animals were fasted overnight and water was provided *ad libitum*. Blood was collected from a femoral vein without using an anticoagulant. Samples were centrifuged within 1 hour after collection and serum was harvested. Serum samples were stored in a freezer, set to maintain -60 to-80°C, until packed on dry ice and shipped to the Sponsor for analysis. Results will reported separately.

Additional Serum Collection

At the scheduled terminal necropsy and the necropsy of Animal No. I05506 (Group 4 male), blood samples (approximately 20 mL) were collected from the vena cava at the time of exsanguination. Samples were collected without using an anticoagulant and centrifuged within 1 hour of collection. Serum was harvested and stored in a freezer, set to maintain -60 to -80°C, until packed on dry ice and shipped to the Sponsor for possible future analysis.

An aliquot (0.8 ml) of the additional serum collection samples collected from all animals from Groups 1, 2, 3, and 4 sacrificed at the terminal necropsy were sent on dry ice by the Sponsor to AniLytics for total triiodothyronine, total thyroxin, free triiodothyronine, and free thyroxin determinations.

Urine and Feces PFOS Level Determination

Urine [at least 2 mL (see Protocol Deviations)] and feces (at least 5 g) were collected overnight on the first day of recovery (Day 184) and on Days 189, 216, 275, 321, and 366 during recovery. In addition, a 24-hour sample of urine and feces was collected before the completion of 52 weeks of recovery. Except for the first day of recovery, animals were not fasted. Samples were stored in a freezer set to maintain -10 to -30°C, until they were packed on dry ice and shipped to the Sponsor. The samples will be analyzed for PFOS. Results will be reported separately.

Additional Fecal Samples

During Week 23, a fresh fecal sample (up to 5 g) was collected from all animals in the control and high-dose groups. Samples were collected in white polypropylene containers after pans were cleaned in the morning to ensure that the fecal samples were not more than 6 hours old (see Protocol Deviations). Samples were packed on dry ice and shipped to the Mayo Clinic for analysis.

Interim Liver Biopsy Samples

A sample of liver (approximately 1 to 2 g) was collected by biopsy from animals in Group 4 only during recovery [Week 57 (Day 393), on the same day as the serum PFOS blood collection]. This sample was divided into four portions as follows.

One subsample was preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin (duplicate slides were prepared), and examined microscopically.

The second subsample was flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until shipped to the Sponsor for analysis (see Protocol Deviations). Results will be reported separately.

The third subsample was processed to block stage for electron microscopic evaluation. The tissue blocks and a hematoxylin and eosin-stained slide for light microscopy were transferred to PAI. Tissues will be processed and evaluated by electron microscopy by PAI. A report will be provided by PAI for inclusion in the final report.

The fourth subsample was flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until transferred to the Sponsor for possible future analysis.

Terminal Liver Biopsy Samples

A sample of liver (approximately 1 g) was collected by biopsy from all animals in Group 3 during recovery [Week 80 (Day 554), one week after the serum PFOS blood collection (see Protocol Deviations)]. This sample was flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until shipped to the Sponsor for analysis. Results will be reported separately.

Anatomic Pathology - Terminal Sacrifice

Necropsy. A necropsy was done on Animal No. I05509 (Group 4 male) that died on Day 155 (Week 23) and Animal No. I05506 (Group 4 male) that was sacrificed in a moribund condition on Day 179 (Week 26). During Week 27 (Days 184 and 185) four animals/sex/group (Groups 1 through 3) and four females and two males (Group 4) were fasted overnight, anesthetized with ketamine and xylazine, weighed, bled for required tests, exsanguinated, and necropsied. Animals were necropsied in random order.

The necropsy included a macroscopic examination of the external surface of the body; all orifices; the cranial cavity; the external surface of the brain; the nasal cavity and paranasal sinuses; cervical tissues and organs; and the thoracic, abdominal, and pelvic cavities and viscera.

Organ Weights. At scheduled and unscheduled sacrifices, the following organs (when present) were weighed; paired organs were weighed separately.

adrenal (2)
brain
epididymis (2)
kidney (2)
liver
ovary (2)
pancreas
testis (2)
thyroid (2) with parathyroid

Organ-to-body weight percentages and organ-to-brain weight ratios were calculated.

Palmitoyl CoA Oxidase Determinations. Representative samples of the right lateral lobe of liver were collected from each animal at the scheduled sacrifice, weighed, flash-frozen in liquid nitrogen, and stored in a freezer, set to maintain -60 to -80°C, until analyzed for palmitoyl CoA oxidase activity.

Cell Proliferation Evaluation. Representative samples of the left lateral lobe of the liver, left and right testes, and pancreas were collected and preserved in zinc formalin. A second set of tissues (representative samples of the left lateral lobe of the liver, left and right testes, and pancreas) preserved in formalin without zinc were also prepared. After fixation, samples were embedded in paraffin and shipped to Pathology Associates International (PAI) for proliferation cell nuclear antigen (PCNA) evaluation, including the examination of slides stained with hematoxylin and eosin (see Protocol Deviations). Results were provided by PAI for inclusion in the final report (Appendix 7).

Liver PFOS Determination. A section of liver (approximately 20 g) was collected from each animal at the scheduled sacrifice, weighed, flash-frozen in liquid nitrogen, and stored in a freezer, set to maintain -60 to -80°C, until shipped with plasma samples to the Sponsor. Results will be reported separately.

Gallbladder and Bile Collection. At the scheduled terminal sacrifice for each animal, bile was collected from the gallbladder, measured, transferred into a cryovial, and flash-frozen in liquid nitrogen. The gallbladder, once emptied, was weighed, and a section (approximately 4 to 5 mm) from the mid-portion was collected. The remaining gallbladder was placed in a cryovial and flash-frozen in liquid nitrogen. The bile and gallbladder samples were stored on dry ice until transferred to a freezer set to maintain

-60 to -80°C. Samples were packed on dry ice and shipped to the Sponsor for possible future analysis.

Tissue Preservation. The following tissues (when present) or representative samples were collected and preserved in 10% neutral-buffered formalin, unless otherwise specified (see Protocol Deviations).

adrenal (2)
aorta
brain
cecum
cervix
colon
duodenum
epididymis (2)
esophagus
eyes [(2) preserved in Davidson's fixative for all sacrificed animals]
femur with bone marrow (articular surface of the distal end)
gallbladder
heart
ileum
jejunum
kidney (2)
lesions
liver
lung
mammary gland
mesenteric lymph node
ovary (2)
pancreas
pituitary
prostate
rectum
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle (2)
skeletal muscle (thigh)
skin
spinal cord (cervical, thoracic, and lumbar)
spleen
sternum with bone marrow
stomach
testis [(2) preserved in Bouin's solution for all sacrificed animals]
thymus
thyroid (2) with parathyroid
trachea
urinary bladder
uterus
vagina

Histopathology. Tissues (as appropriate) were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically from each animal in the control and high-dose groups (see Protocol Deviations for exceptions). In addition, liver and thymus for all animals in the low- and mid-dose groups and spinal cord gray matter from females in the low- and mid-dose groups were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. Other tissues, as appropriate, will be retained for possible future examination.

Bone marrow smears from the sternum of each animal at scheduled and unscheduled sacrifices were prepared, stained with Wright's stain, and retained for possible examination.

Electron Microscopy. A sample of the liver was collected from each animal at the scheduled terminal sacrifice. Tissues were processed into blocks and, along with a hematoxylin and eosin-stained slide, were shipped to PAI for analysis. Results will be provided for inclusion in the final report.

Anatomic Pathology - Recovery Sacrifice

Termination. Remaining animals in Group 1 were donated on Day 549 and remaining animals in Group 3 were transferred to Covance 6329-268 on Day 560. On Day 549, remaining animals in Group 4 were fasted overnight, anesthetized with ketamine and xylazine, weighed, exsanguinated, and necropsied.

The necropsy of the animals in Group 4 included a macroscopic examination of the external surface of the body; all orifices; the cranial cavity; the external surface of the brain; the nasal cavity and paranasal sinuses; cervical tissues and organs; and the thoracic, abdominal, and pelvic cavities and viscera.

Liver Samples. Samples of liver were collected from animals in Group 4 as follows.

One sample was preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin (duplicate slides were prepared), and examined microscopically.

The second sample was flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until shipped to the Sponsor for analysis. Results will be reported separately.

The third sample was processed to block stage for electron microscopic evaluation. The tissue blocks and a hematoxylin and eosin-stained slide for light microscopy were transferred to Pathology Associates International (PAI). Tissues will be processed and evaluated by electron microscopy by PAI. A report will be provided by PAI for inclusion in the final report.

Additional Tissue and Serum Samples. Samples of lung, kidney, spleen, thyroid, brain, abdominal fat, heart, (approximately 3 g each), and bile and serum (each as much as possible) were collected. These samples were flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until shipped to the Sponsor for possible future analysis.

Statistical Analyses

Levene's test (Levene, 1960) was done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance. Comparison tests took variance heterogeneity into consideration.

One-way analysis of variance [ANOVA (Winer, 1971a)] was used (if applicable) to analyze initial body weights, organ weights, palmitoyl CoA oxidase activities, continuous clinical pathology values, and blood hormone determinations. If the ANOVA was significant, Dunnett's t-test (Dunnett, 1964) was used for control versus treated group comparisons.

One-way analysis of covariance [ANCOVA (Winer, 1971b)] was used to analyze body weights, with initial body weights as the covariate. If the ANCOVA was significant, covariate-adjusted means were used for control versus treated group comparisons.

Group comparisons (Groups 2 through 4 versus Group 1) were evaluated at the 5.0%, two-tailed probability level. Only data collected on or after the first day of treatment were analyzed statistically. Statistical analyses were not performed on data collected during recovery.

Record Retention

All raw data, documentation, records, protocol, and specimens generated as a result of this study will be archived in the storage facilities of Covance-Madison for a period of 1 year. One year after the submission of the final report, the Sponsor will determine the final disposition of the materials. All raw data stored on magnetic media and the protocol, study correspondence, and an original copy of the final report will be retained by Covance-Madison.

Within 1 year after submission of the final report, all of the aforementioned materials from the Sponsor's designees (Ani Lytics Inc., 3M E. T. & S, Mayo Clinic, and Pathology Associates International) will be sent to the Sponsor (Andrew Seacat, PhD, 3M) by the Sponsor's designees.

RESULTS AND DISCUSSION

Observation of Animals

Clinical Observations. Clinical observations are summarized in Tables 1, 2, and 3; individual data are presented in Appendix 2. Individual animal fate data are also presented in Appendix 2.

Animal Nos. I05506 and I05509 given 0.75 mg/kg/day (Group 4 males) did not survive to the scheduled terminal sacrifice. All other animals survived to the scheduled study termination. No clinical observations noted in the animals that survived to the terminal sacrifice or recovery were attributable to the administration of PFOS.

Animal No. I05509 (Group 4 male) died after dosing on Day 155 (Week 23). On Day 154 (Week 22) observations of constricted pupil in both eyes and pale gums were noted. Observations noted on Day 155 prior to dosing included few, mucoid, liquid, and black-colored feces and low food consumption. Approximately 15 minutes after dosing, the animal was observed as hypoactive with labored respiration and pale gums. This animal also appeared dehydrated and was cold to the touch. These observations persisted until approximately 30 minutes postdose when the animal was also noted as recumbent. Shortly thereafter, the animal died during an examination by a laboratory animal veterinarian. An enlarged liver was detected by palpation. The cause of death was determined to be pulmonary necrosis with severe acute inflammation.

On Day 179 (Week 26), Animal No. I05506 (Group 4 male) was sacrificed in a moribund condition. Low food consumption was noted on Day 178 (Week 26) and at the a.m. observation interval on Day 179. Approximately 5 to 10 minutes postdose on Day 179, the animal had excessive salivation, labored respiration, and hypoactive and ataxic behavior. With the exception of excessive salivation, these findings continued to be observed approximately 3 hours postdose. The cause of the moribund condition was not determined.

Two additional animals had noteworthy observations during treatment. One female in the group given the control material, Animal No. I05529, was examined by a laboratory animal veterinarian on Day 5 (Week 1) due to observations of dehydration, thin appearance, clear oral and nasal discharge, excessive salivation, and audible respiration. This animal was diagnosed with pneumonia and treated with Lactated Ringer's solution and antibiotics. This animal had recovered by Day 14 (Week 2). Animal No. I05534 (Group 4 female) was diagnosed with a tapeworm infection during Week 23 and was treated with praziquantel. Neither infection was test material-related.

Clinical observations during recovery were typical of laboratory primates.

Ophthalmology. Ophthalmic observations are summarized in Table 4; individual data are presented in Appendix 2.

There were no ophthalmic observations at the Week 26 or Week 52 examinations that were test material-related. Animal No. I05529 (Group 1 female) was noted as having increased myelination of the right optic nerve at the baseline and Week 52 ophthalmic examinations. Because this is a permanent, congenital condition, it was noted at the baseline and recovery examinations only and is not related to treatment with PFOS.

Body Weights. Body weight data are illustrated in Figures 1 and 2 and summarized in Tables 5 and 6; individual data are presented in Appendix 3.

Covariate-adjusted mean (CAM) body weights were slightly lower in males given 0.75 mg/kg/day when compared with males given the control material beginning at Week 21; the difference was significant at Weeks 23 and 27. In females given 0.75 mg/kg/day, CAM body weights were significantly lower at Weeks 11 through 16, 19 through 23, and 25 through 27 when compared with females given the control material. These decreases were likely test material-related.

Differences in body weights were not apparent during the recovery period.

Food Consumption. Food consumption data are summarized in Tables 1, 2, and 3 (Summary of Clinical Observations); individual data are included in the individual clinical observations in Appendix 2.

Low food consumption was noted sporadically for animals in the groups given the control material and 0.03 mg/kg/day. The incidence of low food consumption was generally higher in the groups given 0.15 and 0.75 mg/kg/day as compared to animals given the control material and appeared to be test material-related. During recovery, instances of low food consumption were sporadic and were similar for animals in the control and treated groups.

Clinical Pathology

Hematology, clinical chemistry, and urinalysis data are summarized in Tables 7 through 45; individual data are presented in Appendix 4.

Administration of PFOS was associated with moderately to markedly lower total cholesterol for males and females given 0.75 mg/kg/day and high density lipoprotein cholesterol for males and females given 0.15 or 0.75 mg/kg/day. During the treatment period, the effect on total cholesterol became progressively worse over time. The effect on cholesterol was reversed within 5 weeks of the end of treatment, and the effect on high density lipoprotein cholesterol was reversed within 9 weeks of the end of treatment. Of uncertain relationship to administration of PFOS was lower total bilirubin concentration for males given 0.75 mg/kg/day and higher serum bile acid concentration for males given 0.75 mg/kg/day. These potential effects of the test material were very mild, and neither was considered adverse.

Palmitoyl CoA Oxidase Determination

Palmitoyl CoA oxidase determinations are summarized in Table 46; individual data are presented in Appendix 5.

Results of palmitoyl CoA oxidase determinations were not considered to be related to the test material.

Blood Hormone Determination

Summary and Individual Blood Hormone Data are presented in Appendix 6.

Estradiol values were generally lower on Days 62, 91, and 182 in males given 0.75 mg/kg/day, although because of the variation in the data only the Day 182 value was significant. Estrone values were generally higher in all of the treated females on Days 37, 62, and 91, although because of the variation in the data none of these values were significantly different, and this difference was not apparent on Day 182. Triiodothyronine values were notably lower on Days 91 and 182 in both males and females given 0.15 or 0.75 mg/kg/day. With the single exception on Day 91 of males given 0.15 mg/kg/day, all values were significantly lower. There were several other instances in which the hormone values in treated groups differed from those of controls, but these differences were not consistent over time or between sexes, were not clearly dose-related, and did not appear to be related to the administration of the test material.

During recovery were occasional instances in which the hormone values in treated groups differed slightly from those of controls, but those differences were not consistent over time or between sexes, were not clearly dose-related, and did not appear to be clearly related to the administration of the test material.

Apparent differences in the sexual maturity of both males and females used in this study complicates the interpretation of the hormone data.

Anatomic Pathology

Terminal body weights, absolute organ weights, organ-to-body weight percentages, and organ-to-brain weight ratios are summarized in Table 47; incidences of macroscopic and microscopic observations are summarized in Tables 48 through 51. Individual data are presented in Appendix 5.

Two of four males receiving 0.75 mg/kg/day (high dose) did not survive to the scheduled terminal sacrifice at Week 27. At the terminal sacrifice, females in the group receiving 0.75 mg/kg/day had increased absolute liver weight, liver-to-body weight percentages, and liver-to-brain weight ratios. In males, liver-to-body weight percentages were increased in the high-dose group compared to the control group. Absolute and relative liver weight increases were regarded as test material-related. Among the macroscopic observations, only "mottled" liver was considered test material-related. "Mottled" livers were observed in the two high-dose males and in one high-dose female. Of the two males not surviving until the scheduled terminal sacrifice, one had "mottled" and "large" liver.