PFOS:

A PILOT REPRODUCTION STUDY WITH THE MALLARD

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-105

3M LAB REQUEST NO. U2723

FIFRA Guideline 71-4

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STUDY INITIATION DATE: February 28, 2000

STUDY COMPLETION DATE: December 18, 2003

Submitted to

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Pilot Reproduction Study with the Mallard

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-105

STUDY COMPLETION: December 18, 2003

The study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan Notification No. 3850, Agricultural Production Bureau, 10 August 1984, with the following exceptions:

The study was conducted under multiple protocols. The in-life portion was conducted under one protocol (Wildlife International, Ltd. study number 454-105), and the analytical portions were conducted under two separate protocols (Exygen Research study numbers 023-042 and 023-063). Exygen Research study number 023-042 was initiated with a separate Study Director.

Results of analyses conducted by Exygen Research for study numbers 023-042 and 023-063 are reported separately.

Amendments number 2 and 3 and deviation 1 for the Exygen Research analytical protocol for study number 023-042 do not have the Sponsor's signature to indicate these changes were approved by the Sponsor.

STUDY DIRECTOR:

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Sean P. Gallagher
Senior Biologist, Avian Toxicology

DATE

SPONSOR'S REPRESENTATIVE:

John L. Newsted — 3/24/04

John L. Newsted

DATE

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DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR part 160, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all audits and inspections and the dates any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO: STUDY DIRECTOR	MANAGEMENT
Test Substance Preparation	February 28, 2000	February 28, 2000	March 1, 2000
Sample Preparation	March 1, 2000	March 1, 2000	March 1, 2000
Diet Preparation	March 7, 2000	March 7, 2000	March 7, 2000
Blood Collection	April 11, 2000	April 11, 2000	April 12, 2000
Analytical Data & Draft Report	January 16, 17, 28 & 29, 2003	January 29, 2003	January 31, 2003
Biological Data & Draft Report	January 27-31, February 3, 2003	February 3, 2003	March 12, 2003
Data and Final Report	December 1-3, 2003	December 3, 2003	December 18, 2003

All inspections were study-based unless otherwise noted.

Kimberly A. Hoxter
Quality Assurance Representative

12-18-03
DATE

REPORT APPROVAL

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3M LAB REQUEST NO.: U2723

STUDY DIRECTOR:

Sean P. Gallagher — 12/18/03
Senior Biologist, Avian Toxicology — DATE

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SUMMARY

STUDY: PFOS: A Pilot Reproduction Study with the Mallard

SPONSOR: 3M Corporation

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-105

TEST DATES: Study Initiation – February 28, 2000
Experimental Start – February 29, 2000
Adult Termination – April 11 & July 14, 2000
Biological Termination – July 25, 2000

TEST ANIMALS: Mallard (*Anas platyrhynchos*)

AGE TEST ANIMALS: Approximately 27 weeks of age at the initiation of the test

SOURCE TEST ANIMALS: Whistling Wings, Inc.
113 Washington Street
Hanover, IL 61041-0509
U.S.A.

NOMINAL TEST CONCENTRATIONS: 0, 1.8, 6.2, and 17.6 ppm a.i.

RESULTS: Mallards were exposed to PFOS at dietary concentrations of 0, 1.8, 6.2 and 17.6 ppm a.i. for 6 weeks. The control group and 17.6 ppm a.i. test group were maintained on test diets for an additional 13 weeks. No treatment-related mortalities were observed at any of the concentrations tested. No overt signs of toxicity were noted at the 1.8 or 6.2 ppm a.i. test concentrations. At the 17.6 ppm a.i. test concentration, a single hen was noted with signs of toxicity that may have been related to treatment. There were no apparent treatment-related effects on body weight amongst males and females in the 1.8 and 6.2 ppm a.i. test concentrations. There were mean body weight losses among males and females in the 17.6 ppm a.i. test group that may have been related to treatment. There were no apparent treatment-related effects on feed consumption or egg production in the 1.8, 6.2 or 17.6 ppm a.i. treatment groups. There were no apparent treatment-related effects on any of the reproductive parameters measured. Histopathological examination of selected tissue revealed a higher incidence of testicular regression and adipose tissue microvesiculation for adult males in the 17.6 ppm a.i. group. While these observations may be incidental to treatment, a treatment-related effect could not be precluded. Based upon the results of this study, the no-observed-effect concentration for mallards exposed to PFOS in the diet for six weeks was 6.2 ppm a.i.

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for 3M Corporation at the Wildlife International, Ltd. avian toxicology facility in Easton, Maryland 21601. The biological portion of the test was conducted from February 29, 2000 until July 25, 2000. Raw data generated at Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454-105 in archives located on the Wildlife International, Ltd. site. Biological specimens are stored at 3M Corporation, St. Paul, Minnesota 55133.

OBJECTIVES

The objective of this study was to evaluate the effects upon the adult Mallard (*Anas platyrhynchos*) of dietary exposure to the potassium salt of Perfluorooctane Sulfonic Acid (hereafter referred to as PFOS) over a period of approximately 6 weeks or 19 weeks. Effects on adult health, body weight, and feed consumption were evaluated. In addition, the effects of adult exposure to PFOS on the number of eggs laid, fertility, embryo viability, hatchability and offspring survival were evaluated. Histopathological examination of selected tissues and analyses of blood and tissue samples were also used to evaluate the effects upon adults exposed to PFOS and to their offspring.

EXPERIMENTAL DESIGN

Mallard (20 males and 20 females) were randomly distributed into one control group and three treatment groups. The test concentrations were selected in consultation with the Sponsor, based upon the results of a LC50 study (Wildlife International, Ltd. Project Number 454-102) and additional toxicity information provided by the Sponsor. The original test concentrations selected were 0, 2, 7 and 20 ppm a.i. Following experimental start, the test material was reanalyzed and the final purity was lower than originally reported. Therefore, the actual nominal test concentrations were 0, 1.8, 6.2 and 17.6 ppm a.i.

PFOS Treatment Groups

Group	Nominal Concentration (ppm a.i.)	Pens per Group	Birds per Pen	
			Males	Females
1	(Control) 0	5	1	1
2	1.8	5	1	1
3	6.2	5	1	1
4	17.6	5	1	1

Each treatment and control group contained five pairs of birds with one male and one female per pen. Three treatment groups were fed diets containing either 1.8, 6.2, or 17.6 ppm a.i. of PFOS. The control group was fed diet comparable to the treatment groups, but without the addition of the test substance.

Adult mallards were exposed to PFOS at nominal dietary concentrations of 1.8, 6.2 and 17.6 ppm a.i. for a period of 6 weeks. A control group, fed non-treated diet, was maintained concurrently with the treatment groups. Each treatment and the control group consisted of five pairs of birds, housed with one male and one female per pen. At the end of Week 6, adult birds in the 1.8 and 6.2 ppm a.i. test concentrations were euthanized and subjected to gross necropsy. Test birds in the control group and 17.6 ppm a.i. treatment group were maintained on the appropriate diets until the beginning of Week 20 of the test, at which time they were also euthanized and subjected to gross necropsy. Effects on adult health, body weight, and feed consumption were evaluated as well as effects upon egg production, embryo viability, hatchability and offspring survival for all test concentrations.

The adult birds were observed daily for mortality, abnormal behavior and signs of toxicity. Adult body weights were measured at test initiation, on Weeks 2, 4, 6, 8, 10 and 11, and at adult termination. Feed consumption for each pen was measured over a seven day period each week throughout the test. Necropsies were performed on all adult birds and on 10 offspring from each test concentration. Liver weights were recorded for all necropsied birds. Liver, bile and blood (when available) and feather samples were collected at the time of necropsy for possible analysis. Additional samples of liver, brain, kidney, gonad, proventriculus, gall bladder, adipose tissue, and bursa of fabricius (when available) were fixed in 10% buffered formalin for histopathological examination.

During the test, the number of eggs laid in each pen was recorded to evaluate egg production. Eggs laid in Weeks 1, 3 and 6 of the test were collected and separated into shell, shell membrane, yolk and albumen and stored frozen for possible analysis. Eggs laid during Weeks 5 and 6 (Days 31 to 37) of the test were collected and set for incubation. Embryo viability, hatchability, hatchling health and survivability were examined.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "PFOS: A Pilot Reproduction Study with the Mallard". The protocol was based on procedures outlined in the Environmental Protection Agency's Registration Guidelines *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms,* Subsection 71-4 and the ASTM "Standard Practice for Conducting Reproductive Studies with Avian Species" (1,2).

Test Substance and Internal Standard

The test substance, PFOS, was received from 3M Corporation on October 29, 1998 and was assigned Wildlife International, Ltd. identification number 4675 upon receipt. The test substance was a white powder and was identified as: FC-95; Lot 217. The test material had an original reported purity of 98.9% and had expired at the time of experimental start. An assay of the test material after experimental start indicated a purity of 90.49%. The final assay of the test material indicated a purity of 86.9% and expiration date of August 31, 2006 (Appendix I). The test substance was held under ambient condition in locked storage at the Wildlife International, Ltd. facilities in Easton, Maryland. Concentrations of the test substance in the diet were adjusted to 100% active ingredient based upon the original reported purity of 98.9%. Dietary concentrations are expressed as parts per million active ingredient (ppm a.i.) in the diet based upon the final reported purity of 86.9%.

The internal standard, 4H PFOS, was received from 3M Corporation on July 2, 1998 and was assigned Wildlife International, Ltd. identification number 4526 upon receipt. The internal standard was a granular material identified as: 1H, 1H, 2H, 2H Perfluoroctane Sulfonic Acid; CAS No. 27619-97-2. The internal standard was held under ambient conditions in locked storage at the Wildlife International, Ltd. facilities in Easton, Maryland.

Test Organisms

Fifty-one (24 males and 27 females) pen-reared mallards were purchased from Whistling Wings, Inc., 113 Washington Street, Hanover, IL 61041-0509, U.S.A. At the start of acclimation, the mallards appeared healthy and were phenotypically indistinguishable from wild type. The birds were from the same hatch, approaching their first breeding season and had not been used in any previous testing. At the start of acclimation, a random number generating function in a spreadsheet program was used to randomize pen assignment for each bird. Immediately prior to test initiation, all potential study birds

were examined for physical injuries and general health. Birds that did not appear healthy, either due to injury or inability to acclimate to laboratory conditions, or were outside the weight range for the test, were excluded from the study. All birds were approximately 27 weeks of age at test initiation (first day of exposure to test diet) and ranged in weight from 788 to 1258 grams at test initiation. Sex of the birds was determined by a visual examination of the plumage.

Identification

Adult birds were identified by individual leg bands, each pen was identified with a unique number, and groups of pens were identified by project number and concentration. During the test, the number of eggs laid in each pen was recorded to evaluate egg production. All eggs collected for sampling or incubation were marked with the pen number using a permanent ink marking pen for identification. Hatchlings were initially identified by wing bands so that they could be traced to their parental pen of origin. Wing bands were removed and the offspring were banded with leg bands at approximately four weeks of age.

Avian Feed and Water

All adult birds and their offspring were given feed and water *ad libitum* during acclimation and testing. The basal diet fed to both adults and offspring was formulated to Wildlife International, Ltd. specifications by Agway Inc. (Appendix II, Table 1). The basal ration contained at least 27% protein and 2.5% fat, and no more than 5% fiber.

The basal diet contained approximately 1.1% calcium, derived from feedstuffs and the 0.9% limestone used in the formulation of the basal diet by Agway. While this level of calcium is sufficient for growth and maintenance rations, additional calcium is required in the ration of breeding birds for egg shell formation. Therefore, an additional 5% (w/w) of limestone (approximately 38.5% Ca) was added to the basal diet for the adults. This raised the calcium level in the diet for the breeding birds to approximately 3%, slightly above the minimum recommended for quail (2.3%) and mallard (2.75%) (3). Offspring received basal diet without test substance and without the addition of 5% supplemental limestone.

Water was supplied by the town of Easton public water supply. Neither the adults nor offspring received any form of medication in the feed during the test. Feed and water were analyzed periodically in accordance with Wildlife International, Ltd. Standard Operating Procedures.

Diet Preparation

Test diets were prepared by mixing PFOS into a premix that was used for weekly preparation of the final diet. Control diet and each treated diet were prepared weekly beginning on February 28, 2000 and presented to the birds on Tuesday of each week. Dietary concentrations were adjusted for purity of the test substance and are presented as parts per million active ingredient (ppm a.i.). Details of the weekly preparation of test and control diets are shown in Appendix III.

Diet Sampling

Homogeneity of the test substance in the diet was evaluated by collecting six samples from each of the treated diets and one sample from the control diet on Day 0 of Week 1. Samples were collected from the top, middle and bottom of the left and right sections of the mixing vessel. Control and treatment group diet samples were also collected from the feed troughs on Day 7 of Week 1 to assess stability of the test substance under actual test conditions. Additionally, a sample was collected from the control and treatment group diets during Week 6 of the test to measure/verify test concentrations. The diet samples were stored frozen or transferred immediately to the Wildlife International, Ltd. analytical chemistry facility for analysis.

Analytical Method

The method used for the analysis of PFOS in avian diet was based upon methodology developed at Wildlife International, Ltd. and entitled "Analytical Method Verification for the Determination of PFOS in Avian Diet" (Wildlife International, Ltd. Project No. 454C-110).

Samples were extracted with methanol and diluted in a 50% methanol : 50% NANOpure® water solution containing 0.0100 mg 1H, 1H, 2H, 2H Perfluorooctane Sulfonic Acid (4H PFOS; internal standard)/L and 0.05% formic acid (v/v) so that they fell within the calibration range of the PFOS methodology. A method flow chart is provided in Appendix IV, Figure 1. Concentrations of PFOS in the standards and extracts of the samples were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer SCIEX API 100LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. HPLC separations were achieved using a Keystone Betasil C_{18} analytical column (50 mm × 2 mm I.D., 3-µm particle size). The instrument parameters are summarized in Appendix IV, Table 1.

Calibration standards of PFOS prepared in a 50% methanol : 50% NANOpure® water solution containing 0.0100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v), ranging in concentration from 0.000439 to 0.00439 mg a.i./L (Week 1, Day 0) or 0.000351 to 0.00439 mg a.i./L (Week 1, Day 7, Week 6, Day 0 and the sample re-extraction set), were analyzed with the samples. The same and most prominent peak response for PFOS was utilized to monitor PFOS in all calibration, quality control, and study samples. No attempt was made to quantify PFOS on the basis of individual isomeric components. Linear regression equations were generated using peak area response ratios (PFOS : internal standard) versus the respective concentration ratios (PFOS : internal standard) of the calibration standards. An example of a calibration curve is presented in Appendix IV, Figure 2. The concentration of test substance in the samples was determined by substituting the peak area response ratios of the samples into the applicable linear regression equation. Typical ion chromatograms of low and high-level calibration standards are shown in Appendix IV, Figures 3 and 4, respectively. Examples of calculations are presented in Appendix IV, Table 2.

The method limit of quantitation (LOQ) for the Week 1, Day 0 analysis was set at 0.879 ppm a.i., calculated as the product of the lowest calibration standard analyzed (0.000439 mg a.i./L) and the overall dilution factor of the matrix blank sample (2000 L/Kg). The method LOQ for the Week 1, Day 7 and Week 6, Day 0 analyses and the sample re-extraction set was set at 1.41 ppm a.i., calculated as the product of the lowest standard analyzed (0.000351 mg a.i./L) and the overall dilution factor of the matrix blank samples (4000 L/Kg). Examples of calculations are presented in Appendix IV, Table 2.

Along with the sample analyses, four matrix blanks were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses (Appendix IV, Table 3). A typical ion chromatogram of a matrix blank is presented in Appendix IV, Figure 5.

Avian diet samples were fortified at 0.879, 8.79, and 22.0 ppm a.i. (Week 1, Day 0) or 1.76, 8.79 and 22.0 ppm a.i. (Week 1, Day 7; Week 6, Day 0 and the sample re-extraction set) and analyzed concurrently with the samples to determine the mean procedural recovery. The method yielded mean procedural recoveries of 105%, 104%, 107% and 102%. These values correspond to each sample set analyzed or reanalyzed during the definitive study (Appendix IV, Table 3). Sample measured

concentrations were not corrected for the respective mean procedural recovery of that sample set. A typical ion chromatogram of a matrix fortification is presented in Appendix IV, Figure 6.

Housing and Environmental Conditions

Housing and husbandry practices were conducted so as to adhere to the guidelines established by the National Research Council (4). The adult birds were housed indoors in batteries of pens manufactured by Safeguard Products, Inc. (Model No. 5355), measuring approximately 75 X 90 X 45 cm high. The pens were constructed of vinyl-coated wire mesh. A diagram of the test layout is presented in Appendix V.

Each pen was equipped with a feed trough. Weekly, sufficient feed for the feeding period was placed in the trough for each pen and presented to the birds. During the feeding period additional feed was weighed and added to the troughs as needed. Water was supplied by nipple-type waterers.

Only birds associated with this study were maintained in the study room in order to avoid excessive disturbances. The average temperature in the adult mallard study room during the course of the test was 25.9 ± 0.7°C (SD) with an average relative humidity of 48% ± 17% (SD). The air handling system in the study room was designed to vent up to 15 room air volumes every hour and replace them with fresh air.

The photoperiod in the adult mallard room was maintained by a time clock. The photoperiod during acclimation and the test was 17 hours of light per day to induce egg laying. Throughout the test, the birds received a mean of approximately 202 lux (~ 18.8 ft. candles) of illumination provided by fluorescent lights that closely approximated noon-day sunlight.

Observations

The test birds were acclimated to the facilities and study pens for approximately 4 weeks prior to initiation of the test. During acclimation, all birds were observed daily. Birds exhibiting abnormal behavior or debilitating physical injuries were not used for the test. During the study, all adult birds were observed daily for signs of toxicity or abnormal behavior. Additionally, all offspring were observed daily from hatching until termination. A record was maintained of all mortalities and clinical observations.

Adult Body Weight and Feed Consumption

Adult body weights were measured at test initiation, on Weeks 2, 4, 6, 8, 10, 11 and at adult termination. Feed consumption for each pen was measured weekly throughout the test. Feed consumption was determined by weighing the freshly filled feeder on Day 0, recording the amount of any additional diet added during the week, and weighing the feeder and remaining feed at the end of the feeding period (Day 7). The amount of feed wasted by the birds was not quantified, since the wasted feed was normally scattered and mixed with water and excreta. Therefore, feed consumption is presented as an estimate of total feed consumption. All remaining birds were euthanized three days after the beginning of Week 20. Therefore, no feed consumption estimate was calculated for Week 20.

Adult Blood Collection

At the end of Week 6, blood samples were collected from all surviving birds, when possible. Following collection of blood samples, birds in the 1.8 and 6.2 ppm a.i. treatment groups were euthanized. Additional blood samples were also collected, when possible, from birds in the control group and the 17.6 ppm a.i. treatment group prior to euthanasia at the beginning of Week 20. All blood samples were separated into serum and hemacytes/platelets, stored frozen and shipped to Centre Analytical Laboratories, Inc. for possible analysis.

Adult Necropsy and Tissue Collection

At the conclusion of the exposure period, all surviving adult birds were euthanized by cervical dislocation, necropsied, and stored frozen. At the time of necropsy, tissues were collected for histopathological examination (0 and 17.6 ppm a.i. groups only) and possible analyses. When available, samples of gall bladder, liver, proventriculus, kidneys, brain, gonads, bursa of Fabricius and adipose tissue were fixed in 10% buffered formalin. Histology samples were shipped to EPL in Herndon, VA for histopathology. When available, samples of bile and liver were stored frozen and shipped to Centre Analytical Laboratories, Inc. for possible analysis. Any remaining tissue not fixed for histopathology and feather samples were stored frozen for potential analysis.

Egg Collection and Storage

Eggs laid during a seven day period beginning on the second day of Week 5 of the test were collected daily from test pens and stored in a cold room until incubation. Eggs to be incubated were washed to reduce the possibility of pathogen contamination before storing them in the cold room. Eggs

collected for the first four days were washed by hand prior to storage. The remaining eggs were washed in a commercial egg washer (Kuhl Egg Washer) with a chlorine based detergent (Kuhl Super CD). Water in the washer was warmed to approximately 45°C. The eggs were placed in the wash water and soaked for approximately 15 seconds. The washer's circulation motor was then turned on for approximately three minutes. The eggs were removed from the washer, allowed to cool to approximately room temperature and rinsed with fresh water. The cold room was maintained at a mean temperature of 13.1 ± 0.1°C (SD) with a mean relative humidity of approximately 75% ± 4% (SD). Groups of eggs were identified by an alphabetic lot code. All eggs laid during the week were considered as one lot.

Candling and Incubation

At the end of the weekly interval, all eggs were removed from the cold room, counted and candled with a Speed King (Model No. 32) egg-candling lamp to detect egg shell cracks or abnormal eggs. Cracked or abnormal eggs were recorded and discarded.

All eggs not discarded were placed in a Petersime Incubator (Model No. SP20). In the incubator the temperature was maintained at an average 37.5 ± 0.0°C (SD) with an average wet bulb temperature of 30.6 ± 0.1°C (SD) (relative humidity of approximately 60%). The incubator was equipped with a pulsator fan and blades that produced a mild breathing air movement designed to eliminate intracabinet temperature and humidity variation during incubation. In order to prevent adhesion of the embryo to the shell membrane, the incubator was also equipped with an automatic egg rotation device, designed to rotate the eggs from 50° off of vertical in one direction to 50° off of vertical in the opposite direction (total arc of rotation was 100°) every two hours through Day 24 of incubation. Eggs were candled on Day 14 of incubation to determine embryo viability and on Day 21 to determine embryo survival.

Hatching and Brooding

On Day 24 of incubation, the eggs were placed in a Petersime Hatcher (Model No. SP-6H) and allowed to hatch. Pedigree baskets constructed of galvanized steel wire mesh were used to keep hatchlings separated by parental pen of origin. Eggs were not rotated in the hatcher. The average temperature in the hatching compartment was 37.2 ± 0.0°C (SD), and the average wet bulb temperature was raised to 33.8 ± 0.2°C (SD) (relative humidity of approximately 77%).

All hatchlings, unhatched eggs, and egg shells were removed from the hatcher on Day 27 or 28 of incubation. The group body weight of the surviving hatchlings by pen was determined. Ducklings were wing banded for identification by pen of origin and then routinely housed according to the appropriate parental concentration grouping in brooding pens until approximately 3 weeks of age. All ducklings were moved to pens without supplemental heat, where they were housed approximately 9 weeks. The ducklings were fed untreated diet without the addition of 5% supplemental limestone. At approximately 12 weeks of age, the average body weight by parental pen of all surviving offspring was determined, and the birds were euthanized with carbon dioxide and disposed of by incineration. Those offspring selected for blood and tissue sampling were stored frozen following necropsy and later disposed of by incineration.

Hatchlings were housed in batteries of brooding pens manufactured by Safeguard Products, Inc. Each pen measured approximately 62 X 92 X 25.5 cm high. The walls, floors and ceilings of each pen were constructed of vinyl-coated wire mesh. Thermostats in the brooding compartment of each pen were set to maintain a temperature of approximately 38°C from the time of hatching until the birds were five to seven days of age, when the temperature was adjusted to maintain a temperature of approximately 29°C. Brooding was discontinued once hatchlings were determined to be of sufficient size to thermoregulate. The average ambient room temperature in the room housing offspring was 23.5 ± 0.6°C (SD) with an average relative humidity of 71 ± 11% (SD). All ducklings were removed from brooding pens and transferred to pens without supplemental heat at approximately 3 weeks of age. These pens were the same model used to house the adult birds. The photoperiod for the offspring was maintained by a time clock at 16 hours of light per day.

Offspring Blood and Tissue Collection

Prior to euthanasia of the offspring, blood samples were collected from 10 offspring in each treatment group. All blood samples were separated into serum and hemacytes/platelets, stored frozen, and shipped to the Centre Analytical Laboratories, Inc. for possible analyses. Additionally, tissues from the 10 offspring in each group were collected for histopathological examination and analyses. When available, samples of gall bladder, liver, proventriculus, kidneys, brain, gonads, bursa of Fabricius and adipose tissue were fixed in 10% buffered formalin and shipped to EPL in Herndon, VA for histopathology. When available, samples of bile and liver tissue were stored frozen and shipped to Centre Analytical Laboratories, Inc. for possible analysis.

Statistical Analyses

Upon completion of the test, an Analysis of Variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure (5, 6) was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the individual pens within each experimental group, except body and liver weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation. Two sets of statistical analyses were conducted with the body weight and feed consumption data. One set of analyses only looked at body weight and feed consumption data from the first 6 weeks of the study and evaluated all three treatment groups. The second set of analysis only evaluated the control and 17.6 ppm a.i. treatment groups and examined data for the full duration of the study, 20 weeks. Dunnett's multiple comparison procedure was not considered appropriate to compare the control group to a single treatment group. The student's T-test was used to make statistical comparisons in those instances where only the control group and the 17.6 ppm a.i. treatment group were compared.

1. Adult Body Weight – Individual body weight was measured at test initiation, Weeks 2, 4, 6, 8, 10, 11, and at adult termination. Statistical comparisons were made between the control group and each treatment group at each weighing interval by sex for the first 6 weeks. In addition, statistical comparisons were made between the control group and 17.6 ppm a.i. treatment group for weeks 8, 10, 11, and 20.
2. Adult Feed Consumption – Feed consumption expressed as grams of feed per bird per day was examined by pen weekly during the test. Statistical comparisons were made between the control and each treatment group for weeks 1 through 6. In addition, statistical comparisons were made between the control and 17.6 ppm a.i. treatment group for weeks 7 through 19.
3. Eggs Laid – The number of eggs laid per female per treatment group. For the evaluation of reproductive performance, data was taken from week 5 eggs set.
4. Viable Embryos – The number of live embryos determined at Day 14 by candling.
5. Eggs Cracked of Eggs Laid – The number of eggs determined by candling to be cracked divided by the number of eggs laid, per pen.
6. Viable Embryos of Eggs Set – The number of embryos at the Day 14 candling was divided by the number of eggs set, per pen.

7. Live 3- Week Embryos of Viable Embryos – The number of live embryos at the Day 21 candling was divided by the number of viable embryos, per pen.
8. Hatchlings of 3-Week Embryos – The number of hatchlings removed from the hatcher was divided by the number of live 3-week embryos, per pen.
9. 14-Day Old Survivors of Hatchlings – The number of 14-day old survivors was divided by the number of hatchlings per week, by pen.
10. Hatchlings of Eggs Set – The number of hatchlings was divided by the number of eggs set per week, by pen.
11. 14-Day Old Survivors of Eggs Set – The number of 14-Day old survivors was divided by the number of eggs set per week, by pen.
12. Offspring's Body Weight – The group body weights of surviving hatchlings and 14-day old survivors were measured by parental pen group.
13. Liver Weight – Individual liver weights were measured at adult termination and at the termination of offspring of week 5. Statistical comparisons of adult liver weights were made by sex between the control and treatment groups. Juvenile liver weights were compared by test group, without regard to sex.

RESULTS AND DISCUSSION

Adult mallards were exposed to PFOS at nominal dietary concentrations of 1.8, 6.2 and 17.6 ppm a.i. for a period of 6 weeks. A control group, fed non-treated diet, was maintained concurrently with the treatment groups. Each treatment and the control group consisted of five pairs of birds, housed with one male and one female per pen. At the end of Week 6, adult birds in the 1.8 and 6.2 ppm a.i. test concentrations were euthanized and subjected to gross necropsy. Test birds in the control group and 17.6 ppm a.i. treatment group were maintained on the appropriate diets until the beginning of Week 20 of the test, at which time they were also euthanized and subjected to gross necropsy.

Analytical Results

None of the control samples showed any indication of the presence of the test substance or of the presence of co-eluting substance at the characteristic retention time of the test substance (Table 1). Diet samples were collected from the 1.8, 6.2 and 17.6 ppm a.i. test concentrations on Week 1, Day 0, and were analyzed to evaluate the homogeneity of the test substance in the diet and to verify test substance concentrations. Means and standard deviations for the three test concentrations were 1.8 ± 0.13 ppm a.i.,

6.0 ± 0.65 ppm a.i. and 17.6 ± 1.46 ppm a.i., respectively. The coefficients of variation were 7.2%, 11% and 8.3%, respectively. These values represented 100, 97 and 100% of nominal concentrations (Appendix IV, Table 4). Samples collected during Week 6, Day 0 of the test to verify test substance concentrations for the 1.8, 6.2 and 17.6 ppm a.i. diets had means and standard deviations of 2.0 ± 0.092 ppm a.i., 6.0 ± 0.67 ppm a.i. and 16.8 ± 0.608 ppm a.i., respectively. The coefficients of variation were 4.6%, 11% and 3.6%, respectively. These values represented 111, 97 and 95% of nominal concentrations (Appendix IV, Table 5). Analysis of diet samples collected from feeders after being held at ambient temperature for 7 days averaged 89%, 87% and 105% of the Day 0 values for the 1.8, 6.2 and 17.6 ppm a.i. test concentrations, respectively (Appendix IV, Table 6). A typical ion chromatogram of a test sample is shown in Appendix IV, Figure 7.

Mortalities and Clinical Observations

No adult mortalities occurred in the control group or in any of the treatment groups during the course of the test. Several birds were noted with bumble foot (foot lesions) and feather loss as a result of pen wear and/or penmate aggression during the course of the test. A single bird in the 6.2 ppm a.i treatment group was noted with a head lesion. An incidental clinical sign, lameness, was associated with the bumble foot.

In the 17.6 ppm a.i. treatment group, the hen from pen 218 arched her neck and became rigid when captured for bleeding at test termination. She also exhibited body tremors and rapid blinking and respiration during the bleeding process. Clinical signs appeared to be triggered by the stress of handling. This hen was observed to be normal in appearance and behavior prior to test termination. All other birds in all test groups were normal in appearance and behavior for the duration of the test.

Adult Body Weight and Feed Consumption

When compared to the control group there were no apparent treatment-related effects on adult body weight at the 1.8 or 6.2 ppm a.i. test concentrations and any differences between the control group and 6.2 ppm a.i. test concentration were not statistically significant. There was a statistically significant ($p < 0.05$) reduction in female body weight at the 1.8 ppm a.i. test concentration from Week 2 to Week 4. However, this reduction was not consistent nor dose-responsive and was not considered treatment-related. There were mean body weight losses among males and females in the 17.6 ppm a.i. treatment group that may have been related to treatment. When compared to the control group, mean male body weight for the

17.6 ppm a.i. treatment group was significantly ($p < 0.05$) lower at the Week 10, 11 and 20 body weight intervals. There was also a significant loss of overall mean body weight for males at the 17.6 ppm a.i. test concentration. While there was an overall loss of mean body weight for females in the 17.6 ppm a.i., the difference from the control was not statistically significant ($p > 0.05$). Mean body weight measurements are presented in Table 2, and individual body weight measurements are presented in Appendix VI.

Due to excessive wastage by some birds, feed consumption was variable between pens. However, when compared to the control group, there appeared to be no treatment-related effects on feed consumption at the 1.8, 6.2 or 17.6 ppm a.i. test concentrations. While there were statistically significant ($p < 0.05$) differences between the control group and the 17.6 ppm a.i. treatment group for Weeks 14, 16, 17, 18 and 19, these differences were due to apparent increases in feed consumption for the 17.6 ppm a.i. group. Feed consumption measurements at the 17.6 ppm a.i. test concentration were consistently higher than the control group with a corresponding decrease in body weight, suggesting the increase was the result of feed wastage. Mean feed consumption measurements are shown in Table 3, and feed consumption measurements by pen are presented in Appendix VII.

Gross Necropsy

At the end of Week 6 (Day 42), all adult birds in the 1.8 and 6.2 ppm a.i. treatment groups were euthanized and subjected to gross necropsy. Adult birds in the control and 17.6 ppm a.i. treatment groups were maintained on the appropriate diets until the beginning of Week 20 and were then euthanized and subjected to gross necropsy. All findings observed were considered to be incidental to treatment. Necropsy findings are reported in Table 4 and Appendix VIII.

Study offspring were approximately 12 weeks of age at the time of euthanasia. A subsample of 10 juvenile birds for each test group were subjected to gross necropsy. Necropsy revealed several birds in each treatment level with bumble foot (foot lesions). Additionally, a female offspring in the 17.6 ppm a.i. treatment group was noted with picked and broken feathers and an unkempt appearance. Internally the bird was noted with a retained yolk sac, a remnant of the natal yolk sac. One male offspring in the 17.6 ppm a.i. treatment group was noted with the posterior portion of the left kidney absent. All findings observed were considered to be incidental to treatment.

Histopathology

Light microscopic examination was performed on selected tissues by a board certified pathologist at Experimental Pathology Laboratories, Inc. Herndon, VA. Sections of liver, brain, kidney, gonad, proventriculus, gall bladder, adipose tissues, and bursa of fabricius (when available) were collected from adult test birds and from 10 offspring (approximately 12 weeks old at euthanasia) from each test group for examination. No lesions considered possibly related to treatment were noted in liver, kidney, proventriculus, gall bladder, ovary, brain and bursa of fabricius of adult male and females, or their offspring at any of the concentrations tested. Additionally, there were no lesions considered to be treatment-related noted in adipose tissue of adult females and offspring of both sexes, or in the testes of male offspring.

When compared to the control group, testes of adult males at the 17.6 ppm a.i. test concentration exhibited a higher incidence of features consistent with post-reproductive phase regression. Adult males in the 17.6 ppm a.i. group also exhibited an increased incidence of adipose tissue microvesiculation. The increased incidence of testicular regression and adipose tissue microvesiculation for adult males in the 17.6 ppm a.i. treatment group may be incidental to treatment, but a treatment-related effect could not be discounted. The full pathology report is provided in Appendix IX.

Tissue Analysis

The analysis of the egg, blood and tissue samples collected during the study were conducted by Exygen Research (formerly known as Centre Analytical Laboratories) and are reported separately. Blood and liver analytical results are reported in "Extraction of Potassium Perfluorooctanesulfonate from Quail serum and quail liver for analysis using HPLC-Electrospray/Mass Spectrometry; Centre Study Number 023-41". The egg components analytical results are reported in "Extraction of Potassium Perfluorooctanesulfonate from Egg Membrane, Albumen, and Yolk for analysis using HPLC-Electrospray/Mass Spectrometry; Centre Study Number 023-065".

Reproductive Results

While egg production was highly variable among hens, there were no apparent treatment-related effects upon egg production at any of the concentrations tested. Any differences between the control group and the treatment groups were not statistically significant. When compared to the control group, there was a reduction in egg production at the 6.2 ppm a.i. treatment level. However, this reduction was not dose responsive and was not considered related to treatment. Egg production at the 1.8 and 17.6 ppm a.i. test concentrations was comparable to or exceeded the control group. Mean egg production by concentration is presented in Table 5. Individual egg production data are presented in Appendix X.

When compared to the control group, there were no apparent treatment-related effects on embryo viability, hatchability, hatchling health and survivability at the 1.8, 6.2 or 17.6 ppm a.i. levels. Any differences between the control group and the treatment groups were not statistically significant for any parameter measured. While there were fewer 14-day old survivors per hen produced at the 1.8 and 6.2 ppm a.i. test concentrations, these reductions were due to fewer eggs being set for incubation at these levels. Reproductive data are summarized in Table 6. Reproductive data for individual pens is presented in Appendix XI.

Offspring Body Weights

There were no apparent treatment-related effects upon the body weights of hatchlings in any of the treatment groups and any differences from the control group were not statistically significant. There were also no apparent treatment-related effects upon the body weight of juvenile birds in the 1.8, 6.2 or 17.6 ppm a.i. treatment groups. Offspring body weight data is presented in Table 7 and Appendix XII.

Liver Weights

When compared to the control group, there were no apparent treatment-related effects on adult liver weight at any of the concentrations tested. There was a statistically significant ($p < 0.05$) increase in mean female liver weight at the 1.8 and 6.2 ppm a.i. test concentrations. While mean adult liver weights were higher among males and females at the 1.8 and 6.2 ppm a.i. tests concentrations when compared to the control group, these differences were due to the timing of adult termination for these treatment groups. The 1.8 and 6.2 ppm a.i. test birds were euthanized at the end of Week 6 of the test, during a period of high egg production. The control group and 17.6 ppm a.i. treatment group test birds were euthanized at the beginning of Week 20 of the test by which time egg production had declined dramatically. One

would expect birds in full reproductive condition, particularly females, to have higher levels of fat reserves in the liver and correspondingly higher liver weights. When compared to the control group, there were no apparent treatment-related effects on juvenile liver weight at any of the concentrations tested. While there was a significant increase in mean offspring liver weight for the 1.8 ppm a.i. treatment group, the increase was not dose-responsive and appeared to be related to slightly higher mean body weights for this group. Mean adult and offspring liver weights are presented in Table 8. Individual adult liver weights are presented in Appendix XIII. Individual offspring liver weights are presented in Appendix XIV.

CONCLUSION

Mallards were exposed to PFOS at dietary concentrations of 0, 1.8, 6.2 and 17.6 ppm a.i. for 6 weeks. The control group and 17.6 ppm a.i. test group were maintained on test diets for an additional 13 weeks. No treatment-related mortalities were observed at any of the concentrations tested. No overt signs of toxicity were noted at the 1.8 or 6.2 ppm a.i. test concentrations. At the 17.6 ppm a.i. test concentration, a single hen was noted with signs of toxicity that may have been related to treatment. There were no apparent treatment-related effects on body weight among males and females in the 1.8 and 6.2 ppm a.i. test concentrations. There were mean body weight losses among males and females in the 17.6 ppm a.i. test group that may have been related to treatment. There were no apparent treatment-related effects on feed consumption or egg production in the 1.8, 6.2 or 17.6 ppm a.i. treatment groups. There were no apparent treatment-related effects on any of the reproductive parameters measured. Histopathological examination of selected tissue revealed a higher incidence of testicular regression and adipose tissue microvesiculation for adult males in the 17.6 ppm a.i. group. While these observations may be incidental to treatment, a treatment-related effect could not be precluded. Based upon the results of this study, the no-observed-effect concentration for mallards exposed to PFOS in the diet for six weeks was 6.2 ppm a.i.

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Table 1

Mean Measured Concentrations (ppm a.i.) of PFOS
in Avian Diet from a Mallard Pilot Reproduction Study

Nominal Concentration[1]		Interval: Week 1, Day 0	Interval: Week 1, Day 7	Interval: Week 6, Day 0
Control (0 ppm a.i.)	Measured	< 0.879 [2]	< 1.41	< 1.41
1.8 (ppm a.i.)	Mean Measured	1.8	1.6	2.0
	% Nominal	100	89 [3]	111
6.4 (ppm a.i.)	Mean Measured	6.0	5.2	6.0
	% Nominal	97	87 [3]	97
18.3 (ppm a.i.)	Mean Measured	17.6	18.4	16.8
	% Nominal	100	105 [3]	95

[1] Nominal concentrations and results of diet analysis were corrected for the change in test substance purity from 90.49% to 86.9

[2] The limit of quantitation (LOQ) was equivalent to 0.879 ppm a.i. for Week 1, Day 0 and 1.41 ppm a.i. for Week 1, Day 7 and Week 6, Day 0.

[3] The mean percent of the Day 0 values.

Table 2
Mean Adult Body Weight[1] (g) from a Mallard
Pilot Reproduction Study with PFOS

Experimental Group	Sex		Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6	Change 6-8	Week 8	Change 8-10	Week 10	Change 10-11	Week 11	Change 11-20	Week 20	Change 0-20
Control	M	$\bar{X}$	1120	-36	1084	-2	1082	42	1124	3	19	1142	59	1202	40	1242	-38	1204	83
(0 ppm a.i.)		SD	53	65	59	54	66	16	71	41	32	81	21	79	40	59	95	97	58
	F	$\bar{X}$	998	-36	962	89	1051	1	1052	55	-54	998	26	1025	-10	1015	-10	1005	7
		SD	106	87	70	84	52	59	75	116	31	61	62	18	44	50	59	23	118
1.8	M	$\bar{X}$	1093	-10	1083	-9	1073	63	1136	43	--	--	--	--	--	--	--	--	--
ppm a.i.		SD	70	52	69	21	77	24	78	72	--	--	--	--	--	--	--	--	--
	F	$\bar{X}$	1003	88	1092	-13*	1079	35	1114	110	--	--	--	--	--	--	--	--	--
		SD	92	64	55	57	72	41	91	106	--	--	--	--	--	--	--	--	--
6.2	M	$\bar{X}$	1091	-33	1058	39	1098	21	1118	27	--	--	--	--	--	--	--	--	--
ppm a.i.		SD	31	20	40	37	42	73	63	74	--	--	--	--	--	--	--	--	--
	F	$\bar{X}$	988	66	1054	28	1082	-43	1038	51	--	--	--	--	--	--	--	--	--
		SD	62	69	65	66	105	78	95	111	--	--	--	--	--	--	--	--	--
17.6	M	$\bar{X}$	1095	-41	1053	-4	1049	38	1087	-8	-13	1074	4	1078*	-5	1073*	-31	1042*	-53*
ppm a.i.		SD	107	56	78	14	70	25	51	74	62	24	55	65	18	73	92	94	57
	F	$\bar{X}$	999	23	1022	11	1033	-55	978	-21	17	995	-27	967	-10	958	-60	897	-102
		SD	150	104	138	53	102	59	149	88	62	148	37	172	15	173	98	139	90

[1] Mean ($\bar{X}$) ± standard deviation (SD). The means for body weights and body weight changes are calculated and rounded separately.

-- Data not avalible. Birds euthanized at the end of Week 6.

* Statistically different from the control group at $p < 0.05$.

Table 3

Mean Feed Consumption[1] (g/bird/day) from a Mallard
Pilot Reproduction Study with PFOS

Experimental Group		Weeks																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Control	Mean	146	124	150	178	186	212	200	236	220	211	236	175	139	196	139	123	168	130	170
0 ppm a.i.	SD	28	16	24	37	17	40	53	63	28	71	39	28	19	22	42	28	41	25	44
1.8	Mean	157	150	154	172	160	177	--	--	--	--	--	--	--	--	--	--	--	--	--
ppm a.i.	SD	47	31	43	47	42	50	--	--	--	--	--	--	--	--	--	--	--	--	--
6.2	Mean	145	137	168	191	195	178	--	--	--	--	--	--	--	--	--	--	--	--	--
ppm a.i.	SD	34	39	29	40	78	56	--	--	--	--	--	--	--	--	--	--	--	--	--
17.6	Mean	156	136	200	187	196	234	225	241	253	226	249	218	195	267 *	172	191 *	238 *	183 *	227 *
ppm a.i.	SD	65	38	62	42	57	84	51	79	67	70	69	62	69	49	52	42	51	39	29

[1] Mean ± standard deviation.

-- Data not avalible. Birds euthanized at the end of Week 6.

* Statistically different from the control group at $p < 0.05$.

Table 4

Summary of Gross Pathological Observations from a Mallard
Pilot Reproduction Study with PFOS

Adult Birds Euthanized at 6 Weeks and Test Termination

	Males - Treatment Group (ppm a.i.)				Females - Treatment Group (ppm a.i.)			
	Control	1.8	6.2	17.6	Control	1.8	6.2	17.6
Number of birds	5	5	5	5	5	5	5	5
Molting flight feathers	3	0	0	0	2	0	0	3
Feather loss & abrasions on neck	0	0	0	0	0	0	1	0
Bumble foot	3	1	3	4	3	3	3	1
Impacted feed under tongue	0	0	0	1	0	0	0	0
Abnormally small body size	0	0	0	0	0	0	0	1
Testes small, ~ 1.0-2.0 cm	0	0	0	3	-	-	-	-
Testes small, ~ 2.0-3.0 cm	2	0	0	1	-	-	-	-
Testes small, ~ 3.5 cm	2	0	0	0	-	-	-	-
Regressing ovary	-	-	-	-	0	1	0	0
Regressed ovary	-	-	-	-	4	0	0	5
Egg yolk peritonitus	-	-	-	-	0	1	3	1
Egg remnants in the repro. tract	-	-	-	-	1	0	0	0
Intrabdominal egg	-	-	-	-	0	0	1	0
Air sacculitus	0	0	0	0	0	0	0	1
Not remarkable	0	4	2	0	1	1	1	0

Table 5
Mean Egg Production [1] (Eggs Laid/Hen and Eggs/Hen/Day)
from a Mallard Pilot Reproduction Study with PFOS

Experimental Group		Weeks																							
		1	2	3	4	5	6	Total	E/H/D[2]	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total	E/H/D[1]
Control	Total	17	15	14	17	22	27	112		21	26	13	13	7	5	0	0	0	0	0	0	0	0	85	
0 ppm a.i.	Mean	3	3	3	3	4	5	22	0.53	4	5	3	3	1	1	0	0	0	0	0	0	0	0	17	0.18
	SD	3	3	4	3	3	3	16	0.39	3	3	3	4	3	2	0	0	0	0	0	0	0	0	15	0.16
1.8	Total	11	15	25	20	19	16	106		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
ppm a.i.	Mean	2	3	5	4	4	3	21	0.50	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	SD	3	3	3	4	3	4	18	0.44	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
6.2	Total	5	12	13	12	14	8	64		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
ppm a.i.	Mean	1	2	3	2	3	2	13	0.30	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	SD	2	3	4	3	4	3	17	0.41	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
17.6	Total	15	22	33	31	24	22	147		10	15	15	17	8	7	7	4	3	4	0	0	0	0	90	
ppm a.i.	Mean	3	4	7	6	5	4	29	0.70	2	3	3	3	2	1	1	1	1	1	0	0	0	0	18	0.19
	SD	3	1	1	1	2	3	5	0.11	3	4	3	4	3	3	3	2	1	2	0	0	0	0	23	0.24

[1] Total number of eggs laid and mean number of eggs laid per pen ± standard deviation.

[2] Eggs per hen per day.

-- Data not available. Birds euthanized at the end of Week 6.

Differences between the control group and the treatment group were not statistically significant ($p > 0.05$).

Table 6

Summary of Reproductive Performance (Eggs Set from Week 5)
from a Mallard Pilot Reproduction Study with PFOS

Reproductive Parameter	Experimental Group			
	Control (0 ppm a.i.)	1.8 ppm a.i.	6.2 ppm a.i.	17.6 ppm a.i.
Number of Replicates	5	5	5	5
Eggs Laid	24	18	12	24
Eggs Cracked	0	1	0	1
Eggs Set	24	17	12	23
Viable Embryos	22	17	12	21
Live 3-Week Embryos	22	17	12	21
Hatchlings	21	14	12	18
Offspring Survivors	21	13	12	17
Eggs Laid/Hen	5	4	2	5
Offspring/Hen	4	3	2	3
Eggs Laid/Hen/Day	0.69	0.51	0.34	0.69
Eggs Cracked/Eggs Laid (%)	0	5	0	4
Viable Embryos/Eggs Set (%)	90	100	100	92
Live 3-Week Embryos/Viable (%)	100	100	100	100
Hatchlings/3-Week Embryos (%)	92	82	100	83
Offspring Survivors/Hatchlings (%)	100	94	100	97
Hatchlings/Eggs Set (%)	85	82	100	76
Offspring Survivors/Eggs Set (%)	85	77	100	74
Hatchlings/Hen/Day	0.60	0.40	0.34	0.51
Offspring Survivors/Hen/Day	0.60	0.37	0.34	0.49

Differences between the control group and the treatment groups were not statistically significant ($p > 0.05$)

Table 7

Mean Body Weight[1] (g) of Hatchlings and Surviving Offspring
from a Mallard Pilot Reproduction Study with PFOS

Experimental Group	Hatchlings		Surviving Offspring[2]	
	Mean	SD	Mean	SD
Control (0 ppm a.i.)	28.4	± 2.0	1012	± 72
1.8 ppm a.i.	32.8	± 1.4	1065	± 92
6.2 ppm a.i.	33.3	± 0.4	1036	± 71
17.6 ppm a.i.	35.0	± 1.6	1030	± 35

[1]Mean ± standard deviation.

[2]Offspring were approximately 12 weeks of age at final body weight interval.

Differences between the control group and the treatmeng groups were not statistically significant ($p > 0.05$)

Table 8
Mean Liver Weights[1] (g) from a Mallard
Pilot Reproduction Study with PFOS

Experimental Group	Males Liver Mean	Males Liver SD	Females Liver Mean	Females Liver SD	Offspring[2] Liver Mean	Offspring[2] Liver SD
Control (0 ppm a.i.)	21.336	4.793	21.240	5.382	20.890	3.148
1.8 ppm a.i.	25.336	1.564	39.801	8.832 *	27.661	4.999 *
6.2 ppm a.i.	29.205	4.900	31.938	4.462 *	23.220	4.215
17.6 ppm a.i.	23.411	8.019	20.624	2.947	24.717	5.489

[1] Mean ± standard deviation.

[2] Offspring were approximately 12 weeks of age at time of euthanasia and tissue collection.

* Statistically different from the control group at $p < 0.05$.

Appendix I

Certificate of Analysis

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801 www.centrelab.com
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A
3M Product: PFOS, Lot 217
Reference #: SD-018
Purity: 86.9%

Test Name	Specifications	Result
Purity[1]		**86.9%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS)		
1. Calcium		1. 0.005 wt./wt.%
2. Magnesium		2. 0.001 wt./wt.%
3. Sodium		3. 1.439 wt./wt.%
4. Potassium[2]		4. 6.849 wt./wt.%
5. Nickel		5. <0.001 wt./wt.%
6. Iron		6. 0.005 wt./wt.%
7. Manganese		7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.91 wt./wt.%
Total % Impurity (LC/MS)		8.41 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.33 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable[3]
Inorganic Anions (IC)		
1. Chloride		1. <0.015 wt./wt.%
2. Fluoride		2. 0.59 wt./wt.%
3. Bromide		3. <0.040 wt./wt.%
4. Nitrate		4. <0.009 wt./wt.%
5. Nitrite		5. <0.006 wt./wt.%
6. Phosphate		6. <0.007 wt./wt.%
7. Sulfate[4]		7. 8.76 wt./wt.%
Organic Acids [5] (IC)		
1. TFA		1. <0.1 wt./wt.%
2. PFPA		2. <0.1 wt./wt.%
3. HFBA		3. 0.10 wt./wt.%
4. NFPA		4. 0.28 wt./wt.%
Elemental Analysis[6]:		
1. Carbon	1. Theoretical Value = 17.8%	1. 12.48 wt./wt.%
2. Hydrogen	2. Theoretical Value = 0%	2. 0.244 wt./wt.%
3. Nitrogen	3. Theoretical Value = 0%	3. 1.74 wt./wt.%
4. Sulfur	4. Theoretical Value = 5.95%	4. 8.84 wt./wt.%
5. Fluorine	5. Theoretical Value = 60%	5. 54.1 wt./wt.%

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Page 2

Certificate of Analysis

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INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/06

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/06

[1]Purity = 100% - (sum of metal impurities, 1.45% +LC/MS impurities, 8.41%+Inorganic Fluoride, 0.59%+NMR impurities, 1.905%+organic acid impurities, 0.38%+POAA, 0.33%)

Total impurity from all tests = 13.07%
Purity = 100% - 13.07% = 86.9%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

[4]Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

[5]TFA Trifluoroacetic acid
HFBA Heptafluorobutyric acid
NFPA Nonafluoropentanoic acid
PFPA Pentafluoropropanoic acid

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3K^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

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Certificate of Analysis

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INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.22
C5	1.33
C6	4.72
C7	1.14
Total	**8.41**

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average result from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average result from the C6 and C8 standard curves.

Prepared By: _____ 10/11/01
Charles Simons Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 10/11/01
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

Appendix II
Diet and Supplement Formulations

Table 1
Wildlife International, Ltd. Game Bird Ration[1]

INGREDIENTS	PERCENT (%)
Fine Corn Meal	44.83
Soy Bean Meal, 48% Protein	30.65
Wheat Midds	6.50
Protein Base	6.00
Agway Special, 60% Protein	4.00
Alfalfa Meal, 20% Protein	3.00
Dried Whey	2.50
Ground Limestone	0.90
Eastman CalPhos	0.60
Methionine Premix + Liquid	0.35
Vitamin and Mineral Premix (see below)	0.32
GL Ferm (Fermatco)[2]	0.25
Salt Iodized	0.10
Total	100.00

Vitamin and Mineral Premix, when added at 0.32% of the ration, supplied the following amounts per ton:	Amount Supplied Per Ton:
Vitamin D_3	2,000,000 I.C.U.
Vitamin A	7,000,000 I.U.
Riboflavin	6 grams
Niacin	40 grams
Pantothenic Acid	10 grams
Vitamin B_{12}	8 mgs
Folic Acid	600 mgs
Biotin	64 mgs
Pyridoxine	1.2 grams
Thiamine	1.2 grams
Vitamin E	20,000 I.U.
Vitamin K (Menadione Dimethylpyrimidinol Bisulfite)	5.8 grams
Manganese	102 grams
Zinc	47 grams
Copper	6.8 grams
Iodine	1.5 grams
Iron	51 grams
Selenium	182 mgs

[1] The guaranteed analysis is a minimum of 27% protein, a minimum of 2.5% crude fat and a maximum of 5% crude fiber.

[2] Fermentation By-Products (Source of Unidentified Growth Factors).

Appendix III
Diet Preparation

Premixes for PFOS were prepared on February 28, 2000; April 7, 2000; May 12, 2000; and June 19, 2000. Nominal preparation was as follows:

Control:	No premix required.
1.8 ppm a.i.:	0.3084 g PFOS + 6099.7 g ration
6.2 ppm a.i.:	1.0794 g PFOS + 6098.9 g ration
17.6 ppm a.i.:	3.0839 g PFOS + 6096.9 g ration

Basal ration was weighed into a tared Hobart mixing bowl. Approximately 100 g of ration was transferred to a Waring® blender. The test substance was weighed into a tared weigh boat and a small mortar was taken to crush any areas of consolidation. The test substance was transferred to the Waring® blender and the weigh boat was rinsed three times with ration from the mixing bowl, with the rinse being added to the blender. The blender contents were mixed approximately one minute and transferred to the mixing bowl. The blender was rinsed three times with uncontaminated ration from the bowl, with the rinse being returned to the bowl. The bowl was placed on a Hobart mixer, and the contents were mixed approximately 15 minutes. The premix was weighed into 1000.0 g aliquots, placed in appropriately labeled plastic bags, reweighed, and stored frozen.

As needed, the appropriate premix was incorporated into the final diet as follows:

0 ppm a.i.:	23.75 kg ration + 1.250 kg limestone
1.8 ppm a.i.:	1000 g Premix + 22.75 kg ration + 1.250 kg limestone
6.2 ppm a.i.:	1000 g Premix + 22.75 kg ration + 1.250 kg limestone
17.6 ppm a.i.:	1000 g Premix + 22.75 kg ration + 1.250 kg limestone

The diets were mixed for approximately 20 minutes in a Patterson-Kelly Twin Shell Blender.

Appendix IV

The Analysis of PFOS in Avian Diet

Appendix IV

ANALYTICAL METHODS AND RESULTS

TABLE 1

Typical LC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer SCIEX API 100LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in selective ion monitoring mode (SIM).
ANALYTICAL COLUMN:	Keystone Betasil C_{18} column (50 mm x 2 mm I.D., 3-µm particle size)
OVEN TEMPERATURE:	30°C
STOP TIME:	5.00 minutes
FLOW RATE:	0.220 mL/minute
MOBILE PHASE:	72.0% Methanol : 28.0% NANOpure® Water containing 0.1% Formic Acid
INJECTION VOLUME:	5.0 µL
PFOS RETENTION TIME:	Approximately 3.6 minutes
INTERNAL STANDARD RETENTION TIME:	Approximately 2.6 minutes
PFOS MONITORED MASS:	498.6 amu
INTERNAL STANDARD MONITORED MASS:	426.7 amu

Appendix IV

TABLE 2

CALCULATIONS

The concentration of PFOS found at the instrument was determined using the following equation:

$$\text{PFOS (mg a.i./L) at instrument} = \frac{\text{peak area ratio - (y-intercept)}}{\text{slope}} \times \text{internal standard conc. (mg a.i./L)}$$

Determination of Sample Residues (PFOS)

The concentration, expressed as ppm a.i., for each sample was determined using the following equation:

$$\text{PFOS (ppm a.i.) in sample} = \frac{\text{PFOS (mg a.i./L) at instru.} \times \text{extract final volume (L)} \times \text{dilution factor}}{\text{initial weight (Kg)}}$$

Determination of Limit of Quantitation (LOQ)

The method LOQ, expressed as ppm a.i., was determined using the following equation:

$$\text{LOQ (ppm a.i.)} = \text{lowest standard concentration (mg a.i./L)} \times \text{overall dilution factor of matrix blank*}$$

$$\text{*overall dilution factor of matrix blank sample} = \frac{\text{extract final volume (L)} \times \text{dilution factor}}{\text{initial weight (Kg)}}$$

Fortification Recoveries

The ppm a.i. measured in each sample is divided by the nominal concentration of each sample (fortified level, ppm a.i.). This ratio times 100 is the percent recovery of the method at that level of fortification.

$$\text{\% Recovery} = \frac{\text{ppm a.i. measured in sample}}{\text{ppm a.i. fortified}} \times 100$$

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TABLE 3

Matrix Blanks and Fortifications Analyzed Concurrently with the Samples

Sample			Concentration of PFOS (ppm a.i.)		Percent Recovery	Mean Recovery ($\bar{x}$)
Number (454-105-)	Type	Interval	Fortified[1]	Measured[1,2]		
MAB-1	Matrix Blank	Week 1, Day 0	0.00	< 0.879	-	
MAS-1	Matrix Fortification	Week 1, Day 0	0.879	0.992	113	105
MAS-2	Matrix Fortification	Week 1, Day 0	8.79	8.89	101	
MAS-3	Matrix Fortification	Week 1, Day 0	22.0	22.0	100	
MAB-2	Matrix Blank	Week 1, Day 7	0.00	< 1.41	-	
MAS-4	Matrix Fortification	Week 1, Day 7	1.76	1.97	112	104
MAS-5	Matrix Fortification	Week 1, Day 7	8.79	9.11	104	
MAS-6	Matrix Fortification	Week 1, Day 7	22.0	21.3	97.0	
MAB-3	Matrix Blank	Week 6, Day 0	0.00	< 1.41	-	
MAS-7	Matrix Fortification	Week 6, Day 0	1.76	1.98	113	107
MAS-8	Matrix Fortification	Week 6, Day 0	8.79	9.40	107	
MAS-9	Matrix Fortification	Week 6, Day 0	22.0	22.0	100	
MAB-4	Matrix Blank	Re-extraction Set[4]	0.00	< 1.41	-	
MAS-10	Matrix Fortification	Re-extraction Set[4]	1.76	1.84	105	102
MAS-11	Matrix Fortification	Re-extraction Set[4]	8.79	8.86	101	
MAS-12	Matrix Fortification	Re-extraction Set[4]	22.0	21.7	98.7	

[1] Concentrations were corrected for change in test substance purity (98.9% to 86.9%) per Certificate of Analysis dated October 11, 2001. Nominal test concentrations based upon the new test substance purity are 1.8, 6.2 and 17.6 ppm a.i.

[2] Less than values correspond to limit of quantitation (LOQ).

[3] Results were generated using MacQuan version 1.6 software. Manual calculations may differ slightly since fortified and measured concentrations were corrected for change in test substance purity and rounded for reporting purposes.

[4] Re-extraction of: sample 454-105,105-13 from Week 1, Day 0; samples 454-105-23 and -26 from Week 1, Day 7 and sample 454-105,105-50 from Week 6, Day 0.

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TABLE 4

Homogeneity of PFOS in Avian Diet

Nominal Concentration[1] (ppm a.i.)	Sample I.D. Number (S-454-105-)	Location Sampled in Mixing Vessel	Measured PFOS Concentration[1] (ppm a.i.)	Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Mean Percent of Nominal
1.8	3	Top Left	1.70		
	4	Top Right	1.82		
	5	Middle Left	1.92	$\bar{x}$ = 1.8 ppm a.i	100
	6	Middle Right	1.73	SD = 0.13 ppm a.i.	
	7	Bottom Left	1.77	CV = 7.2%	
	8	Bottom Right	2.05		
6.2	9	Top Left	5.33		
	10	Top Right	5.65		
	11	Middle Left	6.09	$\bar{x}$ = 6.0 ppm a.i.	97
	12	Middle Right	5.81	SD = 0.65 ppm a.i.	
	13	Bottom Left	5.87[2]	CV = 11%	
	14	Bottom Right	7.21		
17.6	15	Top Left	16.5		
	16	Top Right	17.7		
	17	Middle Left	16.5	$\bar{x}$ = 17.6 ppm a.i.	100
	18	Middle Right	20.4	SD = 1.46 ppm a.i.	
	19	Bottom Left	17.0	CV = 8.3%	
	20	Bottom Right	17.6		

[1] Concentrations were corrected for change in test substance purity (98.9% to 86.9%) per Certificate of Analysis dated October 11, 2001. Nominal test concentrations based upon the new test substance purity are 1.8, 6.2 and 17.6 ppm a.i.

[2] Mean result of duplicate re-extraction of original sample.

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TABLE 5

Verification of PFOS Concentrations in Avian Diet

Nominal Concentration[1] (ppm a.i.)	Sample I.D. Number (S-454-105-)	Interval (Day 0 of Week -)	Measured PFOS Concentration[1,2] (ppm a.i.)	Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Mean Percent of Nominal
0	1	1	< 0.879	N/A	-
	2	1	< 0.879		
	43	6	< 1.41	N/A	-
	44	6	< 1.41		
1.8	3-8	1	-	(see Table 4)	100
	45	6	1.94	$\bar{x}$ =2.0 ppm a.i.	111
	46	6	1.94	SD = 0.092 ppm a.i.	
	47	6	2.10	CV = 4.6%	
6.2	9-14	1	-	(see Table 4)	97
	48	6	6.31	$\bar{x}$ = 6.0 ppm a.i.	97
	49	6	6.52	SD = 0.67 ppm a.i.	
	50	6	5.27[3]	CV = 11%	
17.6	15-20	1	-	(see Table 4)	100
	51	6	17.5	$\bar{x}$ = 16.8 ppm a.i.	95
	52	6	16.5	SD = 0.608 ppm a.i.	
	53	6	16.4	CV = 3.6%	

[1] Concentrations were corrected for change in test substance purity (98.9% to 86.9%) per Certificate of Analysis dated October 11, 2001. Nominal test concentrations based upon the new test substance purity are 1.8, 6.2 and 17.6 ppm a.i.
[2] Less than values correspond to limit of quantitation (LOQ).
[3] Mean result of duplicate re-extraction of original sample.

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TABLE 6

Ambient Stability of PFOS in Avian Diet During the Mallard Pilot Reproduction Study

	PFOS Concentration						
	Week 1, Day 0[1]			Week 1, Day 7			
Nominal Concentration[2] (ppm a.i.)	Sample Number (S-454-105-)	Mean Measured (ppm a.i.)	Mean Percent of Nominal	Sample Number (S-454-105-)	Measured[2,3] (ppm a.i.)	Mean Measured (ppm a.i.)	Mean Percent of Day 0
0	1-2	< 0.879	-	32	< 1.41	-	-
				33	< 1.41		
1.8	3-8	1.8	100	34	1.50	1.6	89
				35	1.49		
				36	1.85		
6.2	9-14	6.0	97	37	5.48	5.2	87
				38	5.01		
				39	5.03		
17.6	15-20	17.6	100	40	18.7	18.4	105
				41	18.8		
				42	17.8		

[1] Day 0 values are from homogeneity samples presented in Table 4 and verification samples presented in Table 5.
[2] Concentrations were corrected for change in test substance purity (98.9% to 86.9%) per Certificate of Analysis dated October 11, 2001. Nominal test substance concentrations based upon the new test substance purity are 1.8, 6.2 and 17.6 ppm a.i.
[3] Less than values correspond to limit of quantitation (LOQ).

Appendix IV

METHOD OUTLINE FOR THE ANALYSIS OF PFOS IN AVIAN DIET

Prepare matrix fortification samples in the desired avian feed stock using the dry mix technique.

↓

Weigh 10 g samples of the matrix blank, matrix fortification and test samples into weigh boats and transfer to 8-oz. French square glass bottles. Record weights.

↓

For each sample, measure 100 mL of methanol with a graduated cylinder and transfer into the French square bottle.

↓

Cap bottles and place on shaker table. Allow the samples to shake for a minimum of 30 minutes at approximately 250 rpm.

↓

Vacuum filter with qualitative filter paper and rinse retained feed 3 times with methanol into filtrate.

↓

Transfer the filtrate into a 200-mL volumetric flask and bring to volume with methanol.

↓

Prepare appropriate dilution(s) to bring final concentration into the calibration range of the LCMS methodology. Use methanol for intermediate dilutions, if required. For all final dilutions, use 50% methanol: 50% NANOpure® water dilution solvent containing 0.0100 mg 4HPFOS/L (internal standard) and 0.05% (v/v) formic acid.

↓

Ampulate and submit sample for LCMS analysis.

Figure 1. Analytical method flow chart for the analysis of PFOS in avian diet.

Appendix IV

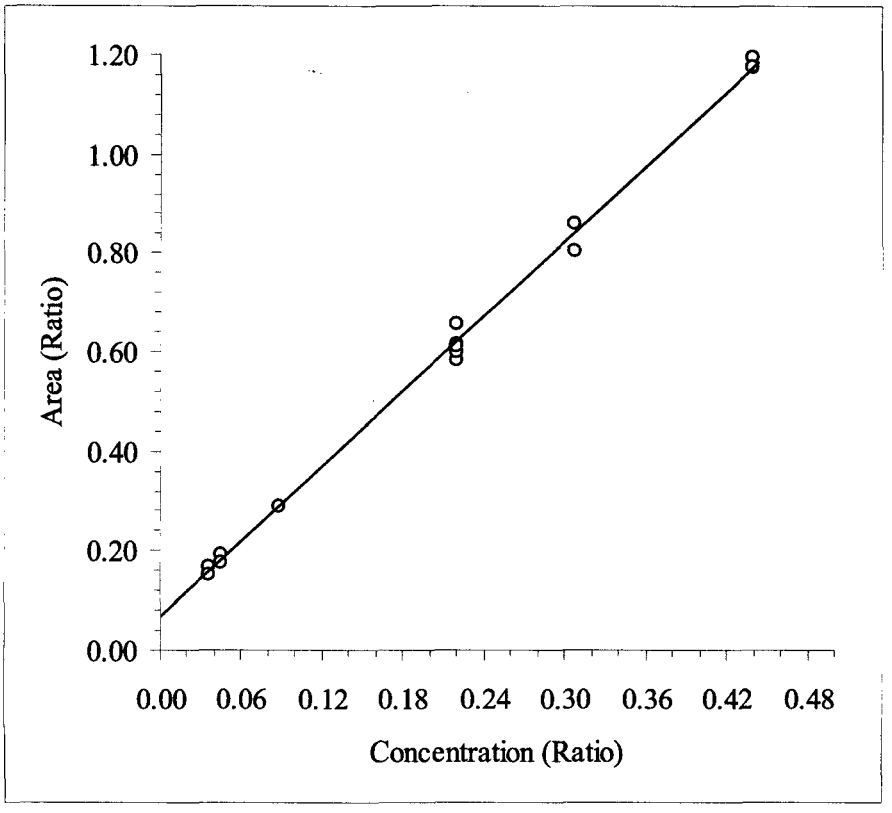

Figure 2. Typical calibration curve for PFOS. Slope = 2.48679; Intercept = 0.07396; r = 0.9985. Curve is weighted (1/x).

Appendix IV

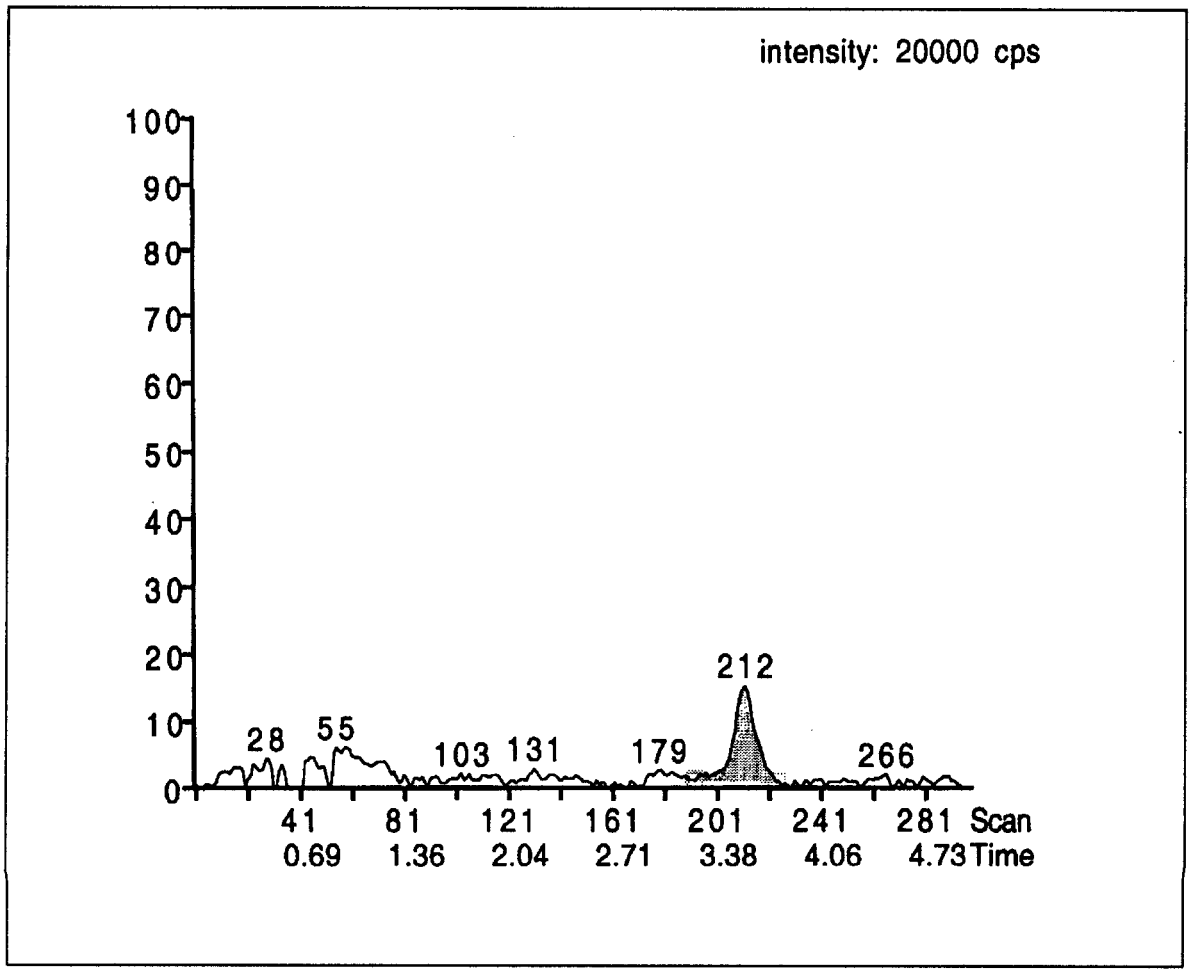

Figure 3. Typical ion chromatogram of a low-level PFOS standard, 0.000351 mg a.i./L.

Appendix IV

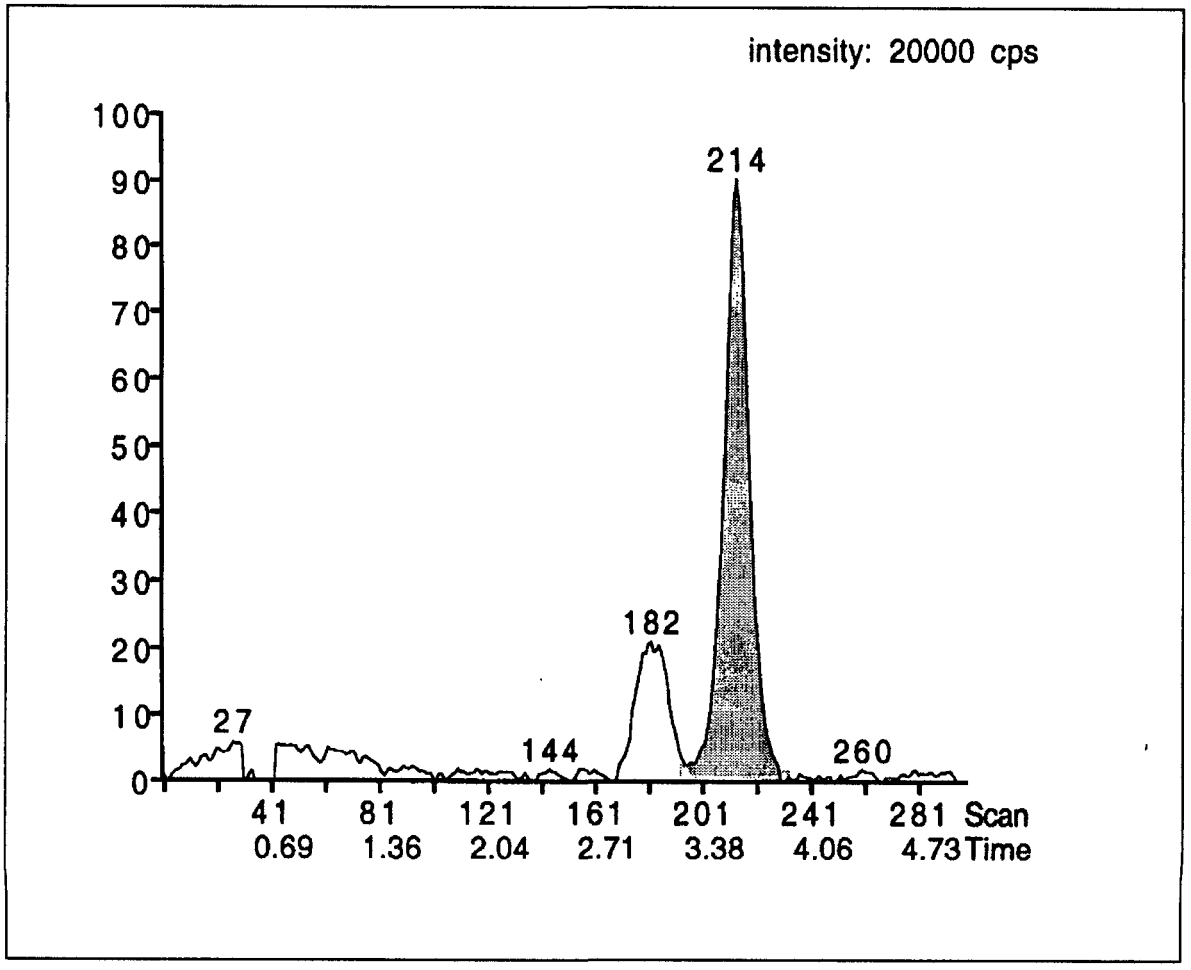

Figure 4. Typical ion chromatogram of a high-level PFOS standard, 0.00439 mg a.i./L.

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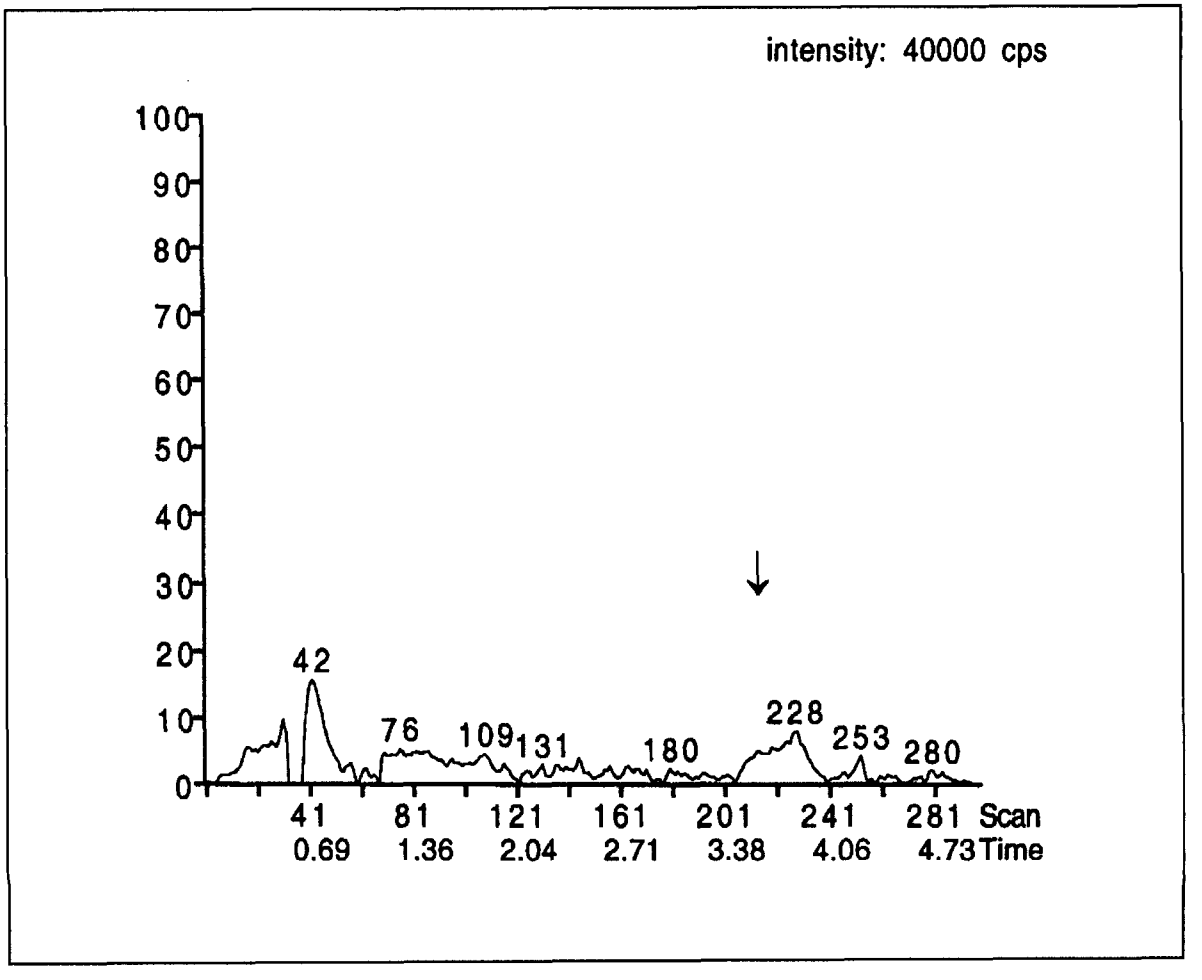

Figure 5. Typical ion chromatogram of a matrix blank sample (454-105-MAB-1). The arrow indicates approximate retention time of PFOS.

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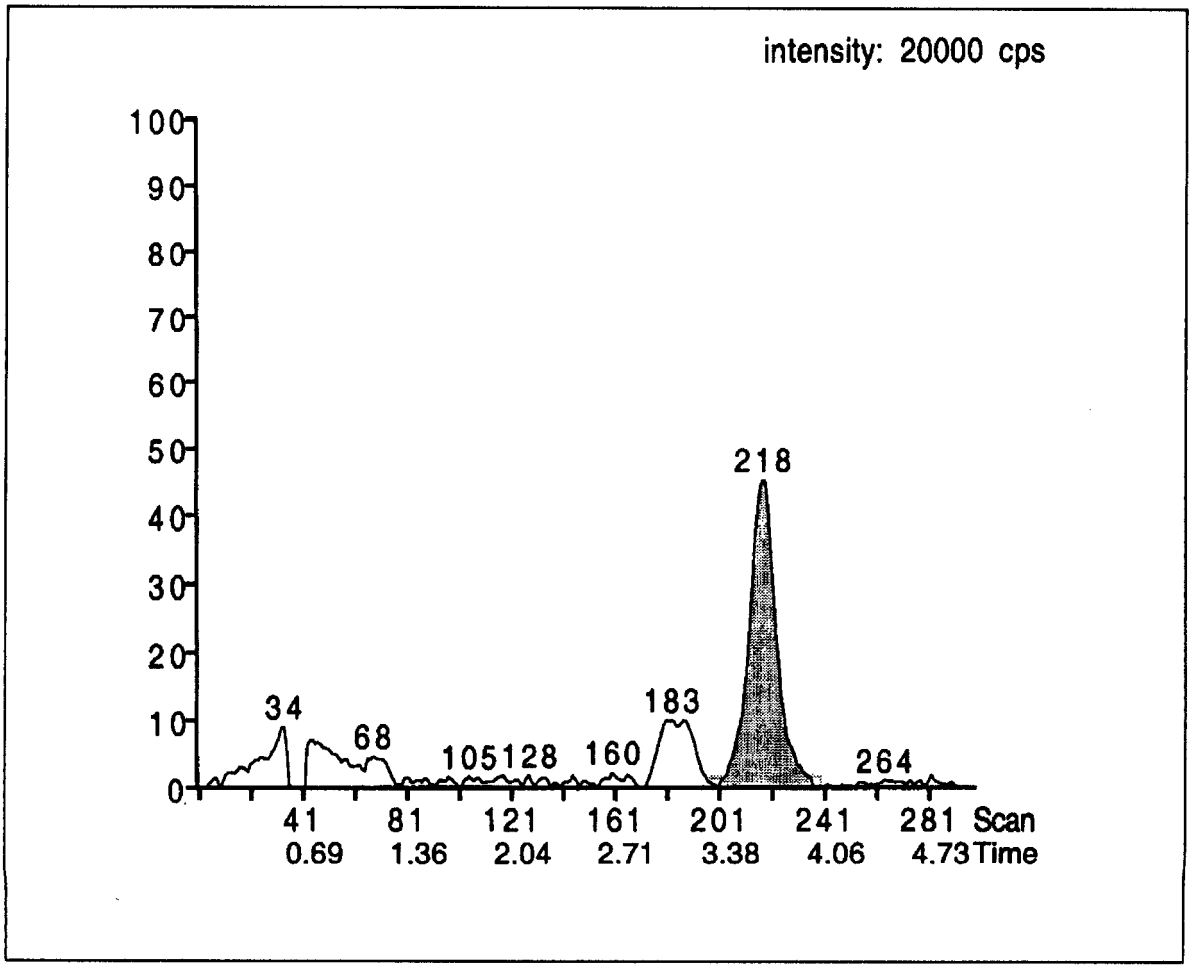

Figure 6. Typical ion chromatogram of a matrix fortification sample (454-105-MAS-9, 22.0 ppm a.i.).

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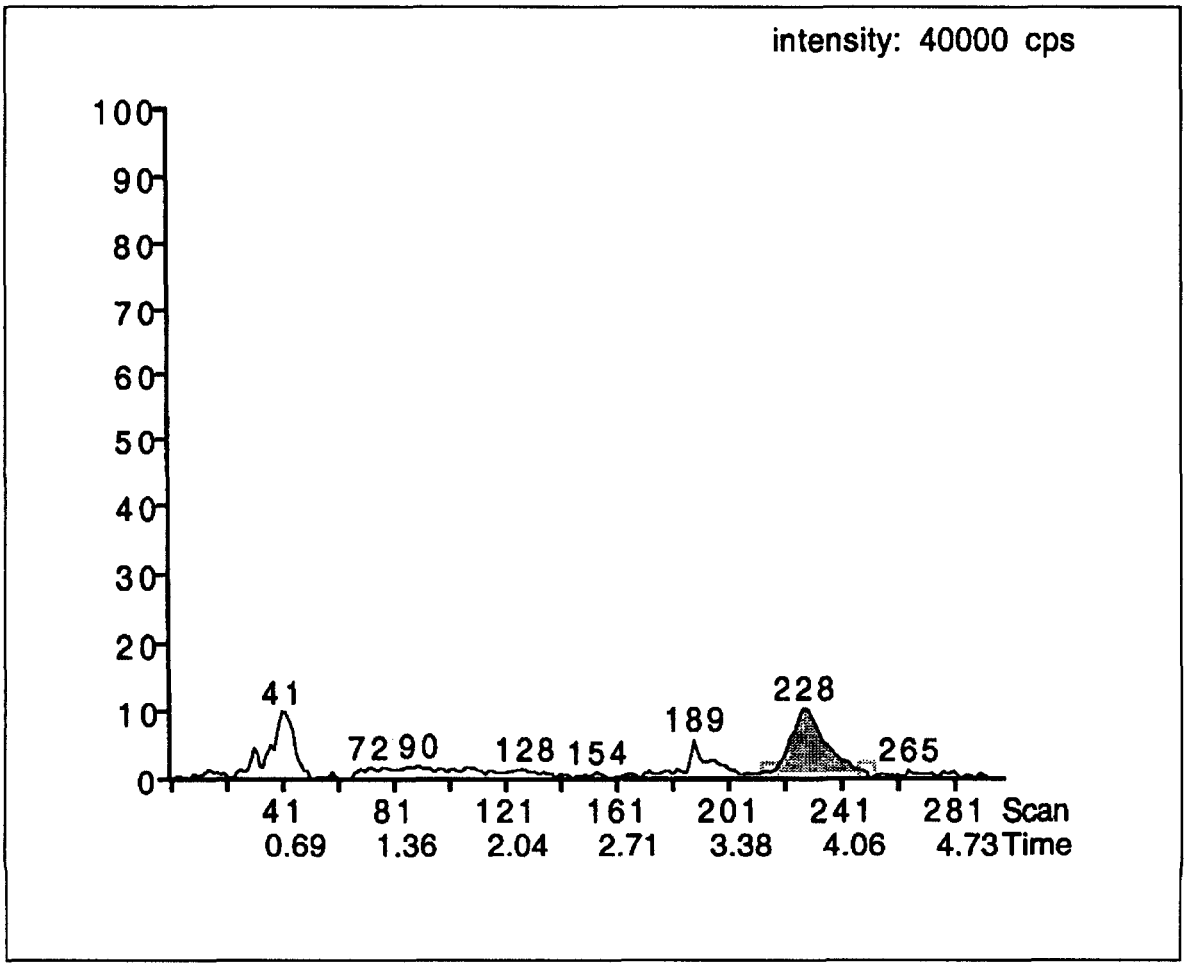
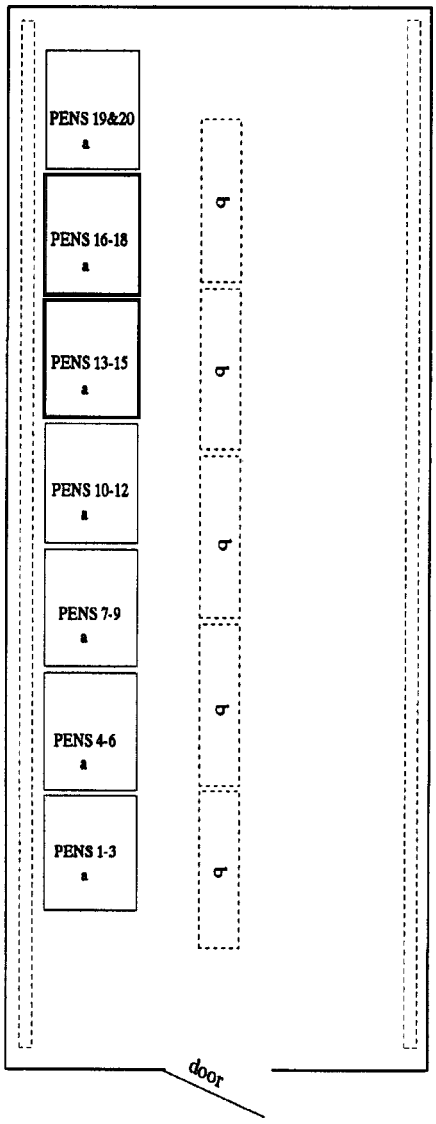


Figure 7. Typical ion chromatogram of an avian diet sample on Day 0 (S-454-105-3, 1.8 ppm a.i.).

Appendix V
Diagram of Test Layout



|—— —| = approx. 1m

= 6" gutter

a = Safeguard Products Inc. Model No. 5355

b = Four X 4 ft. Chroma 50 light bulbs

Appendix VI
Table 1
Adult Body Weight (g) from a Mallard
Pilot Reproduction Study with PFOS

Control (0 ppm a.i.) - Males

Pen	Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6	Change 6-8	Week 8	Change 8-10	Week 10	Change 10-11	Week 11	Change 11-20	Week 20	Change 0-20
201	1080	10	1090	13	1103	29	1132	52	-26	1106	71	1177	44	1221	-136	1085	5
202	1121	-33	1088	13	1101	47	1148	27	16	1164	37	1201	18	1219	44	1263	142
203	1064	-57	1007	-41	966	41	1007	-57	35	1042	80	1122	107	1229	-116	1113	49
204	1198	-133	1065	71	1136	65	1201	3	61	1262	72	1334	10	1344	-58	1286	88
205	1139	33	1172	-68	1104	26	1130	-9	8	1138	36	1174	21	1195	77	1272	133
Mean	1120	-36	1084	-2	1082	42	1124	3	19	1142	59	1202	40	1242	-38	1204	83
SD	53	65	59	54	66	16	71	41	32	81	21	79	40	59	95	97	58

Control (0 ppm a.i.) - Females

Pen	Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6	Change 6-8	Week 8	Change 8-10	Week 10	Change 10-11	Week 11	Change 11-20	Week 20	Change 0-20
201	1074	-172	902	161	1063	-69	994	-80	-47	947	94	1041	-1	1040	-19	1021	-53
202	1027	-50	977	28	1005	-9	996	-31	-14	982	39	1021	-85	936	66	1002	-25
203	1108	-36	1072	-20	1052	96	1148	40	-100	1048	-3	1045	23	1068	-98	970	-138
204	928	28	956	174	1130	-10	1120	192	-44	1076	-65	1011	18	1029	1	1030	102
205	851	52	903	101	1004	-1	1003	152	-64	939	67	1006	-6	1000	2	1002	151
Mean	998	-36	962	89	1051	1	1052	55	-54	998	26	1025	-10	1015	-10	1005	7
SD	106	87	70	84	52	59	75	116	31	61	62	18	44	50	59	23	118

The means for body weights and body weight changes are calculated and rounded separately.

Appendix VI
Table 2
Adult Body Weight (g) from a Mallard
Pilot Reproduction Study with PFOS

1.8 ppm a.i. - Males

Pen	Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6
206	1165	-12	1153	8	1161	74	1235	70
207	1147	-27	1120	-36	1084	44	1128	-19
208	1070	54	1124	6	1130	63	1193	123
209	987	19	1006	-29	977	96	1073	86
210	1095	-85	1010	4	1014	36	1050	-45
Mean	1093	-10	1083	-9	1073	63	1136	43
SD	70	52	69	21	77	24	78	72

1.8 ppm a.i. - Females

Pen	Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6
206	895	178	1073	69	1142	-8	1134	239
207	969	76	1045	-81	964	-2	962	-7
208	1057	11	1068	5	1073	36	1109	52
209	964	122	1086	-6	1080	88	1168	204
210	1132	55	1187	-51	1136	60	1196	64
Mean	1003	88	1092	-13	1079	35	1114	110
SD	92	64	55	57	72	41	91	106

The means for body weights and body weight changes are calculated and rounded separately.

Appendix VI
Table 3
Adult Body Weight (g) from a Mallard
Pilot Reproduction Study with PFOS

6.2 ppm a.i. - Males

Pen	Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6
211	1096	-50	1046	38	1084	58	1142	46
212	1077	-57	1020	51	1071	-55	1016	-61
213	1051	-17	1034	22	1056	128	1184	133
214	1097	-27	1070	92	1162	-24	1138	41
215	1135	-13	1122	-7	1115	-4	1111	-24
Mean	1091	-33	1058	39	1098	21	1118	27
SD	31	20	40	37	42	73	63	74

6.2 ppm a.i. - Females

Pen	Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6
211	985	168	1153	39	1192	8	1200	215
212	1032	39	1071	84	1155	-163	992	-40
213	1049	-21	1028	-79	949	36	985	-64
214	890	85	975	18	993	-27	966	76
215	983	59	1042	78	1120	-71	1049	66
Mean	988	66	1054	28	1082	-43	1038	51
SD	62	69	65	66	105	78	95	111

The means for body weights and body weight changes are calculated and rounded separately.

Appendix VI
Table 4
Adult Body Weight (g) from a Mallard
Pilot Reproduction Study with PFOS

17.6 ppm a.i. - Males

Pen	Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6	Change 6-8	Week 8	Change 8-10	Week 10	Change 10-11	Week 11	Change 11-20	Week 20	Change 0-20
216	1028	-82	946	19	965	48	1013	-15	72	1085	-13	1072	-19	1053	-111	942	-86
217	1258	-111	1147	-13	1134	0	1134	-124	-52	1082	80	1162	8	1170	-8	1162	-96
218	1138	-33	1105	-4	1101	34	1135	-3	-87	1048	-68	980	-12	968	80	1048	-90
219	1062	-10	1052	-6	1046	40	1086	24	18	1104	-6	1098	-22	1076	24	1100	38
220	987	29	1016	-18	998	67	1065	78	-15	1050	28	1078	18	1096	-139	957	-30
Mean	1095	-41	1053	-4	1049	38	1087	-8	-13	1074	4	1078	-5	1073	-31	1042	-53
SD	107	56	78	14	70	25	51	74	62	24	55	65	18	73	92	94	57

17.6 ppm a.i. - Females

Pen	Week 0	Change 0-2	Week 2	Change 2-4	Week 4	Change 4-6	Week 6	Change 0-6	Change 6-8	Week 8	Change 8-10	Week 10	Change 10-11	Week 11	Change 11-20	Week 20	Change 0-20
216	1075	-84	991	43	1034	-126	908	-167	49	957	-29	928	-31	897	54	951	-124
217	895	97	992	-6	986	-76	910	15	95	1005	16	1021	-5	1016	-123	893	-2
218	1112	-95	1017	60	1077	-1	1076	-36	32	1108	-53	1055	9	1064	-191	873	-239
219	788	78	866	32	898	-85	813	25	-48	765	-73	692	-5	687	6	693	-95
220	1125	120	1245	-74	1171	12	1183	58	-44	1139	2	1141	-17	1124	-47	1077	-48
Mean	999	23	1022	11	1033	-55	978	-21	17	995	-27	967	-10	958	-60	897	-102
SD	150	104	138	53	102	59	149	88	62	148	37	172	15	173	98	139	90

The means for body weights and body weight changes are calculated and rounded separately.

Appendix VII
Table 1
Feed Consumption (g/bird/day) from a Mallard
Pilot Reproduction Study with PFOS

Control (0 ppm a.i.)

	Weeks																		
Pen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
201	183	132	178	216	190	262	258	282	229	233	263	223	112	232	125	110	145	116	155
202	123	137	157	164	188	185	163	188	224	190	168	149	129	190	86	111	154	133	151
203	117	135	128	130	157	169	130	149	172	121	240	168	154	182	126	91	121	114	141
204	140	98	164	216	191	244	238	278	237	196	249	166	159	200	158	143	198	116	248
205	167	117	122	165	203	198	209	282	238	317	261	169	139	174	198	159	220	172	155
Mean	146	124	150	178	186	212	200	236	220	211	236	175	139	196	139	123	168	130	170
SD	28	16	24	37	17	40	53	63	28	71	39	28	19	22	42	28	41	25	44

Appendix VII
Table 2
Feed Consumption (g/bird/day) from a Mallard
Pilot Reproduction Study with PFOS

1.8 ppm a.i.

	Weeks					
Pen	1	2	3	4	5	6
206	130	130	174	185	156	151
207	110	109	105	94	90	106
208	189	158	149	167	173	187
209	135	167	127	215	183	228
210	223	188	216	200	199	215
Mean	157	150	154	172	160	177
SD	47	31	43	47	42	50

Appendix VII
Table 3
Feed Consumption (g/bird/day) from a Mallard
Pilot Reproduction Study with PFOS

6.2 ppm a.i.

	Weeks					
Pen	1	2	3	4	5	6
211	137	133	168	176	208	196
212	122	92	187	177	182	127
213	194	186	197	247	312	260
214	110	109	122	143	96	125
215	164	166	168	213	179	181
Mean	145	137	168	191	195	178
SD	34	39	29	40	78	56

Appendix VII
Table 4
Feed Consumption (g/bird/day) from a Mallard
Pilot Reproduction Study with PFOS

17.6 ppm a.i.

	Weeks																		
Pen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
216	121	138	269	209	266	219	266	303	284	221	193	173	188	211	112	146	265	175	216
217	102	96	132	133	135	127	156	150	195	160	248	206	178	234	207	191	163	131	223
218	161	135	218	223	244	267	238	299	306	290	350	324	296	338	222	170	294	228	274
219	130	114	139	150	154	200	190	159	168	155	179	177	104	288	118	259	213	165	195
220	265	198	242	220	180	355	277	293	314	306	275	211	209	266	200	188	254	216	228
Mean	156	136	200	187	196	234	225	241	253	226	249	218	195	267	172	191	238	183	227
SD	65	38	62	42	57	84	51	79	67	70	69	62	69	49	52	42	51	39	29

Appendix VIII
Table 1

Individual Gross Pathological Observations
from a Mallard Pilot Reproduction Study with PFOS
Birds Euthanized at Test Termination

Control (0 ppm a.i.)

Males	Pens				
	201	202	203	204	205
Molting flight feathers	X	X	-	-	X
Bumble foot	-	X	X	X	-
Testes small - ~ 2.0 - 3.0 cm	X	-	-	-	X
Testes small - ~ 3.5 cm	-	X	X	-	-
Not remarkable	-	-	-	-	-

Females	Pens				
	201	202	203	204	205
Molting flight feathers	X	-	-	-	X
Bumble foot	X	-	X	-	X
Regressed ovary	X	-	X	X	X
Egg remnants in the reproductive tract	-	-	-	X	-
Not remarkable	-	X	-	-	-

Appendix VIII
Table 2

Individual Gross Pathological Observations
from a Mallard Pilot Reproduction Study with PFOS
Birds Euthanized at Test Termination

1.8 ppm a.i.

Males	Pens				
	206	207	208	209	210
Bumble foot	-	-	-	-	X
Not remarkable	X	X	X	X	-

Females	Pens				
	206	207	208	209	210
Bumble foot	X	X	-	-	X
Regressing ovary	-	X	-	-	-
Egg yolk peritonitus	-	-	-	X	-
Not remarkable	-	-	X	-	-

Appendix VIII
Table 3

Individual Gross Pathological Observations
from a Mallard Pilot Reproduction Study with PFOS
Birds Euthanized at Test Termination

6.2 ppm a.i.

Males	Pens				
	211	212	213	214	215
Bumble foot	-	X	-	X	X
Not remarkable	X	-	X	-	-

Females	Pens				
	211	212	213	214	215
Feather loss and abrasions on neck	-	-	X	-	-
Bumble foot	X	-	X	-	X
Egg yolk peritonitus	X	-	-	X	-
Extensive egg yolk peritonitus	-	-	X	-	-
Intrabdominal egg	-	-	X	-	-
Not remarkable	-	X	-	-	-

Appendix VIII
Table 4

Individual Gross Pathological Observations
from a Mallard Pilot Reproduction Study with PFOS
Birds Euthanized at Test Termination

17.6 ppm a.i.

Males	Pens				
	216	217	218	219	220
Mass of feed impacted under the tongue	-	-	X	-	-
Bumble foot	X	X	X	X	-
Testes small - ~ 1.0 - 2.0 cm	-	X	X	X	-
Testes small - ~ 2.5 cm	-	-	-	-	X
Not remarkable	-	-	-	-	-

Females	Pens				
	216	217	218	219	220
General condition - abnormally small body[1]	-	-	-	X	-
Molting flight feathers	X	X	-	X	-
Bumble foot	-	-	X	-	-
Air sacculitis	X	-	-	-	-
Slight egg yolk peritonitus	X	-	-	-	-
Regressed ovary	X	X	X	X	X
Not remarkable	-	-	-	-	-

[1] Denotes a small body frame, but does not indicate a thin condition.

Appendix IX

Histopathology Report

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

WILDLIFE INTERNATIONAL, LTD.
PROJECT NUMBER 454-105
EPL PROJECT NUMBER 212-025

PFOS: A PILOT REPRODUCTION STUDY WITH THE MALLARD

PATHOLOGY REPORT

Submitted by:
Experimental Pathology Laboratories, Inc.
P.O. Box 474
Herndon, VA 20172-0474
(703) 471-7060

Submitted to:

Wildlife International, Ltd.
Easton, MD 21601

January 25, 2001

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

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PATHOLOGY SUMMARY

EPL®

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

WILDLIFE INTERNATIONAL, LTD.
STUDY NUMBER 454-105
EPL PROJECT NUMBER 212-025

PFOS: A PILOT REPRODUCTION STUDY
WITH THE MALLARD

PATHOLOGY SUMMARY

Light microscopic examination was performed on sections of selected tissues from adult male and female mallards (*Anas platyrhynchos*) which were untreated or which received various concentrations of the test article (Perfluorooctanesulfonic acid, potassium salt [PFOS]) in the feed for six to 19 weeks. Selected tissues from untreated approximately 12-week-old offspring of the adult mallards were also examined by light microscopy. The objective of this study is to evaluate the effects upon adult mallards (*Anas platyrhynchos*) of dietary exposure to a test substance over at least a six-week period. The experimental design was as follows:

Group *	Adults		Offspring **	
	Males	Females	Males	Females
Control (0 ppm a. i.)	5	5	5	5
1.8 ppm a. i. PFOS	5	5	4	6
6.2 ppm a. i. PFOS	5	5	4	6
17.6 ppm a. i. PFOS	5	5	5	5

* Mallards in the control and 17.6 ppm a.i. groups were treated for 19 weeks; mallards in the 1.8 and 6.2 ppm a.i. groups were treated for six weeks.
** Offspring in all groups did not receive the test article.

MATERIALS AND METHODS

At the completion of the various study intervals, all adult mallards were euthanized, and necropsies were performed by Wildlife International, Ltd.

Selected offspring were euthanized at approximately 12 weeks of age using carbon dioxide gas, and samples for histopathological evaluation were collected by Wildlife International, Ltd. Selected tissues from adults and offspring were fixed in 10% neutral buffered formalin and sent to Experimental Pathology Laboratories, Inc. (EPL®), where they were embedded in paraffin and made into hematoxylin and eosin-stained sections on glass microscope slides. The following tissues from adults and offspring were examined by light microscopy as available: liver, gallbladder, proventriculus, kidney, brain, gonad (ovary or testis), bursa of Fabricius, and adipose tissue. In some cases, nonprotocol-required tissues were sectioned along with adjacent protocol-required tissues; these were evaluated, and microscopic findings were recorded at the discretion of the pathologist.

All tissues required by protocol are presented in the Histopathology Incidence Tables. Microscopic findings for each tissue examined from each mallard are listed in the Histopathology Incidence Tables. Microscopic changes were graded one to five depending on severity. Nongradable findings are listed as present (P) and tissues with extensive autolysis are listed as (A). All findings for all animals are summarized by sex, age group, and treatment group in the Summary Incidence Tables, together with the total number of animals in each group for which the tissues were examined.

A tabulation of gross observations noted at necropsy with the corresponding microscopic change, if appropriate, is presented in the Correlation of Gross and Microscopic Findings tables. The entries in these tables were transcribed from the Gross Necropsy records provided by Wildlife International, Ltd.

RESULTS

All adults and offspring survived to the end of their respective study intervals. No effects considered possibly related to test article (PFOS) administration were noted in liver, kidney, gallbladder, proventriculus, ovary, brain, and/or bursa of Fabricius of male and female adult mallards or their offspring, in the adipose tissue of adult females and offspring of both sexes, or in the testes of offspring males.

Testes of adult males exhibited several features most consistent with post-reproductive phase regression, a normal physiological phenomenon, including aspermia, decreased spermatogenesis, and/or decreased seminiferous tubule diameter. Group differences in the extent of these findings were evident.

Aspermia, or complete absence of mature spermatozoa, was noted only in the testes of 3/5 17.6 ppm a.i. adult males, while decreased spermatogenesis, characterized by lower numbers of mature spermatozoa than expected in actively reproductive testes, was seen in 1/5 17.6 ppm a.i. males but in 4/5 control adult males. While seminiferous tubules from both control and 17.6 ppm a.i. adult males had decreased diameter, the mean decrease in 17.6 ppm a.i. adult males was more pronounced than in controls. Correspondingly, the size range of 17.6 ppm a.i. testes recorded at necropsy was 1.0-2.5 cm, lower than the 2.0-3.5 cm range recorded for control testes.

Overall, these findings are suggestive of more advanced testicular regression in the 17.6 ppm a.i. adult males compared to controls. This may have been a fortuitous event accentuated by the small group sizes, but the possibility of a test article effect cannot be entirely ruled out.

All offspring testes examined were listed as immature because they lacked all evidence of spermatogenesis, had very small seminiferous tubules with absent or narrow lumens, and had germinal epithelium with decreased cell layers

(compared to adult testes). This morphology was consistent with normal physiological immaturity in young (approximately 12-week-old) birds rather than a pathological effect.

Ovaries of many control and 17.6 ppm a.i. adult females exhibited fibrosis and/or decreased follicular diameter. Decreased follicular diameter corresponded to grossly observed "regressed ovary." Affected follicles were smaller and had shorter stalks than follicles in reproductively active ovaries. Fibrosis was characterized by a few nodular foci of mature connective tissue scattered through the ovarian cortex. These ovarian findings were most consistent with early post-reproductive phase regression, a normal physiological phenomenon. Since the incidence and degree of these findings were similar in control and 17.6 ppm a.i. female adult groups, they were considered incidental and unrelated to treatment.

Ovaries of all offspring females were characterized by numerous oocytes and very small, nonpedunculated follicles completely embedded in a prominent ovarian cortex. This morphology is consistent with normal physiological immaturity in young (approximately 12-week-old) birds rather than a pathological effect.

Adipose tissue of adults and offspring consisted of sheets of large white adipocytes with single large lipid droplets, among which were often scattered a few adipocytes which contained multiple, smaller, discrete vacuoles. While almost all samples of adipose tissue exhibited a few of these smaller adipocytes, microvesiculation was diagnosed only when the numbers of these cells were increased. Microvesiculation was noted in both control and treated adult and offspring mallards, and was usually a very subtle, minimal finding. A clearly increased incidence was noted only in 17.6 ppm a.i. adult males compared to control adult males. This increase may have been a fortuitous event accentuated

by small group sizes or possible variability in adipose tissue sampling sites, but the possibility of a test article effect cannot be entirely ruled out.

Incidences of adipose tissue microvesiculation in male and female offspring did not exhibit clear, dose-related increases, and were considered incidental and unrelated to treatment.

Several additional microscopic findings (described below) were noted in adult and/or offspring mallards from control and treated groups, including mononuclear cell infiltrates in various tissues; kidney mineralization; ureteral lamina propria cyst; and involution of the bursa of Fabricius. Incidences and mean severity were similar when groups from each generational cohort were compared with each other. For these reasons, these findings were considered incidental and unrelated to test article (PFOS) administration.

Mononuclear cell infiltrates in various tissues consisted of discrete foci of small lymphocytes with occasional monocytes and macrophages. Scattered clusters of coarsely to finely dark basophilic material in the renal cortex denoted kidney mineralization. In one 6.2 ppm a.i. offspring female, a section of ureter fortuitously sectioned along with kidney exhibited a single lamina propria cyst lined by flattened epithelium and filled with amorphous, pale basophilic material. Only one adult mallard (a control male) had a bursa of Fabricius available for microscopic evaluation. The bursa in this individual exhibited the age-related involution expected in adult birds. The bursa of Fabricius was present and unremarkable in all male and female offspring.

Additional findings were also noted in the liver of adults and/or offspring. Liver pigment deposition consisted of dust-like, brown to golden-brown pigment granules present in hepatocyte cytoplasm, Kupffer cells, and/or periportal areas; the identity of the pigment was not determined, but may have been related to bile. The liver of one adult control female exhibited amyloid deposition consisting

of amorphous, pale bluish-violet material which filled and distended the hepatic sinusoids, and compressed adjacent hepatic cords. Minimal focal hepatocellular degeneration/necrosis occurred only in a few control and treated offspring. It consisted of a few small discrete foci in which hepatocytes were pale to hypereosinophilic, had indistinct outlines, and fragmented or condensed nuclei. Hepatocellular degeneration/necrosis was considered incidental and unrelated to treatment.

In adults and offspring, liver fatty change denoted variably sized, sharply demarcated, clear vacuoles (consistent with fat accumulation) in the hepatocellular cytoplasm. Liver hepatocellular vacuolization denoted multiple, usually small, confluent, poorly demarcated vacuoles which often resulted in a "lace-like" appearance of the hepatocellular cytoplasm. When present in most hepatocytes, these changes were designated "diffuse," and when present in a few scattered areas, they were designated as "focal."

Hepatocellular fatty change occurred sporadically at low incidences and similar severity in control and treated adults and offspring, and was considered incidental. In adults and offspring, hepatocellular vacuolization had similar incidences when control and treated groups of each sex were compared, but marginal, not always dose-related increases in severity were sometimes noted. It seems more likely that these very minor differences were more related to biological variation accentuated by small group sizes rather than being test article effects.

CONCLUSIONS AND SUMMARY

No lesions considered possibly related to test article (PFOS) administration were noted in liver, kidney, proventriculus, gallbladder, ovary, brain, and/or bursa of Fabricius, brain of adult male and female mallards or their

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offspring, in the adipose tissue of adult females and offspring of both sexes, or in the testes of offspring males.

Testes of adult males exhibited several features most consistent with post-reproductive phase regression, a normal physiological phenomenon, including aspermia, decreased spermatogenesis, and/or decreased seminiferous tubule diameter. Group differences in the extent of these findings were evident, with aspermia occurring only in the high dose (17.6 ppm a.i.) adult males, and the degree of decrease in seminiferous tubule diameter being generally more pronounced in 17.6 ppm a.i. adult males than in control adult males. Overall, these findings are suggestive of more advanced testicular regression in the 17.6 ppm a.i. adult males compared to controls. Adult males in the 17.6 ppm a.i. group also exhibited clearly increased incidences of adipose tissue microvesiculation. These testicular and adipose tissue findings in 17.6 ppm a.i. adult males may have been fortuitous events accentuated by the small group sizes, but the possibility that they were related to test article administration cannot be entirely ruled out.

The few other changes in various tissues of adult and/or offspring mallards from control and treated groups were considered incidental and unrelated to test article (PFOS) administration.

Margarita M. Gruebbel

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Veterinary Pathologist
1/25/01

MMG/lcp

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: PFOS: A Pilot Reproduction Study with the Mallard

Client Study: 454-105 EPL Project Coordinator: Dr. Margarita M. Gruebbel

EPL Project Number: 212-025 EPL Pathologist: Dr. Margarita M. Gruebbel

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Project Coordinator and Management are indicated below.

	Dates	
Area Inspected	Inspection	Reporting
EPL Project Sheets	9/11/00; 10/3/00	9/11/00; 10/3/00
Project Setup	9/28/00; 10/3/00	9/28/00; 10/3/00
Histology Setup	10/3/00	10/3/00
Data Review	11/13,15/00	11/13,15/00
Draft Report	12/13,14,27/00	12/14,27/00
Final Report	1/29/01	1/29/01

Date of last quarterly facility inspection 9/00

Patricia L. Runge
EPL Quality Assurance Unit

1/29/01
Date

SUMMARY INCIDENCE TABLES

ADULT SACRIFICE

SUMMARY INCIDENCE TABLE

454-105
Adult Sacrifice
Male Mallard

	GROUP CONTROL	GROUP 17.6				
ADIPOSE TISSUE (NO. EXAMINED)	(5)	(5)				
Infiltrate, Mononuclear Cell						
Microvesiculation	1	5				
BRAIN (NO. EXAMINED)	(5)	(5)				
BURSA OF FABRICIUS (NO.						
EXAMINED)	(1)					
Involution	1					
GALLBLADDER (NO. EXAMINED)	(5)	(5)				
Infiltrate, Mononuclear Cell	4	3				
KIDNEY (NO. EXAMINED)	(5)	(5)				
Infiltrate, Mononuclear Cell		2				
Mineralization						
LIVER (NO. EXAMINED)	(5)	(5)				
Amyloid Deposition						
Hepatocyte(s), Fatty Change,						
Diffuse	1					
Hepatocyte(s), Fatty Change,						
Focal	1					
Hepatocyte(s), Vacuolization,						
Diffuse	2	4				
Infiltrate, Mononuclear Cell	5	5				
Pigment Deposition	4	2				
PROVENTRICULUS (NO. EXAMINED)	(5)	(5)				
Infiltrate, Mononuclear Cell	5	5				
TESTIS (NO. EXAMINED)	(5)	(5)				
Aspermia		3				
Seminiferous Tubules,						
Decreased Diameter	4	5				
Spermatogenesis, Decreased	3	1				

SUMMARY INCIDENCE TABLE

454-105
Adult Sacrifice
Female Mallard

	GROUP CONTROL	GROUP 17.6				
ADIPOSE TISSUE (NO. EXAMINED)	(5)	(5)				
Infiltrate, Mononuclear Cell	1	2				
Microvesiculation	2	2				
BRAIN (NO. EXAMINED)	(5)	(5)				
BURSA OF FABRICIUS (NO.						
EXAMINED)						
Involution						
GALLBLADDER (NO. EXAMINED)	(5)	(5)				
Infiltrate, Mononuclear Cell	5	4				
KIDNEY (NO. EXAMINED)	(5)	(5)				
Infiltrate, Mononuclear Cell	4	4				
Mineralization		1				
LIVER (NO. EXAMINED)	(5)	(5)				
Amyloid Deposition	1					
Hepatocyte(s), Fatty Change,						
Diffuse	1					
Hepatocyte(s), Fatty Change,						
Focal	1					
Hepatocyte(s), Vacuolization,						
Diffuse	3	4				
Infiltrate, Mononuclear Cell	5	5				
Pigment Deposition		1				
OVARY (NO. EXAMINED)	(5)	(5)				
Fibrosis	3	2				
Follicles, Decreased Diameter	4	5				
PROVENTRICULUS (NO. EXAMINED)	(5)	(5)				
Infiltrate, Mononuclear Cell	5	4				

HISTOPATHOLOGY INCIDENCE TABLES

ADULT SACRIFICE

HISTOPATHOLOGY INCIDENCE TABLE

454-105
Adult Sacrifice
Male Mallard

	GROUP CONTROL						GROUP 17.6				
ANIMAL	19961	19963	19965	19967	19969		19991	19993	19995	19997	19999
ADIPOSE TISSUE	X		X	X	X						
Infiltrate, Mononuclear Cell											
Microvesiculation		1					1	1	2	1	1
BRAIN	X	X	X	X	X		X	X	X	X	X
BURSA OF FABRICIUS		N	N	N	N		N	N	N	N	N
Involution	4										
GALLBLADDER	X						X			X	
Infiltrate, Mononuclear Cell		1	2	1	1			1	1		1
KIDNEY	Xm	Xm	Xm	Xm	Xm		m	Xm	X	m	Xm
Infiltrate, Mononuclear Cell							1			1	
Mineralization											
LIVER											
Amyloid Deposition											
Hepatocyte(s), Fatty Change, Diffuse		1									
Hepatocyte(s), Fatty Change, Focal					1						
Hepatocyte(s), Vacuolization, Diffuse			2	2			2	3	2		2
Infiltrate, Mononuclear Cell	1	1	1	1	2		1	1	1	1	2
Pigment Deposition	2	1	1		2					1	2
PROVENTRICULUS											
Infiltrate, Mononuclear Cell	1	1	1	1	1		1	1	1	1	1
TESTIS	m	m	m	Xm	m		m	m	m	m	m
Aspermia								P	P	P	
Seminiferous Tubules, Decreased Diameter	2	2	1		3		1	4	5	5	2
Spermatogenesis, Decreased	4	2			4						2

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

454-105
Adult Sacrifice
Female Mallard

	GROUP CONTROL						GROUP 17.6				
ANIMAL	19626	19644	19666	19688	19707		19992	19944	19966	19988	20070
ADIPOSE TISSUE		X	X				X		X		
Infiltrate, Mononuclear Cell				1				1			1
Microvesiculation	1				1					1	1
BRAIN	X	X	X	X	X		X	X	X	X	X
BURSA OF FABRICIUS	N	N	N	N	N		N	N	N	N	N
Involution											
GALLBLADDER								X			
Infiltrate, Mononuclear Cell	1	1	1	1	1		1		1	1	1
KIDNEY	X	m	m	m	m		m	m	m		
Infiltrate, Mononuclear Cell		1	1	1	1		1		1	2	1
Mineralization								1			
LIVER											
Amyloid Deposition					2						
Hepatocyte(s), Fatty Change, Diffuse					1						
Hepatocyte(s), Fatty Change, Focal			1								
Hepatocyte(s), Vacuolization, Diffuse	2	3		1			3	3	3	2	
Infiltrate, Mononuclear Cell	2	1	1	2	1		1	1	1	1	2
Pigment Deposition									1		
OVARY		X									
Fibrosis	3			3	1				1	1	
Follicles, Decreased Diameter	3		3	3	3		3	2	3	3	3
PROVENTRICULUS									X		
Infiltrate, Mononuclear Cell	1	4	3	2	2		4	1		1	1

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

ADULT SACRIFICE

454-105
Adult Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Males Group Identification: CONTROL - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
1961	EXTERNAL	Molting flight feathers	No Comment Required
	TESTES	~3.0 cm	Seminiferous Tubules, Decreased Diameter (TESTIS)
1963	EXTERNAL	Bumble foot left foot	No Comment Required
	EXTERNAL	Molting flight feathers	No Comment Required
	TESTES	~3.5 cm	Seminiferous Tubules, Decreased Diameter (TESTIS)
1965	EXTERNAL	Bumble foot both feet	No Comment Required
	TESTES	~3.5 cm	Seminiferous Tubules, Decreased Diameter (TESTIS)
1967	EXTERNAL	Bumble foot right foot	No Comment Required
1969	EXTERNAL	Molting flight feathers	No Comment Required
	TESTES	~2.0 cm	Seminiferous Tubules, Decreased Diameter (TESTIS)

454-105
Adult Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Males Group Identification: 17.6 - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
1991	EXTERNAL	Bumble foot both feet	No Comment Required
1993	EXTERNAL	Bumble foot both feet	No Comment Required
	TESTES	~1.5 cm	Seminiferous Tubules, Decreased Diameter (TESTIS)
1995	EXTERNAL	Bumble foot both feet	No Comment Required
	GI TRACT	Mass of feed impacted under the tongue	No Comment Required
	TESTES	~2.0 cm	Seminiferous Tubules, Decreased Diameter (TESTIS)
1997	EXTERNAL	Bumble foot both feet	No Comment Required
	TESTES	~1.0 cm	Seminiferous Tubules, Decreased Diameter (TESTIS)
1999	TESTES	~2.5 cm	Seminiferous Tubules, Decreased Diameter (TESTIS)

454-105
Adult Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Females Group Identification: CONTROL - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
1962	EXTERNAL	Bumble foot both feet	No Comment Required
	EXTERNAL	Molting flight feathers	No Comment Required
	OVARY	Regressed ovary	Follicles, Decreased Diameter; Fibrosis
1966	EXTERNAL	Bumble foot both feet	No Comment Required
	OVARY	Regressed ovary	Follicles, Decreased Diameter
1968	OVARY	Regressed ovary	Follicles, Decreased Diameter
	REPRODUCTIVE	Egg remnant	No Comment Required
1970	EXTERNAL	Bumble foot both feet	No Comment Required
	EXTERNAL	Molting flight feathers	No Comment Required
	OVARY	Regressed ovary	Follicles, Decreased Diameter

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Females Group Identification: 17.6 - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
1992	EXTERNAL	Molting flight feather	No Comment Required
	RESPIRATORY	Air sacculitis	No Comment Required
	ABDOMINAL CAVITY	Egg yolk peritonitis (slight)	No Comment Required
	OVARY	Regressed ovary	Follicles, Decreased Diameter
1994	EXTERNAL	Molted flight feathers	No Comment Required
	OVARY	Ovary regressed	Follicles, Decreased Diameter
1996	EXTERNAL	Bumble foot both feet	No Comment Required
	OVARY	Regressed ovary	Follicles, Decreased Diameter
1998	EXTERNAL	Molting flight feathers	No Comment Required
	GENERAL CONDITION	Abnormally small	No Comment Required
	OVARY	Regressed ovary	Follicles, Decreased Diameter
2000	OVARY	Regressed ovary	Follicles, Decreased Diameter

SUMMARY INCIDENCE TABLES

OFFSPRING SACRIFICE

SUMMARY INCIDENCE TABLE

454-105
Offspring Sacrifice
Male Mallard

	GROUP CONTROL	GROUP 1.8	GROUP 6.2	GROUP 17.6		
ADIPOSE TISSUE (NO. EXAMINED)	(5)	(4)	(4)	(5)		
Infiltrate, Mononuclear Cell						
Microvesiculation	1			2		
BRAIN (NO. EXAMINED)	(5)	(4)	(4)	(5)		
Infiltrate, Mononuclear Cell		1				
BURSA OF FABRICIUS (NO. EXAMINED)	(5)	(4)	(4)	(5)		
GALLBLADDER (NO. EXAMINED)	(5)	(4)	(4)	(5)		
Infiltrate, Mononuclear Cell	3	3	2	2		
KIDNEY (NO. EXAMINED)	(5)	(4)	(4)	(5)		
Infiltrate, Mononuclear Cell	4	4	2	4		
LIVER (NO. EXAMINED)	(5)	(4)	(4)	(5)		
Hepatocytes, Degeneration/Necrosis, Focal		1	1	1		
Hepatocytes, Fatty Change, Diffuse				1		
Hepatocytes, Fatty Change, Focal	1		1			
Hepatocytes, Vacuolization, Diffuse	4	4	3	4		
Infiltrate, Mononuclear Cell	5	4	3	4		
Pigment Deposition			1			
PROVENTRICULUS (NO. EXAMINED)	(5)	(4)	(4)	(5)		
Infiltrate, Mononuclear Cell	3	4	4	5		
TESTIS (NO. EXAMINED)	(4)	(4)	(4)	(5)		
Immature	3	4	4	5		
Infiltrate, Mononuclear Cell	2	2		2		

SUMMARY INCIDENCE TABLE

454-105
Offspring Sacrifice
Female Mallard

	GROUP CONTROL	GROUP 1.8	GROUP 6.2	GROUP 17.6		
ADIPOSE TISSUE (NO. EXAMINED)	(5)	(6)	(6)	(5)		
Infiltrate, Mononuclear Cell		1				
Microvesiculation		2	2	2		
BRAIN (NO. EXAMINED)	(5)	(6)	(6)	(5)		
Infiltrate, Mononuclear Cell						
BURSA OF FABRICIUS (NO. EXAMINED)	(5)	(6)	(6)	(5)		
GALLBLADDER (NO. EXAMINED)	(5)	(6)	(6)	(5)		
Infiltrate, Mononuclear Cell	2	5	5	1		
KIDNEY (NO. EXAMINED)	(5)	(6)	(6)	(5)		
Infiltrate, Mononuclear Cell	3	6	6	3		
LIVER (NO. EXAMINED)	(5)	(6)	(6)	(5)		
Hepatocytes, Degeneration/Necrosis, Focal	1					
Hepatocytes, Fatty Change, Diffuse						
Hepatocytes, Fatty Change, Focal	1					
Hepatocytes, Vacuolization, Diffuse	3	5	4	5		
Infiltrate, Mononuclear Cell	4	6	6	5		
Pigment Deposition			1			
OVARY (NO. EXAMINED)	(5)	(6)	(6)	(5)		
Immature	5	6	6	5		
Infiltrate, Mononuclear Cell		1	1			
PROVENTRICULUS (NO. EXAMINED)	(5)	(6)	(6)	(5)		
Infiltrate, Mononuclear Cell	4	5	5	5		
URETER (NO. EXAMINED)			(1)			
Lamina Propria, Cyst(s)			1			

HISTOPATHOLOGY INCIDENCE TABLES

OFFSPRING SACRIFICE

HISTOPATHOLOGY INCIDENCE TABLE

454-105
Offspring Sacrifice
Male Mallard

	GROUP CONTROL					GROUP 1.8				GROUP 6.2			
ANIMAL	23005	23007	23008	23141	23116	23123	23128	23131	23134	23138	23139	23413	23443
ADIPOSE TISSUE		X	X	X	X	X	X	X	X	X	X	X	X
Infiltrate, Mononuclear Cell													
Microvesiculation	1												
BRAIN	X	X	X	X	X	X		X	X	X	X	X	X
Infiltrate, Mononuclear Cell							1						
BURSA OF FABRICIUS	X	X	X	X	X	X	X	X	X	X	X	X	X
GALLBLADDER			A		X		A					A	A
Infiltrate, Mononuclear Cell	1	1		1		1		1	1	1	1		
KIDNEY	m	m	Xm	m	m	m	m	m	m	m	m	Xm	Xm
Infiltrate, Mononuclear Cell	1	1		1	2	1	1	1	1	1	2		
LIVER													
Hepatocytes, Degeneration/Necrosis, Focal								1		1			
Hepatocytes, Fatty Change, Diffuse													
Hepatocytes, Fatty Change, Focal		1										2	
Hepatocytes, Vacuolization, Diffuse	2		2	1	2	2	1	2	2	3	2	2	
Infiltrate, Mononuclear Cell	1	1	1	1	1	1	1	1	1		1	1	1
Pigment Deposition												1	
PROVENTRICULUS	X				X								
Infiltrate, Mononuclear Cell		1	1	1		1	1	1	2	1	1	1	1
TESTIS	m	m	m	N									
Immature	P		P		P	P	P	P	P	P	P	P	P
Infiltrate, Mononuclear Cell	1	1					1		2				

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m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

454-105
Offspring Sacrifice
Male Mallard

GROUP
17.6

ANIMAL	23554	23557	23560	23603
ADIPOSE TISSUE		X	X	X
Infiltrate, Mononuclear Cell				
Microvesiculation	1		1	
BRAIN	X	X	X	X
Infiltrate, Mononuclear Cell				
BURSA OF FABRICIUS	X	X	X	X
GALLBLADDER	A	A	A	
Infiltrate, Mononuclear Cell				1
KIDNEY	m	m	m	m
Infiltrate, Mononuclear Cell	1	1	1	1
LIVER				
Hepatocytes,				
Degeneration/Necrosis, Focal		1		
Hepatocytes, Fatty Change,				
Diffuse				2
Hepatocytes, Fatty Change,				
Focal				
Hepatocytes, Vacuolization,				
Diffuse	2	2	2	
Infiltrate, Mononuclear Cell	1	1		1
Pigment Deposition				
PROVENTRICULUS				
Infiltrate, Mononuclear Cell	1	1	1	1
TESTIS				
Immature	P	P	P	P
Infiltrate, Mononuclear Cell			1	1

ANIMAL	23563
ADIPOSE TISSUE	X
Infiltrate, Mononuclear Cell	
Microvesiculation	
BRAIN	X
Infiltrate, Mononuclear Cell	
BURSA OF FABRICIUS	X
GALLBLADDER	
Infiltrate, Mononuclear Cell	1
KIDNEY	Xm
Infiltrate, Mononuclear Cell	
LIVER	
Hepatocytes,	
Degeneration/Necrosis, Focal	
Hepatocytes, Fatty Change,	
Diffuse	
Hepatocytes, Fatty Change,	
Focal	
Hepatocytes, Vacuolization,	
Diffuse	3
Infiltrate, Mononuclear Cell	1
Pigment Deposition	
PROVENTRICULUS	
Infiltrate, Mononuclear Cell	1
TESTIS	
Immature	P
Infiltrate, Mononuclear Cell	

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HISTOPATHOLOGY INCIDENCE TABLE

454-105
Offspring Sacrifice
Female Mallard

	GROUP CONTROL					GROUP 1.8						GROUP 6.2					
ANIMAL	23001	23002	23003	23017	23020	23022	23025	23026	23030	23032	23033	23035	23036	23037	23042	23044	23046
ADIPOSE TISSUE	X	X	X	X	X	X	X				X		X	X		X	X
Infiltrate, Mononuclear Cell										1							
Microvesiculation								2	1			1			1		
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Infiltrate, Mononuclear Cell																	
BURSA OF FABRICIUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GALLBLADDER	A		X		A				A						X		
Infiltrate, Mononuclear Cell		1		1		1	1	1		1	1	1	1	1		1	1
KIDNEY	Xm	m	Xm	m	m	m	m	m	m	m	m	m	m	m	m	m	m
Infiltrate, Mononuclear Cell		1		1	1	1	1	1	1	1	1	1	1	1	1	2	1
LIVER																	
Hepatocytes, Degeneration/Necrosis, Focal					1												
Hepatocytes, Fatty Change, Diffuse																	
Hepatocytes, Fatty Change, Focal		2															
Hepatocytes, Vacuolization, Diffuse	1		1		1	3	2	3	3		3	2	2		3		1
Infiltrate, Mononuclear Cell	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1
Pigment Deposition														1			
OVARY																	
Immature	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Infiltrate, Mononuclear Cell						1								1			
PROVENTRICULUS					X		X							X			
Infiltrate, Mononuclear Cell	1	1	1	1		1		1	1	1	1	1	1		1	3	1
URETER																	
Lamina Propria, Cyst(s)														P			

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HISTOPATHOLOGY INCIDENCE TABLE

454-105
Offspring Sacrifice
Female Mallard

GROUP
17.6

ANIMAL	23478	23488	23535	23585	23595
ADIPOSE TISSUE			X	X	X
Infiltrate, Mononuclear Cell					
Microvesiculation	3	1			
BRAIN	X	X	X	X	X
Infiltrate, Mononuclear Cell					
BURSA OF FABRICIUS	X	X	X	X	X
GALLBLADDER		A	X	X	X
Infiltrate, Mononuclear Cell	1				
KIDNEY	m	m	Xm	m	Xm
Infiltrate, Mononuclear Cell	1	1		1	
LIVER					
Hepatocytes, Degeneration/Necrosis, Focal					
Hepatocytes, Fatty Change, Diffuse					
Hepatocytes, Fatty Change, Focal					
Hepatocytes, Vacuolization, Diffuse	3	3	2	1	3
Infiltrate, Mononuclear Cell	1	1	1	1	1
Pigment Deposition					
OVARY					
Immature	P	P	P	P	P
Infiltrate, Mononuclear Cell					
PROVENTRICULUS					
Infiltrate, Mononuclear Cell	1	2	1	1	1

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m=missing one paired organ u=unscheduled sac./death

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

OFFSPRING SACRIFICE

454-105
Offspring Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Males Group Identification: CONTROL - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
2305	EXTERNAL	Bumble foot both feet	No Comment Required
2308	EXTERNAL	Bumble foot both feet	No Comment Required
2314	EXTERNAL	Bumble foot both feet	No Comment Required
2316	EXTERNAL	Bumble foot both feet	No Comment Required

454-105
Offspring Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Males Group Identification: 1.8 - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
2323	EXTERNAL	Bumble foot both feet	No Comment Required

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Males Group Identification: 6.2 - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
2338	EXTERNAL	Bumble foot both feet	No Comment Required
2339	EXTERNAL	Bumble foot both feet	No Comment Required

454-105
Offspring Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Males Group Identification: 17.6 - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
2354	EXTERNAL	Bumble foot both feet	No Comment Required

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Females Group Identification: 1.8 - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
2322	EXTERNAL	Bumble foot both feet	No Comment Required
2325	EXTERNAL	Bumble foot both feet	No Comment Required
2332	EXTERNAL	Bumble foot both feet	No Comment Required
2333	EXTERNAL	Bumble foot both feet	No Comment Required

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Females Group Identification: 6.2 - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
2335	EXTERNAL	Bumble foot both feet	No Comment Required
2336	EXTERNAL	Bumble foot both feet	No Comment Required
2342	EXTERNAL	Bumble foot left foot	No Comment Required
2344	EXTERNAL	Bumble foot both feet	No Comment Required

454-105
Offspring Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Females Group Identification: 17.6 - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
2347	EXTERNAL	Picked and broken feathers	No Comment Required
	EXTERNAL	General unkept condition	No Comment Required
	ABDOMINAL CAVITY	Retained yolk sac	No Comment Required
2348	EXTERNAL	Slight bumble foot both feet	No Comment Required

Appendix X
Table 1
Egg Production (eggs laid/hen/week) from a Mallard
Pilot Reproduction Study with PFOS

Control (0 ppm a.i.)

	Weeks																							
Pen	1	2	3	4	5	6	Total	E/H/D[1]	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total	E/H/D[1]
201	6	4	0	1	5	6	22	0.52	3	6	1	0	0	0	0	0	0	0	0	0	0	0	10	0.11
202	7	7	7	7	7	7	42	1.00	6	8	6	5	1	0	0	0	0	0	0	0	0	0	26	0.28
203	4	4	7	6	5	7	33	0.79	6	4	0	0	0	0	0	0	0	0	0	0	0	0	10	0.11
204	0	0	0	0	0	0	0	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
205	0	0	0	3	5	7	15	0.36	6	8	6	8	6	5	0	0	0	0	0	0	0	0	39	0.41
Total	17	15	14	17	22	27	112		21	26	13	13	7	5	0	0	0	0	0	0	0	0	85	
Mean	3	3	3	3	4	5	22	0.53	4	5	3	3	1	1	0	0	0	0	0	0	0	0	17	0.18
SD	3	3	4	3	3	3	16	0.39	3	3	3	4	3	2	0	0	0	0	0	0	0	0	15	0.16

[1] Eggs laid per hen per day

Appendix X
Table 2
Egg Production (eggs laid/hen/week) from a Mallard
Pilot Reproduction Study with PFOS

1.8 ppm a.i.

	Weeks							
Pen	1	2	3	4	5	6	Total	E/H/D[1]
206	0	0	7	7	6	1	21	0.50
207	0	2	4	0	0	0	6	0.14
208	5	7	7	7	7	7	40	0.95
209	0	0	0	0	0	0	0	0.00
210	6	6	7	6	6	8	39	0.93
Total	11	15	25	20	19	16	106	
Mean	2	3	5	4	4	3	21	0.50
SD	3	3	3	4	3	4	18	0.44

[1] Eggs laid per hen per day

Appendix X
Table 3
Egg Production (eggs laid/hen/week) from a Mallard
Pilot Reproduction Study with PFOS

6.2 ppm a.i.

	Weeks							
Pen	1	2	3	4	5	6	Total	E/H/D[1]
211	0	0	0	0	0	0	0	0.00
212	1	4	7	6	8	6	32	0.76
213	0	1	0	0	0	0	1	0.02
214	0	0	0	0	0	0	0	0.00
215	4	7	6	6	6	2	31	0.74
Total	5	12	13	12	14	8	64	
Mean	1	2	3	2	3	2	13	0.30
SD	2	3	4	3	4	3	17	0.41

[1] Eggs laid per hen per day

Appendix X
Table 4
Egg Production (eggs laid/hen/week) from a Mallard
Pilot Reproduction Study with PFOS

17.6 ppm a.i.

	Weeks																						
Pen	1	2	3	4	5	6	Total	E/H/D[1]	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total	E/H/D[1]
216	5	6	7	6	6	2	32	0.76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
217	0	4	5	7	6	1	23	0.55	2	7	7	7	7	7	7	4	3	4	0	0	0	0	55	0.59
218	7	4	7	6	2	7	33	0.79	1	7	4	3	0	0	0	0	0	0	0	0	0	0	15	0.16
219	0	3	7	5	6	5	26	0.62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
220	3	5	7	7	4	7	33	0.79	7	1	4	7	1	0	0	0	0	0	0	0	0	0	20	0.21
Total	15	22	33	31	24	22	147		10	15	15	17	8	7	7	4	3	4	0	0	0	0	90	
Mean	3	4	7	6	5	4	29	0.70	2	3	3	3	2	1	1	1	1	1	0	0	0	0	18	0.19
SD	3	1	1	1	2	3	5	0.11	3	4	3	4	3	3	3	2	1	2	0	0	0	0	23	0.24

[1] Eggs laid per hen per day

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Page 1
Reproductive Performance by Pen
from a Mallard Pilot Reproduction Study with PFOS

Table 1
Reproductive Data (Count) By Pen

Parameter	Control (0 ppm a.i.)						1.8 ppm a.i.						6.2 ppm a.i.						17.6 ppm a.i.					
Pens	201	202	203	204	205	Total	206	207	208	209	210	Total	211	212	213	214	215	Total	216	217	218	219	220	Total
Eggs Laid	5	7	6	0	6	24	4	0	7	0	7	18	0	8	0	0	4	12	7	5	3	5	4	24
Eggs Cracked	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1
Eggs Set	5	7	6	0	6	24	4	0	7	0	6	17	0	8	0	0	4	12	7	5	3	4	4	23
Viable Embryos	3	7	6	0	6	22	4	0	7	0	6	17	0	8	0	0	4	12	6	5	3	4	3	21
Live 3-Wk Embryos	3	7	6	0	6	22	4	0	7	0	6	17	0	8	0	0	4	12	6	5	3	4	3	21
Hatchlings	2	7	6	0	6	21	3	0	5	0	6	14	0	8	0	0	4	12	6	4	2	4	2	18
Offspring Survivors	2	7	6	0	6	21	3	0	5	0	5	13	0	8	0	0	4	12	5	4	2	4	2	17

Appendix XI
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Reproductive Performance by Pen
from a Mallard Pilot Reproduction Study with PFOS

Table 2
Eggs Laid / Hen / Day

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Eggs Laid	Days	Eggs/ Hen/Day	Eggs Laid	Days	Eggs/ Hen/Day	Eggs Laid	Days	Eggs/ Hen/Day	Eggs Laid	Days	Eggs/ Hen/Day
1	5	7	0.71	4	7	0.57	0	7	0.00	7	7	1.00
2	7	7	1.00	0	7	0.00	8	7	1.14	5	7	0.71
3	6	7	0.86	7	7	1.00	0	7	0.00	3	7	0.43
4	0	7	0.00	0	7	0.00	0	7	0.00	5	7	0.71
5	6	7	0.86	7	7	1.00	4	7	0.57	4	7	0.57
Total	24			18			12			24		
Mean	5		0.69	4		0.51	2		0.34	5		0.69
SD	3		0.40	4		0.50	4		0.51	1		0.21

Table 3
Eggs Cracked / Eggs Laid (%)

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Eggs Cracked	Eggs Laid	%	Eggs Cracked	Eggs Laid	%	Eggs Cracked	Eggs Laid	%	Eggs Cracked	Eggs Laid	%
1	0	5	0	0	4	0	0	0	-	0	7	0
2	0	7	0	0	0	-	0	8	0	0	5	0
3	0	6	0	0	7	0	0	0	-	0	3	0
4	0	0	-	0	0	-	0	0	-	1	5	20
5	0	6	0	1	7	14	0	4	0	0	4	0
Total	0	24		1	18		0	12		1	24	
Mean	0	5	0	0	4	5	0	2	0	0	5	4
SD	0	3	0	0	4	8	0	4	0	0	1	9

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Page 3
Reproductive Performance by Pen
from a Mallard Pilot Reproduction Study with PFOS

Table 4
Viable Embryos / Eggs Set (%)

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Viable Embryos	Eggs Set	%	Viable Embryos	Eggs Set	%	Viable Embryos	Eggs Set	%	Viable Embryos	Eggs Set	%
1	3	5	60	4	4	100	0	0	-	6	7	86
2	7	7	100	0	0	-	8	8	100	5	5	100
3	6	6	100	7	7	100	0	0	-	3	3	100
4	0	0	-	0	0	-	0	0	-	4	4	100
5	6	6	100	6	6	100	4	4	100	3	4	75
Total	22	24		17	17		12	12		21	23	
Mean	4	5	90	3	3	100	2	2	100	4	5	92
SD	3	3	20	3	3	0	4	4	0	1	2	11

Table 5
Live 3-Week Embryos / Viable Embryos (%)

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Live 3-Week	Viable	%	Live 3-Week	Viable	%	Live 3-Week	Viable	%	Live 3-Week	Viable	%
1	3	3	100	4	4	100	0	0	-	6	6	100
2	7	7	100	0	0	-	8	8	100	5	5	100
3	6	6	100	7	7	100	0	0	-	3	3	100
4	0	0	-	0	0	-	0	0	-	4	4	100
5	6	6	100	6	6	100	4	4	100	3	3	100
Total	22	22		17	17		12	12		21	21	
Mean	4	4	100	3	3	100	2	2	100	4	4	100
SD	3	3	0	3	3	0	4	4	0	1	1	0

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Page 4
Reproductive Performance by Pen
from a Mallard Pilot Reproduction Study with PFOS

Table 6
Hatchlings / Live 3-Week Embryos (%)

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Hatch	Live 3-Week	%	Hatch	Live 3-Week	%	Hatch	Live 3-Week	%	Hatch	Live 3-Week	%
1	2	3	67	3	4	75	0	0	-	6	6	100
2	7	7	100	0	0	-	8	8	100	4	5	80
3	6	6	100	5	7	71	0	0	-	2	3	67
4	0	0	-	0	0	-	0	0	-	4	4	100
5	6	6	100	6	6	100	4	4	100	2	3	67
Total	21	22		14	17		12	12		18	21	
Mean	4	4	92	3	3	82	2	2	100	4	4	83
SD	3	3	17	3	3	16	4	4	0	2	1	17

Table 7
Surviving Offspring / Hatchlings

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Offspring Surv.	Hatch	%	Offspring Surv.	Hatch	%	Offspring Surv.	Hatch	%	Offspring Surv.	Hatch	%
1	2	2	100	3	3	100	0	0	-	5	6	83
2	7	7	100	0	0	-	8	8	100	4	4	100
3	6	6	100	5	5	100	0	0	-	2	2	100
4	0	0	-	0	0	-	0	0	-	4	4	100
5	6	6	100	5	6	83	4	4	100	2	2	100
Total	21	21		13	14		12	12		17	18	
Mean	4	4	100	3	3	94	2	2	100	3	4	97
SD	3	3	0	3	3	10	4	4	0	1	2	7

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Reproductive Performance by Pen
from a Mallard Pilot Reproduction Study with PFOS

Table 8
Hatchlings / Eggs Set (%)

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Hatch	Eggs Set	%	Hatch	Eggs Set	%	Hatch	Eggs Set	%	Hatch	Eggs Set	%
1	2	5	40	3	4	75	0	0	-	6	7	86
2	7	7	100	0	0	-	8	8	100	4	5	80
3	6	6	100	5	7	71	0	0	-	2	3	67
4	0	0	-	0	0	-	0	0	-	4	4	100
5	6	6	100	6	6	100	4	4	100	2	4	50
Total	21	24		14	17		12	12		18	23	
Mean	4	5	85	3	3	82	2	2	100	4	5	76
SD	3	3	30	3	3	16	4	4	0	2	2	19

Table 9
Surviving Offspring / Eggs Set (%)

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Offspring Surv	Eggs Set	%	Offspring Surv	Eggs Set	%	Offspring Surv	Eggs Set	%	Offspring Surv	Eggs Set	%
1	2	5	40	3	4	75	0	0	-	5	7	71
2	7	7	100	0	0	-	8	8	100	4	5	80
3	6	6	100	5	7	71	0	0	-	2	3	67
4	0	0	-	0	0	-	0	0	-	4	4	100
5	6	6	100	5	6	83	4	4	100	2	4	50
Total	21	24		13	17		12	12		17	23	
Mean	4	5	85	3	3	77	2	2	100	3	5	74
SD	3	3	30	3	3	6	4	4	0	1	2	18

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Reproductive Performance by Pen
from a Mallard Pilot Reproduction Study with PFOS

Table 10
Hatchlings / Hen / Day

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Hatch	Days	Hatch/ Hen/Day	Hatch	Days	Hatch/ Hen/Day	Hatch	Days	Hatch/ Hen/Day	Hatch	Days	Hatch/ Hen/Day
1	2	7	0.29	3	7	0.43	0	7	0.00	6	7	0.86
2	7	7	1.00	0	7	0.00	8	7	1.14	4	7	0.57
3	6	7	0.86	5	7	0.71	0	7	0.00	2	7	0.29
4	0	7	0.00	0	7	0.00	0	7	0.00	4	7	0.57
5	6	7	0.86	6	7	0.86	4	7	0.57	2	7	0.29
Total	21			14			12			18		
Mean	4		0.60	3		0.40	2		0.34	4		0.51
SD	3		0.43	3		0.40	4		0.51	2		0.24

Table 11
Surviving Offspring / Hen / Day

	Control (0 ppm a.i.)			1.8 ppm a.i.			6.2 ppm a.i.			17.6 ppm a.i.		
Replicate	Offspring Surv	Days	Offspring/ Hen/Day	Offspring Surv	Days	Offspring/ Hen/Day	Offspring Surv	Days	Offspring/ Hen/Day	Offspring Surv	Days	Offspring/ Hen/Day
1	2	7	0.29	3	7	0.43	0	7	0.00	5	7	0.71
2	7	7	1.00	0	7	0.00	8	7	1.14	4	7	0.57
3	6	7	0.86	5	7	0.71	0	7	0.00	2	7	0.29
4	0	7	0.00	0	7	0.00	0	7	0.00	4	7	0.57
5	6	7	0.86	5	7	0.71	4	7	0.57	2	7	0.29
Total	21			13			12			17		
Mean	4		0.60	3		0.37	2		0.34	3		0.49
SD	3		0.43	3		0.36	4		0.51	1		0.19

Appendix XII
Mean Offspring Body Weight (g)
from a Mallard Pilot Reproduction Study with PFOS

Mean Hatchling Body Weight (g)

Replicate	Control (0 ppm a.i.)	1.8 ppm a.i.	6.2 ppm a.i.	17.6 ppm a.i.
1	26.5	34.3	--	34.8
2	30.6	--	33.6	33.3
3	29.7	31.6	--	37.5
4	--	--	--	34.8
5	26.8	32.3	33.0	34.5
Mean	28.4	32.8	33.3	35.0
SD	2.0	1.4	0.4	1.6

Mean Surviving Offspring[1] Body Weight (g)

Replicate	Control (0 ppm a.i.)	1.8 ppm a.i.	6.2 ppm a.i.	17.6 ppm a.i.
1	1024	995	--	985
2	1050	--	1086	1051
3	977	1170	--	1056
4	--	--	--	1059
5	999	1031	986	998
Mean	1012	1065	1036	1030
SD	31	92	71	35

[1] Offspring were approximately 12 weeks of age at final body weight interval.

-- No offspring available.

Appendix XIII
Page 1
Adult Liver Weight (g) from a Mallard
Pilot Reproduction Study with PFOS

Control (0 ppm a.i.)

	Male	Female
Pen	Liver	Liver
201	18.774	19.198
202	28.913	30.570
203	19.874	19.405
204	22.680	16.724
205	16.441	20.303
Mean	21.336	21.240
SD	4.793	5.382

1.8 ppm a.i.

	Male	Female
Pen	Liver	Liver
206	27.814	46.860
207	24.713	31.905
208	25.891	35.551
209	23.983	33.155
210	24.280	51.532
Mean	25.336	39.801
SD	1.564	8.832

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Adult Liver Weight (g) from a Mallard
Pilot Reproduction Study with PFOS

6.2 ppm a.i.

	Male	Female
Pen	Liver	Liver
211	27.724	36.008
212	25.304	30.427
213	32.723	37.337
214	35.818	27.942
215	24.458	27.977
Mean	29.205	31.938
SD	4.900	4.462

17.6 ppm a.i.

	Male	Female
Pen	Liver	Liver
216	18.845	21.358
217	34.189	21.871
218	29.108	24.518
219	20.358	17.355
220	14.556	18.018
Mean	23.411	20.624
SD	8.019	2.947

Appendix XIV

Offspring[1] Liver Weight (g) from a Mallard
Pilot Reproduction Study with PFOS

Control (0 ppm a.i.)		1.8 ppm a.i.		6.2 ppm a.i.		17.6 ppm a.i.	
Pen	Liver	Pen	Liver	Pen	Liver	Pen	Liver
201	21.940	206	24.539	212	29.540	216	28.132
201	21.925	206	33.706	212	25.845	216	19.205
202	17.608	206	24.375	212	21.894	217	30.096
202	19.435	208	26.543	212	27.367	217	28.920
203	17.948	208	26.833	212	24.851	218	34.326
203	19.424	208	27.320	212	18.934	218	23.248
203	24.555	210	28.403	215	23.160	219	23.808
205	16.544	210	26.273	215	25.229	219	22.787
205	24.035	210	38.193	215	15.702	220	19.251
205	25.485	210	20.424	215	19.680	220	17.399
Mean	20.890		27.661		23.220		24.717
SD	3.148		4.999		4.215		5.489

[1] Offspring were approximately 12 weeks of age at the time of euthanasia and tissue collection.

Appendix XV

Changes To Study Protocol

This study was conducted in accordance with the study protocol signed on February 28, 2000 and the following amendments and deviations:

1. The protocol was amended to indicate eggs would be held refrigerated until separated for sampling and eliminated the separation of the shell membrane from the shell.

2. The protocol was amended to reduce the number of eggs collected for analysis from all eggs, to eggs collected during weeks 1, 3 and 6 of the test. Eggs collected during Weeks 2, 4 and 5 will be disposed of by incineration.

3. The protocol was amended to change the test substance purity from 98.9% to 90.49%. Correspondingly, the test concentrations were changed from 0, 2, 7 and 20 ppm a.i to 0, 1.8, 6.4 and 18.3 ppm a.i.

4. The protocol was amended to indicate for a seven day period beginning March 31 (early Week 5), eggs would be collected daily for incubation, hatching and rearing of offspring. The amendment also detailed the conditions of egg storage, incubation, housing and brooding of hatchlings. Additionally the amendment indicated hatchlings would be uniquely identified and weighed at hatch and at 14 days of age, and listed reproductive parameters to be measured.

5. The necropsy section of the protocol was amended to indicate at termination samples would be collected from all remaining study adults and from 10 offspring in each test group for histopathological examination. Any remaining tissue not fixed for histopathology will be stored frozen for potential analysis.

6. The protocol was amended to extend the adult portion of the study for the control group and 18.3 ppm a.i. treatment group at least four weeks. During the extension, the number of eggs laid for each pen would be recorded and eggs would be disposed of. Attempts would also be made to collect blood samples from the control group and 18.3 ppm a.i. treatment group birds at the end of Week 6. Adult test birds in the 1.8 and 6.4 ppm a.i. treatment groups will be euthanized at the end of Week 6. Additionally, the amendment required collection of feather samples at the time of gross necropsy.

7. The protocol was amended to indicate the raw data and report would be audited by the Quality Assurance Unit and a Good Laboratory Practice compliant final report would be prepared. Additionally, the Sponsor's representative was changed to John Newsted, and his address was added.

8. The protocol was amended to indicate analyses of egg, blood and tissue samples will be reported separately. Results of egg, blood and tissue analyses may be amended to the biological results at a later date.

9. The protocol was amended to add statistical analyses of data to determine statistically significant differences between groups.

10. Study offspring were not euthanized , weighed and disposed of at 14 days of age. Instead, offspring were raised to approximately 12 weeks of age prior to being euthanized, weighed, sampled and stored frozen. A protocol amendment was not prepared in a timely manner for this change.

11. The test substance purity changed from 90.49% to 86.9%. Correspondingly, the test concentrations changed from 0, 1.8, 6.4 and 18.3 ppm a.i. to 0, 1.8, 6.2 and 17.6 ppm a.i. A protocol amendment was not produced in a timely manner for this change.

12. Additional adult body weight measurements were taken at Weeks 6, 8, 10 and 11 of the test. The amendment to extend the test inadvertently did not specify additional body weight measurements.

13. Eggs laid on July 8, 2000 (Day 4 of Week 19) were inadvertently not counted and collected. Instead, eggs laid on July 8, 2000 were counted and collected with eggs laid on the following day.

14. Wing bands were removed from study offspring at approximately five weeks of age and offspring were reidentified with leg bands. The protocol indicated offspring would be identified with wing bands.

Appendix XVI
Personnel Involved in Study

The following key personnel were involved in the conduct or management of this study:

Avian Toxicology

(1) Mark Jaber, Wildlife Toxicologist

(2) Joann B. Beavers, Director, Avian Toxicology

(3) Linda R. Mitchell, Manager of Ecotox Operations

(4) Diana Temple, Laboratory Supervisor

(5) Sean P. Gallagher, Senior Biologist, Avian Toxicology

Chemistry

(1) Willard B. Nixon, Ph.D., Director of Chemistry

(2) Raymond L. Van Hoven, Ph.D., Scientist, Analytical Chemistry