PFOS:
A REPRODUCTION STUDY WITH THE MALLARD

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-109

3M LAB REQUEST NO. U2723

FIFRA Guideline 71-4

OECD Guideline 206

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STUDY INITIATION DATE: January 22, 2001

STUDY COMPLETION DATE: August 21, 2003

Submitted to

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St. Paul, Minnesota 55106

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Reproduction Study with the Mallard

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-109

STUDY COMPLETION: August 21, 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:

Complete signed and dated reports for blood and tissue analyses conducted by Exygen Research and 3M Corporation are not included in the report. Summary tables are included as appendices and full reports will be submitted separately.

The study was conducted under multiple protocols. The in-life and 3M analytical portions were conducted under one protocol (Wildlife International, Ltd. study number 454-109), and the Exygen Research analytical portions were conducted under two additional separate protocols (Exygen Research study number 023-066 and 023-070).

Analyses of egg contents conducted under Exygen Research study number 023-070 were not conducted in accordance with Good Laboratory Practice Standards, and results of these analyses are reported separately.

The analytical phase performed by Exygen Research under study number 023-066 was performed in compliance with EPA TSCA GLP (40 CFR Part 792).

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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:

		DATE REPORTED TO:	
ACTIVITY	DATE CONDUCTED	STUDY DIRECTOR	MANAGEMENT
Test substance preparation	January 22, 2001	January 22, 2001	January 23, 2001
Matrix fortification	January 30, 2001	January 31, 2001	January 31, 2001
Body weight and environmental conditions	February 6, 2001	February 7, 2001	February 9, 2001
Hatchling body weight	May 24, 2001	May 24, 2001	May 25, 2001
14-day old body weight	May 31, 2001	May 31, 2001	May 31, 2001
Egg shell thickness measurements	July 5, 2001	July 5, 2001	July 6, 2001
Candling	July 6, 2001	July 6, 2001	July 25, 2001
ARDS data entry check	August 14 & 15, 2001	August 15, 2001	August 15, 2001
Analytical data and draft report	October 29, November 2 & 5, 2001	November 5, 2001	November 9, 2001
Biological data and draft report	December 5, 7, 10-12, 2001	December 12, 2001	March 11, 2002
Final report	August 20, 2003	August 20, 2003	August 21, 2003

Robert N. McGee
Quality Assurance Representative

August 21, 2003
DATE

REPORT APPROVAL

SPONSOR: 3M Corporation

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3M LAB REQUEST NO. U2723

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SUMMARY

STUDY: PFOS: A Reproduction Study with the Mallard

SPONSOR: 3M Corporation

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-109

TEST DATES: Study Initiation – January 22, 2001
Experimental Start – January 23, 2001
Photostimulation – April 4, 2001
First Eggs Set – April 13, 2001
Adult Termination – June 20-21, 2001
Analytical Termination – April 11, 2002
Biological Termination – July 26, 2001
Experimental Termination – April 11, 2002

TEST ANIMALS: Mallard (*Anas platyrhynchos*)

AGE TEST ANIMALS: 24 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, 10, 50, and 150 ppm a.i.

RESULTS: There were treatment-related mortalities, overt signs of toxicity and treatment-related effects upon adult body weight at the 50 and 150 ppm a.i. test concentrations. There were also treatment-related effects upon feed consumption at the 150 ppm a.i. test concentration. The 50 and 150 ppm a.i. test concentrations were terminated prior to the reproductive phase of the test. There were no treatment-related mortalities, overt signs of toxicity, or treatment-related effects upon body weight, feed consumption or reproductive performance at the 10 ppm a.i. test concentration. However, there was an increased incidence of small testes for males in the 10 ppm a.i. test concentration that may have been related to treatment. Based on the effects observed at the 10 ppm a.i test concentration, the no-observed-effect concentration for mallards exposed to PFOS in the diet was determined to be less than 10 ppm a.i., the lowest concentration tested during this study.

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 January 23, 2001 until July 26, 2001. Raw data generated at Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454-109 in archives located on the Wildlife International, Ltd. site. Biological specimens are stored at 3M Corporation, St. Paul, MN 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 21 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, offspring survival, and egg shell thickness 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 then offspring.

EXPERIMENTAL DESIGN

Mallard (80 males and 80 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 pilot reproduction study (Wildlife International, Ltd. Project Number 454-105) and additional toxicity information provided by the Sponsor.

PFOS Treatment Groups

Group	Nominal Concentration (ppm a.i.)	Pens per Group	Extra Pens - Hematology	Birds per Pen	
				Males	Females
1	(Control) 0	16	4	1	1
2	10	16	4	1	1
3	50	16	4	1	1
4	150	16	4	1	1

Each treatment and control group contained 16 pairs of birds with one male and one female per pen. Four additional pairs of birds were added to each test level (for a total of 20 pairs per level) and maintained for collection of blood samples during the test. Three treatment groups were fed diets containing either 10, 50, or 150 ppm a.i. of PFOS. The control group was fed diet comparable to the treatment groups, but without the addition of the test substance.

Because overt signs of toxicity and treatment-related mortalities were noted at the 150 ppm a.i. test concentration, the dietary concentration for the 150 ppm a.i. treatment level was reduced to 20 ppm a.i. of PFOS at the beginning of Week 3 of the test. The 20 ppm a.i. level was terminated at the beginning of Week 5 of the test due to the limited recovery of birds at this test concentration. For reporting purposes, the 150/20 ppm a.i. test concentration will be referred to as the 150 ppm a.i. concentration. Adults in the 50 ppm a.i. test concentrations were euthanized at the end of Week 7 of the test due to treatment-related mortality and overt signs of toxicity. Therefore, the test was completed with the control group and the 10 ppm a.i. test concentration only.

All adult birds were observed daily throughout the test for signs of toxicity or abnormal behavior. Adult body weights were measured at test initiation, on Weeks 2, 4, 6, 8, and at adult termination and feed consumption was measured weekly throughout the test. At the beginning of Week 11, the photoperiod was increased to induce egg production. Following the start of egg production, eggs were set weekly for incubation. Weekly, eggs were selected by indiscriminate draw for egg shell thickness measurement and all remaining eggs were candled prior to incubation to detect egg shell cracks or abnormal eggs. Eggs were also candled twice during incubation to detect infertile eggs or embryo mortality. On Day 24 of incubation, the eggs were placed in a hatcher and allowed to hatch. Once hatching was completed, hatchlings were removed from the hatcher and the group body weight of the hatchlings by pen was determined. At 14 days of age, the average body weight by parental pen of all surviving offspring was determined. Upon completion of the test, statistical analyses were performed to determine statistically significant differences between groups.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "PFOS: A 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; OECD Guideline 206; and the ASTM "*Standard Practice for Conducting Reproductive Studies with Avian Species*" (1,2,3).

Test Substance

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 a reported purity of 86.9% and expiration date of August 31, 2006 (Appendix I). The test substance was held under ambient conditions 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. Therefore, dietary concentrations are expressed as parts per million active ingredient (ppm a.i.) in the diet.

Test Organisms

Approximately 84 pairs of pen-reared mallard were purchased from Whistling Wings, Inc., 113 Washington Street, Hanover, IL 61041-0509, U.S.A. At the start of acclimation, the mallard were apparently healthy and 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 24 weeks of age at test initiation (first day of exposure to test diet) and ranged in weight from 781 to 1444 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. All eggs laid during the study

were marked with the pen number using a permanent ink marking pen for identification. Hatchlings were identified by wing bands so that they could be traced to their parental pen of origin.

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). 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%) (4). 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 of the three treated diets were prepared weekly beginning on January 23, 2001 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, when available, during Weeks 2, 3, 4, 8, 12, 16 and 20 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). Three modifications from the cited methodology were incorporated into the present study. These changes were (1) the use of high performance liquid chromatography with triple quadrupole mass detection (HPLC/MS/MS) rather than single quadrupole mass detection to reduce potential matrix interferences, (2) the elimination of the internal standardization procedure because an appropriate internal standard could not be identified, and (3) the use of a shorter (50 mm vs.100 mm) analytical chromatography column to expedite analysis time.

Samples were extracted with methanol, vacuum filtered, diluted in methanol, and then diluted in a 50:50 methanol: NANOpure® water dilution solvent 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 HPLC/MS/MS using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph interfaced with a Perkin-Elmer API 3000 Mass Spectrometer operated in multiple reaction monitoring (MRM) detection mode. The mass spectrometer was 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) fitted with a Keystone Javelin C_{18} Guard Cartridge (20 mm × 2 mm I.D.). The instrument parameters are summarized in Appendix IV, Table 1.

Calibration standards were prepared in 50:50 methanol: NANOpure® water by appropriate dilutions of a 1.00 mg a.i./L stock solution of PFOS in methanol. The calibration standards, ranging in concentration from 0.00100 to 0.0100 mg a.i./L, were analyzed with each sample set. 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 the peak area responses versus the respective concentrations of the calibration standards. An example of a calibration curve is presented in Appendix IV, Figure 2. The concentration of PFOS in avian feed samples was determined by substituting the peak areas into the applicable linear regression equation. Typical ion chromatograms of low and high calibration standards are shown in Appendix IV, Figures 3 and 4, respectively. Examples of equations used in calculations are presented in Appendix IV, Table 2.

The instrument limit of detection (LOD) for these analyses was 10.0 pg on-column, calculated from the product of the injection volume (10.0 µL) and the lowest standard concentration 1.00 µg a.i./L = 1.00 pg a.i./µL. The method limit of quantitation (LOQ) for these analyses was 2.00 ppm a.i., calculated as the product of the lowest calibration standard analyzed (0.001 mg a.i./L) and the overall dilution factor of the matrix blank sample (2000 L/Kg). Examples of calculations are presented in Appendix IV, Table 2.

Along with the sample analyses, seven 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 the following levels and analyzed concurrently with the samples to determine the mean procedural recovery. The fortification levels were 5.00, 50.0, and 200 ppm a.i. for sampling intervals Week 1, Day 0; Week 1, Day 7; Week 2, Day 0; and Week 3, Day 0. The fortification levels were 5.00, 25.0, and 100 ppm a.i. for sampling interval Week 4, Day 0. The fortification levels were 5.00 and 25.0 ppm a.i. for sampling intervals Week 8, Day 0; Week 12, Day 0; Week 16, Day 0; and Week 20, Day 0. The method yielded mean procedural recoveries of 101%, 101%, 102%, 100% and 99%, respectively, for the 5.00, 25.0, 50.0, 100, and 200 ppm a.i. fortification levels (Appendix IV, Table 3). Sample measured concentrations were not corrected for the mean procedural recoveries. A typical ion chromatogram of a matrix fortification is presented in Appendix IV, Figure 6.

Study Phases

The primary phases of the study and their approximate durations were:

1. Acclimation - 3 weeks.
2. Pre-photostimulation - 10 weeks.
3. Pre-egg laying (with photostimulation) - 0 weeks.
4. Egg laying - Approximately 10 weeks.
5. Post-adult termination (final incubation, hatching, and 14-day offspring rearing period) - 5 weeks.

Housing and Environmental Conditions

Housing and husbandry practices were conducted so as to adhere to the guidelines established by the National Research Council (5). 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 21.7°C ± 1.1°C (SD) with an average relative humidity of 40% ± 19% (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 first 10 weeks of the test was eight hours or less of light per day. The photoperiod was increased to 17 hours of light per day at the beginning of Week 11 to induce egg laying and was maintained at that length until the adult birds were euthanized. Throughout the test, the birds received a mean of approximately 188 lux (~ 17 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 3 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 14 days of age. 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, and at adult termination. Body weights were not measured during egg laying because of the possible adverse effects handling may have on egg production.

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.

Adult Blood Collection

Four additional pairs of adult birds were added to each test level to be maintained for collection of blood samples during the test. These pairs received the same diet and were handled in the same manner as other pairs in their test group. However, because of the potential stress of the blood collection procedures, data from the four additional pairs were not used to evaluate treatment-related effects. Blood samples were collected from birds in these designated pairs, when possible, at five week intervals throughout the test. At the time of adult termination, blood samples were collected from all surviving birds, when possible, prior to euthanasia. All blood samples were separated into serum and hemacytes/platelets, stored frozen and shipped to the sponsor or sponsor's designate for possible analysis.

Adult Necropsy and Tissue Collection

When the remaining birds from 150/20 pm a.i. treatment group were euthanized at the end of the Week 4, no necropsies were performed. All other adult birds that died or were euthanized during the

course of the study were subjected to a gross necropsy. At the conclusion of the exposure period, all surviving adult birds were euthanized by cervical dislocation, necropsied, and disposed of by incineration. At the time of necropsy, tissues were collected for histopathological examination 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 and shipped to EPL in Herndon, VA for histopathology. When available, samples of bile, liver, proventriculus, kidneys, brain, reproductive organs and adipose tissue were stored frozen and shipped to the Sponsor for possible analysis. Fecal samples were also collected, when available, from the lower digestive tract and stored frozen for possible analysis.

Egg Collection and Storage

Eggs were collected daily from all pens, when available. Eggs to be incubated were washed to reduce the possibility of pathogen contamination before storing them in the cold room. 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 eggs were then stored in a cold room until incubation. The cold room was maintained at a mean temperature of 13.5°C ± 0.2°C (SD) with a mean relative humidity of approximately 69% ± 6% (SD). Groups of eggs were identified by an alphabetic lot code. All eggs laid in a weekly interval 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 eggs selected by indiscriminate draw for egg shell thickness measurement. The remaining eggs were 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 or used for egg shell thickness measurements were placed in a Petersime Incubator (Model No. SP20). In the incubator the temperature was maintained at an average 37.5°C ± 0.0°C (SD) with an average wet bulb temperature of 30.5°C ± 0.3°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 13 or 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°C ± 0.0°C (SD), and the average wet bulb temperature was raised to 33.8°C ± 0.2°C (SD) (relative humidity of approximately 80%).

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. Hatchlings were wing banded for identification by pen of origin and then routinely housed according to the appropriate parental concentration grouping in brooding pens until 14 days of age. The hatchlings were fed untreated diet without the addition of 5% supplemental limestone. At 14 days of age, the average body weight by parental pen of all surviving ducklings was determined. The ducklings were euthanized with carbon dioxide and 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 maintain a temperature of approximately 29°C. The average ambient room temperature was 24.2°C ± 1.2°C (SD) with an average relative humidity of 77% ± 15% (SD). The photoperiod for the hatchlings was maintained by a time clock at 16 hours of light per day.

Offspring Blood and Tissue Collection

Prior to euthanasia of the last group of offspring (Lot J), blood samples were collected from 10 offspring in both the control group and 10 ppm a.i. treatment group. All blood samples were separated into serum and hemacytes/platelets, stored frozen and shipped to the sponsor for possible analyses. Additionally, tissues from the 10 offspring in each group were collected for histopathological examination. 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, liver, proventriculus, kidneys, brain, reproductive organs and adipose tissue were stored frozen and shipped to the sponsor for possible analyses. Fecal samples were also collected from pans below the floor of brooders housing Lot J offspring and stored frozen for possible analysis. A single composite sample was collected for the control group and a single composite sample was collected for the 10 ppm a.i. treatment group.

Egg Shell Thickness Measurements and Egg Components Collection

Weekly throughout the egg laying period one egg was collected, when available, from each of the test pens. Eggs were collected from odd numbered pens during odd numbered weeks (1,3,5, etc.) and from the even numbered pens during the even numbered weeks (2,4,6, etc.). The eggs from weeks 1, 2 and 10 were separated into albumen, yolk, shell and membrane and all components were stored frozen. Eggs collected during weeks 3-9 were opened at the waist and their contents removed. The egg contents were separated into albumen and yolk and the yolk was stored frozen. All egg samples were transferred to the Sponsor for possible analysis. The shells from Weeks 3 through 9 were allowed to air dry for at least one week at room temperature prior to being measured for thickness. The average thickness of the dried shell plus the membrane was determined by measuring five points around the waist of the egg using a micrometer. Measurements were made to the nearest 0.002 mm.

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 (6,7) 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 adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation (see Appendix VI for

reproductive parameters). With the exception of liver weight, statistical analysis of the data was performed using Avian Reproduction Data System (ARDS) Software; a validated software package developed by Wildlife International, Ltd. (8). Liver weights were compared by Student's t-test using Microsoft Excel. Each of the following parameters was analyzed statistically:

1. Adult Body Weight - Individual body weight was measured at test initiation, Weeks 2, 4, 6, 8 and at adult termination. Statistical comparisons were made between the control group and each treatment group at each weighing interval by sex.
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.
3. Eggs Laid of Maximum Laid - The number of eggs laid per female divided by the largest number of eggs laid by any one female. This transformation was used to convert the number of eggs laid to a percentile value less than or equal to 100.
4. Eggs Cracked of Eggs Laid - The number of eggs determined by candling to be cracked divided by the number of eggs laid, per pen.
5. Viable Embryos of Eggs Set - The number of viable embryos at the Day 14 candling was divided by the number of eggs set, per pen.
6. 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.
7. 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.
8. 14-Day Old Survivors of Hatchlings - The number of 14-day old survivors was divided by the number of hatchlings per week, by pen.
9. Hatchlings of Eggs Set - The number of hatchlings was divided by the number of eggs set per week, by pen.
10. 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.
11. Hatchlings of Maximum Set - The number of hatchlings per female divided by the largest number of eggs set from any one female. This transformation was used to convert the number of hatchlings to a percentile value equal to or less than 100.

12. 14-Day Old Survivors of Maximum Set - The number of 14-day old survivors per pen divided by the largest number of eggs set.
13. Egg Shell Thickness - The average egg shell thickness of indiscriminately selected eggs per pen was measured.
14. Offspring's Body Weight - The group body weights of surviving hatchlings and 14-day old survivors were measured by parental pen group.
15. Liver Weight – Individual liver weights were measured at adult termination and at termination of selected Lot J offspring. Statistical comparisons were made by sex between the control group and 10 ppm a.i. group.

RESULTS AND DISCUSSION

Mature mallards received PFOS at nominal dietary concentrations of 10, 50, and 150 ppm a.i. Due to extensive overt signs of toxicity and treatment-related mortalities in the 50 and 150 ppm a.i. treatment groups, the experimental design was modified. Adults in the 50 ppm a.i. test concentrations were euthanized at the end of Week 7 of the test. The dietary concentration for the 150 ppm a.i. treatment level was reduced to 20 ppm a.i. of PFOS at the beginning of Week 3 of the test. The 20 ppm a.i. level was terminated at the beginning of Week 5 of the test due to the limited recovery of birds at this level. Therefore, the test was completed with the control group and 10 ppm a.i. test concentrations only. The control group was fed diet comparable to the treatment groups, but without the addition of the test substance.

Diet Analytical Results

None of the control samples showed any indication of the presence of the test substance or of the presence of co-eluting substances at the characteristic retention time of the test substance. Diet samples were collected from the 10, 50 and 150 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 9.75 ± 0.313 ppm a.i., 50.1 ± 0.981 ppm a.i. and 147 ± 4.31 ppm a.i., respectively. The coefficients of variation were 3.21%, 1.96% and 2.93%, respectively. These values represented 98%, 100%, and 98% of nominal concentrations (Appendix IV, Table 4).

Samples collected throughout the test to verify test substance concentrations for the 10, 20, 50, and 150 ppm a.i. diets had means and standard deviations of 10.2 ± 0.553 ppm a.i., 20.8 ± 2.14 ppm a.i., 50.9 ± 2.04 ppm a.i, and 161 ± 15.6 ppm a.i., respectively. The coefficients of variation were 5.44%, 10.3%, 4.01% and 9.66%, respectively. These values represented 102, 104, 102, and 108% of nominal concentrations (Appendix IV, Table 5). Analysis of diet samples collected from feeders after being held at ambient temperature for 7 days averaged 100%, 101% and 97% of the Day 0 values for the 10, 50 and 150 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. Mean weekly measured concentrations for each test group are presented in Table 1.

Mortalities

While there was one incidental mortality in the control group, there were no mortalities in the 10 ppm a.i. treatment group. Three incidental mortalities and ten treatment-related mortalities occurred in the 50 ppm a.i. treatment group and four treatment-related mortalities occurred in the 150 ppm a.i. treatment group.

The single incidental mortality in the control group was a male (Pen 210) that died during Week 15 of the test. Gross necropsy of the drake revealed foot lesions on both feet. Internally, hemorrhages were noted in the myocardium, the abdominal air sacs were cloudy and the spleen was enlarged and mottled. Additionally, the liver was firm, mottled and pale, the kidneys were slightly pale and a small amount of clear fluid was noted in the abdominal cavity. Necropsy of the bird's penmate at termination of the adult portion of the study revealed a regressed ovary, but was otherwise unremarkable.

Three incidental and ten treatment-related mortalities occurred in the 50 ppm a.i. treatment group and four treatment-related moralities occurred in the 150 ppm a.i. treatment group. Necropsy findings for the treatment related mortalities in the 50 and 150 ppm a.i. test concentrations were similar. Common findings included thin or emaciated condition with a loss of muscle mass and prominent keel, small spleen and pale kidneys. All remaining birds at the 150 ppm a.i. test concentration were euthanized at the beginning of Week 5. Due to the shortened nature of the exposure period, necropsies were not performed. All remaining birds at the 50 ppm a.i. test concentration were euthanized at the beginning of Week 8 of the test, and necropsies were performed following euthanasia. Necropsy findings are summarized in Table 2.

Clinical Observations

No overt signs of toxicity were observed at the 10 ppm a.i. test concentration. With the exception of foot lesions and occasional lameness, all birds in the control group and 10 ppm a.i. treatment group were normal in appearance and behavior throughout the test.

In the 150 ppm a.i. and 50 ppm a.i. treatment groups, signs of toxicity were first noted at the end of Week 2. One male in the 50 ppm a.i. treatment group and several birds in the 150 pm a.i. treatment group exhibited signs of toxicity induced by the stress of handling during the Week 2 body weight measurements. In several cases, birds convulsed during body weight measurements. Signs of toxicity in both the 50 and 150 ppm a.i. treatment groups continued to be observed until the birds were euthanized. Clinical signs of toxicity observed included depression, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, ruffled appearance, thin appearance, lower limb weakness, lethargy, lacrimation and convulsions.

Adult Body Weight

There were no apparent treatment-related effects upon adult body weight at the 10 ppm a.i. test concentration, and any differences between the control group and the 10 ppm a.i. test concentration were not statistically significant at any of the body weight intervals. However, there were marked treatment-related reductions in mean body weight at both the 50 and 150 ppm a.i. test concentrations.

With the exception of the mean male body weight at the Week 2 interval, all differences between the control group and birds in the 50 ppm a.i. treatment group were statistically significant at $p < 0.01$. At the 150 ppm a.i. test concentration, mean male and female body weights were statistically different from the control group at $p < 0.01$ at both the Week 2 and Week 4 body weight intervals. Between the Week 2 and Week 4 body weight intervals, a weight gain was noted for birds at the 150 ppm a.i. test concentration. However, the weight gain observed was a reflection of the reduction of the concentration of PFOS in the diet to 20 ppm a.i. during Week 3 of the test. Mean body weight measurements are presented in Table 3, and Figures 1 and 2. Individual body weight measurements are presented in Appendix VII.

Adult Feed Consumption

There were no apparent treatment-related effects upon feed consumption at the 10 or 50 ppm a.i. test concentrations. No statistically significant differences between the control group and 10 or 50 ppm a.i. treatment groups were observed at any of the feed consumption intervals. However, treatment-related reductions in feed consumption were observed at the 150 ppm a.i. test concentration during Weeks 1 and 2. The reductions were statistically significant at $p < 0.05$ during Week 1 and at $p < 0.01$ during Week 2. The dietary concentration for the 150 ppm a.i. treatment group was reduced to 20 ppm a.i. during Week 3 of the test. There was a statistically significant ($p < 0.01$) increase in feed consumption at the 150 (20) ppm a.i. test concentration during Weeks 3 and 4 of the test. Mean feed consumption measurements are shown in Table 4 and Figure 3. Feed consumption measurements by pen are presented in Appendix VIII.

Adult Necropsy and Liver Weights

All surviving adults in the control group and the 10 ppm a.i. treatment group were subjected to gross necropsy following adult termination. When compared to the control group, there was an increased incidence of males with small testes (approximate length < 3.5 cm) in the 10 ppm a.i. treatment group for which a treatment-related effect could not be precluded. Two males in the control group were noted with small testes, compared to seven males in the 10 ppm a.i. group. All other findings were considered unrelated to treatment. Necropsy findings are reported in Table 5 and Appendix IX.

When compared to the control group, there were no treatment-related effects upon the mean weights of adult livers at the 10 ppm a.i. test concentration. Any difference between the 10 ppm a.i. treatment group and the control group was not statistically significant. Mean adult liver weights are presented in Table 6, while individual adult liver weights are presented in Appendix X.

Histopathology

No lesions considered related to test article (PFOS) administration were noted in the liver, kidney, adipose tissue, brain, and proventriculus of adult and offspring males and females, bursa of Fabricius in offspring males and females, ovaries of adult and offspring females and testes of male offspring. The increase in the number of adult males in the 10 ppm a.i. treated group with reduced testicular size (with or without a corresponding microscopic observation), suggests that PFOS may have accelerated early post-reproductive phase regression, a normal physiological phenomenon. Any potential test-article effects on the bursa of Fabricius in adult males and females could not be determined since samples were not

submitted for evaluation. In addition, any potential test-article effects on the gallbladder from adults and offspring could not be definitively determined due to the extensive autolysis that precluded any accurate diagnosis. The few other changes in various tissues of adult and/or offspring mallards from control and 10 ppm a.i. treated groups were considered incidental and unrelated to test article (PFOS) administration. The full histopathology report is provided in Appendix XI.

Adult Liver and Sera

Adult blood samples (both red blood cells and sera) collected through Week 15 of the test were analyzed by Exygen Research. The results for analysis of samples collected for this study are reported together with results for the northern bobwhite study run concurrently with the same test concentrations. Summary results of blood analyses performed for this study are presented in Exygen report Tables X through XXIV located in Appendix XIIA The full report for analysis of blood samples by Exygen Research is located under Exygen Study Number 023-066. "Extraction of Potassium Perfluorooctanesulfonate from Red Blood Cells and Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry".

Blood and liver samples collected at the time of adult termination for the control group and 10 ppm a.i. test concentration were shipped to 3M Environmental Laboratory for analysis. Summary results of sera and liver analyses conducted by 3M Environmental Laboratory are presented in 3M report Tables 1, 2 and 10 through 15 located in Appendix XIIB. The full report for analysis of sera and liver samples collected at the time of adult termination is located in 3M Environmental Laboratory report E01-1256. "Analytical Phase Report for PFOS: A Reproduction Study with the Mallard".

Reproductive Results

There were no treatment-related effects upon reproductive performance at the 10 ppm a.i. test concentration as compared to the controls. When compared to the control group, there were no statistically significant differences in any of the reproductive parameters measured in the 10 ppm a.i. test concentration. Summaries of the reproductive data are presented in Tables 7 and 8, and in Figures 4 and 5. Reproductive parameters by pen are presented in Appendix XIII and XIV.

Egg Shell Thickness

There were no apparent treatment related effects upon egg shell thickness at the 10 ppm a.i. test concentration. When compared to the control group, there were no statistically significant differences in egg shell thickness in the 10 ppm a.i. treatment group. Egg shell thickness data are presented in Table 9 and Appendix XV.

Offspring Body Weights

There were no apparent treatment related effects upon offspring body weight at the 10 ppm a.i. test concentration. When compared to the control group, there were no statistically significant differences in the body weight of hatchlings or 14-day old survivors from the 10 ppm a.i. treatment group. Offspring body weight data are presented in Table 10, and Appendices XVI and XVII.

Offspring Liver Weights

There were no treatment-related effects upon the mean weights of offspring livers at the 10 ppm a.i. test concentration. Any difference between the 10 ppm a.i. treatment group and the control group was not statistically significant. Mean offspring liver weights are presented in Table 6, while individual offspring liver weights are presented in Appendix XVIII.

Offspring Observations

There were incidental observations of neck curl, weakened condition and foot/leg lesions and associated lameness, for offspring in both the control group and 10 ppm a.i. treatment group. At the 10 ppm a.i. test concentration, a general observation was made that the offspring from Lots C and D were sluggish, with a slight reduced reaction to external stimuli. None of the offspring in the Lots proceeding or following Lots C and D were noted as sluggish or with a reduced reaction to external stimuli. Due to the isolated nature of the clinical signs of toxicity noted, these observations were not considered treatment related. All other offspring were normal in appearance and behavior throughout the test.

Offspring Liver and Sera

Blood and liver samples collected at the time of offspring termination for the control group and 10 ppm a.i. test concentration were shipped to 3M Environmental Laboratory for analysis. Summary results of sera and liver analyses conducted by 3M Environmental Laboratory are presented in 3M report Tables 1, 2 and 10 through 15 located in Appendix XIIB. The full report for analysis of sera and liver samples

collected at the time of juvenile termination is located in 3M Environmental Laboratory report E01-1256. "Analytical Phase Report for PFOS: A Reproduction Study with the Mallard".

CONCLUSION

There were no treatment-related mortalities, overt signs of toxicity, or treatment-related effects upon body weight, feed consumption or reproductive performance at the 10 ppm a.i. test concentration. However, there was an increased incidence of small testes for males in the 10 ppm a.i. test concentration that may have been related to treatment. There were treatment-related mortalities, overt signs of toxicity and treatment-related effects upon adult body weight at the 50 and 150 ppm a.i. test concentrations. There were also treatment-related effects feed consumption at the 150 ppm a.i. test concentration. The 50 and 150 ppm a.i. test concentrations were terminated prior to the reproductive phase of the test. Based on the effect observed at the 10 ppm a.i test concentration, the no-observed-effect concentration for mallards exposed to PFOS in the diet was determined to be less than 10 ppm a.i., the lowest concentration tested during this study.

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Table 1

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

Nominal Concentration (ppm a.i.)		Interval								
		Week 1		Week 2	Week 3	Week 4	Week 8	Week 12	Week 16	Week 20
		Day 0	Day 7[1]	Day 0	Day 0	Day 0	Day 0	Day 0	Day 0	Day 0
0	Measured	<2.00[2]	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
10	Mean Measured									
	% Nominal	98[3]	100[4]	103	101	97	109	101	101	99
50	Mean Measured									
	% Nominal	100[3]	101[4]	97	104	104	-	-	-	-
150	Mean Measured									
	% Nominal	98[3]	97[4]	108	96[5]	112[5]	-	-	-	-

[1] Feed samples were collected from feed troughs at the end of Week 1 to assess stability of the test substance under actual test conditions.

[2] <LOQ indicates the value was less than the limit of quantitation.

[3] The mean of homogeneity samples collected at Week 1, Day 0.

[4] The percent of the mean values for Week 1, Day 0.

[5] The 150 ppm a.i. treated diet was reduced to 20 ppm a.i. during this interval.

- No values available, test group terminated.

Table 2
Summary of Gross Pathological Observations
from a Mallard Reproduction Study with PFOS
Adult Birds Found Dead/Euthanized during the Test

	Males			Females	
	Control (0 ppm a.i.)	50 [1] ppm a.i.	150 [2] ppm a.i.	50 [1] ppm a.i.	150 [2] ppm a.i.
Number of birds	1	17	2	18	2
Abrasion on mandible	0	1	0	0	0
Foot/leg lesions	1	3	0	0	0
Thin	0	4	0	3	0
Emaciated	0	0	1	2	1
Loss of muscle mass	0	1	1	3	1
Prominent keel	0	0	1	2	1
Breast muscle - hematoma and loose blood	0	1	0	0	0
Heart - flaccid	0	0	0	1	0
Heart - pale	0	3	0	1	0
Heart - petechial to ecchymatic hemorrhages	1	0	0	0	0
Left lung - yellow plaques on margins	0	1	0	0	0
Lungs - pale	0	1	0	0	0
Abdominal air sacs - cloudy	1	0	0	0	0
Spleen - small and/or pale	0	7	0	5	0
Spleen - enlarged and mottled	1	0	0	0	0
Liver - firm, pale and mottled	1	0	0	0	0
Abdominal cavity - autolysis	0	1	0	0	2
Abdominal cavity - small amount of clear fluid	1	0	0	0	0
Abdominal cavity - blood in left thoracic air sac	0	1	0	0	0
Gizzard contents - bile-stained	0	3	0	2	1
Small intestines - small white ulcers (polyps)	0	1	0	0	0
Intestinal contents - pasty	0	0	0	0	1
Intestinal contents - firm	0	1	0	1	0
Kidneys - pale	1	4	0	0	1
Not remarkable	0	7	1	10	0

[1] Remaining birds in the 50 ppm a.i. test concentration were euthanized at the end of Week 7 of the test. Gross necropsy observations for birds from which blood samples were collected are not reported.

[2] Remaining birds in the 150 ppm a.i. test concentration were euthanized at the beginning of Week 5. Gross necropsies were not performed at the termination of the 150 ppm a.i. test concentration.

Table 3

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

Experimental Group (ppm a.i.)	Sex	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
Control	Male	1084	7	1091	23	1114	13	1128	-3	1125	52	1164	92
	Female	984	4	988	14	1002	19	1020	12	1033	122	1155	171
10	Male	1111	10	1121	7	1127	17	1144	7	1151	-24	1127	17
	Female	982	22	1004	18	1022	19	1041	9	1049	95	1144	162
50	Male	1104	-57	1047	-64	983**	-66	916**	-	-	-	-	-
	Female	956	-83	873**	-60	813**	-31	781**	-	-	-	-	-
150	Male	1107	-195	912**	88	1016**	-	-	-	-	-	-	-
	Female	985	-206	780**	98	880**	-	-	-	-	-	-	-

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

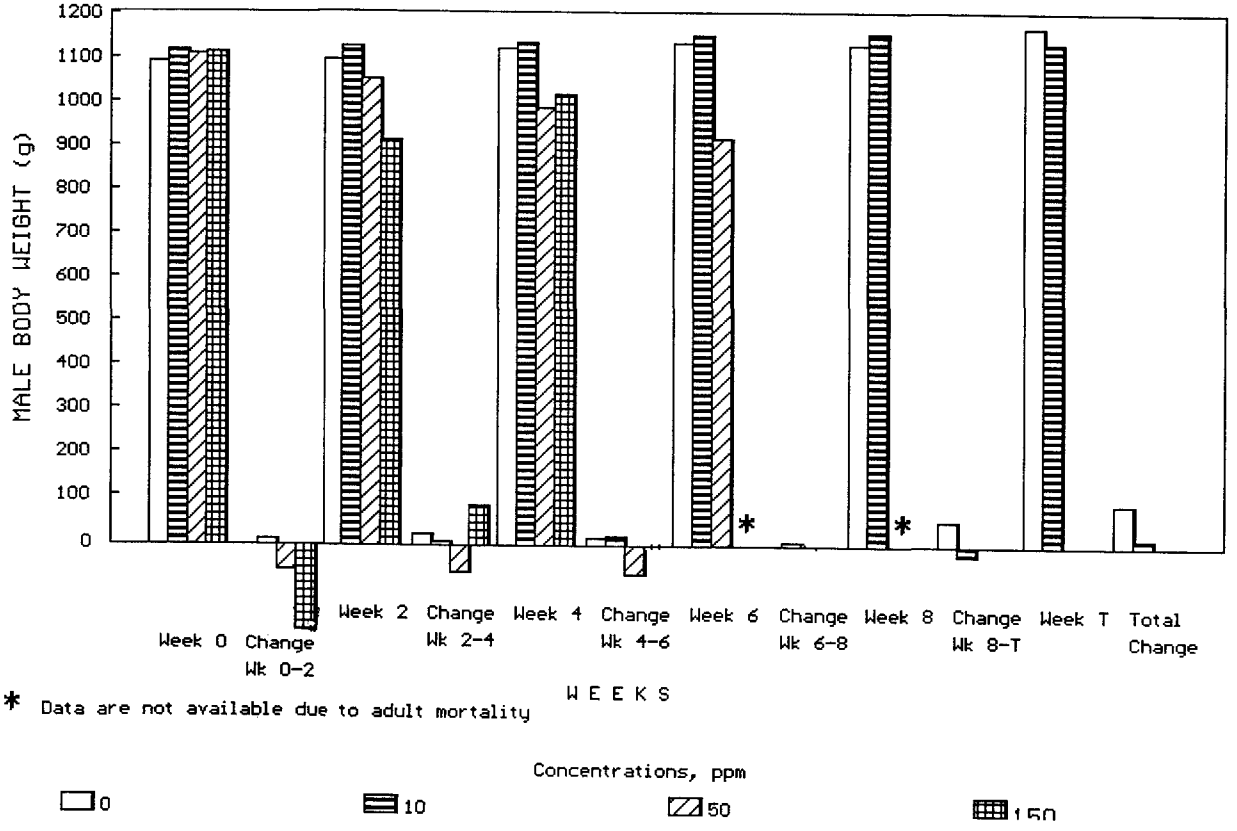
- Data are not available due to adult mortality.

** Significantly different from the control at $p < 0.01$

[1] Only surviving birds were included in the calculations for each body weight interval.

Figure 1

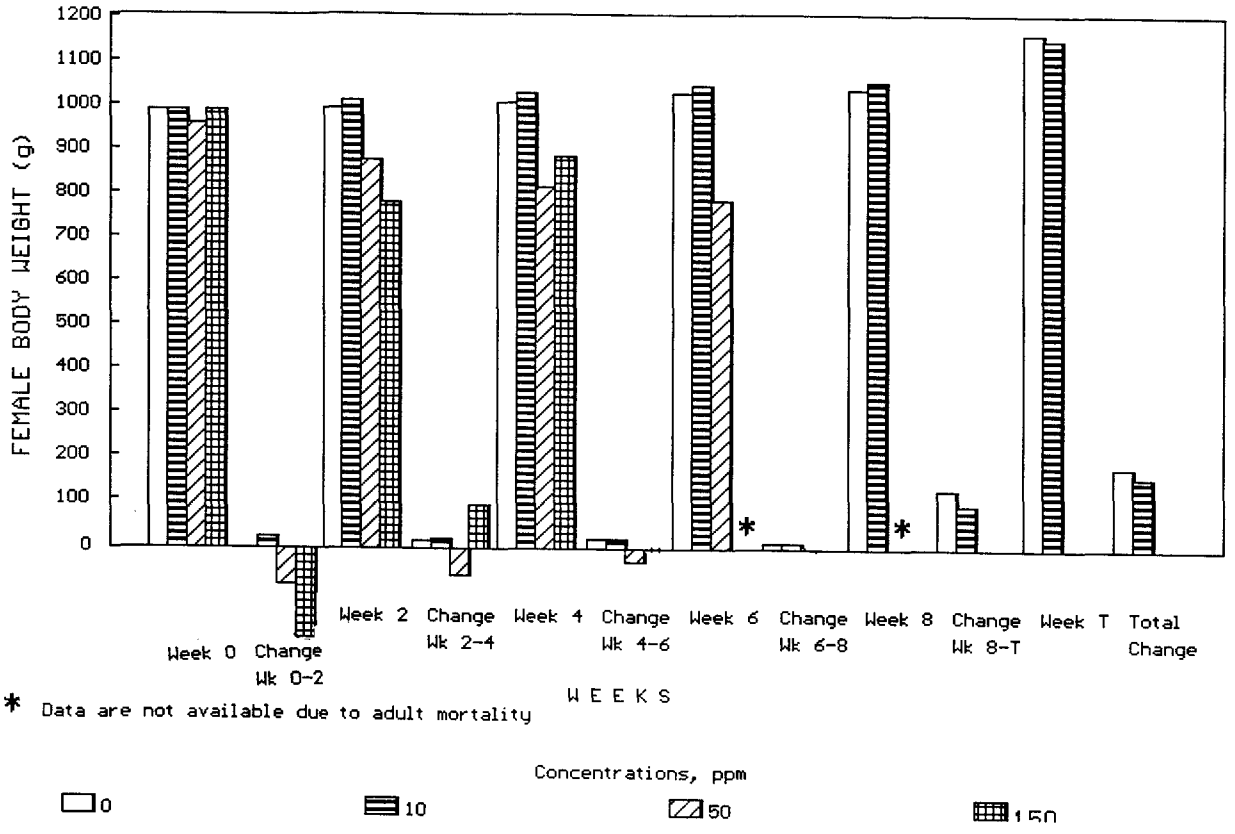
Mean Adult Male Body Weight (g) from a Mallard Reproduction Study with PFOS



* Data are not available due to adult mortality

Figure 2

Mean Adult Female Body Weight (g) from a Mallard Reproduction Study with PFOS



* Data are not available due to adult mortality

Table 4

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

Experimental Group (ppm a.i.)	WEEKS																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	102	122	119	134	124	133	133	134	135	132	135	178	185	173	178	178	191	212	194	195	205
10	119	146	134	146	137	145	148	145	139	138	129	158	183	175	175	172	180	188	178	189	193
50	101	114	118	129	116	124	112	-	-	-	-	-	-	-	-	-	-	-	-	-	-
150	73*	75**	166**	194**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality

* Significantly different from the control at $p < 0.05$

** Significantly different from the control at $p < 0.01$

Figure 3
Mean Feed Consumption (g/bird/day) from a Mallard Reproduction Study with PFOS

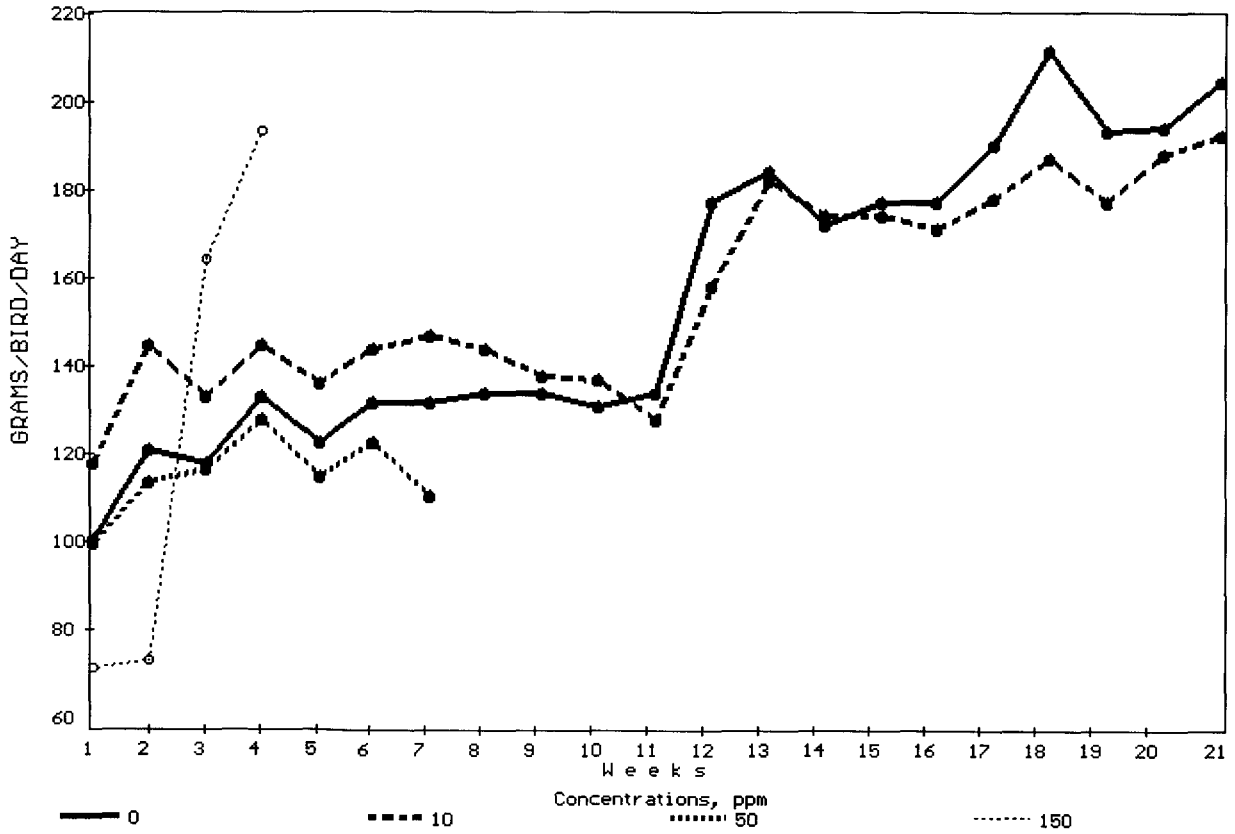


Table 5
Summary of Gross Pathological Observations
from a Mallard Reproduction Study with PFOS
Adult Birds Euthanized at Test Termination[1]

	Males		Females	
	Control (0 ppm a.i.)	10 ppm a.i.	Control (0 ppm a.i.)	10 ppm a.i.
Number of birds	15	16	16	16
Foot/leg lesions	4	4	8	13
Breast blister	0	0	0	2
Cranium - swollen and bruised	0	0	0	1
Heart - pericardial sac fluid-filled	0	0	0	2
Spleen - enlarged and mottled	0	0	1	0
Spleen - enlarged and firm	0	0	0	1
Liver - firm, pale and mottled	0	0	1	0
Abdominal cavity - yolk remnants	-	-	1	0
Abdominal cavity - slight egg yolk peritonitis	-	-	1	0
Abdominal cavity - egg yolk peritonitis	-	-	2	3
Abdominal cavity - slight old egg yolk peritonitis	-	-	5	0
Small intestines - area of hyperemia	0	0	2	0
Kidneys - pale	0	0	1	0
Testes - small (~ 1.5-3.5 cm)	2	6	-	-
Reproductive tract - multiple fluid filled cysts	-	-	1	0
Ovary - regressing	-	-	1	0
Ovary - regressed	-	-	4	4
Ovary - cystic follicles	-	-	1	1
Ovary - hemorrhagic follicles	-	-	1	0
Not remarkable	9	8	2	0

[1] Gross necropsy observations for birds maintained for collection of blood samples during the test are not reported.

Table 6

Mean Liver Weights[1] (g)

from a Mallard Reproduction Study with PFOS

Experimental Group	Adult Males		Adult Females		Offspring Males		Offspring Females	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control 0 ppm a.i.	29.422	± 3.864	41.648	± 10.512	14.534	± 4.679	14.951	± 2.326
10 ppm a.i.	30.601	± 4.633	42.207	± 10.268	13.652	± 4.398	14.068	± 2.143
50 ppm a.i.	-	-	-	-	-	-	-	-
150 ppm a.i.	-	-	-	-	-	-	-	-

[1]Mean ± standard deviation.

- Data not available due to mortality.

The above differences from the control group were not statistically significant (Student's t-test).

Table 7

Summary of Reproductive Performance from a Mallard Reproduction Study with PFOS

Reproductive Parameter	Experimental Group (ppm a.i.)			
	Control	10	50	150
Number of Replicates	15	16	-	-
Total Eggs Laid	719	877	-	-
Eggs Cracked	10	10	-	-
Eggs Set	644	781	-	-
Viable Embryos	567	664	-	-
Live 3-Week Embryos	564	653	-	-
Hatchlings	460	522	-	-
14-Day Old Survivors	454	507	-	-
Eggs Laid/Hen	48	55	-	-
Eggs Laid/Hen/Day[1]	0.67	0.76	-	-
14-Day Old Survivors/Hen	30	32	-	-

[1] Based on 72 days of eggs production.

- Data are not available due to adult mortality

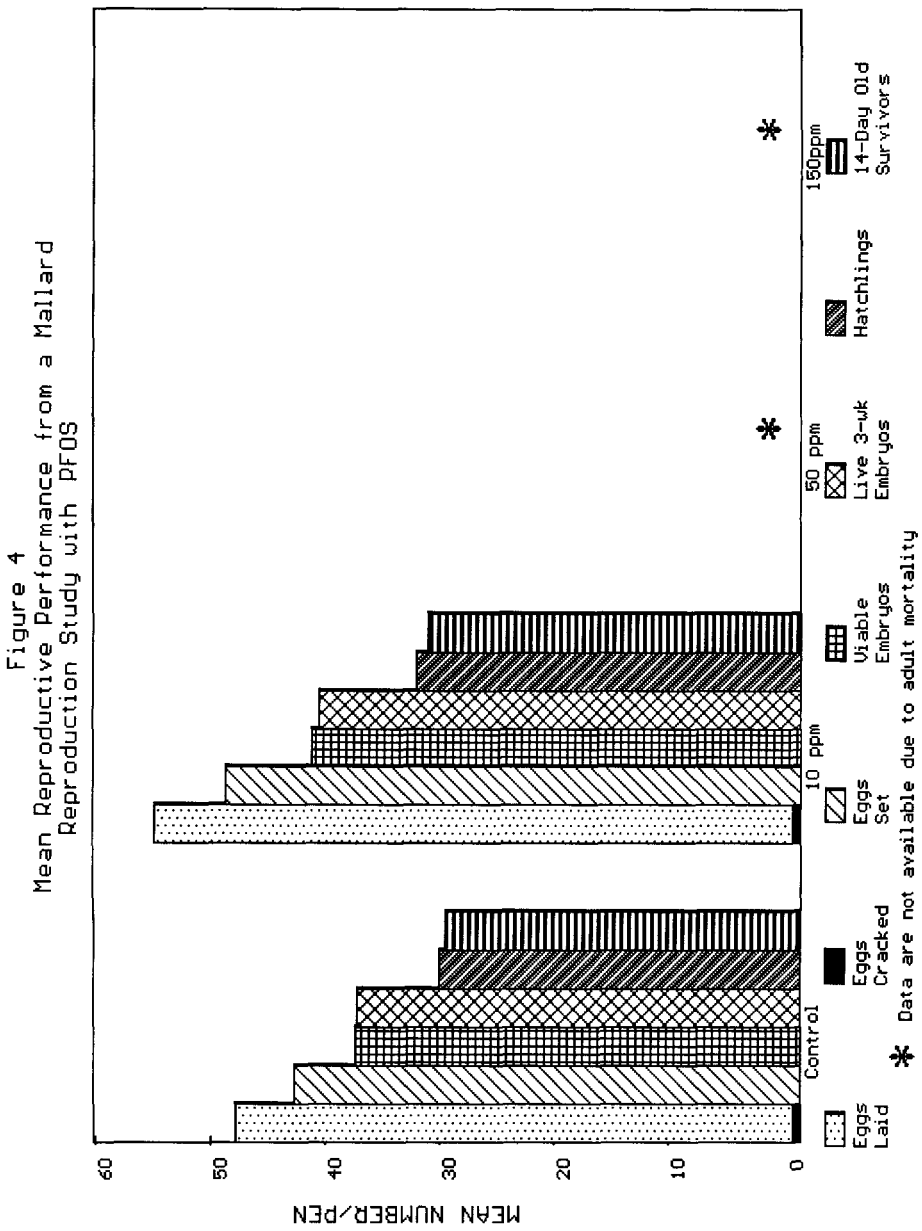


Figure 4
Mean Reproductive Performance from a Mallard
Reproduction Study with PFOS
MEAN NUMBER/PEN
0
10
20
30
40
50
60
Control
10 ppm
50 ppm
150ppm
Eggs Laid
Eggs Cracked
Eggs Set
Viable Embryos
Live 3-wk Embryos
Hatchlings
14-Day Old Survivors
* Data are not available due to adult mortality

Table 8

Summary of Reproductive Performance, Normalized as Percentages (%) from a Mallard Reproduction Study with PFOS[1]

	Experimental Group (ppm a.i.)			
Reproductive Parameter	Control	10	50	150
Number of Replicates	15	16	N/A	N/A
Total Eggs Laid[2]	719	877	N/A	N/A
Eggs Laid/Maximum Laid (%)	67	76	N/A	N/A
Eggs Cracked/Eggs Laid (%)	2	1	N/A	N/A
Viable Embryos/Eggs Set (%)	88	87	N/A	N/A
Live 3-Week Embryos/Viable Embryos (%)	100	98	N/A	N/A
Hatchlings/Live 3-Week Embryos (%)	81	80	N/A	N/A
14-Day Old Survivors/Hatchlings (%)	99	97	N/A	N/A
Hatchlings/Eggs Set (%)	71	69	N/A	N/A
14-Day Old Survivors/Eggs Set (%)	70	67	N/A	N/A
Hatchlings/Maximum Set (%)	48	51	N/A	N/A
14-Day Old Survivors/Maximum Set (%)	47	50	N/A	N/A

N/A – No eggs available due to adult mortality.

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

[1] Values represent pen means for experimental group. Values for each pen are presented in Appendices VII and VIII.

[2] Represents the total number of eggs laid in each group.

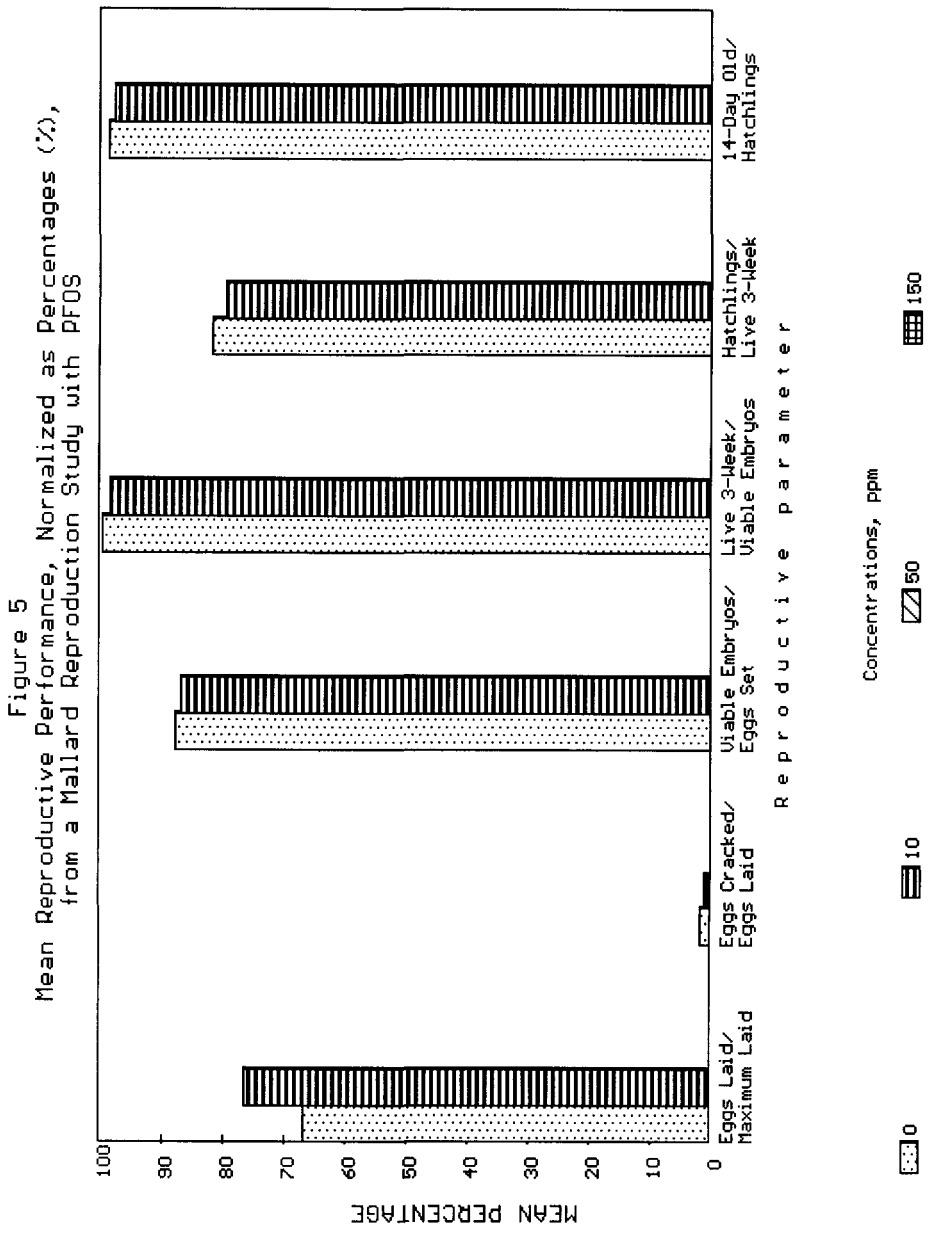


Figure 5
Mean Reproductive Performance, Normalized as Percentages (%),
from a Mallard Reproduction Study with PFOS
MEAN PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
Eggs Laid/
Maximum Laid
Eggs Cracked/
Eggs Laid
Viable Embryos/
Eggs Set
Live 3-Week/
Viable Embryos
Hatchlings/
Live 3-Week
14-Day Old/
Hatchlings
Reproductive parameter
Concentrations, ppm
0
10
50
150

Table 9

Mean Eggshell Thickness Measurements (mm)
from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)	No. Eggs Measured	Shell Thickness Mean (± SD)
Control	49	0.392 ± 0.034
10	54	0.396 ± 0.014
50	-	- -
150	-	- -

- Data are not available due to adult mortality

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

Table 10

Mean Body Weight (g) of Hatchlings and 14-Day Old Survivors from a Mallard Reproduction Study with PFOS

Experimental Group (PPM A.I.)	Hatchlings		14-Day Old Survivors	
	Number	Mean (± SD)	Number	Mean (± SD)
Control	459	35 ± 3	454	287 ± 28
10	521	37 ± 2	507	282 ± 23
50	0		0	
150	0		0	

The number of hatchlings weighed may differ from the total number of hatchlings since those hatchlings found dead were not weighed.

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

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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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, $C_8F_{17}SO_3^-K^+$ (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 II
Diet and Supplement Formulations

Table 2
Vitamins and Electrolytes Concentrate

Water Soluble Powder		
GUARANTEED ANALYSIS	Per 4 oz.	Per lb.
Vitamin A	2,5000,000	10,000,000 IU
Vitamin D	1,000,000	4,000,000 ICU
Vitamin E	1,000	4,000 IU
Riboflavin	750	3,000 Mg
d-Pantothenic Acid	1,250	5,000 Mg
Niacin	2,500	10,000 Mg
Vitamin B-12	2.5	10.0 Mg
MSBC	1,000	4,000 Mg
Folic Acid	65	260 Mg
Thiamine HC1	250	1,000 Mg
Pyridoxine Hydrochloride	250	1,000 Mg
Ascorbic Acid	3,750	15,000 Mg

INGREDIENTS:
Vitamin A Supplement, D-Activated Animal Sterol (source of Vitamin D_3), Alpha Tocopheryl Acetate (source of Vitamin E). Riboflavin Supplement, d-Calcium Pantothenate, Niacin Supplement, Vitamin B-12 Supplement, Menadione Sodium Bisulfite (source of Vitamin K), Folic Acid, Thiamine HC1, Pyridoxine Hydrochloride, Ascorbic Acid, Sodium Chloride, Calcium Chloride, Magnesium Sulfate, Ferric Ammonium Citrate, Potassium Chloride and Dextrose.

MIXING PROCEDURE:
The vitamin and electrolyte mix was prepared as a ration of approximately 2 grams of Durvet vitamins and electrolytes to approximately 1 gallon of water.

Appendix III
Diet Preparation

Premixes for PFOS were prepared on January 22, 2001; February 6 and 12, 2001; March 2, 6 and 27, 2001; April 12, 2001; May 3 and 23, 2001; and June 4, 2001. Nominal preparation was as follows:

Control:	No premix required.
10 ppm a.i.:	2.5633 g PFOS + 8097.4 g ration
50 ppm a.i.:	12.8165 g PFOS + 8087.2 g ration
150 ppm a.i.:	38.4494 g PFOS + 8061.6 g ration
20 ppm a.i.	5.1266 g PFOS + 8094.9 g ration

Basal ration, 5000.0 g, was weighed into a tared Hobart mixing bowl. The remaining ration was weighed and set aside. The test substance was weighed into a tared weigh boat. Approximately 100 g of set aside ration was placed in a Waring® blender. The test substance was transferred to the Waring® blender and the weigh boat was rinsed three times with set aside ration, 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 set aside ration with the rinse being added to the bowl. The remaining set aside ration was added to the bowl, the bowl was placed on a Hobart mixer, and the contents were mixed approximately 15 minutes. If not used immediately after mixing, 2000.0 g aliquots of the premix were weighed, 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.:	52.25 kg ration + 2.750 kg limestone
10 ppm a.i.:	2000 g Premix + 50.25 kg ration + 2.750 kg limestone
50 ppm a.i.:	2000 g Premix + 50.25 kg ration + 2.750 kg limestone
150 ppm a.i.:	2000 g Premix + 50.25 kg ration + 2.750 kg limestone
20 ppm a.i.	2000 g Premix + 50.25 kg ration + 2.750 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 HPLC/MS/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 3000 Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in multiple ion reaction monitoring (MRM) mode.
ANALYTICAL COLUMN:	Keystone Betasil C_{18} column (50 mm × 2 mm I.D., 3-µm particle size)
GUARD COLUMN:	Keystone Javelin C_{18} column (20 mm × 2 mm I.D.)
OVEN TEMPERATURE:	40°C
STOP TIME:	5.00 minutes
FLOW RATE:	250 µL/minute
MOBILE PHASE:	70.0% Methanol: 30.0% NANOpure® Water containing 0.1% Formic Acid
INJECTION VOLUME:	10.0 µL
PFOS RETENTION TIME:	Approximately 4.0 minutes
PFOS MONITORED MASS:	499.0 amu → 99.1 amu

APPENDIX IV

Table 2

Calculations

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

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

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:

LOQ (ppm a.i.) = lowest standard concentration (mg a.i./L) × 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.

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

APPENDIX IV

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 (454-109-)	Type	Interval	Fortified[1]	Measured[1,2]		
MAB-1	Matrix Blank	Week 1, Day 0	0.00	< 2.00	-	
MAS-1	Matrix Fortification	Week 1, Day 0	5.00	4.85	97	102
MAS-2	Matrix Fortification	Week 1, Day 0	50.0	52.8	106	
MAS-3	Matrix Fortification	Week 1, Day 0	200	206	103	
MAB-2	Matrix Blank	Week 2, Day 0	0.00	< 2.00	-	
MAS-4	Matrix Fortification	Week 2, Day 0	5.00	4.55	91	94
MAS-5	Matrix Fortification	Week 2, Day 0	50.0	47.4	95	
MAS-6	Matrix Fortification	Week 2, Day 0	200	189	95	
MAB-3	Matrix Blank	Week 3, Day 0	0.00	< 2.00	-	
MAS-7	Matrix Fortification	Week 3, Day 0	5.00	5.50	110	105
MAS-8	Matrix Fortification	Week 3, Day 0	50.0	52.0	104	
MAS-9	Matrix Fortification	Week 3, Day 0	200	200	100	
MAB-4	Matrix Blank	Week 4, Day 0	0.00	< 2.00	-	
MAS-10	Matrix Fortification	Week 4, Day 0	5.00	5.20	104	103
MAS-11	Matrix Fortification	Week 4, Day 0	25.0	26.1	104	
MAS-12	Matrix Fortification	Week 4, Day 0	100	100	100	

[1] Less than values correspond to limit of quantitation (LOQ).
[2] Results were generated using MacQuan version 1.6 software. Manual calculations may differ slightly.

APPENDIX IV

Table 3 (Continued)

Matrix Blanks and Fortifications Analyzed Concurrently with the Samples

Sample			Concentration of PFOS (ppm a.i.)		Percent Recovery	Mean Recovery ($\bar{x}$)
Number (454-109-)	Type	Interval	Fortified[1]	Measured[1,2]		
MAB-5	Matrix Blank	Week 8, Day 0	0.00	< 2.00	-	
MAS-13	Matrix Fortification	Week 8, Day 0	5.00	5.40	108	109
MAS-14	Matrix Fortification	Week 8, Day 0	25.0	27.5	110	
MAB-6	Matrix Blank	Week 12, Day 0	0.00	< 2.00	-	
MAS-15	Matrix Fortification	Week 12, Day 0	5.00	5.21	104	101
MAS-16	Matrix Fortification	Week 12, Day 0	25.0	24.4	98	
MAS-17[3]	Matrix Fortification	Week 16, Day 0	5.26	5.39	102	100
MAS-18	Matrix Fortification	Week 16, Day 0	25.0	24.5	98	
MAB-7	Matrix Blank	Week 20, Day 0	0.00	< 2.00	-	
MAS-19	Matrix Fortification	Week 20, Day 0	5.00	4.86	97	97
MAS-20	Matrix Fortification	Week 20, Day 0	25.0	24.0	96	

[1] Less than values correspond to limit of quantitation (LOQ).
[2] Results were generated using MacQuan version 1.6 software. Manual calculations may differ slightly.
[3] Week 16 samples were held and batched with Week 20 samples. Matrix fortifications were prepared to verify stability. A storage matrix blank was not required.

APPENDIX IV

Table 4

Homogeneity of PFOS in Avian Diet

Nominal Concentration (ppm a.i.)	Sample I.D. Number (454-109-)	Location Sampled in Mixing Vessel	Measured PFOS Concentration (ppm a.i.)	Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Mean Percent of Nominal
10	2	Top Left	9.69		
	3	Top Right	9.47		
	4	Middle Left	9.87	$\bar{x}$ = 9.75 ppm a.i	98
	5	Middle Right	10.2	SD = 0.313 ppm a.i.	
	6	Bottom Left	9.35	CV = 3.21%	
	7	Bottom Right	9.93		
50	8	Top Left	49.9		
	9	Top Right	48.9		
	10	Middle Left	51.7	$\bar{x}$ = 50.1 ppm a.i.	100
	11	Middle Right	50.4	SD = 0.981 ppm a.i.	
	12	Bottom Left	50.3	CV = 1.96%	
	13	Bottom Right	49.3		
150	14	Top Left	151		
	15	Top Right	153		
	16	Middle Left	143	$\bar{x}$ = 147 ppm a.i.	98
	17	Middle Right	143	SD = 4.31 ppm a.i.	
	18	Bottom Left	144	CV = 2.93%	
	19	Bottom Right	147		

APPENDIX IV

Table 5

Verification of PFOS Concentrations in Avian Diet

Nominal Concentration (ppm a.i.)	Sample I.D. Number (454-109-)	Interval (Day 0 of Week -)	Measured PFOS Concentration[1] (ppm a.i.)	Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Percent of Nominal	Mean Percent of Nominal
0	34	2	< 2.00			
	41	3	< 2.00			
	48	4	< 2.00			
	55	8	< 2.00	-	-	-
	60	12	< 2.00			
	65	16	< 2.00			
	70	20	< 2.00			
10	35	2	11.0		110	
	36	2	9.68		97	
	42	3	9.53		95	
	43	3	10.7		107	
	49	4	9.70		97	
	50	4	9.60		96	
	56	8	10.6	$\bar{x}$ = 10.2 ppm a.i.	106	102
	57	8	11.2	SD = 0.553 ppm a.i.	112	
	61	12	10.4	CV = 5.44%	104	
	62	12	9.86		99	
	66	16	9.82		98	
	67	16	10.4		104	
	71	20	10.1		101	
	72	20	9.69		97	

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

APPENDIX IV

Table 5 (Continued)

Verification of PFOS Concentrations in Avian Diet

Nominal Concentration (ppm a.i.)	Sample I.D. Number (454-109-)	Interval (Day 0 of Week -)	Measured PFOS Concentration[1] (ppm a.i.)	Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Percent of Nominal	Mean Percent of Nominal
20	46	3	18.6		93	
	47	3	19.8	$\bar{x}$ = 20.8 ppm a.i.	99	104
	53	4	23.6	SD = 2.14 ppm a.i.	118	
	54	4	21.1	CV = 10.3%	106	
50	37	2	48.7		97	
	38	2	48.4		97	
	44	3	51.1	$\bar{x}$ = 50.9 ppm a.i.	102	102
	45	3	53.1	SD = 2.04 ppm a.i.	106	
	51	4	51.1	CV = 4.01%	102	
	52	4	53.1		106	
150	39	2	172	$\bar{x}$ = 161 ppm a.i.	115	108
	40	2	150	SD = 15.6 ppm a.i.	100	
				CV = 9.66%		

APPENDIX IV

Table 6

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

	PFOS Concentration						
	Week 1, Day 0[1]			Week 1, Day 7			
Nominal Concentration (ppm a.i.)	Sample Number (454-109-)	Mean Measured (ppm a.i.)	Mean Percent of Nominal	Sample Number (454-109-)	Measured[2] (ppm a.i.)	Mean Measured (ppm a.i.)	Mean Percent of Day 0
0	1	<2.00	-	27	< 2.00	<2.00	-
10	2-7	9.75	98	28 29	9.75 9.67	9.71	100
50	8-13	50.1	100	30 31	53.4 48.1	50.8	101
150	14-19	147	98	32 33	141 144	143	97

[1] Day 0 values are from homogeneity samples presented in Table 4.
[2] 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 HPLC/MS/MS methodology. Use methanol for intermediate dilutions, if required. For all final dilutions, use 50% methanol : 50% NANOpure® water dilution solvent.

↓

Ampulate and submit sample for HPLC/MS/MS analysis.

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

APPENDIX IV

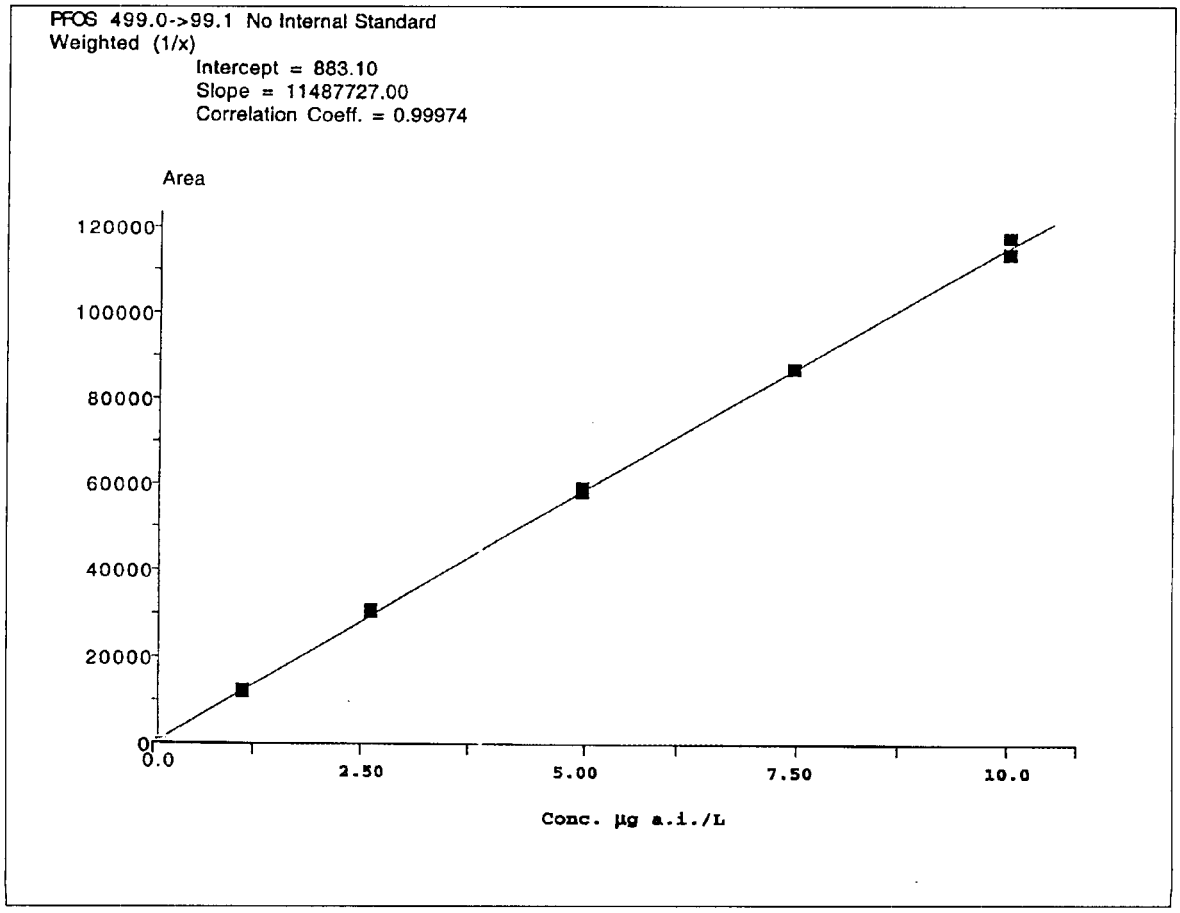


Figure 2. Typical calibration curve for PFOS. Slope = 11487727; Intercept = 883.10; r = 0.99974. Curve is weighted (1/x).

APPENDIX IV

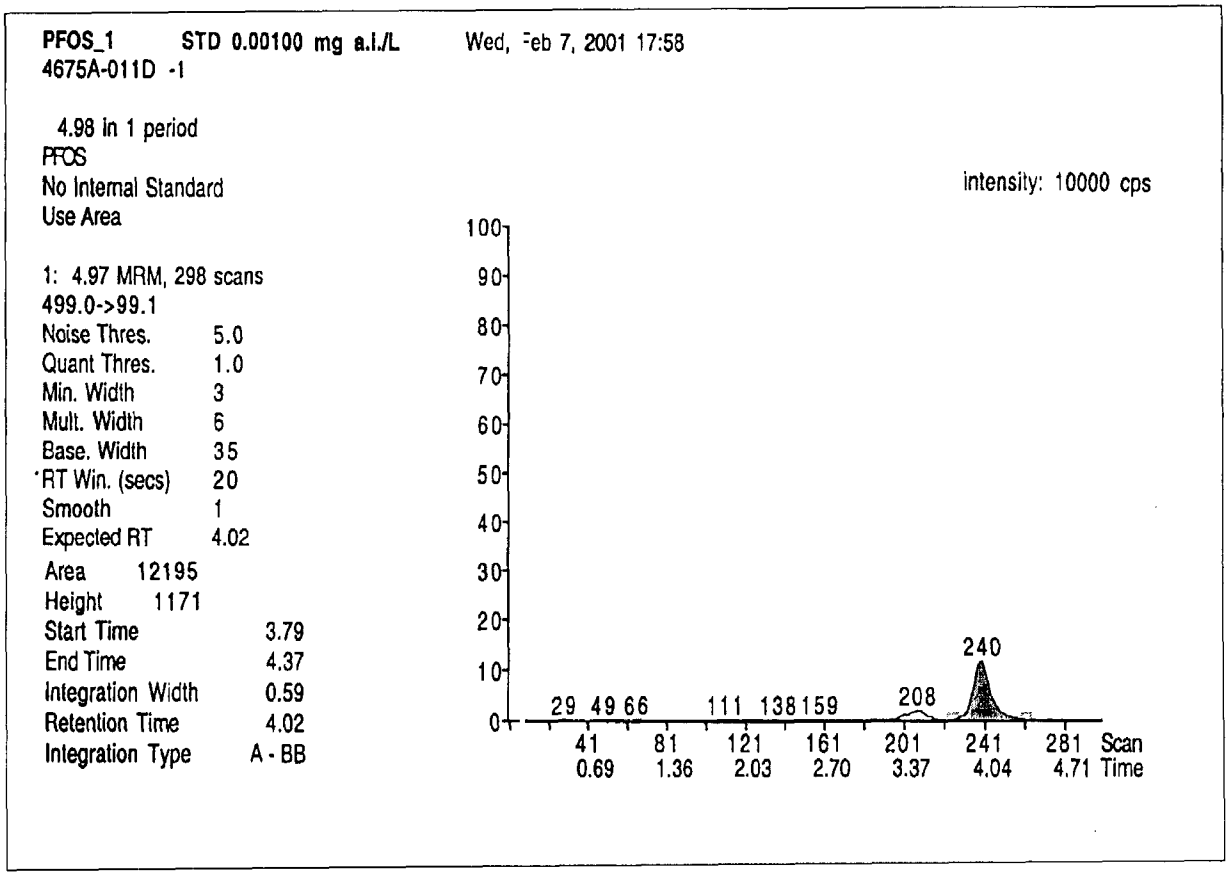


Figure 3. Typical ion chromatogram of a low PFOS calibration standard, 0.00100 mg a.i./L. (Monitored mass 499.0 → 99.1).

APPENDIX IV

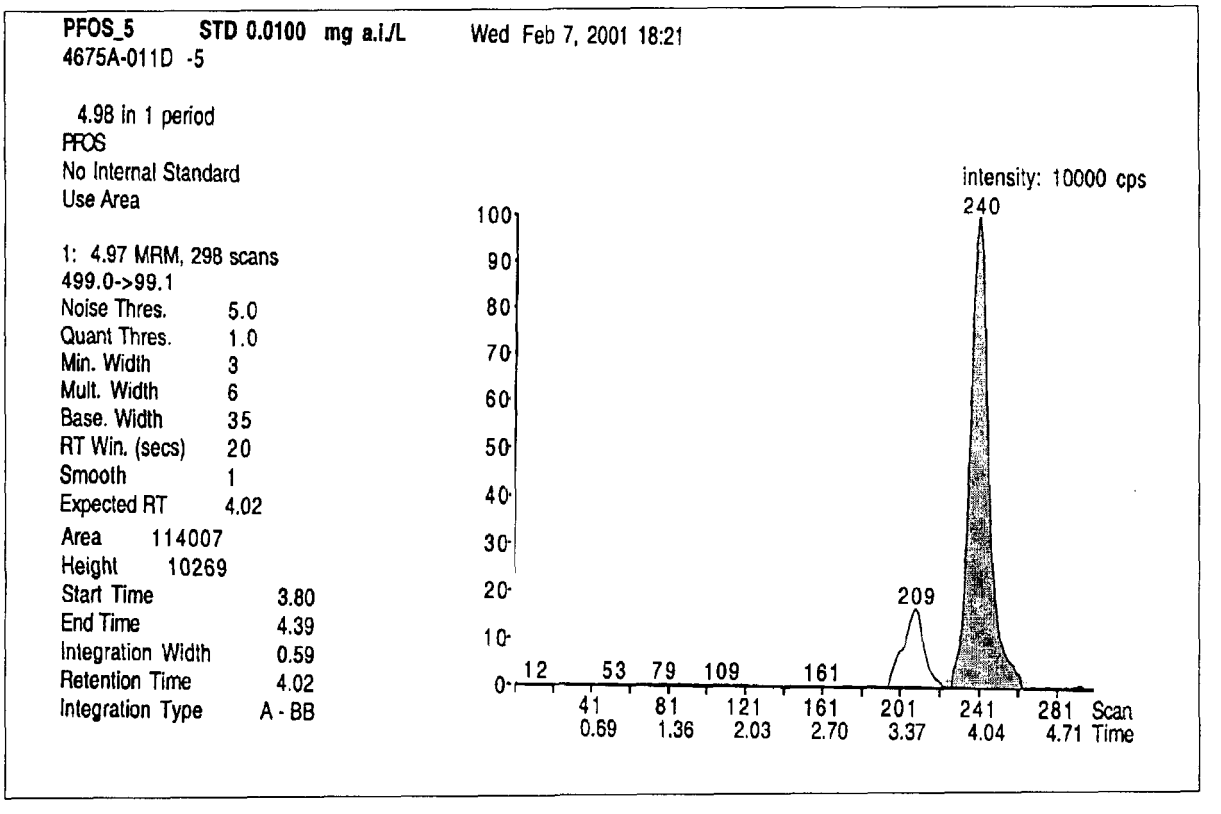


Figure 4. Typical ion chromatogram of a high PFOS calibration standard, 0.0100 mg a.i./L. (Monitored mass 499.0 → 99.1).

APPENDIX IV

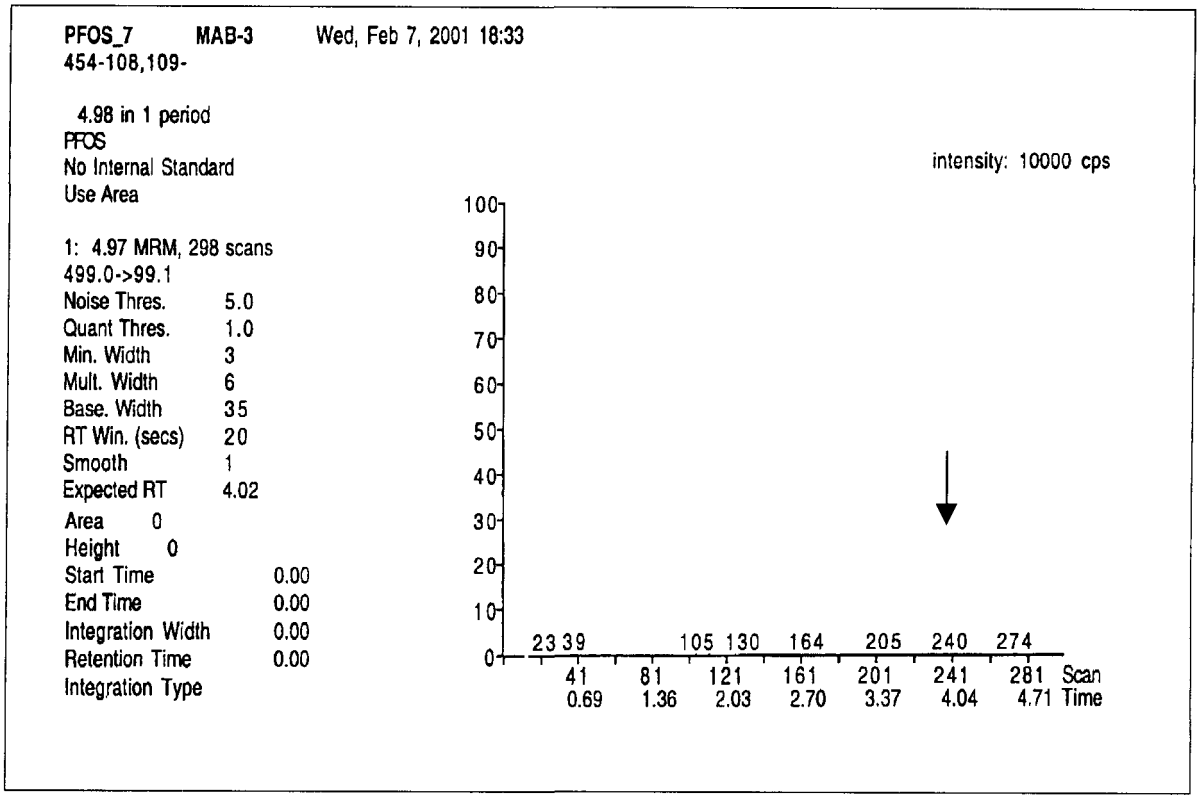


Figure 5. Typical ion chromatogram of a matrix blank sample (454-109-MAB-3). The arrow indicates the approximate retention time of PFOS.
(Monitored mass 499.0 → 99.1).

APPENDIX IV

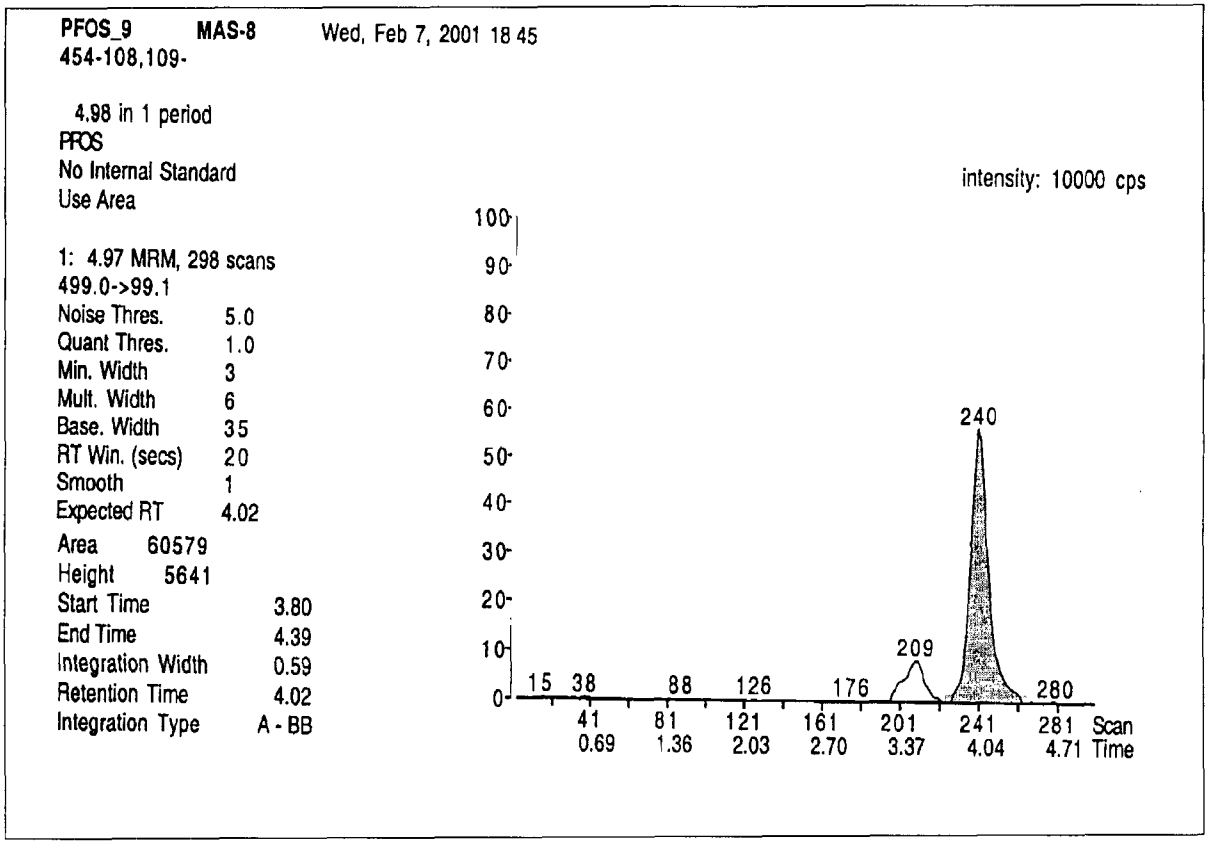


Figure 6. Typical ion chromatogram of a matrix fortification sample (454-109-MAS-8, 50.0 ppm a.i.)
(Monitored mass 499.0 → 99.1).

APPENDIX IV

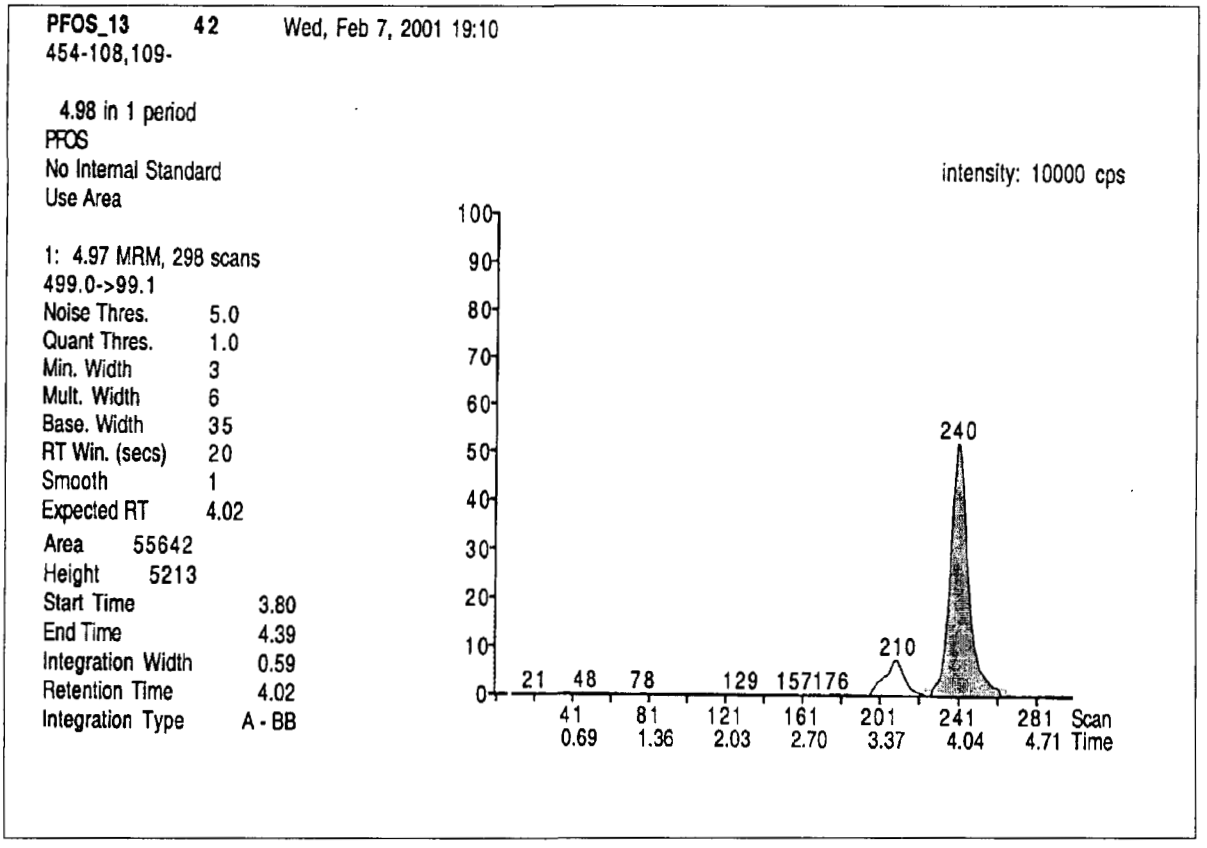
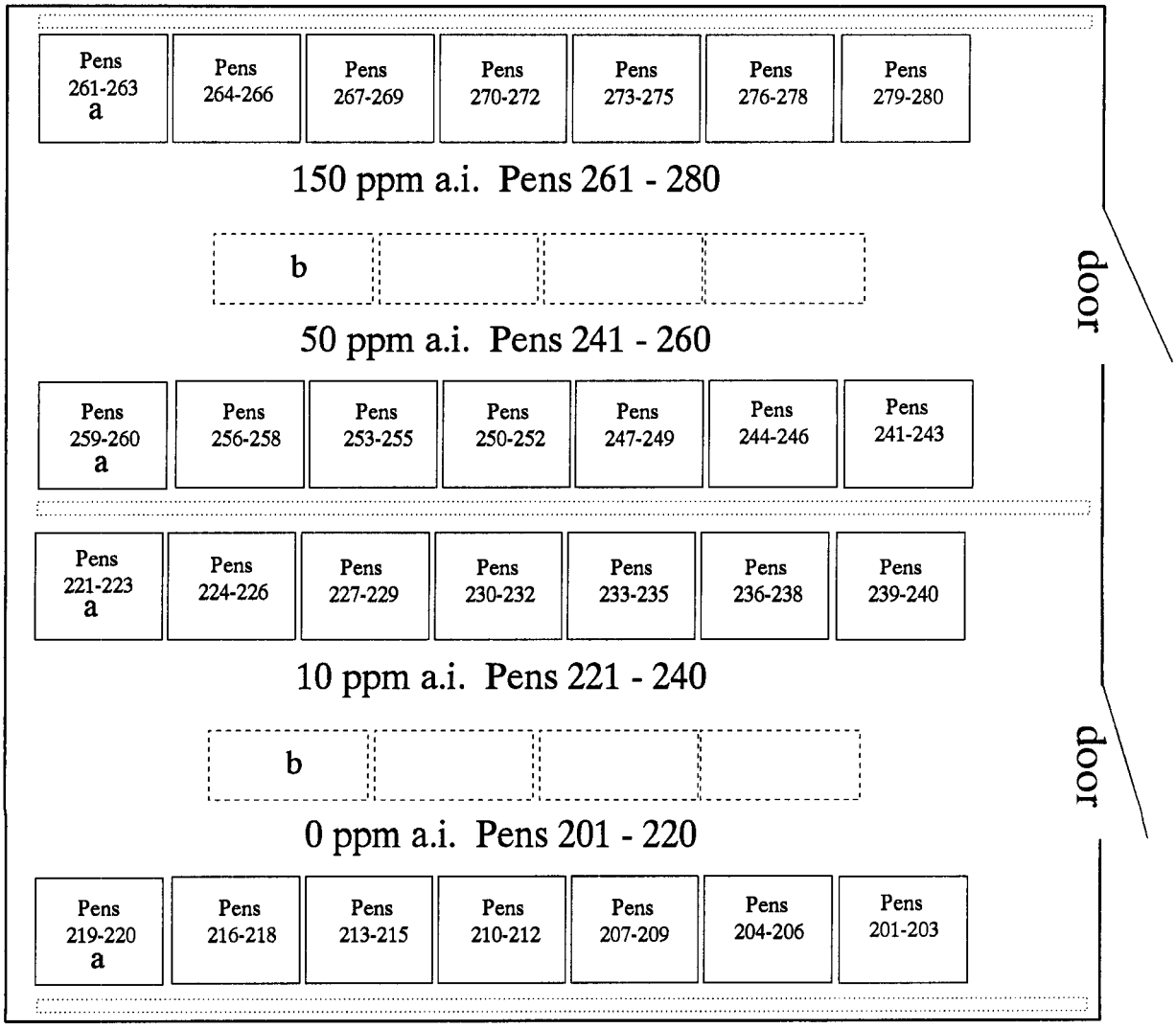


Figure 7. Typical ion chromatogram of an avian diet sample on Day 0 (454-109-42, 10.0 ppm a.i.). Dilution factor = 2000X.
(Monitored mass 499.0 → 99.1).

Appendix V
Diagram of Test Layout
from a Mallard Reproduction Study with PFOS

Room 56



= approx. 1m

= 6" gutter

a = Safeguard Products Inc. Model No. 5355

b = Four X 4 ft. Chroma 50 light bulbs

Note: Pens 217-220, 237-240, 257-260 and 277-280 were used to house birds used for blood sampling.

Appendix VI

Reproductive Parameters

1. Eggs Laid

   Definition - The number of eggs produced during the breeding season.

   Sensitivity - This is a parameter that is frequently affected by chemicals that cause reproductive impairment. It is also highly sensitive to the general conditions under which the test is conducted, and may be adversely affected by improper diet, crowding, excessive disturbances, etc.

2. Eggs Cracked

   Definition - Eggs determined to have cracked shells when inspected with a candling lamp. Fine cracks cannot be detected without utilizing a candling lamp, and if undetected, will bias the data by adversely affecting embryo development.

   Sensitivity - This parameter is not frequently affected as cracking is directly related to shell thickness, and few chemicals have caused egg shell thinning. Improper pen design, overcrowding, and pecking by the birds can also increase egg cracking.

3. Eggs Set

   Definition - All eggs placed under incubation, i.e., total eggs laid minus cracked eggs, mechanically damaged eggs, and those selected for egg shell thickness analysis. The reason for presenting this parameter is to establish a base of reference for the following parameter - viable embryos.

4. Viable Embryos

   Definition - Eggs in which fertilization has occurred and embryonic development has begun. This is determined by candling the eggs 13-14 days after the initiation of incubation. It is difficult to distinguish between infertility and an early embryonic death.

   Sensitivity - This is a frequently affected parameter with infertility or embryonic mortality caused by an unfavorable environment for fertilization in the oviduct, impotent males, or chemical residue in the egg.

Appendix VI
Reproductive Parameters

Page 2

5. Live Three-Week Embryos

Definition - These are embryos that are developing normally after three weeks of incubation. This is determined by candling the eggs.

Sensitivity - This parameter is seldom affected.

6. Hatchlings

Definition - Embryos that mature, pip the shell, and liberate themselves from their eggs on Day 27 or 28 of incubation.

Sensitivity - This is a frequently affected parameter which is also highly sensitive to the conditions of incubation, such as rate and angle of rotation, humidity, and temperature.

- Humidity: If too dry, ducklings will stick to their shells.

- Temperature: If too hot and humid, ducklings will grow too fast and be too large for their shells; thereby not having the intra-egg mobility necessary to pip their shells.

7. Body Weight - Hatchlings

Definition - The average weight of hatchlings by parental pen of origin, taken immediately upon removal from the hatcher. Hatchlings from each weekly lot of eggs are weighed. Mean weight for each week and mean weight by pen are reported.

Sensitivity - This is an occasionally affected parameter and may reflect some residual or latent toxic effects from chemical residue in the egg.

8. 14-Day Old Survivors

Definition - Birds that survive brooding for two weeks following hatch.

Sensitivity - This is a seldom affected parameter and is probably more indicative of the conditions under which the birds were reared in battery brooders than the chemical to which the adults were exposed.

Appendix VI

Reproductive Parameters

Page 3

9. Body Weight - 14-Day Old Survivors

Definition - The average body weight of the 14-day old survivors by parental pen of origin, taken upon removal from the brooder unit at 14 days of age. Fourteen-day old survivors from each weekly lot of eggs are weighed. Mean weight for each week and mean weight by pen are reported.

Sensitivity - This parameter is seldom affected, and more closely reflects the rearing practices utilized.

10. Egg Shell Thickness

Definition - The thickness of the shell and the membrane of the egg at its equator after having been opened, washed, and dried for at least one week at room temperature. Egg shell thickness measurements for each egg measured, and pen means are reported.

Sensitivity - This is a seldom affected parameter; however, measurements must be taken very carefully to ensure accuracy. Possible mechanical errors include membrane curling at the measurement surface, and calcium deposits on the measurement surface.

Appendix VI

Replicate Identification

Experimental Group	Report Replicate Number	Report Pen Number	Raw Data Pen Number	Raw Data Band Number	
				Male	Female
Control	1	201	201	329	330
0 ppm a.i.	2	202	202	331	332
	3	203	203	333	334
	4	204	204	335	336
	5	205	205	337	338
	6	206	206	339	340
	7	207	207	341	342
	8	208	208	343	344
	9	209	209	345	346
	10	210	210	347	348
	11	211	211	349	350
	12	212	212	351	352
	13	213	213	353	354
	14	214	214	355	356
	15	215	215	357	358
	16	216	216	359	360

Appendix VI

Replicate Identification

Experimental Group	Report Replicate Number	Report Pen Number	Raw Data Pen Number	Raw Data Band Number	
				Male	Female
Treatment	1	217	221	369	370
10 ppm a.i.	2	218	222	371	372
	3	219	223	373	374
	4	220	224	375	376
	5	221	225	377	378
	6	222	226	379	380
	7	223	227	381	382
	8	224	228	383	384
	9	225	229	385	386
	10	226	230	387	388
	11	227	231	389	390
	12	228	232	391	392
	13	229	233	393	394
	14	230	234	395	396
	15	231	235	397	398
	16	232	236	399	400

Appendix VII - Table 1

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

(Control, Males)

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
201	1098	-11	1087	15	1102	-12	1090	-47	1043	119	1162	64
202	1061	-56	1005	22	1027	34	1061	27	1088	96	1184	123
203	994	-1	993	24	1017	43	1060	-15	1045	130	1175	181
204	1077	56	1133	19	1152	-18	1134	5	1139	53	1192	115
205	1094	-25	1069	75	1144	-19	1125	-54	1071	144	1215	121
206	1061	-15	1046	23	1069	-4	1065	-13	1052	99	1151	90
207	1078	25	1103	21	1124	-18	1106	6	1112	42	1154	76
208	1073	12	1085	33	1118	51	1169	22	1191	-78	1113	40
209	1126	-38	1088	23	1111	42	1153	-5	1148	29	1177	51
210	1264	5	1269	26	1295	18	1313	-5	1308	-	-	-
211	1050	43	1093	-5	1088	0	1088	-3	1085	-13	1072	22
212	1018	59	1077	1	1078	13	1091	12	1103	0	1103	85
213	997	41	1038	36	1074	44	1118	25	1143	-30	1113	116
214	1164	-21	1143	21	1164	22	1186	-4	1182	85	1267	103
215	1139	0	1139	24	1163	1	1164	5	1169	-68	1101	-38
216	1057	31	1088	16	1104	13	1117	1	1118	167	1285	228
Mean	1084	7	1091	23	1114	13	1128	-3	1125	52	1164	92
SD	67	34	64	17	65	24	63	22	68	77	60	64

The means for body weights and body weight changes are calculated and rounded separately.
- Data are not available due to adult mortality.

Appendix VII - Table 2

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

(Control, Females)

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
201	905	35	940	25	965	-19	946	-4	942	249	1191	286
202	857	-11	846	32	878	21	899	33	932	101	1033	176
203	860	21	881	6	887	19	906	6	912	246	1158	298
204	1024	26	1050	46	1096	8	1104	-7	1097	205	1302	278
205	924	25	949	-4	945	34	979	39	1018	-46	972	48
206	1025	-3	1022	10	1032	29	1061	24	1085	250	1335	310
207	1057	-40	1017	27	1044	-11	1033	14	1047	121	1168	111
208	1092	36	1128	1	1129	43	1172	15	1187	227	1414	322
209	1086	-3	1083	10	1093	31	1124	-7	1117	93	1210	124
210	968	32	1000	3	1003	32	1035	5	1040	-52	988	20
211	930	23	953	33	986	9	995	5	1000	214	1214	284
212	986	-14	972	1	973	23	996	20	1016	150	1166	180
213	1215	-48	1167	12	1179	26	1205	21	1226	28	1254	39
214	863	-4	859	7	866	18	884	1	885	102	987	124
215	983	15	998	-11	987	6	993	14	1007	55	1062	79
216	962	-20	942	21	963	31	994	18	1012	12	1024	62
Mean	984	4	988	14	1002	19	1020	12	1033	122	1155	171
SD	98	26	90	15	90	17	94	14	94	103	134	109

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

Appendix VII - Table 3

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

(10 PPM A.I., Males)

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
217	966	22	988	-10	978	22	1000	-72	928	43	971	5
218	1273	-2	1271	12	1283	20	1303	0	1303	-84	1219	-54
219	1153	-57	1096	12	1108	-11	1097	30	1127	171	1298	145
220	1193	53	1246	-34	1212	-2	1210	38	1248	68	1316	123
221	1038	10	1048	-14	1034	14	1048	29	1077	24	1101	63
222	1148	31	1179	18	1197	27	1224	-15	1209	-59	1150	2
223	1049	7	1056	24	1080	-4	1076	-4	1072	-63	1009	-40
224	1003	2	1005	-20	985	2	987	-13	974	-25	949	-54
225	1146	44	1190	-9	1181	25	1206	-7	1199	40	1239	93
226	1148	-13	1135	43	1178	16	1194	5	1199	-35	1164	16
227	1097	-11	1086	3	1089	61	1150	55	1205	-116	1089	-8
228	1231	73	1304	12	1316	32	1348	26	1374	-230	1144	-87
229	1079	9	1088	15	1103	-11	1092	55	1147	-34	1113	34
230	1081	-5	1076	26	1102	22	1124	-30	1094	2	1096	15
231	1121	-2	1119	5	1124	39	1163	29	1192	-136	1056	-65
232	1042	3	1045	21	1066	23	1089	-21	1068	54	1122	80
Mean	1111	10	1121	7	1127	17	1144	7	1151	-24	1127	17
SD	82	30	94	20	96	19	101	34	115	95	105	69

The means for body weights and body weight changes are calculated and rounded separately.
Differences between control and this treatment group were not significant ($p>0.05$).

Appendix VII - Table 4

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

(10 PPM A.I., Females)

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
217	1071	93	1164	63	1227	-61	1166	38	1204	11	1215	144
218	890	18	908	-2	906	19	925	22	947	237	1184	294
219	984	-36	948	33	981	18	999	-4	995	167	1162	178
220	1083	12	1095	23	1118	141	1259	34	1293	23	1316	233
221	1068	-13	1055	5	1060	-11	1049	-23	1026	86	1112	44
222	1079	-20	1059	30	1089	-23	1066	-24	1042	136	1178	99
223	1148	54	1202	27	1229	46	1275	53	1328	-29	1299	151
224	852	-7	845	11	856	4	860	2	862	126	988	136
225	1000	87	1087	8	1095	-16	1079	-25	1054	129	1183	183
226	1025	29	1054	-22	1032	-4	1028	5	1033	26	1059	34
227	988	33	1021	7	1028	41	1069	-11	1058	200	1258	270
228	1028	44	1072	34	1106	-25	1081	-3	1078	144	1222	194
229	827	21	848	42	890	59	949	38	987	154	1141	314
230	970	44	1014	-3	1011	86	1097	39	1136	45	1181	211
231	879	7	886	-13	873	13	886	23	909	76	985	106
232	824	-14	810	37	847	15	862	-23	839	-18	821	-3
Mean	982	22	1004	18	1022	19	1041	9	1049	95	1144	162
SD	101	37	117	22	123	48	126	27	138	79	128	92

The means for body weights and body weight changes are calculated and rounded separately.
Differences between control and this treatment group were not significant ($p > 0.05$).

Appendix VII - Table 5

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

(50 PPM A.I., Males)

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
233	1013	-39	974	-48	926	-79	847	-	-	-	-	-
234	1039	-46	993	-21	972	2	974	-	-	-	-	-
235	1444	-62	1382	-134	1248	-130	1118	-	-	-	-	-
236	1023	-38	985	-62	923	-182	741	-	-	-	-	-
237	997	-50	947	-11	936	-28	908	-	-	-	-	-
238	1196	-88	1108	-100	1008	-29	979	-	-	-	-	-
239	1367	-84	1283	-77	1206	-54	1152	-	-	-	-	-
240	1084	-74	1010	-62	948	-109	839	-	-	-	-	-
241	1018	-69	949	-43	906	-58	848	-	-	-	-	-
242	1180	-83	1097	-98	999	-44	955	-	-	-	-	-
243	1142	-89	1053	-66	987	-	-	-	-	-	-	-
244	1009	-10	999	-47	952	-74	878	-	-	-	-	-
245	1088	-42	1046	-57	989	-36	953	-	-	-	-	-
246	998	-42	956	-77	879	-38	841	-	-	-	-	-
247	936	-5	931	-21	910	-68	842	-	-	-	-	-
248	1129	-95	1034	-98	936	-67	869	-	-	-	-	-
Mean	1104	-57	1047	-64	983**	-66	916**	-	-	-	-	-
SD	139	28	124	33	102	46	109	-	-	-	-	-

The means for body weights and body weight changes are calculated and rounded separately.
- Data are not available due to adult mortality.
** Significantly different from the control at $p < 0.01$

Appendix VII - Table 6

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

(50 PPM A.I., Females)

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
233	939	-106	833	-56	777	1	778	-	-	-	-	-
234	919	-75	844	-51	793	0	793	-	-	-	-	-
235	978	-81	897	-90	807	-76	731	-	-	-	-	-
236	981	-90	891	-36	855	-27	828	-	-	-	-	-
237	969	-145	824	-40	784	-69	715	-	-	-	-	-
238	951	-73	878	-27	851	-41	810	-	-	-	-	-
239	880	-60	820	-25	795	26	821	-	-	-	-	-
240	887	-75	812	-58	754	-37	717	-	-	-	-	-
241	1002	-105	897	-50	847	-103	744	-	-	-	-	-
242	928	-19	909	-71	838	12	850	-	-	-	-	-
243	1034	-98	936	-89	847	-15	832	-	-	-	-	-
244	963	-106	857	-38	819	-51	768	-	-	-	-	-
245	849	-76	773	-79	694	-39	655	-	-	-	-	-
246	904	-108	796	-84	712	-39	673	-	-	-	-	-
247	1109	-56	1053	-101	952	-6	946	-	-	-	-	-
248	995	-54	941	-65	876	-38	838	-	-	-	-	-
Mean	956	-83	873**	-60	813**	-31	781**	-	-	-	-	-
SD	64	29	69	24	63	34	74	-	-	-	-	-

The means for body weights and body weight changes are calculated and rounded separately.
- Data are not available due to adult mortality.
** Significantly different from the control at $p < 0.01$

Appendix VII - Table 7

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

(150 PPM A.I., Males)

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
249	1128	-145	983	145	1128	-	-	-	-	-	-	-
250	1024	-242	782	276	1058	-	-	-	-	-	-	-
251	1057	-198	859	72	931	-	-	-	-	-	-	-
252	1177	-246	931	113	1044	-	-	-	-	-	-	-
253	898	-208	690	-	-	-	-	-	-	-	-	-
254	1229	-263	966	102	1068	-	-	-	-	-	-	-
255	1012	-239	773	118	891	-	-	-	-	-	-	-
256	1094	-185	909	-	-	-	-	-	-	-	-	-
257	1222	-174	1048	-27	1021	-	-	-	-	-	-	-
258	1276	-228	1048	122	1170	-	-	-	-	-	-	-
259	1218	-158	1060	-6	1054	-	-	-	-	-	-	-
260	1060	-152	908	88	996	-	-	-	-	-	-	-
261	1178	-209	969	55	1024	-	-	-	-	-	-	-
262	1050	-132	918	31	949	-	-	-	-	-	-	-
263	1008	-192	816	50	866	-	-	-	-	-	-	-
264	1073	-143	930	90	1020	-	-	-	-	-	-	-
Mean	1107	-195	912**	88	1016**	-	-	-	-	-	-	-
SD	103	41	106	73	85	-	-	-	-	-	-	-

The means for body weights and body weight changes are calculated and rounded separately.
- Data are not available due to adult mortality.
** Significantly different from the control at $p < 0.01$

Appendix VII - Table 8

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

(150 PPM A.I., Females)

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Change Week 6-8	Week 8	Change Week 8-Term	Test Term	Total Change
249	857	-182	675	-	-	-	-	-	-	-	-	-
250	832	-200	632	198	830	-	-	-	-	-	-	-
251	891	-143	748	88	836	-	-	-	-	-	-	-
252	903	-218	685	127	812	-	-	-	-	-	-	-
253	892	-159	733	124	857	-	-	-	-	-	-	-
254	972	-205	767	44	811	-	-	-	-	-	-	-
255	1113	-205	908	135	1043	-	-	-	-	-	-	-
256	1091	-238	853	-	-	-	-	-	-	-	-	-
257	1027	-266	761	134	895	-	-	-	-	-	-	-
258	912	-178	734	103	837	-	-	-	-	-	-	-
259	1056	-257	799	86	885	-	-	-	-	-	-	-
260	1159	-236	923	106	1029	-	-	-	-	-	-	-
261	1061	-208	853	-3	850	-	-	-	-	-	-	-
262	1116	-248	868	21	889	-	-	-	-	-	-	-
263	781	-136	645	166	811	-	-	-	-	-	-	-
264	1104	-212	892	46	938	-	-	-	-	-	-	-
Mean	985	-206	780**	98	880**	-	-	-	-	-	-	-
SD	119	39	95	56	76	-	-	-	-	-	-	-

The means for body weights and body weight changes are calculated and rounded separately.
- Data are not available due to adult mortality.
** Significantly different from the control at $p < 0.01$

Appendix VIII - Table 1

Feed Consumption (g/bird/day) from a Mallard Reproduction Study with PFOS (Control)

	WEEKS																				
Pen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
201	96	100	122	138	144	132	138	155	138	158	152	174	169	168	164	157	162	181	183	211	204
202	76	82	102	110	90	95	104	107	95	96	79	172	191	171	112	123	129	146	129	155	145
203	93	106	115	132	91	111	92	115	121	115	139	184	225	252	236	200	253	324	232	223	292
204	154	159	133	141	124	144	138	116	129	125	158	179	212	158	165	147	164	168	151	176	170
205	76	89	81	102	92	111	110	91	129	133	163	168	175	188	240	275	243	243	259	260	318
206	104	127	130	140	126	148	151	148	139	131	141	195	157	141	139	136	166	166	166	164	167
207	96	107	105	114	104	111	118	110	102	96	105	150	170	108	131	144	140	157	164	162	180
208	85	155	121	159	168	187	162	175	164	151	114	226	239	214	181	217	242	263	191	239	234
209	182	174	187	236	195	231	235	230	252	196	227	180	185	171	192	223	224	327	245	245	227
210	86	101	91	100	106	107	97	108	92	103	96	143	165	127	143	185	210	215	185	115	143
211	108	148	103	125	115	124	125	116	125	108	123	178	166	144	156	159	144	145	130	180	169
212	136	149	152	147	141	152	158	174	149	168	171	193	217	195	205	174	220	265	246	226	267
213	78	119	106	114	113	116	123	130	119	126	117	156	148	162	153	137	155	152	132	168	150
214	67	102	100	103	97	103	101	107	105	114	102	228	179	168	182	168	160	214	261	212	224
215	113	131	145	160	170	156	170	151	180	164	166	188	215	210	241	244	242	230	253	173	201
216	78	109	106	122	107	105	101	120	128	123	114	137	151	187	204	159	205	202	179	209	191
Mean	102	122	119	134	124	133	133	134	135	132	135	178	185	173	178	178	191	212	194	195	205
SD	31	27	26	33	31	36	37	36	39	29	37	26	28	35	40	43	43	59	48	39	53

Appendix VIII - Table 2

Feed Consumption (g/bird/day) from a Mallard Reproduction Study with PFOS (10 PPM A.I.)

	WEEKS																				
Pen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
217	121	146	93	134	117	110	129	126	136	132	149	171	157	140	136	133	125	129	122	139	145
218	94	112	107	137	133	131	134	134	131	136	171	166	223	196	215	235	221	232	228	205	233
219	117	159	125	152	145	163	172	181	166	157	196	173	220	188	158	164	195	178	198	190	218
220	206	238	205	172	183	229	264	229	245	190	134	226	224	209	234	206	214	242	259	263	274
221	84	117	109	122	108	133	133	124	124	136	120	176	218	195	189	186	190	216	122	194	190
222	107	136	129	149	133	138	135	130	124	123	104	146	193	166	140	127	140	172	158	169	161
223	169	206	191	232	189	171	166	156	151	150	131	143	173	197	221	192	218	216	215	188	216
224	124	116	121	166	99	110	126	113	115	113	117	126	134	128	113	135	138	134	123	133	131
225	137	171	156	129	144	147	129	124	108	111	93	132	169	171	176	145	149	160	138	163	170
226	103	104	109	133	117	113	113	116	101	122	134	138	168	146	148	136	159	150	143	163	186
227	90	116	115	143	126	138	133	140	134	132	109	148	168	160	168	173	172	172	155	172	155
228	153	199	182	180	163	169	178	177	194	163	103	168	177	162	141	134	144	136	135	139	187
229	94	115	123	139	131	149	155	154	136	134	115	184	212	229	226	198	203	219	200	212	240
230	109	149	146	126	149	154	150	148	136	148	169	166	200	246	250	273	256	276	237	252	225
231	98	129	112	118	148	145	146	164	136	149	119	132	155	129	142	153	171	196	247	221	190
232	93	123	115	109	111	126	102	103	86	118	99	142	143	144	144	165	175	173	175	219	174
Mean	119	146	134	146	137	145	148	145	139	138	129	158	183	175	175	172	180	188	178	189	193
SD	33	39	33	30	26	29	37	32	38	21	29	25	30	35	42	41	37	43	48	39	38

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

Appendix VIII - Table 3

Feed Consumption (g/bird/day) from a Mallard Reproduction Study with PFOS (50 PPM A.I.)

	WEEKS																				
Pen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
233	110	121	96	96	80	75	93	-	-	-	-	-	-	-	-	-	-	-	-	-	-
234	73	108	96	81	87	125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
235	116	123	159	153	123	112	109	-	-	-	-	-	-	-	-	-	-	-	-	-	-
236	159	180	155	144	122	77	109	-	-	-	-	-	-	-	-	-	-	-	-	-	-
237	90	98	98	122	129	119	134	-	-	-	-	-	-	-	-	-	-	-	-	-	-
238	91	93	103	117	118	136	110	-	-	-	-	-	-	-	-	-	-	-	-	-	-
239	79	86	100	101	125	136	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-
240	117	122	144	148	166	138	156	-	-	-	-	-	-	-	-	-	-	-	-	-	-
241	116	105	129	134	84	88	87	-	-	-	-	-	-	-	-	-	-	-	-	-	-
242	107	149	149	195	163	136	87	-	-	-	-	-	-	-	-	-	-	-	-	-	-
243	77	90	81	102	95	158	63	-	-	-	-	-	-	-	-	-	-	-	-	-	-
244	92	133	141	132	99	182	254	-	-	-	-	-	-	-	-	-	-	-	-	-	-
245	114	110	114	130	109	123	95	-	-	-	-	-	-	-	-	-	-	-	-	-	-
246	72	92	107	148	131	161	81	-	-	-	-	-	-	-	-	-	-	-	-	-	-
247	96	131	109	124	90	113	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-
248	104	91	114	139	132	107	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	101	114	118	129	116	124	112	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SD	22	25	24	27	26	29	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Appendix VIII - Table 4

Feed Consumption (g/bird/day) from a Mallard Reproduction Study with PFOS (150 PPM A.I.)

	WEEKS																				
Pen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
249	64	67	116	198	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250	56	34	163	165	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
251	118	118	198	185	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
252	80	72	172	149	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
253	53	71	134	108	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
254	39	45	215	171	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
255	53	58	222	285	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
256	42	47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
257	62	50	63	190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
258	70	77	171	161	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
259	70	52	106	133	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
260	43	69	133	166	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
261	166	142	175	194	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
262	83	94	149	225	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
263	56	51	164	165	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
264	109	159	300	411	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	73*	75**	166**	194**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SD	33	36	56	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

* Significantly different from the control at $p < 0.05$

** Significantly different from the control at $p < 0.01$

Appendix IX - Table 1
Individual Gross Pathological Observations
from a Mallard Reproduction Study with PFOS
Adult Birds Euthanized at Test Termination
Control (0 ppm a.i.)

Males	Pens															
	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216
Foot/leg lesions	X	X	X	-	-	-	-	-	-	a	-	-	X	-	-	-
Testes - small (~ 1.5-3.5 cm)	-	-	-	-	X	-	-	-	-	a	-	-	-	X	-	-
Not remarkable	-	-	-	X	-	X	X	X	X	a	X	X	-	-	X	X

Females	Pens															
	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216
Foot/leg lesions	-	X	-	X	X	X	-	X	X	-	-	X	-	-	-	X
Spleen - enlarged and mottled	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-
Liver - firm, pale and mottled	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-
Abdominal cavity - yolk remnants	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-
Abdominal cavity - slight egg yolk peritonitis	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abdominal cavity - egg yolk peritonitis	-	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-
Abdominal cavity - slight old egg yolk peritonitis	-	-	X	-	X	-	-	X	-	-	-	-	-	-	X	X
Small intestines - area of hyperemia	X	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Kidneys - pale	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-
Reproductive tract - multiple fluid filled cysts	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-
Ovary - regressing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
Ovary - regressed	-	-	-	-	X	-	-	-	-	X	-	-	X	X	-	-
Ovary - cystic follicles	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
Ovary - hemorrhagic follicles	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
Not remarkable	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-	-

a - Bird found dead during the test. Necropsy findings are presented in Table 2.

Appendix IX - Table 2
Individual Gross Pathological Observations
from a Mallard Reproduction Study with PFOS
Adult Birds Euthanized at Test Termination
10 ppm a.i.

Males	Pens															
	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232
Foot/leg lesions	-	X	X	-	-	-	X	-	-	-	-	X	-	-	-	-
Testes - small (~ 1.5-3.5 cm)	-	-	X	X	-	-	-	-	-	X	-	X	-	-	X	X
Not remarkable	X	-	-	-	X	X	-	X	X	-	X	-	X	X	-	-

Females	Pens															
	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232
Foot/leg lesions	X	X	-	X	X	X	X	X	X	-	X	-	X	X	X	X
Breast blister	-	X	-	-	-	-	-	-	-	-	-	-	-	X	-	-
Cranium - swollen and bruised	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Heart - pericardial sac fluid-filled	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-
Spleen - enlarged and firm	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-
Abdominal cavity - egg yolk peritonitis	-	-	-	-	-	-	-	-	-	-	-	-	-	X	X	X
Ovary - regressed	-	-	-	-	-	-	-	-	-	X	-	X	-	-	X	X
Ovary - cystic follicles	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-
Not remarkable	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix X - Table 1
Adult Liver Weights (g)
from a Mallard Reproduction Study with PFOS
Control (0 ppm a.i.)

	Male	Female
Pen	Liver	Liver
201	31.051	36.870
202	32.528	36.336
203	33.915	46.728
204	25.209	45.564
205	33.438	57.924
206	28.829	46.002
207	28.689	38.662
208	27.090	59.838
209	25.919	52.441
210	-	21.509
211	27.129	45.440
212	30.090	47.038
213	38.599	37.995
214	27.888	25.939
215	26.030	34.229
216	24.921	33.845
Mean	29.422	41.648
SD	3.864	10.512

- Data not reported due to mortality.

Appendix X - Table 2
Adult Liver Weights (g)
from a Mallard Reproduction Study with PFOS
10 ppm a.i.

	Male	Female
Pen	Liver	Liver
221	24.717	61.115
222	30.663	36.454
223	42.118	42.668
224	27.070	54.869
225	32.793	41.660
226	25.430	41.253
227	25.203	47.171
228	27.580	44.828
229	28.447	48.620
230	33.253	28.720
231	30.606	48.247
232	36.870	30.419
233	29.664	42.544
234	30.874	52.126
235	29.018	32.634
236	35.314	21.982
Mean	30.601	42.207
SD	4.663	10.268

The above differences from the control group were not statistically significant (Student's t-test).

Appendix XI

Histopathology Report

EPL®

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

WILDLIFE INTERNATIONAL, LTD.
STUDY NUMBER 454-109
EPL PROJECT NUMBER 212-028

PFOS: A REPRODUCTION STUDY WITH THE MALLARD

PATHOLOGY REPORT

Submitted by:

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December 27, 2001

EPL®

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

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PATHOLOGY SUMMARY

EPL®

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

WILDLIFE INTERNATIONAL, LTD.
STUDY NUMBER 454-109
EPL PROJECT NUMBER 212-028

PFOS: A REPRODUCTION STUDY WITH THE MALLARD

PATHOLOGY SUMMARY

Light microscopic examination was performed on sections of selected tissues from adult male and female mallard ducks (*Anas platyrhynchos*) which were untreated or which received 10 ppm a.i. of the test article (Perfluorooctane Sulfonic Acid, Potassium Salt [PFOS]) in the feed for approximately 21 weeks. Selected tissues from untreated 14-day post-hatching 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 the test substance over at least a 21-week period. The experimental design was as follows:

Experimental Group	Adults[a]		Offspring[b]	
	Males	Females	Males	Females
Control (0 ppm)	20	20	3	7
10 ppm a.i.	20	20	3	7

[a]Adults were euthanized after receiving the test substance in the diet for approximately 21 weeks.
[b]Offspring did not receive the test substance in the diet. All offspring were euthanized on Day 14 post-hatching.

MATERIALS AND METHODS

At the completion of the study interval, all surviving adult mallards were euthanized. Selected offspring were also euthanized at approximately 14 days of age. Necropsies were performed on all birds by Wildlife International, Ltd.

Tissues were collected by Wildlife International, Ltd. from adults and offspring, fixed in 10% neutral buffered formalin, and sent to Experimental Pathology Laboratories, Inc (EPL®), where they were trimmed, embedded in paraffin, and made into hematoxylin and eosin-stained sections on glass microscope slides. The following tissues from adult and offspring mallards were examined by light microscopy as available: liver, gallbladder, proventriculus, kidney, brain, gonad (ovary or testis), bursa of Fabricius, and adipose tissue.

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 (including autolysis such that a diagnostic interpretation could not be rendered) are listed as present (P). 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 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 observations in these tables were transcribed from the Gross Necropsy records provided by Wildlife International, Ltd.

RESULTS

Except for one control (3235) adult male, 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 the liver, kidney, adipose tissue, brain, and proventriculus of adult and offspring males and females, bursa of Fabricius in offspring males and females, ovaries of adult and

offspring females and testes of male offspring. Any potential test article effects on the bursa of Fabricius in adult males and females could not be determined since no samples were submitted for evaluation. The bursa of Fabricius typically regresses prior to sexual maturity. In addition, any potential test-article effects on the gallbladder from adults and offspring could not be definitively determined due to the extensive autolysis, which precluded any accurate diagnosis.

In two (2) control and seven (7) 10 ppm a.i. adult males, the testes were observed macroscopically as being reduced in size (< 3.5 cm) when compared to other birds. Of these birds, one control (3225) and four treated birds (3261, 3275, 3279, 3287), exhibited decreased spermatogenesis, characterized by a reduction in the number or absence of maturing/mature spermatozoa in the seminiferous tubules. The other control (3243) and one treated bird (3295) exhibited degeneration/atrophy of the seminiferous tubules characterized by an absence of mature sperm and a decrease in the number of cell layers of the germinal epithelium. The remaining two of seven 10 ppm a.i. males (3263, 3285) with macroscopically noted reduced testicular size and the balance of the control and 10 ppm a.i. males exhibited normal spermatogenesis, characterized by the presence of numerous spermatids and mature spermatozoa in the seminiferous tubules.

Reduced testicular size, with or without microscopic evidence of decreased spermatogenesis, is a feature most consistent with early post-reproductive phase regressions, a normal physiological phenomenon. The degeneration/atrophy noted in the control and treated adult birds may simply represent a later stage of this normal change. However, the increased incidence of reduced size (with or without a corresponding microscopic observation) of the testes in 10 ppm a.i. adult males, when compared to controls (7 vs. 2), suggests

that treatment may have accelerated the normal post-reproductive phase regression.

Testes of all offspring examined were listed as immature because they lacked all evidence of spermatogenesis, had small seminiferous tubules with absent or narrow lumens, and had germinal epithelium with decreased cell layers (compared to adult testes). This morphology is consistent with normal physiological immaturity expected in 14 day-old birds.

Ovaries of adult females exhibited normal oogenesis, characterized by numerous ova in various stages of follicular development. In five (5) controls and five (5) 10 ppm a.i. adult females, the ovaries were indicated macroscopically as "regressed" or "regressing." Microscopically, those ovaries were no different from their cohorts, except that there may have been a reduction in the number of fully developed ova. If present, this change was not recorded microscopically, since it was considered to be consistent with early post-reproductive phase regression, a normal physiological phenomenon. Since the number of females affected was the same in both the control and 10 ppm a.i. groups, there was no indication that treatment had any effect on this phenomena.

All ovaries of offspring females were characterized by numerous oocytes and very small, nonpedunculated follicles completely embedded in a prominent ovarian cortex. This morphology is consistent with the normal physiological immaturity expected in 14 day-old birds.

Vacuolization in the liver was characterized by the presence of vacuoles within the hepatocellular cytoplasm which varied from, usually small, poorly demarcated vacuoles, giving a "lace-like" appearance to the hepatocellular cytoplasm, to variably sized, sharply demarcated, clear vacuoles in the hepatocellular cytoplasm. While special stains were not employed to identify the

material in the vacuoles, the latter morphology was consistent with fat accumulation.

Hepatocellular vacuolization was observed in the livers of adults and offspring of both sexes. Although there was a slight increase in the incidence of this change in 10 ppm a.i. treated adult males when compared to controls (20 vs. 12), there was no substantial difference in the mean severity between the groups. In addition, the change was not observed in treated male offspring or at a significant difference in treated adult or offspring females when compared to their respective controls. Since this finding was commonly observed in the livers from control birds, it was considered to be incidental and not related to test article administration. The apparent increased incidence in the treated adult males was probably due to biological and/or distributional variability.

Additional incidental findings also noted in the liver included liver pigment deposition in adult males, consisting of dust-like, brown to golden-brown pigment granules within the hepatocellular cytoplasm. The identity of the pigment was not determined, but it may have been biliary in origin. Also, the liver of two control adult males and one control adult female exhibited amyloid deposition consisting of amorphous pale eosinophilic material, which filled the hepatic sinusoids. In the one control male that died (3235), the liver parenchyma was almost completely replaced by the deposited amyloid.

Also noted in the liver, at a low incidence in control and/or treated male and female adults and offspring, were foci of hepatocellular degeneration/ necrosis. These lesions were generally single, small, discrete foci of pale to hypereosinophilic hepatocytes with indistinct, fragmented, or condensed nuclei. Although there was generally a slight predominance of the lesion in treated birds, when compared to controls, it was considered incidental and not related to

treatment because of the low incidence, limited distribution, and relatively low severity (generally minimal to slight).

Additional microscopic findings (described below) noted in adult and/or offspring mallards from control and treated groups included mononuclear cell infiltrates in various tissues, kidney nephropathy, and in the proventriculus, hyperplasia of the glandular epithelium. Generally, in both adult and offspring males and females, there was either no significant difference in the incidence of these lesions between control and treated groups, or the incidence was variable, with some lesions occurring at a higher incidence in controls when compared to treated birds, and other lesions having the opposite distribution. In addition, there was no significant difference in the mean severity of these lesions in treated birds when compared to controls. For these reasons, these findings were considered incidental and unrelated to test article (PFOS) administration.

Mononuclear cell infiltrates, observed in various tissues, consisted of foci of lymphocytes and plasma cells generally accompanied by a few heterophils as a minor component. Nephropathy in the kidneys of both control and treated adult males and females was characterized by the presence of basophilic and/or regenerative tubules in the renal cortex.

Hyperplasia of the proventricular glandular epithelium in adult females was generally multifocal in distribution, and characterized by increased numbers and/or basophilic staining of the glandular epithelium. In many cases, there was minimal compression of adjacent normal glandular epithelium. The pathogenesis of this lesion was not determined. However, since the incidence was not significantly different between controls and treated birds, it was considered incidental and not related to treatment.

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CONCLUSIONS AND SUMMARY

No lesions considered possibly related to test article (PFOS) administration were noted in the liver, kidney, adipose tissue, brain, and proventriculus of adult and offspring males and females, bursa of Fabricius in offspring males and females, ovaries of adult and offspring females and testes of male offspring. The increase in the number of adult males in the 10 ppm a.i. treated group with reduced testicular size (with or without a corresponding microscopic observation), suggests that PFOS may have accelerated early post-reproductive phase regression, a normal physiological phenomenon. Any potential test-article effects on the bursa of Fabricius in adult males and females could not be determined since no samples were submitted for evaluation. In addition, any potential test-article effects on the gallbladder from adults and offspring could not be definitively determined due to the extensive autolysis that precluded any accurate diagnosis. The few other changes in various tissues of adult and/or offspring mallards from control and 10 ppm a.i. treated groups were considered incidental and unrelated to test article (PFOS) administration.

F. A. Ruecker

FREDERICK A. RUECKER, DVM, MS, Diplomate, ACVP
Veterinary Pathologist

1/27/03

Date

FAR/lcp

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

COMPLIANCE STATEMENT

Client Name	Wildlife International, Ltd.	EPL Project Coordinator	Dr. Margarita M. Gruebbel
Client Study	454-109	EPL Pathologist	Dr. Frederick A. Ruecker
Species	Mallard	EPL Project Number	212-028
Study Title	PFOS: A Reproduction Study with the Mallard		
Test Article	Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS)		

The histopathology portions of the above-referenced study were conducted in compliance with the Good Laboratory Practice regulations of the Environmental Protection Agency as stipulated by 40 CFR 160.

Margarita M. Gruebbel

EPL Project Coordinator

Jan 28, 2013

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: PFOS: A Reproduction Study with the Mallard

Client Study: 454-109 EPL Project Coordinator: Dr. Margarita M. Gruebbel

EPL Project Number: 212-028 EPL Pathologist: Dr. Frederick A. Ruecker

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.

Area Inspected	Dates	
	Inspection	Reporting
EPL Project Sheets	10/11/01; 10/15/01	10/11/01; 10/15/01
Project Setup	10/12/01	10/12/01
Histology Setup	10/19/01	10/19/01
Data Review	11/6,7/01	11/6,7/01
Draft Report	11/30/01; 12/4/01	12/4/01
Final Report	1/28/03	1/28/03

Date reported to Study Director/Management 1/28/03

Date of last quarterly facility inspection 11/02

Jan L. Snyder
EPL Quality Assurance Unit

1/28/03
Date

ADULT
SUMMARY INCIDENCE TABLES

454-109
Adult Sacrifice
Male Mallard

SUMMARY INCIDENCE TABLE

	GROUP CONTROL	GROUP 10-ppmai				
ADIPOSE TISSUE (NO. EXAMINED)	(20)	(20)				
Edema	1					
Foreign Body Granuloma						
Infiltrate, Mononuclear Cell		2				
Inflammation	1					
BRAIN (NO. EXAMINED)	(20)	(20)				
Coccoid Organisms,						
Intravascular	1					
Infiltrate, Mononuclear Cell,						
Perivascular	1					
Malacia	1					
Vasculitis/Perivasculitis	1					
BURSA OF FABRICIUS (NO.						
EXAMINED)						
GALLBLADDER (NO. EXAMINED)	(20)	(20)				
Autolysis Precludes Diagnosis	20	20				
KIDNEY (NO. EXAMINED)	(20)	(20)				
Amyloid Deposition						
Coccoid Organisms	1					
Infiltrate, Mononuclear Cell	6	1				
Inflammation, Acute	1					
Inflammation, Granulomatous	1					
Mineralization						
Nephropathy	2	3				
LIVER (NO. EXAMINED)	(20)	(20)				
Amyloid Deposition	2					
Degeneration/Necrosis,						
Hepatocellular		2				
Hyperplasia, Bile Ducts						
Infiltrate, Mixed Inflammatory						
Cell						
Infiltrate, Mononuclear Cell	20	20				
Intracytoplasmic Brown						
Pigment, Hepatocytes	8	7				
Mononuclear Cell Infiltrate						
Vacuolization, Hepatocellular	12	20				
PROVENTRICULUS (NO. EXAMINED)	(20)	(20)				
Atrophy, Glandular Epithelium						

454-109
Adult Sacrifice
Male Mallard

SUMMARY INCIDENCE TABLE

	GROUP CONTROL	GROUP 10-ppmai				
PROVENTRICULUS (CONTINUED)						
Hyperplasia, Glandular						
Epithelium, Multifocal						
Infiltrate, Mononuclear Cell	2	5				
Vasculitis/Perivasculitis	1					
TESTIS (NO. EXAMINED)	(20)	(20)				
Coccoid Organisms,						
Intravascular	1					
Degeneration/Atrophy,						
Seminiferous Tubules	1	1				
Dilatation, Seminiferous						
Tubules		1				
Infiltrate, Mononuclear Cell	3	5				
Reduced Spermatogenesis	1	4				
Vasculitis/Perivasculitis	1					

454-109
Adult Sacrifice
Female Mallard

SUMMARY INCIDENCE TABLE

	GROUP CONTROL	GROUP 10-ppmai				
ADIPOSE TISSUE (NO. EXAMINED)	(20)	(20)				
Edema						
Foreign Body Granuloma	1					
Infiltrate, Mononuclear Cell		1				
Inflammation	4	1				
BRAIN (NO. EXAMINED)	(20)	(20)				
Coccoid Organisms,						
Intravascular						
Infiltrate, Mononuclear Cell,						
Perivascular						
Malacia						
Vasculitis/Perivasculitis						
BURSA OF FABRICIUS (NO.						
EXAMINED)						
GALLBLADDER (NO. EXAMINED)	(20)	(19)				
Autolysis Precludes Diagnosis	20	19				
KIDNEY (NO. EXAMINED)	(20)	(20)				
Amyloid Deposition	1					
Coccoid Organisms						
Infiltrate, Mononuclear Cell	6	6				
Inflammation, Acute						
Inflammation, Granulomatous						
Mineralization		1				
Nephropathy	3	5				
LIVER (NO. EXAMINED)	(20)	(20)				
Amyloid Deposition	1					
Degeneration/Necrosis,						
Hepatocellular	1	3				
Hyperplasia, Bile Ducts	1					
Infiltrate, Mixed Inflammatory						
Cell		1				
Infiltrate, Mononuclear Cell	17	15				
Intracytoplasmic Brown						
Pigment, Hepatocytes						
Mononuclear Cell Infiltrate	1					
Vacuolization, Hepatocellular	17	16				
OVARY (NO. EXAMINED)	(20)	(20)				
Amyloid Deposition	1					
Atrophy	1					

454-109
Adult Sacrifice
Female Mallard

SUMMARY INCIDENCE TABLE

	GROUP CONTROL	GROUP 10-ppmai				
OVARY (CONTINUED)						
Inflammation	1	1				
PROVENTRICULUS (NO. EXAMINED)	(20)	(20)				
Atrophy, Glandular Epithelium	1	1				
Hyperplasia, Glandular						
Epithelium, Multifocal	3	4				
Infiltrate, Mononuclear Cell	5	6				
Vasculitis/Perivasculitis						

ADULT

HISTOPATHOLOGY INCIDENCE TABLES

HISTOPATHOLOGY INCIDENCE TABLE

454-109
Adult Sacrifice
Male Mallard

	GROUP CONTROL																			
ANIMAL	32117	32191	32213	32235	32257	32279	32291	32313	32333	32u35	32357	32391	32413	32435	32457	32491	32513	32535	32557	32591
ADIPOSE TISSUE	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Edema										2										
Foreign Body Granuloma																				
Infiltrate, Mononuclear Cell																				
Inflammation										1										
BRAIN	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X
Coccoid Organisms, Intravascular										P										
Infiltrate, Mononuclear Cell, Perivascular								1												
Malacia										2										
Vasculitis/Perivasculitis										4										
BURSA OF FABRICIUS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
GALLBLADDER	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Autolysis Precludes Diagnosis	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
KIDNEY	m	Xm	m	m	Xm	m	Xm	Xm	m	m	Xm	X	Xm	Xm	X	m	Xm	Xm	m	Xm
Amyloid Deposition																				
Coccoid Organisms										P										
Infiltrate, Mononuclear Cell	2		1	1		1										1			1	
Inflammation, Acute										3										
Inflammation, Granulomatous										2										
Mineralization																				
Nephropathy						1			1											
LIVER																				
Amyloid Deposition	3									5										
Degeneration/Necrosis, Hepatocellular																				
Hyperplasia, Bile Ducts																				
Infiltrate, Mixed Inflammatory Cell																				
Infiltrate, Mononuclear Cell	1	1	1	1	1	2	1	1	1	3	1	1	1	1	1	1	1	1	1	1
Intracytoplasmic Brown Pigment, Hepatocytes		1		1			1	1						1	1	1				2
Mononuclear Cell Infiltrate																				
Vacuolization, Hepatocellular	2	2	2		1			1	2		1		1	1		1	2		1	

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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

454-109
Adult Sacrifice
Male Mallard

GROUP: CONTROL

ANIMAL	32117	32119	32121	32223	32225	32227	32229	32231	32233	32235	32237	32239	32241	32443	32445	32447	32449	32551	32553	32555
PROVENTRICULUS	X		X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X
Atrophy, Glandular Epithelium																				
Hyperplasia, Glandular Epithelium, Multifocal																				
Infiltrate, Mononuclear Cell		1							1											
Vasculitis/Perivasculitis										2										
TESTIS	Xm	Xm	Xm	Xm	m	m	Xm	Xm	Xm	m	Xm	Xm	Xm	m	Xm	Xm	m	Xm	Xm	Xm
Coccoid Organisms, Intravascular										P										
Degeneration/Atrophy, Seminiferous Tubules														4						
Dilatation, Seminiferous Tubules																				
Infiltrate, Mononuclear Cell						1								1			1			
Reduced Spermatogenesis					3															
Vasculitis/Perivasculitis										1										

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P=Present B=Benign M=Malignant
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HISTOPATHOLOGY INCIDENCE TABLE

454-109
Adult Sacrifice
Male Mallard

GROUP
10-ppmai

ANIMAL	32571	32599	32616	32633	32655	32677	32699	32711	32733	32755	32777	32799	32811	32833	32855	32877	32899	32911	32933	32955
ADIPOSE TISSUE	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X	X
Edema																				
Foreign Body Granuloma																				
Infiltrate, Mononuclear Cell														1		1				
Inflammation																				
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coccoid Organisms, Intravascular																				
Infiltrate, Mononuclear Cell, Perivascular																				
Malacia																				
Vasculitis/Perivasculitis																				
BURSA OF FABRICIUS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
GALLBLADDER	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Autolysis Precludes Diagnosis	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
KIDNEY	Xm	m	Xm	Xm	m	Xm	Xm	Xm	Xm	m	Xm	Xm	Xm	Xm	Xm	Xm	m	Xm	Xm	Xm
Amyloid Deposition																				
Coccoid Organisms																				
Infiltrate, Mononuclear Cell										1										
Inflammation, Acute																				
Inflammation, Granulomatous																				
Mineralization																				
Nephropathy		2			1												1			
LIVER																				
Amyloid Deposition																				
Degeneration/Necrosis, Hepatocellular			1						1											
Hyperplasia, Bile Ducts																				
Infiltrate, Mixed Inflammatory Cell																				
Infiltrate, Mononuclear Cell	1	2	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1
Intracytoplasmic Brown Pigment, Hepatocytes	2	2		2	1			1			1			1						
Mononuclear Cell Infiltrate																				
Vacuolization, Hepatocellular	2	3	2	1	1	2	1	1	1	2	2	1	2	2	1	1	2	2	1	1

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

454-109
Adult Sacrifice
Male Mallard

GROUP
10-ppmai

ANIMAL	32571	32591	32613	32663	32685	32667	32699	32711	32733	32755	32777	32799	32811	32833	32855	32877	32899	32911	32933	32955
PROVENTRICULUS	X	X	X	X		X	X			X	X		X	X	X	X	X		X	X
Atrophy, Glandular Epithelium																				
Hyperplasia, Glandular Epithelium, Multifocal																				
Infiltrate, Mononuclear Cell					2			1	1			1						1		
Vasculitis/Perivasculitis																				
TESTIS	Xm	Xm	m	Xm	Xm	Xm	m	Xm	m	m	Xm	m	Xm	Xm	m	m	Xm	m	Xm	m
Coccoid Organisms, Intravascular																				
Degeneration/Atrophy, Seminiferous Tubules																				5
Dilatation, Seminiferous Tubules							1													
Infiltrate, Mononuclear Cell									1	1		1			1			1		
Reduced Spermatogenesis			3							2		3				3				
Vasculitis/Perivasculitis																				

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5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

454-109
Adult Sacrifice
Female Mallard

GROUP
CONTROL

ANIMAL	32118	32120	32202	32224	32226	32248	32300	32322	32343	32363	32383	32404	32422	32444	32466	32488	32505	32522	32545	32566
ADIPOSE TISSUE	X	X	X		X	X	X	X		X		X		X		X	X	X	X	X
Edema																				
Foreign Body Granuloma											P									
Infiltrate, Mononuclear Cell																				
Inflammation				1					2				2		2					
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coccoid Organisms, Intravascular																				
Infiltrate, Mononuclear Cell, Perivascular																				
Malacia																				
Vasculitis/Perivasculitis																				
BURSA OF FABRICIUS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
GALLBLADDER	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Autolysis Precludes Diagnosis	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
KIDNEY	X		Xm	m	m	Xm		m	m	m	Xm	Xm	X	m	m	X	m	Xm	Xm	Xm
Amyloid Deposition					3															
Coccoid Organisms																				
Infiltrate, Mononuclear Cell				1				1	1					1	1		1			
Inflammation, Acute																				
Inflammation, Granulomatous																				
Mineralization																				
Nephropathy		1					1			1										
LIVER																				
Amyloid Deposition					3															
Degeneration/Necrosis, Hepatocellular																			1	
Hyperplasia, Bile Ducts					2															
Infiltrate, Mixed Inflammatory Cell																				
Infiltrate, Mononuclear Cell	1		1	1		1	1	1	1	1	1	1	1	1	1	1		1	1	1
Intracytoplasmic Brown Pigment, Hepatocytes																				
Mononuclear Cell Infiltrate					3															
Vacuolization, Hepatocellular	2	1	2	1	2	1	1	4	3		2	2	1		2	1	1		2	2

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5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

454-109
Adult Sacrifice
Female Mallard

GROUP: CONTROL

ANIMAL	32118	32202	32224	32246	32268	32308	32332	32346	32368	32402	32442	32446	32468	32502	32524	32546
OVARY	X	X	X	X		X	X	X	X	X	X	X		X	X		X	X	X	X
Amyloid Deposition					2															
Atrophy													4							
Inflammation																3				
PROVENTRICULUS	X		X			X	X	X	X	X	X	X		X		X	X		X	X
Atrophy, Glandular Epithelium													2							
Hyperplasia, Glandular Epithelium, Multifocal		2			1													2		
Infiltrate, Mononuclear Cell		1		1	1								2		1					
Vasculitis/Perivasculitis																				

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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

454-109
Adult Sacrifice
Female Mallard

GROUP
10-ppmai

ANIMAL	32581	32602	32624	32646	32668	32701	32724	32746	32780	32802	32846	32868	32890	32912	32946
ADIPOSE TISSUE	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Edema																			
Foreign Body Granuloma																			
Infiltrate, Mononuclear Cell								1											
Inflammation		1																	
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coccoid Organisms, Intravascular																			
Infiltrate, Mononuclear Cell, Perivascular																			
Malacia																			
Vasculitis/Perivasculitis																			
BURSA OF FABRICIUS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
GALLBLADDER	A	A	N	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Autolysis Precludes Diagnosis	P	P		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
KIDNEY	Xm	Xm		m	Xm	Xm	Xm	Xm	Xm	m	X	m	m	Xm	Xm	m	m	Xm	m	m
Amyloid Deposition																			
Coccoid Organisms																			
Infiltrate, Mononuclear Cell			1	1						1		1	1					1	
Inflammation, Acute																			
Inflammation, Granulomatous																			
Mineralization															1				
Nephropathy				1						2					2	1			1
LIVER																			
Amyloid Deposition																			
Degeneration/Necrosis, Hepatocellular							2				2			1					
Hyperplasia, Bile Ducts																			
Infiltrate, Mixed Inflammatory Cell		2																	
Infiltrate, Mononuclear Cell	1			1	1		1	1		1	1	1		1	1	1	1	1	1
Intracytoplasmic Brown Pigment, Hepatocytes																			
Mononuclear Cell Infiltrate																			
Vacuolization, Hepatocellular	2		1	1	2	1		1	2	1	1	1	3	1	1		1	2	1

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P=Present B=Benign M=Malignant
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HISTOPATHOLOGY INCIDENCE TABLE

454-109
Adult Sacrifice
Female Mallard

GROUP
10-ppmai

ANIMAL	32580	32606	32624	32646	32668	32670	32672	32674	32676	32678	32680	32682	32684	32686	32688	32690	32692	32694	32696
OVARY	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Amyloid Deposition																			
Atrophy																			
Inflammation		1																	
PROVENTRICULUS	X		X			X	X				X	X				X	X	X		
Atrophy, Glandular Epithelium								1											
Hyperplasia, Glandular Epithelium, Multifocal		1							1					1					1
Infiltrate, Mononuclear Cell				1	1					1			1		1			1	
Vasculitis/Perivasculitis																			

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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ADULT

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

454-109
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
3225	TESTES	Approximately 3.0 cm	Reduced Spermatogenesis (TESTIS)
3243	TESTES	Approximately 2.0 cm	Degeneration/Atrophy, Seminiferous Tubules (TESTIS)

454-109
Adult Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Males Group Identification: CONTROL - Unscheduled Sac./Death

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
3235	KIDNEY	Slightly pale	No Correlating Lesion

III-2

454-109
Adult Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Males Group Identification: 10-ppmai - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
3261	TESTES	Approximately 3.0 cm	Reduced Spermatogenesis (TESTIS)
3263	TESTES	Approximately 3.0 cm	No Correlating Lesion (TESTIS)
3275	TESTES	Approximately 3.0 cm	Reduced Spermatogenesis (TESTIS)
3279	TESTES	Approximately 3.0 cm	Reduced Spermatogenesis (TESTIS)
3285	TESTES	Approximately 3.5 cm	No Correlating Lesion (TESTIS)
3287	TESTES	Approximately 3.0 cm	Reduced Spermatogenesis (TESTIS)
3295	TESTES	1.5 cm	Degeneration/Atrophy, Seminiferous Tubules (TESTIS)

454-109
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
3226	LIVER	Firm, pale, mottled	Amyloid Deposition
	KIDNEY	Pale	Amyloid Deposition
	OVARY	Regressed ovary	No Correlating Lesion
3228	OVARY	Multiple fluid-filled cysts	No Correlating Lesion
3236	OVARY	Regressed ovary	No Correlating Lesion
3242	OVARY	Regressed ovary	Atrophy
	OVARY	Cystic follicles	No Correlating Lesion
3244	OVARY	Regressed ovary	No Correlating Lesion
3246	OVARY	Regressing ovary	No Correlating Lesion
3248	OVARY	Hemorrhagic follicle	Inflammation

454-109
Adult Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Mallard Sex: Females Group Identification: 10-ppmai - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
3276	OVARY	Regressed ovary	No Correlating Lesion
3280	OVARY	Regressed ovary	No Correlating Lesion
3284	OVARY	Cystic follicles	No Correlating Lesion
3286	OVARY	Regressed ovary	No Correlating Lesion
3288	OVARY	Regressed ovary	No Correlating Lesion
3290	OVARY	Regressed ovary	No Correlating Lesion

OFFSPRING

SUMMARY INCIDENCE TABLES

454-109
Offspring Sacrifice
Male Mallard

SUMMARY INCIDENCE TABLE

	GROUP CONTROL	GROUP 10-ppmai				
ADIPOSE TISSUE (NO. EXAMINED)	(3)	(3)				
Infiltrate, Mononuclear Cell						
BRAIN (NO. EXAMINED)	(3)	(3)				
BURSA OF FABRICIUS (NO.						
EXAMINED)	(3)	(3)				
GALLBLADDER (NO. EXAMINED)	(3)	(3)				
Autolysis Precludes Diagnosis	3	2				
KIDNEY (NO. EXAMINED)	(3)	(3)				
Infiltrate, Mononuclear Cell						
LIVER (NO. EXAMINED)	(3)	(3)				
Degeneration/Necrosis,						
Hepatocellular, Acute		1				
Infiltrate, Mononuclear Cell	2	1				
Vacuolization, Hepatocellular	3					
PROVENTRICULUS (NO. EXAMINED)	(3)	(3)				
Infiltrate, Mononuclear Cell	1	3				
TESTIS (NO. EXAMINED)	(2)	(3)				
Immature	2	3				

454-109
Offspring Sacrifice
Female Mallard

SUMMARY INCIDENCE TABLE

	GROUP CONTROL	GROUP 10-ppmai				
ADIPOSE TISSUE (NO. EXAMINED)	(7)	(7)				
Infiltrate, Mononuclear Cell		1				
BRAIN (NO. EXAMINED)	(7)	(7)				
BURSA OF FABRICIUS (NO.						
EXAMINED)	(7)	(7)				
GALLBLADDER (NO. EXAMINED)	(6)	(6)				
Autolysis Precludes Diagnosis	4	4				
KIDNEY (NO. EXAMINED)	(7)	(7)				
Infiltrate, Mononuclear Cell	3					
LIVER (NO. EXAMINED)	(7)	(7)				
Degeneration/Necrosis,						
Hepatocellular, Acute	3	2				
Infiltrate, Mononuclear Cell	5	3				
Vacuolization, Hepatocellular	3	2				
OVARY (NO. EXAMINED)	(7)	(7)				
Immature	7	7				
PROVENTRICULUS (NO. EXAMINED)	(7)	(7)				
Infiltrate, Mononuclear Cell	5	6				

OFFSPRING
HISTOPATHOLOGY INCIDENCE TABLES

HISTOPATHOLOGY INCIDENCE TABLE

454-109
Offspring Sacrifice
Male Mallard

	GROUP CONTROL			GROUP 10-ppmai		
ANIMAL	9578	9591	9606	9625	9636	9642
ADIPOSE TISSUE	X	X	X	X	X	X
Infiltrate, Mononuclear Cell						
BRAIN	X	X	X	X	X	X
BURSA OF FABRICIUS	X	X	X	X	X	X
GALLBLADDER	A	A	A	X	A	A
Autolysis Precludes Diagnosis	P	P	P		P	P
KIDNEY	Xm	Xm	Xm	Xm	Xm	Xm
Infiltrate, Mononuclear Cell						
LIVER				X		
Degeneration/Necrosis, Hepatocellular, Acute					1	
Infiltrate, Mononuclear Cell	1	1				1
Vacuolization, Hepatocellular	1	1	1			
PROVENTRICULUS	X		X			
Infiltrate, Mononuclear Cell		1		2	1	1
TESTIS	N					
Immature		P	P	P	P	P

EPL
Experimental Pathology Laboratories, Inc.

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

454-109
Offspring Sacrifice
Female Mallard

	GROUP CONTROL							GROUP 10-ppmai						
ANIMAL	95733	95833	95892	95908	96033	96103		96293	96333	96364	96448	96545	96594	
ADIPOSE TISSUE	X	X	X	X	X	X	X		X	X	X	X	X	X
Infiltrate, Mononuclear Cell								1						
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BURSA OF FABRICIUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GALLBLADDER	X	X	A	A	A	A	N	N	X	A	A	A	X	A
Autolysis Precludes Diagnosis			P	P	P	P				P	P	P		P
KIDNEY	Xm	Xm	Xm	Xm	m	m	m	Xm	Xm	Xm	Xm	Xm	Xm	Xm
Infiltrate, Mononuclear Cell					1	1	1							
LIVER								X						
Degeneration/Necrosis, Hepatocellular, Acute			1	3		2					1	1		
Infiltrate, Mononuclear Cell	1	1	1		1		1			1	1			1
Vacuolization, Hepatocellular	1	1					1		1				1	
OVARY														
Immature	P	P	P	P	P	P	P	P	P	P	P	P	P	P
PROVENTRICULUS	X			X									X	
Infiltrate, Mononuclear Cell		1	1		1	2	1	1	2	1	1	1		1

EPL
Experimental Pathology Laboratories, Inc.

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

Appendix XIIA

The Analysis of PFOS in Adult Red Blood Cells and Sera

Exygen Study No.: 023-066

Table X. Summary of PFOS in Mallard Red Blood Cell Samples at 0 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk5	0107230	052101B	5/21/01	5/22/01	NQ
454-109-3249-Wk5*	0107230	052101B	5/21/01	5/22/01	NQ
454-109-3251-Wk5	0107231	052101B	5/21/01	5/22/01	0.00197
454-109-3251-Wk5*	0107231	052101B	5/21/01	5/22/01	0.00167
454-109-3252-Wk5	0107232	052101B	5/21/01	5/22/01	NQ
454-109-3252-Wk5*	0107232	052101B	5/21/01	5/22/01	NQ
454-109-3253-Wk5	0107233	052101B	5/21/01	5/22/01	NQ
454-109-3253-Wk5*	0107233	052101B	5/21/01	5/22/01	NQ
454-109-3254-Wk5	0107234	052101B	5/21/01	5/22/01	NQ
454-109-3254-Wk5*	0107234	052101B	5/21/01	5/22/01	NQ
454-109-3255-Wk5	0107235	052101B	5/21/01	5/22/01	0.00143
454-109-3255-Wk5*	0107235	052101B	5/21/01	5/22/01	NQ
454-109-3256-Wk5	0107236	052101B	5/21/01	5/22/01	NQ
454-109-3256-Wk5*	0107236	052101B	5/21/01	5/22/01	NQ
				AVERAGE:	**0.000401**
				STANDARD DEVIATION:	**0.000706**

* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

LOQ = 0.01 µg/mL for red blood cells and serum

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

Exygen Study No.: 023-066

Table XI. Summary of PFOS in Mallard Red Blood Cell Samples at 10 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk5	0107237	052101BD	5/21/01	5/23/01	24.4
454-109-3289-Wk5*	0107237	052101BD	5/21/01	5/23/01	24.7
454-109-3290-Wk5	0107238	052101BD	5/21/01	5/23/01	11.2
454-109-3290-Wk5*	0107238	052101BD	5/21/01	5/23/01	10.0
454-109-3291-Wk5	0107239	052101BD	5/21/01	5/23/01	11.8
454-109-3291-Wk5*	0107239	052101BD	5/21/01	5/23/01	12.3
454-109-3292-Wk5	0107240	052101BD	5/21/01	5/23/01	15.1
454-109-3292-Wk5*	0107240	052101BD	5/21/01	5/23/01	13.1
454-109-3293-Wk5	0107241	052101BD	5/21/01	5/23/01	30.1
454-109-3293-Wk5*	0107241	052101BD	5/21/01	5/23/01	30.8
454-109-3294-Wk5	0107242	052101BD	5/21/01	5/23/01	4.88
454-109-3294-Wk5*	0107242	052101BD	5/21/01	5/23/01	3.95
454-109-3295-Wk5	0107243	052101BD	5/21/01	5/23/01	18.9
454-109-3295-Wk5*	0107243	052101BD	5/21/01	5/23/01	18.8
454-109-3296-Wk5	0107244	052101BD	5/21/01	5/23/01	26.4
454-109-3296-Wk5*	0107244	052101BD	5/21/01	5/23/01	28.5
				AVERAGE:	**17.8**
				STANDARD DEVIATION:	**8.80**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Exygen Study No.: 023-066

Table XII. Summary of PFOS in Mallard Red Blood Cell Samples at 50 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3329-Wk5	0107245	052201CD	5/22/01	5/24/01	58.9
454-109-3329-Wk5*	0107245	052201CD	5/22/01	5/24/01	62.2
454-109-3330-Wk5	0107246	052201CD	5/22/01	5/24/01	112
454-109-3330-Wk5*	0107246	052201CD	5/22/01	5/24/01	123
				AVERAGE:	**89**
				STANDARD DEVIATION:	**33**

Table XIII. Summary of PFOS in Mallard Red Blood Cell Samples at 0 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk10	0107250	052201A	5/22/01	5/23/01	NQ
454-109-3249-Wk10*	0107250	052201A	5/22/01	5/23/01	NQ
454-109-3250-Wk10	0107251	052201A	5/22/01	5/23/01	NQ
454-109-3250-Wk10*	0107251	052201A	5/22/01	5/23/01	NQ
454-109-3251-Wk10	0107252	052201A	5/22/01	5/23/01	NQ
454-109-3251-Wk10*	0107252	052201A	5/22/01	5/23/01	NQ
454-109-3252-Wk10	0107253	052201A	5/22/01	5/23/01	NQ
454-109-3252-Wk10*	0107253	052201A	5/22/01	5/23/01	NQ
454-109-3253-Wk10	0107254	052201A	5/22/01	5/23/01	NQ
454-109-3253-Wk10*	0107254	052201A	5/22/01	5/23/01	NQ
454-109-3254-Wk10	0107255	052201A	5/22/01	5/23/01	NQ
454-109-3254-Wk10*	0107255	052201A	5/22/01	5/23/01	NQ
454-109-3255-Wk10	0107256	052201A	5/22/01	5/23/01	NQ
454-109-3255-Wk10*	0107256	052201A	5/22/01	5/23/01	NQ
454-109-3256-Wk10	0107257	052201A	5/22/01	5/23/01	NQ
454-109-3256-Wk10*	0107257	052201A	5/22/01	5/23/01	0.00245
				AVERAGE:	**0.000200**
				STANDARD DEVIATION:	**0.000600**

* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

LOQ = 0.01 µg/mL for red blood cells and serum

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

Exygen Study No.: 023-066

Table XIV. Summary of PFOS in Mallard Red Blood Cell Samples at 10 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk10	0107258	052201AD	5/22/01	5/23-24/01	19.3
454-109-3289-Wk10*	0107258	052201AD	5/22/01	5/23-24/01	19.6
454-109-3290-Wk10	0107259	052201AD	5/22/01	5/23-24/01	20.4
454-109-3290-Wk10*	0107259	052201AD	5/22/01	5/23-24/01	20.2
454-109-3291-Wk10	0107260	052201AD	5/22/01	5/23-24/01	14.1
454-109-3291-Wk10*	0107260	052201AD	5/22/01	5/23-24/01	14.9
454-109-3292-Wk10	0107261	052201AD	5/22/01	5/23-24/01	67.3
454-109-3292-Wk10*	0107261	052201AD	5/22/01	5/23-24/01	66.0
454-109-3293-Wk10	0107262	052201AD	5/22/01	5/23-24/01	33.5
454-109-3293-Wk10*	0107262	052201AD	5/22/01	5/23-24/01	34.2
454-109-3294-Wk10	0107263	052201AD	5/22/01	5/23-24/01	60.8
454-109-3294-Wk10*	0107263	052201AD	5/22/01	5/23-24/01	58.9
454-109-3295-Wk10	0107264	052201AD	5/22/01	5/23-24/01	16.1
454-109-3295-Wk10*	0107264	052201AD	5/22/01	5/23-24/01	16.0
454-109-3296-Wk10	0107265	052201AD	5/22/01	5/23-24/01	11.6
454-109-3296-Wk10*	0107265	052201AD	5/22/01	5/23-24/01	11.7
				AVERAGE:	**30.3**
				STANDARD DEVIATION:	**20.7**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Exygen Study No.: 023-066

Table XV. Summary of PFOS in Mallard Red Blood Cell Samples at 0 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk15	0107266	052201B	5/22/01	5/23/01	NQ
454-109-3249-Wk15*	0107266	052201B	5/22/01	5/23/01	NQ
454-109-3250-Wk15	0107267	052201B	5/22/01	5/23/01	NQ
454-109-3250-Wk15*	0107267	052201B	5/22/01	5/23/01	NQ
454-109-3251-Wk15	0107268	052201B	5/22/01	5/23/01	0.00179
454-109-3251-Wk15*	0107268	052201B	5/22/01	5/23/01	0.00188
454-109-3252-Wk15	0107269	052201B	5/22/01	5/23/01	NQ
454-109-3252-Wk15*	0107269	052201B	5/22/01	5/23/01	NQ
454-109-3253-Wk15	0107270	052201B	5/22/01	5/23/01	NQ
454-109-3253-Wk15*	0107270	052201B	5/22/01	5/23/01	NQ
454-109-3254-Wk15	0107271	052201B	5/22/01	5/23/01	ND
454-109-3254-Wk15*	0107271	052201B	5/22/01	5/23/01	ND
454-109-3255-Wk15	0107272	052201B	5/22/01	5/23/01	NQ
454-109-3255-Wk15*	0107272	052201B	5/22/01	5/23/01	NQ
454-109-3256-Wk15	0107273	052201B	5/22/01	5/23/01	NQ
454-109-3256-Wk15*	0107273	052201B	5/22/01	5/23/01	NQ
454-109-3236-Wk15	0107274	052201B	5/22/01	5/23/01	NQ
454-109-3236-Wk15*	0107274	052201B	5/22/01	5/23/01	NQ
				AVERAGE:	**0.000243**
				STANDARD DEVIATION:	**0.000613**

* Duplicate injection

ND = No peak detected
NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

LOQ = 0.01 µg/mL for red blood cells and serum

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) and for ND, zero was used to calculate the average and standard deviation.

Exygen Study No.: 023-066

Table XVI. Summary of PFOS in Mallard Red Blood Cell Samples at 10 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk15	0107275	052201BD	5/22/01	5/23-24/01	53.5
454-109-3289-Wk15*	0107275	052201BD	5/22/01	5/23-24/01	48.2
454-109-3290-Wk15	0107276	052201BD	5/22/01	5/23-24/01	3.98
454-109-3290-Wk15*	0107276	052201BD	5/22/01	5/23-24/01	3.44
454-109-3291-Wk15	0107277	052201BD	5/22/01	5/23-24/01	60.9
454-109-3291-Wk15*	0107277	052201BD	5/22/01	5/23-24/01	61.4
454-109-3292-Wk15	0107278	052201BD	5/22/01	5/23-24/01	9.65
454-109-3292-Wk15*	0107278	052201BD	5/22/01	5/23-24/01	8.79
454-109-3293-Wk15	0107279	052201BDR	5/22/01	5/24/01	113
454-109-3293-Wk15*	0107279	052201BDR	5/22/01	5/24/01	116
454-109-3294-Wk15	0107280	052201BDR	5/22/01	5/24/01	1.54
454-109-3294-Wk15*	0107280	052201BDR	5/22/01	5/24/01	1.61
454-109-3295-Wk15	0107281	052201BDR	5/22/01	5/24/01	8.03
454-109-3295-Wk15*	0107281	052201BDR	5/22/01	5/24/01	6.56
454-109-3296-Wk15	0107282	052201BD	5/22/01	5/23-24/01	0.623
454-109-3296-Wk15*	0107282	052201BD	5/22/01	5/23-24/01	0.505
				AVERAGE:	**31.1**
				STANDARD DEVIATION:	**39.8**

Table XVII. Summary of PFOS in Mallard Red Blood Cell Samples at 50 ppm a.i. for TERM

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3297-TERM	0107283	052201CD	5/22/01	5/24/01	275
454-109-3297-TERM*	0107283	052201CD	5/22/01	5/24/01	272
454-109-3298-TERM	0107284	052201BDR	5/22/01	5/24/01	406
454-109-3298-TERM*	0107284	052201BDR	5/22/01	5/24/01	418
				AVERAGE:	**343**
				STANDARD DEVIATION:	**80**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Exygen Study No.: 023-066

Table XVIII. Summary of PFOS in Mallard Serum Samples at 0 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk5	0107303	072001A	7/20/01	7/21/01	0.00414
454-109-3249-Wk5*	0107303	072001A	7/20/01	7/21/01	0.00417
454-109-3251-Wk5	0107304	072001A	7/20/01	7/21/01	0.00566
454-109-3251-Wk5*	0107304	072001A	7/20/01	7/21/01	0.00599
454-109-3252-Wk5	0107305	072001A	7/20/01	7/21/01	0.00878
454-109-3252-Wk5*	0107305	072001A	7/20/01	7/21/01	0.00943
454-109-3253-Wk5	0107306	072001A	7/20/01	7/21/01	0.00516
454-109-3253-Wk5*	0107306	072001A	7/20/01	7/21/01	0.00499
454-109-3254-Wk5	0107307	072001A	7/20/01	7/21/01	0.00432
454-109-3254-Wk5*	0107307	072001A	7/20/01	7/21/01	0.00444
454-109-3255-Wk5	0107308	072001A	7/20/01	7/21/01	0.00462
454-109-3255-Wk5*	0107308	072001A	7/20/01	7/21/01	0.00392
454-109-3256-Wk5	0107309	072001A	7/20/01	7/21/01	0.00544
454-109-3256-Wk5*	0107309	072001A	7/20/01	7/21/01	0.00516
				AVERAGE:	**0.00544**
			STANDARD DEVIATION:		**0.00167**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Exygen Study No.: 023-066

Table XIX. Summary of PFOS in Mallard Serum Samples at 10 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk5	0107310	072001AD	7/20/01	7/22/01	128
454-109-3289-Wk5*	0107310	072001AD	7/20/01	7/22/01	120
454-109-3290-Wk5	0107311	072001AD	7/20/01	7/22/01	79.6
454-109-3290-Wk5*	0107311	072001AD	7/20/01	7/22/01	94.2
454-109-3291-Wk5	0107312	072001AD	7/20/01	7/22/01	104
454-109-3291-Wk5*	0107312	072001AD	7/20/01	7/22/01	110
454-109-3292-Wk5	0107313	072001AD	7/20/01	7/22/01	83.4
454-109-3292-Wk5*	0107313	072001AD	7/20/01	7/22/01	85.8
454-109-3293-Wk5	0107314	072001AD	7/20/01	7/22/01	59.4
454-109-3293-Wk5*	0107314	072001AD	7/20/01	7/22/01	76.6
454-109-3294-Wk5	0107315	072001AD	7/20/01	7/22/01	60.6
454-109-3294-Wk5*	0107315	072001AD	7/20/01	7/22/01	57.5
454-109-3295-Wk5	0107316	072001AD	7/20/01	7/22/01	73.3
454-109-3295-Wk5*	0107316	072001AD	7/20/01	7/22/01	76.9
				AVERAGE:	**86.4**
				STANDARD DEVIATION:	**22.2**

Table XX. Summary of PFOS in Mallard Serum Samples at 50 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3329-Wk5	0107317	072001AD	7/20/01	7/22/01	376
454-109-3329-Wk5*	0107317	072001AD	7/20/01	7/22/01	400
454-109-3330-Wk5	0107318	072001AD	7/20/01	7/22/01	320
454-109-3330-Wk5*	0107318	072001AD	7/20/01	7/22/01	290
				AVERAGE:	**347**
				STANDARD DEVIATION:	**50**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Exygen Study No.: 023-066

Table XXI. Summary of PFOS in Mallard Serum Samples at 0 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk10	0107322	072001B	7/20/01	7/21-22/01	0.00271
454-109-3249-Wk10*	0107322	072001B	7/20/01	7/21-22/01	0.00372
454-109-3250-Wk10	0107323	072001B	7/20/01	7/21-22/01	0.00726
454-109-3250-Wk10*	0107323	072001B	7/20/01	7/21-22/01	0.00666
454-109-3251-Wk10	0107324	072001B	7/20/01	7/21-22/01	0.00659
454-109-3251-Wk10*	0107324	072001B	7/20/01	7/21-22/01	0.00530
454-109-3252-Wk10	0107325	072001B	7/20/01	7/21-22/01	0.00899
454-109-3252-Wk10*	0107325	072001B	7/20/01	7/21-22/01	0.00940
454-109-3253-Wk10	0107326	072001B	7/20/01	7/21-22/01	0.00517
454-109-3253-Wk10*	0107326	072001B	7/20/01	7/21-22/01	0.00482
454-109-3254-Wk10	0107327	072001B	7/20/01	7/21-22/01	ND
454-109-3254-Wk10*	0107327	072001B	7/20/01	7/21-22/01	ND
454-109-3255-Wk10	0107328	072001B	7/20/01	7/21-22/01	0.00337
454-109-3255-Wk10*	0107328	072001B	7/20/01	7/21-22/01	0.00403
454-109-3256-Wk10	0107329	072001B	7/20/01	7/21-22/01	0.00419
454-109-3256-Wk10*	0107329	072001B	7/20/01	7/21-22/01	0.00384
				AVERAGE:	**0.00476**
				STANDARD DEVIATION:	**0.00266**

* Duplicate injection

ND = No peak detected
NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

LOQ = 0.01 µg/mL for red blood cells and serum

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) and for ND, zero was used to calculate the average and standard deviation.

Exygen Study No.: 023-066

Table XXII. Summary of PFOS in Mallard Serum Samples at 10 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk10	0107330	072001BD	7/20/01	7/22/01	145
454-109-3289-Wk10*	0107330	072001BD	7/20/01	7/22/01	147
454-109-3290-Wk10	0107331	072001BD	7/20/01	7/22/01	32.7
454-109-3290-Wk10*	0107331	072001BD	7/20/01	7/22/01	32.5
454-109-3291-Wk10	0107332	072001BD	7/20/01	7/22/01	93.5
454-109-3291-Wk10*	0107332	072001BD	7/20/01	7/22/01	102
454-109-3292-Wk10	0107333	072001BD	7/20/01	7/22/01	107
454-109-3292-Wk10*	0107333	072001BD	7/20/01	7/22/01	111
454-109-3293-Wk10	0107334	072001BD	7/20/01	7/22/01	132
454-109-3293-Wk10*	0107334	072001BD	7/20/01	7/22/01	136
454-109-3294-Wk10	0107335	072001BD	7/20/01	7/22/01	85.3
454-109-3294-Wk10*	0107335	072001BD	7/20/01	7/22/01	86.4
454-109-3295-Wk10	0107336	072001BD	7/20/01	7/22/01	123
454-109-3295-Wk10*	0107336	072001BD	7/20/01	7/22/01	120
454-109-3296-Wk10	0107337	072001BD	7/20/01	7/22/01	114
454-109-3296-Wk10*	0107337	072001BD	7/20/01	7/22/01	112
				AVERAGE:	**105**
			STANDARD DEVIATION:		**33.8**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

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Table XXIII. Summary of PFOS in Mallard Serum Samples at 0 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3236-Wk15	0107338	071901A	7/19/01	7/19-20/01	ND
454-109-3236-Wk15*	0107338	071901A	7/19/01	7/19-20/01	ND
454-109-3249-Wk15	0107339	071901A	7/19/01	7/19-20/01	0.00729
454-109-3249-Wk15*	0107339	071901A	7/19/01	7/19-20/01	0.0102
454-109-3250-Wk15	0107340	071901A	7/19/01	7/19-20/01	ND
454-109-3250-Wk15*	0107340	071901A	7/19/01	7/19-20/01	ND
454-109-3251-Wk15	0107341	071901A	7/19/01	7/19-20/01	0.00466
454-109-3251-Wk15*	0107341	071901A	7/19/01	7/19-20/01	0.00441
454-109-3252-Wk15	0107342	071901A	7/19/01	7/19-20/01	ND
454-109-3252-Wk15*	0107342	071901A	7/19/01	7/19-20/01	ND
454-109-3253-Wk15	0107343	071901A	7/19/01	7/19-20/01	0.00649
454-109-3253-Wk15*	0107343	071901A	7/19/01	7/19-20/01	0.00723
454-109-3254-Wk15	0107344	071901A	7/19/01	7/19-20/01	ND
454-109-3254-Wk15*	0107344	071901A	7/19/01	7/19-20/01	ND
454-109-3255-Wk15	0107345	071901A	7/19/01	7/19-20/01	0.0233
454-109-3255-Wk15*	0107345	071901A	7/19/01	7/19-20/01	0.00699
454-109-3256-Wk15	0107346	071901A	7/19/01	7/19-20/01	0.0195
454-109-3256-Wk15*	0107346	071901A	7/19/01	7/19-20/01	ND
				AVERAGE:	**0.000503**
			STANDARD DEVIATION:		**0.00616**

* Duplicate injection

ND = No peak detected
NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

LOQ = 0.01 µg/mL for red blood cells and serum

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) and for ND, zero was used to calculate the average and standard deviation.

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Table XXIV. Summary of PFOS in Mallard Serum Samples at 10 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk15	0107347	071901AD	7/19/01	7/20-21/01	152
454-109-3289-Wk15*	0107347	071901AD	7/19/01	7/20-21/01	162
454-109-3290-Wk15	0107348	071901AD	7/19/01	7/20-21/01	10.1
454-109-3290-Wk15*	0107348	071901AD	7/19/01	7/20-21/01	8.75
454-109-3291-Wk15	0107349	071901AD	7/19/01	7/20-21/01	152
454-109-3291-Wk15*	0107349	071901AD	7/19/01	7/20-21/01	142
454-109-3292-Wk15	0107350	071901AD	7/19/01	7/20-21/01	12.1
454-109-3292-Wk15*	0107350	071901AD	7/19/01	7/20-21/01	11.6
454-109-3293-Wk15	0107351	071901AD	7/19/01	7/20-21/01	128
454-109-3293-Wk15*	0107351	071901AD	7/19/01	7/20-21/01	132
454-109-3294-Wk15	0107352	071901AD	7/19/01	7/20-21/01	6.73
454-109-3294-Wk15*	0107352	071901AD	7/19/01	7/20-21/01	4.52
454-109-3295-Wk15	0107353	071901AD	7/19/01	7/20-21/01	117
454-109-3295-Wk15*	0107353	071901AD	7/19/01	7/20-21/01	116
454-109-3296-Wk15	0107354	071901AD	7/19/01	7/20-21/01	9.52
454-109-3296-Wk15*	0107354	071901AD	7/19/01	7/20-21/01	11.3
				AVERAGE:	**73.5**
				STANDARD DEVIATION:	**67.3**

Table XXV. Summary of PFOS in Mallard Serum Samples at 50 ppm a.i. for TERM

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3297-TERM	0107355	072001AD	7/20/01	7/22/01	685
454-109-3297-TERM*	0107355	072001AD	7/20/01	7/22/01	601
454-109-3298-TERM	0107356	072001AD	7/20/01	7/22/01	664
454-109-3298-TERM*	0107356	072001AD	7/20/01	7/22/01	601
				AVERAGE:	**638**
				STANDARD DEVIATION:	**43**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Appendix XIIB

The Analysis of PFOS in Adult and Juvenile Liver and Sera

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SUMMARY

The concentration of the $C_8F_{17}SO_3^-$ anion of perfluorooctane sulfonate, potassium salt (PFOS, $C_8F_{17}SO_3^-K^+$) was determined in the liver and sera of adult mallards and their offspring in both a control group and a 10ppm dosed group from Wildlife feeding study #454-109. The following tables summarize the $C_8F_{17}SO_3^-$ concentrations found in the liver and sera of study samples. Extracted-matrix PFOS standards are used to establish the HPLC-ES/MS/MS instrument calibration curve for $C_8F_{17}SO_3^-$. Concentrations of $C_8F_{17}SO_3^-$ are reported after correction for the purity and the potassium contribution to the mass of the PFOS reference substance.

Table 1 summarizes all study sample data. Table 2 summarizes study data after removing certain results that have been statistically determined to be outliers utilizing the Dixon Q-Test.

Table 1. Summary Table, $C_8F_{17}SO_3^-$ Concentration in Sera and Liver

Sampling Event	Number of Sera Samples	Average $C_8F_{17}SO_3^-$ Conc. Sera	Range of Results	Number of Liver Samples	Average $C_8F_{17}SO_3^-$ Conc. Liver	Range of Results
Male Control Group	18	NA	<100 ng/mL (14 of 18) 107-1400 ng/mL range (4 of 18)	20	<50 ng/g	NA
Female Control Group	20	NA	<100 ng/mL (15 of 20) 166-2680 ng/mL range (5 of 20)	20	<50 ng/g	NA
Male 10ppm Group	20	89700 ng/mL	11300-210000 ng/mL	20	60900 ng/g	24200-125000 ng/g
Female 10ppm Group	20	20300 ng/mL	6350-91400 ng/mL	20	10800 ng/g	2480-30700 ng/g
Male Offspring Control Group	3	NA	<100 ng/mL (2 of 3) 103 ng/mL (1 of 3)	3	<50 ng/g	NA
Female Offspring Control Group	7	NA	<100 ng/mL (7 of 7)	7	<50 ng/g	NA
Male Offspring 10ppm Group	3	4410 ng/mL	3670 - 5450 ng/mL	3	3170 ng/g	2200-4640 ng/g
Female Offspring 10ppm Group	7	4960 ng/mL	3420 - 6760 ng/mL	7	3620 ng/g	2240-5930 ng/g

For individual sample values see Tables 10 to 13 and Attachment B.

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Table 2. Summary Table, $C_8F_{17}SO_3^-$ Concentration in Sera and Liver with Outliers Removed

Sampling Event	Number of Sera Samples	Average $C_8F_{17}SO_3^-$ Conc. Sera	Range of Results	Number of Liver Samples	Average $C_8F_{17}SO_3^-$ Conc. Liver	Range of Results
Male Control Group	18*	*	*	20*	*	*
Female Control Group	17	NA	<100 ng/mL (15 of 17) 166-172 ng/mL range (2 of 17)	20*	*	*
Male 10ppm Group	18	87300 ng/mL	68700-108000 ng/mL	20*	*	*
Female 10ppm Group	19	16600 ng/mL	6350-42800 ng/mL	20*	*	*
Male Offspring Control Group	3*	*	*	3*	*	*
Female Offspring Control Group	7*	*	*	7*	*	*
Male Offspring 10ppm Group	3*	*	*	3*	*	*
Female Offspring 10ppm Group	7*	*	*	7*	*	*

* No outliers removed from this data set. Refer to Table 1 for results. For individual sample values see Tables 10 to 13 and Attachment B.

PURPOSE

The purpose of the analytical phase of this study is to quantify $C_8F_{17}SO_3^-$ in the liver and sera of both control and dosed mallards and in their offspring.

REFERENCE SUBSTANCES

Table 3. Reference Substances

Reference Substance	PFOS	PFDA
Source	3M Internal	Oakwood Products, Inc.
Expiration Date	08/31/2006	12/01/2010
Storage Conditions	Frozen -20°C +/- 10°C	Frozen -20°C +/- 10°C
3M Laboratory Identification Number	SD-018, Lot # 217	SD-036
Physical Description	White Crystalline Powder	White Solid
Purity	86.9%	98%

PFDA is nonadecafluorodecanoic acid, or Perfluorodecanoic acid, $C_{10}F_{19}O_2H$ and is used solely to monitor for gross instrument failure and not as an internal standard for quantitation.

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- **Absolute Recoveries.** The absolute recovery of the QC samples is determined by comparing the results of the extracted quality control spikes quantitated versus an extracted matrix curve and a solvent curve. The sera spikes quantitate higher using a solvent curve than when using a matrix-extracted curve. The low spikes are 11.1% higher, the medium spikes 8.3% higher, and the high spikes 8.3% higher. The 9.5% average higher quantitation of the three levels of spikes using a solvent curve versus an extracted matrix curve demonstrates that it is appropriate to use matrix-extracted standards. The same trend is observed for liver samples. The extracted liver matrix curves are 12.2% higher, 7.8% higher, and 5.6% higher respectively for the low, medium and high spikes of the liver matrix, again demonstrating that it is appropriate to use matrix-extracted standards.
- **Internal Standard:** The internal standard (PFDA) was added to all samples and standards. PFDA was not used for quantitation, but was used to monitor for gross instrument failure.
- **Instrument Response Calibration.** HPLC-ES/MS/MS instrument response was calibrated using the concentration of the $C_8F_{17}SO_3$ anion of perfluorooctane sulfonate, potassium salt (PFOS) that was adjusted for purity and the potassium ion contribution to the mass of PFOS used to prepare stock solutions. Any future calculations requiring concentrations to be expressed as a percentage of dietary in-take must adjust the dosed amounts as well.

DATA SUMMARY

Tables 10 through 13 summarize individual sample data. Representative chromatograms are presented in Attachment C. The table displays $C_8F_{17}SO_3^-$ concentrations for individual injections. All data are corrected for purity of the PFOS reference substance used for the extracted-matrix calibration curve.

Table 10. $C_8F_{17}SO_3^-$ Data Summary, Female Mallard Sera (Adult and Offspring)

3M SAMPLE NUMBER	SAMPLE DESCRIPTION	AMOUNT OF SERA USED (µL)	ADDITIONAL DILUTION	CONC. OF $C_8F_{17}SO_3^-$ IN SAMPLE (NG/ML OF SERA)
E01-1256-28663	454-109-3218, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28665	454-109-3220, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28667	454-109-3222, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28669	454-109-3224, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28670	454-109-3226, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28672	454-109-3228, 0 PPM, Female, Adult	0.5	1	2680 ●
E01-1256-28674	454-109-3230, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28676	454-109-3232, 0 PPM, Female, Adult	0.5	1	172
E01-1256-28678	454-109-3234, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28679	454-109-3236, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28681	454-109-3238, 0 PPM, Female, Adult	0.5	1	166
E01-1256-28683	454-109-3240, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28685	454-109-3242, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28687	454-109-3244, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28689	454-109-3246, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28691	454-109-3248, 0 PPM, Female, Adult	0.5	1	398 ●
E01-1256-28693	454-109-3250, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28695	454-109-3252, 0 PPM, Female, Adult	0.5	1	757 ●

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3M Sample Number	Sample Description	Amount of Serum Used (mL)	Additional Dilution	Conc. of $C_8F_{17}SO_2^-$ in Sample (ng/mL of Serum)
E01-1256-28697	454-109-3254, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28699	454-109-3256, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28701	454-109-3258, 10 PPM, Female, Adult	0.5	1	13600
E01-1256-28703	454-109-3260, 10 PPM, Female, Adult	0.5	1	9420
E01-1256-28705	454-109-3262, 10 PPM, Female, Adult	0.5	1	17000
E01-1256-28707	454-109-3264, 10 PPM, Female, Adult	0.5	1	6350
E01-1256-28709	454-109-3266, 10 PPM, Female, Adult	0.5	1	7900
E01-1256-28711	454-109-3268, 10 PPM, Female, Adult	0.5	1	6730
E01-1256-28713	454-109-3270, 10 PPM, Female, Adult	0.5	10	91400•
E01-1256-28715	454-109-3272, 10 PPM, Female, Adult	0.5	1	7710
E01-1256-28717	454-109-3274, 10 PPM, Female, Adult	0.5	10	29400
E01-1256-28719	454-109-3276, 10 PPM, Female, Adult	0.5	10	40600
E01-1256-28721	454-109-3278, 10 PPM, Female, Adult	0.5	1	9840
E01-1256-28723	454-109-3280, 10 PPM, Female, Adult	0.2	10	32200
E01-1256-28725	454-109-3282, 10 PPM, Female, Adult	0.5	1	10800
E01-1256-28727	454-109-3284, 10 PPM, Female, Adult	0.5	1	6570
E01-1256-28729	454-109-3286, 10 PPM, Female, Adult	0.5	10	28900
E01-1256-28731	454-109-3288, 10 PPM, Female, Adult	0.5	10	42800
E01-1256-28733	454-109-3290, 10 PPM, Female, Adult	0.5	1	16000
E01-1256-28735	454-109-3292, 10 PPM, Female, Adult	0.5	1	10400
E01-1256-28737	454-109-3294, 10 PPM, Female, Adult	0.5	1	7660
E01-1256-28739	454-109-3296, 10 PPM, Female, Adult	0.5	1	10800
E01-1256-28740	454-109-9583, 0 PPM, Female, Offspring	0.15	1	<100
E01-1256-28742	454-109-9589, 0 PPM, Female, Offspring	0.2	1	<100
E01-1256-28743	454-109-9592, 0 PPM, Female, Offspring	0.2	1	<100
E01-1256-28744	454-109-9598, 0 PPM, Female, Offspring	0.1	1	<100
E01-1256-28745	454-109-9603, 0 PPM, Female, Offspring	0.2	1	<100
E01-1256-28747	454-109-9610, 0 PPM, Female, Offspring	0.2	1	<100
E01-1256-28748	454-109-9573, 0 PPM, Female, Offspring	0.05	1	<100
E01-1256-28751	454-109-9629, 10 PPM, Female, Offspring	0.1	1	5130
E01-1256-28752	454-109-9633, 10 PPM, Female, Offspring	0.1	1	3420
E01-1256-28753	454-109-9634, 10 PPM, Female, Offspring	0.5	1	6760
E01-1256-28754	454-109-9645, 10 PPM, Female, Offspring	0.1	1	3750
E01-1256-28755	454-109-9648, 10 PPM, Female, Offspring	0.2	1	4900
E01-1256-28758	454-109-9654, 10 PPM, Female, Offspring	0.1	1	5450
E01-1256-28759	454-109-9659, 10 PPM, Female, Offspring	0.1	1	5350

• These values were rejected based on the results of the Dixon's Q-Test, 90% confidence interval, for the rejection of outliers

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Table 11. $C_8F_{17}SO_3^-$ Data Summary, Male Mallard Sera (Adult and Offspring)

3M Sample Number	Sample Description	Amount of Sera Used (mL)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/mL of Sera)
E01-1256-28662	454-109-3217, 0 PPM, Male, Adult	0.5	1	107
E01-1256-28664	454-109-3219, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28666	454-109-3221, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28668	454-109-3223, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28671	454-109-3227, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28673	454-109-3229, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28675	454-109-3231, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28677	454-109-3233, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28680	454-109-3237, 0 PPM, Male, Adult	0.3	1	<100
E01-1256-28682	454-109-3239, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28684	454-109-3241, 0 PPM, Male, Adult	0.5	1	1400
E01-1256-28686	454-109-3243, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28688	454-109-3245, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28690	454-109-3247, 0 PPM, Male, Adult	0.5	1	162
E01-1256-28692	454-109-3249, 0 PPM, Male, Adult	0.5	1	1070
E01-1256-28694	454-109-3251, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28696	454-109-3253, 0 PPM, Male, Adult	0.1	1	<100
E01-1256-28698	454-109-3255, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28700	454-109-3257, 10 PPM, Male, Adult	0.5	10	78800
E01-1256-28702	454-109-3259, 10 PPM, Male, Adult	0.2	10	83300
E01-1256-28704	454-109-3261, 10 PPM, Male, Adult	0.5	10	68700
E01-1256-28706	454-109-3263, 10 PPM, Male, Adult	0.06	100	210000•
E01-1256-28708	454-109-3265, 10 PPM, Male, Adult	0.2	10	93800
E01-1256-28710	454-109-3267, 10 PPM, Male, Adult	0.5	10	75700
E01-1256-28712	454-109-3269, 10 PPM, Male, Adult	0.5	10	80600
E01-1256-28714	454-109-3271, 10 PPM, Male, Adult	0.5	10	11300•
E01-1256-28716	454-109-3273, 10 PPM, Male, Adult	0.5	10	106000
E01-1256-28718	454-109-3275, 10 PPM, Male, Adult	0.5	10	80500
E01-1256-28720	454-109-3277, 10 PPM, Male, Adult	0.5	10	80400
E01-1256-28722	454-109-3279, 10 PPM, Male, Adult	0.5	10	103000
E01-1256-28724	454-109-3281, 10 PPM, Male, Adult	0.5	10	74300
E01-1256-28726	454-109-3283, 10 PPM, Male, Adult	0.5	10	91800
E01-1256-28728	454-109-3285, 10 PPM, Male, Adult	0.5	10	108000
E01-1256-28730	454-109-3287, 10 PPM, Male, Adult	0.3	10	78700
E01-1256-28732	454-109-3289, 10 PPM, Male, Adult	0.3	10	97900
E01-1256-28734	454-109-3291, 10 PPM, Male, Adult	0.3	10	73500
E01-1256-28736	454-109-3293, 10 PPM, Male, Adult	0.5	10	96800
E01-1256-28738	454-109-3295, 10 PPM, Male, Adult	0.5	10	99700
E01-1256-28741	454-109-9591, 0 PPM, Male, Offspring	0.1	1	<100

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3M Sample Number	Sample Description	Amount of Sera Used (mL)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/mL of Sera)
E01-1256-28746	454-109-9606, 0 PPM, Male, Offspring	0.2	1	103
E01-1256-28749	454-109-9578, 0 PPM, Male, Offspring	0.2	1	<100
E01-1256-28750	454-109-9625, 10 PPM, Male, Offspring	0.2	1	5450
E01-1256-28756	454-109-9636, 10 PPM, Male, Offspring	0.1	1	3670
E01-1256-28757	454-109-9642, 10 PPM, Male, Offspring	0.2	1	4110

• These values were rejected based on the results of the Dixon's Q-Test, 90% confidence interval, for the rejection of outliers.

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Table 12. $C_8F_{17}SO_3^-$ Data Summary, Female Mallard Liver (Adult and Offspring)

3M Sample Number	Sample Description	Amount of Liver Weighed (g)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/g of Liver)
E01-1326-29482	0 ppm a.i. 3218 F Liver, adult	1.0095	1	<50
E01-1326-29583	0 ppm a.i. 3220 F Liver, adult	1.0944	1	<50
E01-1326-29601	0 ppm a.i. 3222 F Liver, adult	1.0435	1	<50
E01-1326-29619	0 ppm a.i. 3224 F Liver, adult	1.0425	1	<50
E01-1326-29637	0 ppm a.i. 3226 F Liver, adult	1.0703	1	<50
E01-1326-29655	0 ppm a.i. 3228 F Liver, adult	1.0526	1	<50
E01-1326-29673	0 ppm a.i. 3230 F Liver, adult	1.0411	1	<50
E01-1326-29691	0 ppm a.i. 3232 F Liver, adult	1.1704	1	<50
E01-1326-29709	0 ppm a.i. 3234 F Liver, adult	1.1290	1	<50
E01-1326-29727	0 ppm a.i. 3236 F Liver, adult	1.0800	1	<50
E01-1326-29745	0 ppm a.i. 3238 F Liver, adult	1.1170	1	<50
E01-1326-29763	0 ppm a.i. 3240 F Liver, adult	1.0086	1	<50
E01-1326-29781	0 ppm a.i. 3242 F Liver, adult	1.0907	1	<50
E01-1326-29799	0 ppm a.i. 3244 F Liver, adult	1.0291	1	<50
E01-1326-29817	0 ppm a.i. 3246 F Liver, adult	1.1294	1	<50
E01-1326-29835	0 ppm a.i. 3248 F Liver, adult	1.1075	1	<50
E01-1326-29853	0 ppm a.i. 3250 F Liver, adult	1.1709	1	<50
E01-1326-29871	0 ppm a.i. 3252 F Liver, adult	1.0761	1	<50
E01-1326-29889	0 ppm a.i. 3254 F Liver, adult	1.1344	1	<50
E01-1326-29907	0 ppm a.i. 3256 F Liver, adult	1.0669	1	<50
E01-1326-29925	10 ppm a.i. 3258 F Liver, adult	1.0034	1	7080
E01-1326-29943	10 ppm a.i. 3260 F Liver, adult	1.1331	1	6780
E01-1326-29961	10 ppm a.i. 3262 F Liver, adult	1.1294	10	13600
E01-1326-29980	10 ppm a.i. 3264 F Liver, adult	1.1829	1	6080
E01-1326-29998	10 ppm a.i. 3266 F Liver, adult	1.0487	1	5380
E01-1326-30016	10 ppm a.i. 3268 F Liver, adult	1.1214	1	5730
E01-1326-30034	10 ppm a.i. 3270 F Liver, adult	1.0751	10	13200
E01-1326-30052	10 ppm a.i. 3272 F Liver, adult	1.0658	1	5800
E01-1326-30070	10 ppm a.i. 3274 F Liver, adult	1.1226	10	13800
E01-1326-30088	10 ppm a.i. 3276 F Liver, adult	1.0953	10	30700
E01-1326-30106	10 ppm a.i. 3278 F Liver, adult	1.1956	1	5960
E01-1326-30124	10 ppm a.i. 3280 F Liver, adult	1.0600	1	2480
E01-1326-30142	10 ppm a.i. 3282 F Liver, adult	1.0275	1	4560
E01-1326-30160	10 ppm a.i. 3284 F Liver, adult	1.0915	1	6330
E01-1326-30178	10 ppm a.i. 3286 F Liver, adult	1.0657	10	28200
E01-1326-30196	10 ppm a.i. 3288 F Liver, adult	1.0330	10	24100
E01-1326-30214	10 ppm a.i. 3290 F Liver, adult	1.0352	10	19800
E01-1326-30232	10 ppm a.i. 3292 F Liver, adult	1.0658	1	4600
E01-1326-30250	10 ppm a.i. 3294 F Liver, adult	1.0627	1	4760

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3M Sample Number	Sample Description	Amount of Liver Weighed (g)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/g of Liver)
E01-1326-30268	10 ppm a.i. 3296 F Liver, adult	1.1238	1	6330
E01-1367-31295	0 ppm a.i. 9583 F Liver, offspring	1.1308	1	<50
E01-1367-31307	0 ppm a.i. 9589 F Liver, offspring	1.0005	1	<50
E01-1367-31313	0 ppm a.i. 9592 F Liver, offspring	1.0390	1	<50
E01-1367-31319	0 ppm a.i. 9598 F Liver, offspring	1.1204	1	<50
E01-1367-31325	0 ppm a.i. 9603 F Liver, offspring	1.1550	1	<50
E01-1367-31337	0 ppm a.i. 9610 F Liver, offspring	1.0217	1	<50
E01-1367-31343	0 ppm a.i. 9573 F Liver, offspring	1.0122	1	<50
E01-1367-31362	10 ppm a.i. 9629 F Liver, offspring	1.1676	1	2950
E01-1367-31368	10 ppm a.i. 9633 F Liver, offspring	1.0510	1	2240
E01-1367-31374	10 ppm a.i. 9634 F Liver, offspring	1.1638	1	4520
E01-1367-31380	10 ppm a.i. 9645 F Liver, offspring	1.1510	1	2580
E01-1367-31386	10 ppm a.i. 9648 F Liver, offspring	1.1125	1	3500
E01-1367-31404	10 ppm a.i. 9654 F Liver, offspring	1.0770	1	5930
E01-1367-31410	10 ppm a.i. 9659 F Liver, offspring	1.0581	1	3580

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Table 13. $C_8F_{17}SO_3^-$ Data Summary, Male Mallard Liver (Adult and Offspring)

3M Sample Number	Sample Description	Amount of Liver Weighed (g)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/g of Liver)
E01-1326-29473	0 ppm a.i. 3217 M Liver, adult	1.1169	1	<50
E01-1326-29574	0 ppm a.i. 3219 M Liver, adult	1.1847	1	<50
E01-1326-29592	0 ppm a.i. 3221 M Liver, adult	1.1181	1	<50
E01-1326-29610	0 ppm a.i. 3223 M Liver, adult	1.0788	1	<50
E01-1326-29628	0 ppm a.i. 3225 M Liver, adult	1.1043	1	<50
E01-1326-29646	0 ppm a.i. 3227 M Liver, adult	1.0486	1	<50
E01-1326-29664	0 ppm a.i. 3229 M Liver, adult	1.0336	1	<50
E01-1326-29682	0 ppm a.i. 3231 M Liver, adult	1.0165	1	<50
E01-1326-29700	0 ppm a.i. 3233 M Liver, adult	1.0395	1	<50
E01-1326-29718	0 ppm a.i. 3235 M Liver, adult	1.0373	1	<50
E01-1326-29736	0 ppm a.i. 3237 M Liver, adult	1.1679	1	<50
E01-1326-29754	0 ppm a.i. 3239 M Liver, adult	1.1493	1	<50
E01-1326-29772	0 ppm a.i. 3241 M Liver, adult	1.0619	1	<50
E01-1326-29790	0 ppm a.i. 3243 M Liver, adult	1.0263	1	<50
E01-1326-29808	0 ppm a.i. 3245 M Liver, adult	1.1541	1	<50
E01-1326-29826	0 ppm a.i. 3247 M Liver, adult	1.0813	1	<50
E01-1326-29844	0 ppm a.i. 3249 M Liver, adult	1.0263	1	<50
E01-1326-29862	0 ppm a.i. 3251 M Liver, adult	1.0088	1	<50
E01-1326-29880	0 ppm a.i. 3253 M Liver, adult	1.0294	1	<50
E01-1326-29898	0 ppm a.i. 3255 M Liver, adult	1.0926	1	<50
E01-1326-29916	10 ppm a.i. 3257 M Liver, adult	1.0654	10	72100
E01-1326-29934	10 ppm a.i. 3259 M Liver, adult	1.1316	10	49200
E01-1326-29952	10 ppm a.i. 3261 M Liver, adult	1.1330	10	70100
E01-1326-29970	10 ppm a.i. 3263 M Liver, adult	1.1404	100	125000
E01-1326-29989	10 ppm a.i. 3265 M Liver, adult	1.1480	10	61700
E01-1326-30007	10 ppm a.i. 3267 M Liver, adult	1.0620	10	49900
E01-1326-30025	10 ppm a.i. 3269 M Liver, adult	1.1687	10	57900
E01-1326-30043	10 ppm a.i. 3271 M Liver, adult	1.0313	10	63000
E01-1326-30061	10 ppm a.i. 3273 M Liver, adult	1.1399	10	85000
E01-1326-30079	10 ppm a.i. 3275 M Liver, adult	1.1143	10	54900
E01-1326-30097	10 ppm a.i. 3277 M Liver, adult	1.0062	10	53900
E01-1326-30115	10 ppm a.i. 3279 M Liver, adult	1.0380	10	69000
E01-1326-30133	10 ppm a.i. 3281 M Liver, adult	1.0669	10	49900
E01-1326-30151	10 ppm a.i. 3283 M Liver, adult	1.0955	10	67100
E01-1326-30169	10 ppm a.i. 3285 M Liver, adult	1.1981	10	55500
E01-1326-30187	10 ppm a.i. 3287 M Liver, adult	1.0500	10	52600
E01-1326-30205	10 ppm a.i. 3289 M Liver, adult	1.0251	10	59300
E01-1326-30223	10 ppm a.i. 3291 M Liver, adult	1.0441	10	46500
E01-1326-30241	10 ppm a.i. 3293 M Liver, adult	1.0979	10	24200
E01-1326-30259	10 ppm a.i. 3295 M Liver, adult	1.0815	10	50400

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3M Sample Number	Sample Description	Portion of Liver Extracted (g)	Dilution Factor	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/g of Liver)
E01-1367-31301	0 ppm a.i. 9591 M Liver, offspring	1.0411	1	<50
E01-1367-31331	0 ppm a.i. 9606 M Liver, offspring	1.1314	1	<50
E01-1367-31349	0 ppm a.i. 9578 M Liver, offspring	1.0413	1	<50
E01-1367-31356	10 ppm a.i. 9625 M Liver, offspring	1.0166	1	4640
E01-1367-31392	10 ppm a.i. 9636 M Liver, offspring	1.1367	1	2200
E01-1367-31398	10 ppm a.i. 9642 M Liver, offspring	1.1252	1	2680

STATEMENT OF CONCLUSION

Sera results from the male control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 14 of 18 mallards (<100ng/mL) and quantifiable concentrations of $C_8F_{17}SO_3^-$ in 4 of 18 (range 107-1400ng/mL, Table 14). The sera results from the female control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 15 of 17 birds (<100ng/mL) and quantifiable concentrations of $C_8F_{17}SO_3^-$ in 2 of 17 (range 166-172ng/mL, Table 15). Analytical results for eighteen sera samples from the 10ppm dosed male group showed an average of 87300ng/mL $C_8F_{17}SO_3^-$ (range 68700-108000ng/mL, Table 15). Analytical results for nineteen sera samples from the 10ppm dosed female group showed an average of 16600ng/mL $C_8F_{17}SO_3^-$ (range 6350-42800ng/mL, Table 15).

Sera results from the male offspring control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 2 of 3 birds (<100ng/mL) and a quantifiable concentration in 1 of 3 (103 ng/mL, Table 14). The sera results from the female offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 7 of 7 birds (<100ng/mL, Table 14). Analytical results for three sera samples from the 10ppm dosed male offspring group showed an average of 4410ng/mL (range 3670-5450ng/mL, Table 14). Analytical results for seven sera samples from the 10ppm dosed female offspring group showed an average of 4960ng/mL (range 3420-6760ng/mL, Table 14).

Liver results from the male control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 20 of 20 birds (<50ng/g, Table 14). The liver results from the female control group also showed no detectable levels of $C_8F_{17}SO_3^-$ in 20 of 20 birds (<50ng/g, Table 14). Analytical results for twenty liver samples from the 10ppm dosed male group showed an average of 60900ng/g $C_8F_{17}SO_3^-$ (range 24200-125000ng/g, Table 14). Analytical results for twenty liver samples from the 10ppm dosed female group showed an average of 10800ng/g $C_8F_{17}SO_3^-$ (range 2480-30700ng/g, Table 14).

Liver results from the male offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 3 of 3 birds (<50ng/g, Table 14). The liver results from the female offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 7 of 7 birds (<50ng/g, Table 14). Analytical results for three liver samples from the 10ppm dosed male offspring group showed an average of 3170ng/g (range 2200-4640ng/g, Table 14). Analytical results for seven liver samples from the 10ppm dosed female offspring group showed an average of 3620ng/g (range 2240-5930ng/g, Table 14).

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Table 14. Summary Table, $C_8F_{17}SO_3^-$ Concentration in Sera and Liver

Sampling Event	Number of Sera Samples	Average $C_8F_{17}SO_3^-$ Conc. Sera	Range of Results	Number of Liver Samples	Average $C_8F_{17}SO_3^-$ Conc. Liver	Range of Results
Male Control Group	18	NA	<100 ng/mL (14 of 18) 107-1400 ng/mL range (4 of 18)	20	<50 ng/g	NA
Female Control Group	20	NA	<100 ng/mL (15 of 20) 166-2680 ng/mL range (5 of 20)	20	<50 ng/g	NA
Male 10ppm Group	20	89700 ng/mL	11300-210000 ng/mL	20	60900 ng/g	24200-125000 ng/g
Female 10ppm Group	20	20300 ng/mL	6350-91400 ng/mL	20	10800 ng/g	2480-30700 ng/g
Male Offspring Control Group	3	NA	<100 ng/mL (2 of 3) 103 ng/mL (1 of 3)	3	<50 ng/g	NA
Female Offspring Control Group	7	NA	<100 ng/mL (7 of 7)	7	<50 ng/g	NA
Male Offspring 10ppm Group	3	4410 ng/mL	3670 - 5450 ng/mL	3	3170 ng/g	2200-4640 ng/g
Female Offspring 10ppm Group	7	4960 ng/mL	3420 - 6760 ng/mL	7	3620 ng/g	2240–5930 ng/g

For individual samples values see Tables 10 to 13 and Attachment B.

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Table 15. Summary Table, $C_8F_{17}SO_3^-$ Concentration in Sera and Liver with Outliers Removed

Sampling Event	Number of Sera Samples	Average $C_8F_{17}SO_3^-$ Conc. Sera	Range of Results	Number of Liver Samples	Average $C_8F_{17}SO_3^-$ Conc. Liver	Range of Results
Male Control Group	18*	*	*	20*	*	*
Female Control Group	17	NA	<100 ng/mL (15 of 17) 166-172 ng/mL range (2 of 17)	20*	*	*
Male 10ppm Group	18	87300 ng/mL	68700-108000 ng/mL	20*	*	*
Female 10ppm Group	19	16600 ng/mL	6350-42800 ng/mL	20*	*	*
Male Offspring Control Group	3*	*	*	3*	*	*
Female Offspring Control Group	7*	*	*	7*	*	*
Male Offspring 10ppm Group	3*	*	*	3*	*	*
Female Offspring 10ppm Group	7*	*	*	7*	*	*

* No outliers removed from this data set. Refer to Table 14 for results. For individual samples values see Tables 10 to 13 and Attachment B.

Statistical Methods

Statistical methods included calculating means, standard deviations, % coefficient of variation, linear regressions, percent recoveries, and the Dixons Q-Test. The Dixons Q-Test, used for the rejection of outliers, has values ordered from lowest to highest. A Q-value is determined from the difference of a result and the next closest result that is divided by the difference of the highest and lowest value in the group of values. A critical Q-value is determined based on the number of samples in the group and the desired confidence interval. If the Q-value is greater than the critical Q-value then the value will be rejected.

Statement of Data Quality

The only measurement is the quality control and matrix spike results. Accuracy (the observed amount in the QC and MS samples, divided by the amount spiked, expressed as a percent) was found to range from 79.7% to 172%; while precision, expressed as the standard deviation of the recoveries, was found to range from 0.717% to 15.7%.

Reproductive Performance by Pen

Appendix XIII - Table 1

Eggs Laid / Maximum Laid (%)

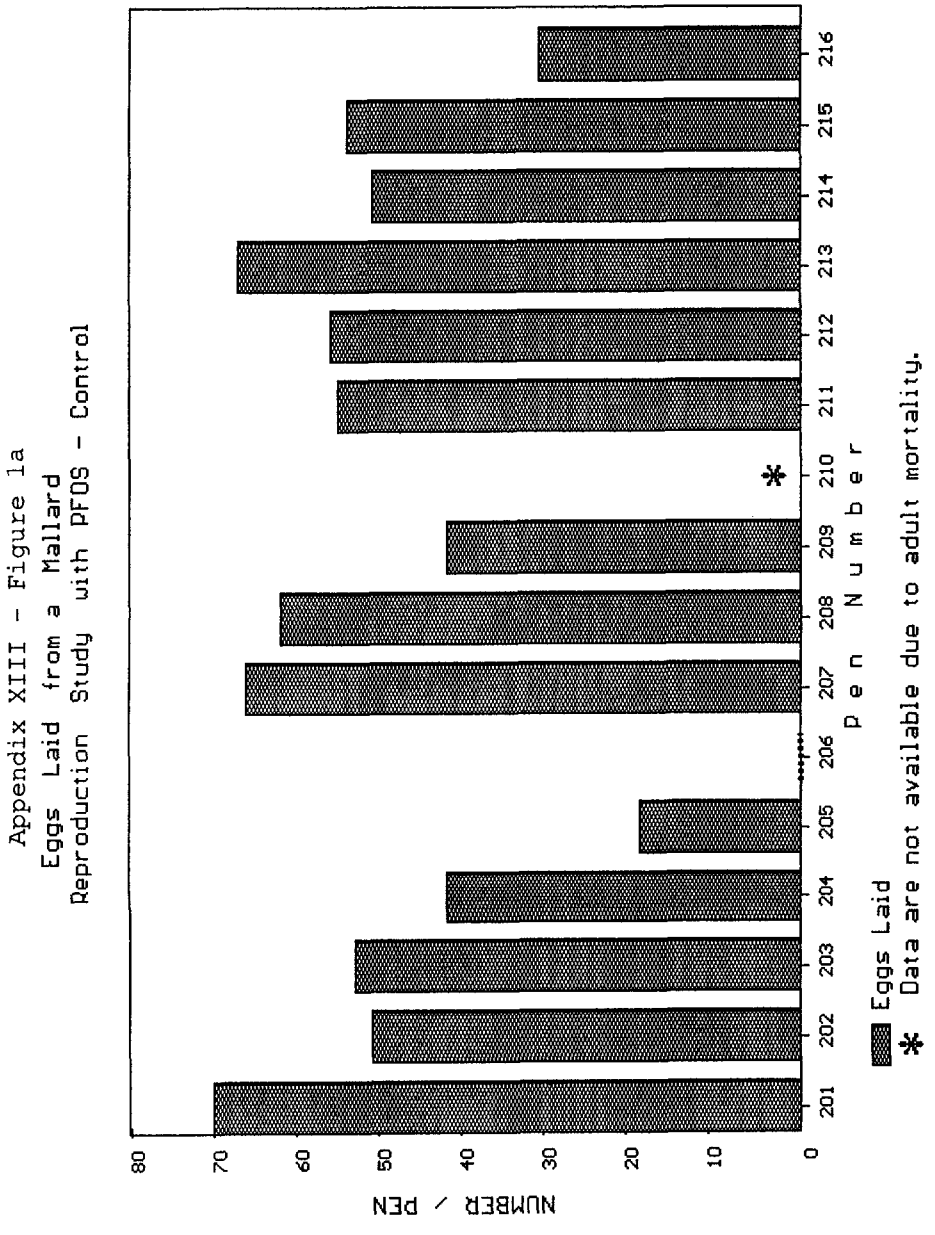
from a Mallard Reproduction Study with PFOS

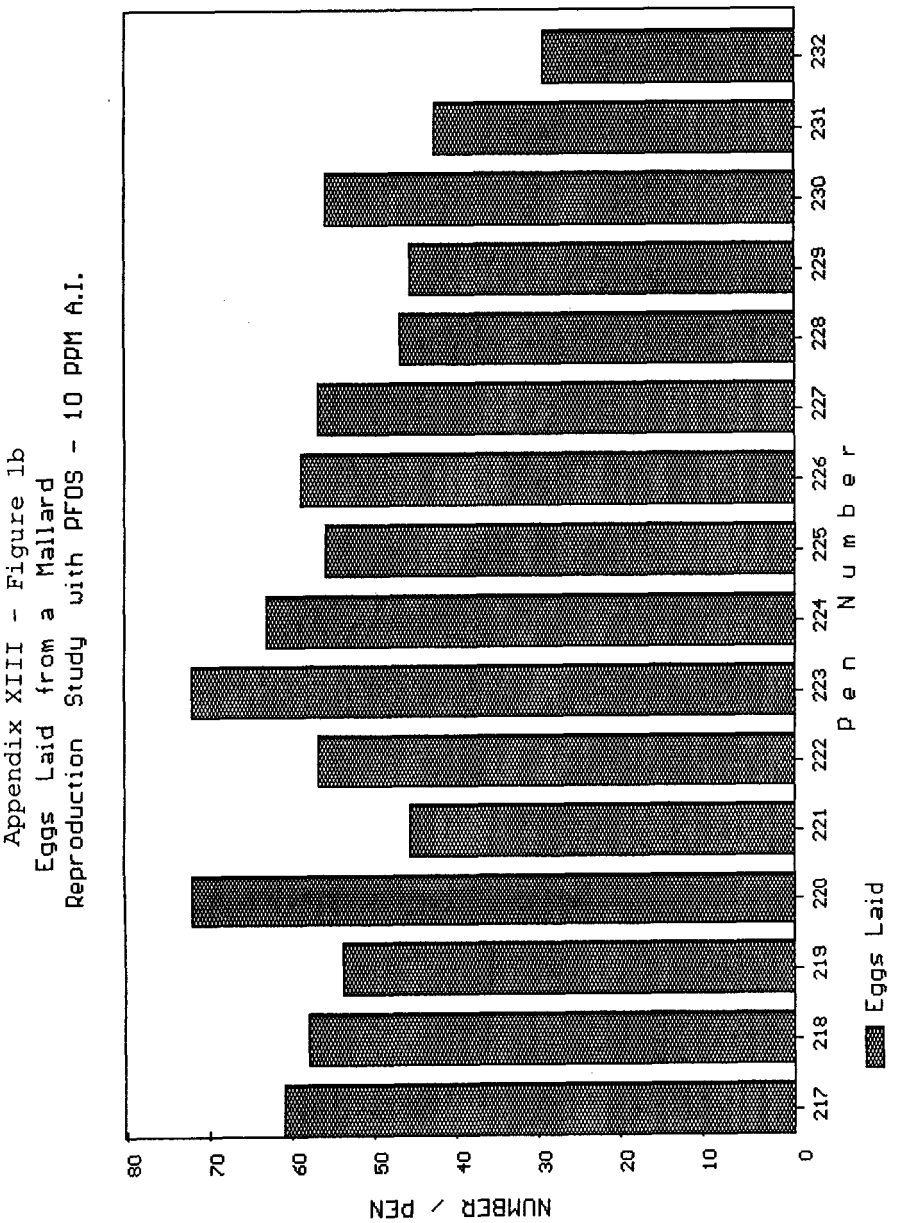
	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	Eggs Laid	Max. Laid	%	Arcsin Trans.	Eggs Laid	Max. Laid	%	Arcsin Trans.	Eggs Laid	Max. Laid	%	Arcsin Trans.	Eggs Laid	Max. Laid	%	Arcsin Trans.
1	70	72	97	80.41	61	72	85	66.99	-	-	-	-	-	-	-	-
2	51	72	71	57.31	58	72	81	63.83	-	-	-	-	-	-	-	-
3	53	72	74	59.09	54	72	75	60.00	-	-	-	-	-	-	-	-
4	42	72	58	49.80	72	72	100	90.00	-	-	-	-	-	-	-	-
5	19	72	26	30.91	46	72	64	53.06	-	-	-	-	-	-	-	-
6	0	72	0	0.00	57	72	79	62.84	-	-	-	-	-	-	-	-
7	66	72	92	73.22	72	72	100	90.00	-	-	-	-	-	-	-	-
8	62	72	86	68.12	63	72	88	69.30	-	-	-	-	-	-	-	-
9	42	72	58	49.80	56	72	78	61.87	-	-	-	-	-	-	-	-
10	-	-	-	-	59	72	82	64.85	-	-	-	-	-	-	-	-
11	55	72	76	60.93	57	72	79	62.84	-	-	-	-	-	-	-	-
12	56	72	78	61.87	47	72	65	53.90	-	-	-	-	-	-	-	-
13	67	72	93	74.72	46	72	64	53.06	-	-	-	-	-	-	-	-
14	51	72	71	57.31	56	72	78	61.87	-	-	-	-	-	-	-	-
15	54	72	75	60.00	43	72	60	50.61	-	-	-	-	-	-	-	-
16	31	72	43	41.01	30	72	42	40.20	-	-	-	-	-	-	-	-
Total	719	1080			877	1152			-	-			-	-		
Mean	48	72	67	54.97	55	72	76	62.83	-	-	-	-	-	-	-	-
SD	19	0	26	19.87	11	0	15	12.87	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Appendix XIII - Figure 1a
Eggs Laid from a Mallard
Reproduction Study with PFOS - Control




Appendix XIII - Figure 1b
Eggs Laid from a Mallard
Reproduction Study with PFOS - 10 PPM A.I.
NUMBER / PEN
0
10
20
30
40
50
60
70
80
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
Pen Number
Eggs Laid

Reproductive Performance by Pen

Appendix XIII - Table 2

Eggs Cracked / Eggs Laid (%)

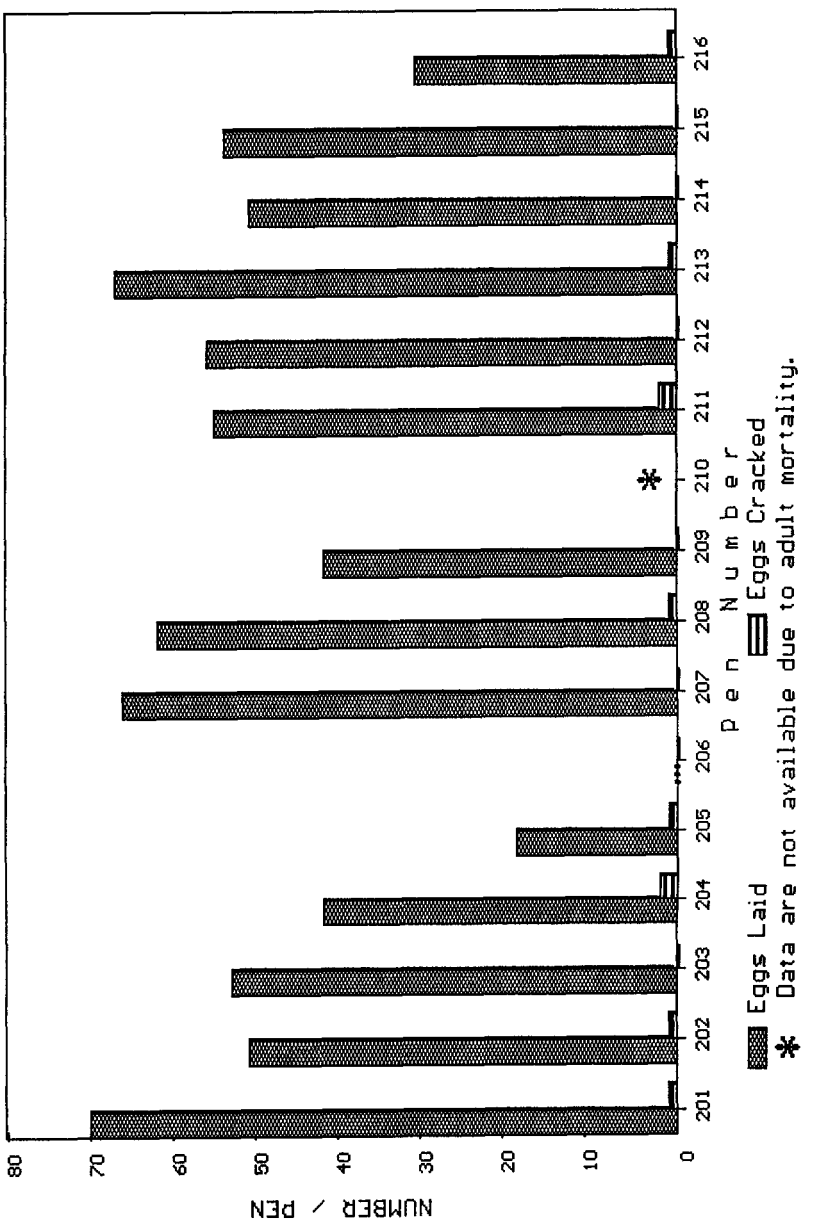
from a Mallard Reproduction Study with PFOS

	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	Eggs Crack	Eggs Laid	%	Arcsin Trans.	Eggs Crack	Eggs Laid	%	Arcsin Trans.	Eggs Crack	Eggs Laid	%	Arcsin Trans.	Eggs Crack	Eggs Laid	%	Arcsin Trans.
1	1	70	1	6.86	0	61	0	0.00	-	-	-	-	-	-	-	-
2	1	51	2	8.05	0	58	0	0.00	-	-	-	-	-	-	-	-
3	0	53	0	0.00	0	54	0	0.00	-	-	-	-	-	-	-	-
4	2	42	5	12.60	4	72	6	13.63	-	-	-	-	-	-	-	-
5	1	19	5	13.26	0	46	0	0.00	-	-	-	-	-	-	-	-
6	0	0			0	57	0	0.00	-	-	-	-	-	-	-	-
7	0	66	0	0.00	3	72	4	11.78	-	-	-	-	-	-	-	-
8	1	62	2	7.30	0	63	0	0.00	-	-	-	-	-	-	-	-
9	0	42	0	0.00	3	56	5	13.38	-	-	-	-	-	-	-	-
10	-	-	-	-	0	59	0	0.00	-	-	-	-	-	-	-	-
11	2	55	4	10.99	0	57	0	0.00	-	-	-	-	-	-	-	-
12	0	56	0	0.00	0	47	0	0.00	-	-	-	-	-	-	-	-
13	1	67	1	7.02	0	46	0	0.00	-	-	-	-	-	-	-	-
14	0	51	0	0.00	0	56	0	0.00	-	-	-	-	-	-	-	-
15	0	54	0	0.00	0	43	0	0.00	-	-	-	-	-	-	-	-
16	1	31	3	10.35	0	30	0	0.00	-	-	-	-	-	-	-	-
Total	10	719			10	877			-	-			-	-		
Mean	1	48	2	5.46	1	55	1	2.43	-	-	-	-	-	-	-	-
SD	1	19	2	5.26	1	11	2	5.23	-	-	-	-	-	-	-	-

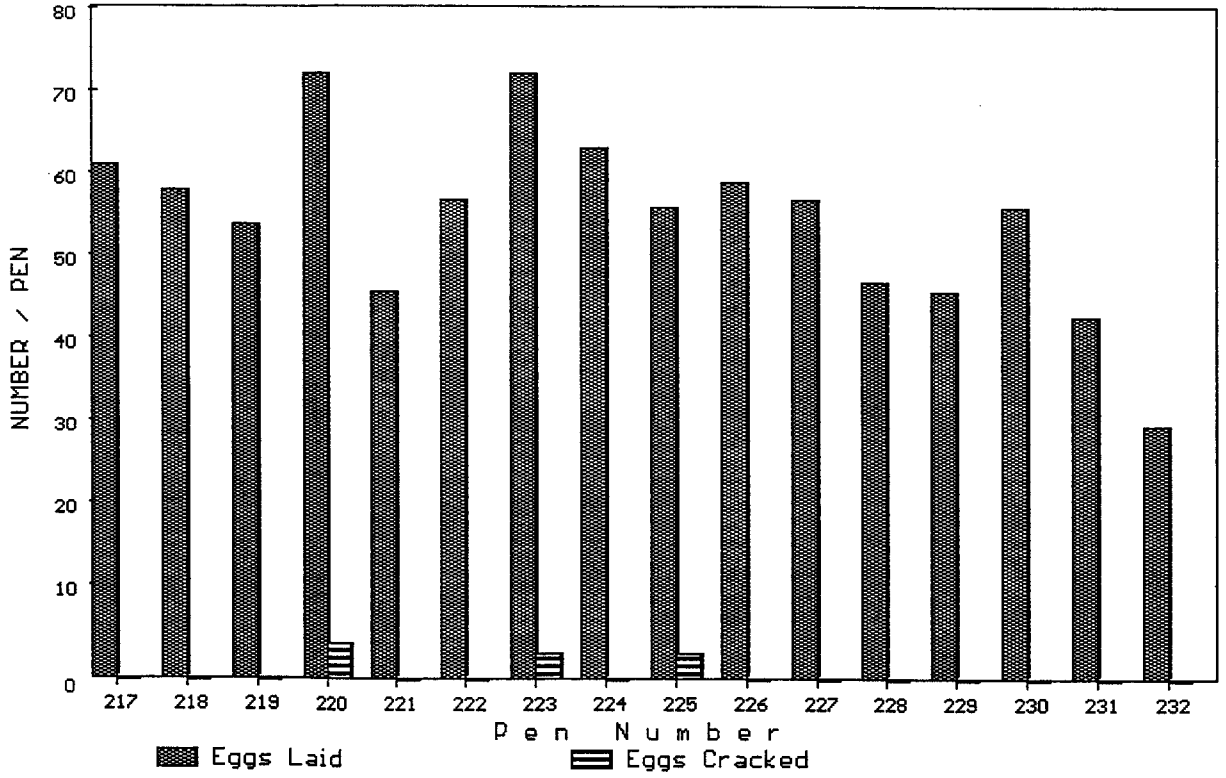
- Data are not available due to adult mortality.

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

Appendix XIII - Figure 2a
Eggs Cracked / Eggs Laid from a Mallard
Reproduction Study with PFOS - Control



Appendix XIII - Figure 2b
Eggs Cracked / Eggs Laid from a Mallard
Reproduction Study with PFOS - 10 PPM A.I.



Reproductive Performance by Pen

Appendix XIII - Table 3

Viable Embryos / Eggs Set (%)

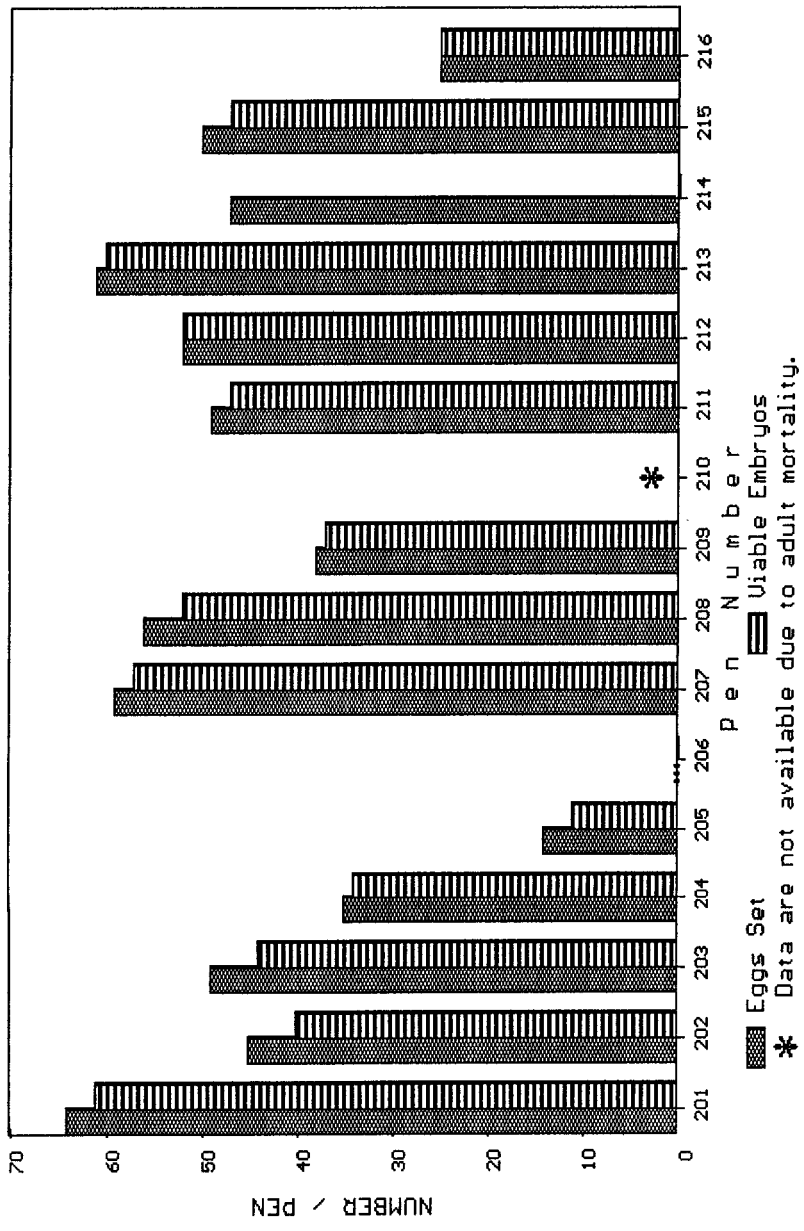
from a Mallard Reproduction Study with PFOS

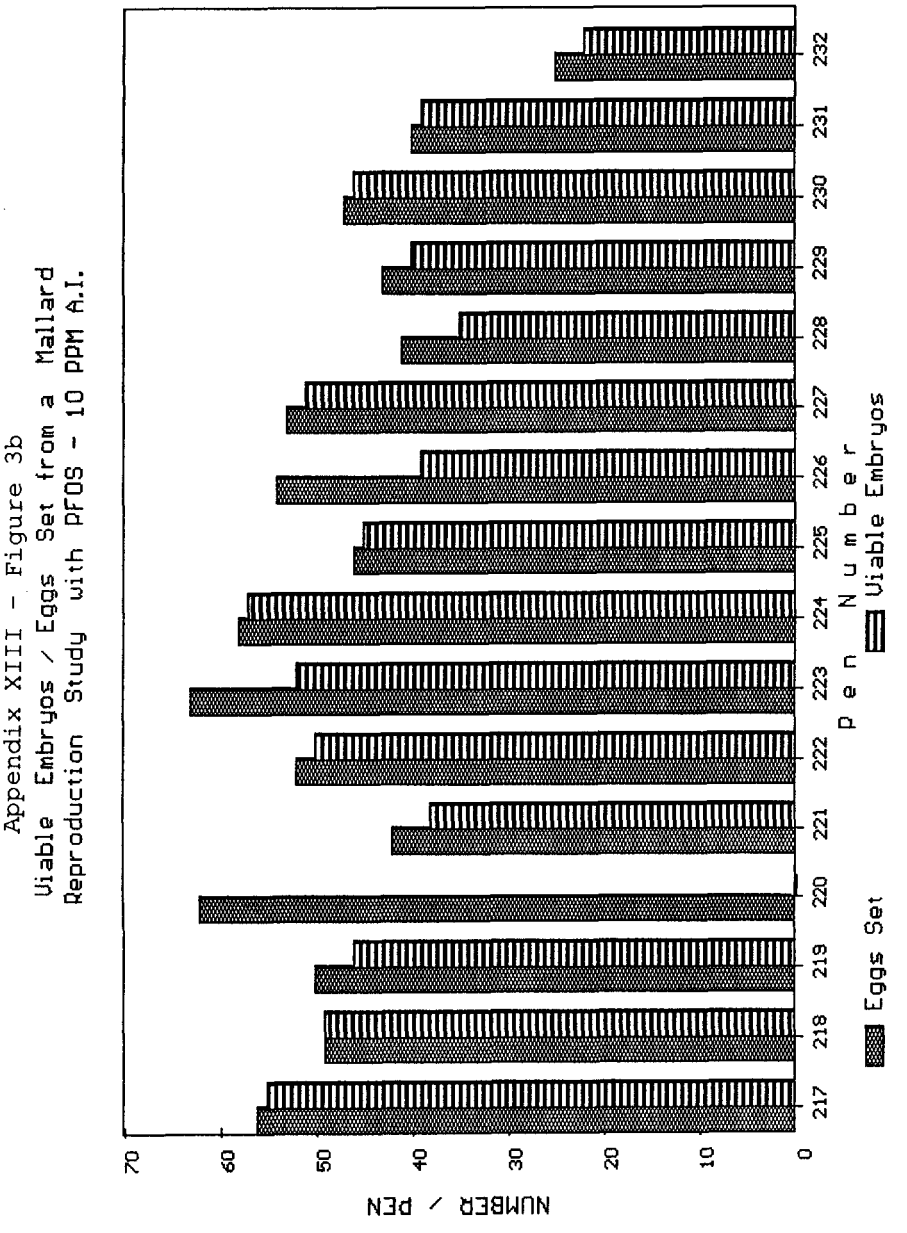
	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	Viable Embr.	Eggs Set	%	Arcsin Trans.	Viable Embr.	Eggs Set	%	Arcsin Trans.	Viable Embr.	Eggs Set	%	Arcsin Trans.	Viable Embr.	Eggs Set	%	Arcsin Trans.
1	61	64	95	77.50	55	56	98	82.32	-	-	-	-	-	-	-	-
2	40	45	89	70.53	49	49	100	90.00	-	-	-	-	-	-	-	-
3	44	49	90	71.37	46	50	92	73.57	-	-	-	-	-	-	-	-
4	34	35	97	80.27	0	62	0	0.00	-	-	-	-	-	-	-	-
5	11	14	79	62.42	38	42	90	72.02	-	-	-	-	-	-	-	-
6	0	0			50	52	96	78.69	-	-	-	-	-	-	-	-
7	57	59	97	79.39	52	63	83	65.30	-	-	-	-	-	-	-	-
8	52	56	93	74.50	57	58	98	82.45	-	-	-	-	-	-	-	-
9	37	38	97	80.66	45	46	98	81.52	-	-	-	-	-	-	-	-
10	-	-	-	-	39	54	72	58.19	-	-	-	-	-	-	-	-
11	47	49	96	78.34	51	53	96	78.80	-	-	-	-	-	-	-	-
12	52	52	100	90.00	35	41	85	67.51	-	-	-	-	-	-	-	-
13	60	61	98	82.64	40	43	93	74.68	-	-	-	-	-	-	-	-
14	0	47	0	0.00	46	47	98	81.61	-	-	-	-	-	-	-	-
15	47	50	94	75.82	39	40	97	80.90	-	-	-	-	-	-	-	-
16	25	25	100	90.00	22	25	88	69.73	-	-	-	-	-	-	-	-
Total	567	644			664	781			-	-			-	-		
Mean	38	43	88	72.39	42	49	87	71.08	-	-	-	-	-	-	-	-
SD	20	18	26	22.06	14	10	24	20.55	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Appendix XIII - Figure 3a
Viable Embryos / Eggs Set from a Mallard
Reproduction Study with PFOS - Control




Appendix XIII - Figure 3b
Viable Embryos / Eggs Set from a Mallard
Reproduction Study with PFOS - 10 PPM A.I.
NUMBER / PEN
0
10
20
30
40
50
60
70
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
Pen Number
Eggs Set
Viable Embryos

Reproductive Performance by Pen

Appendix XIII- Table 4

Live 3-Week Embryos / Viable Embryos (%)

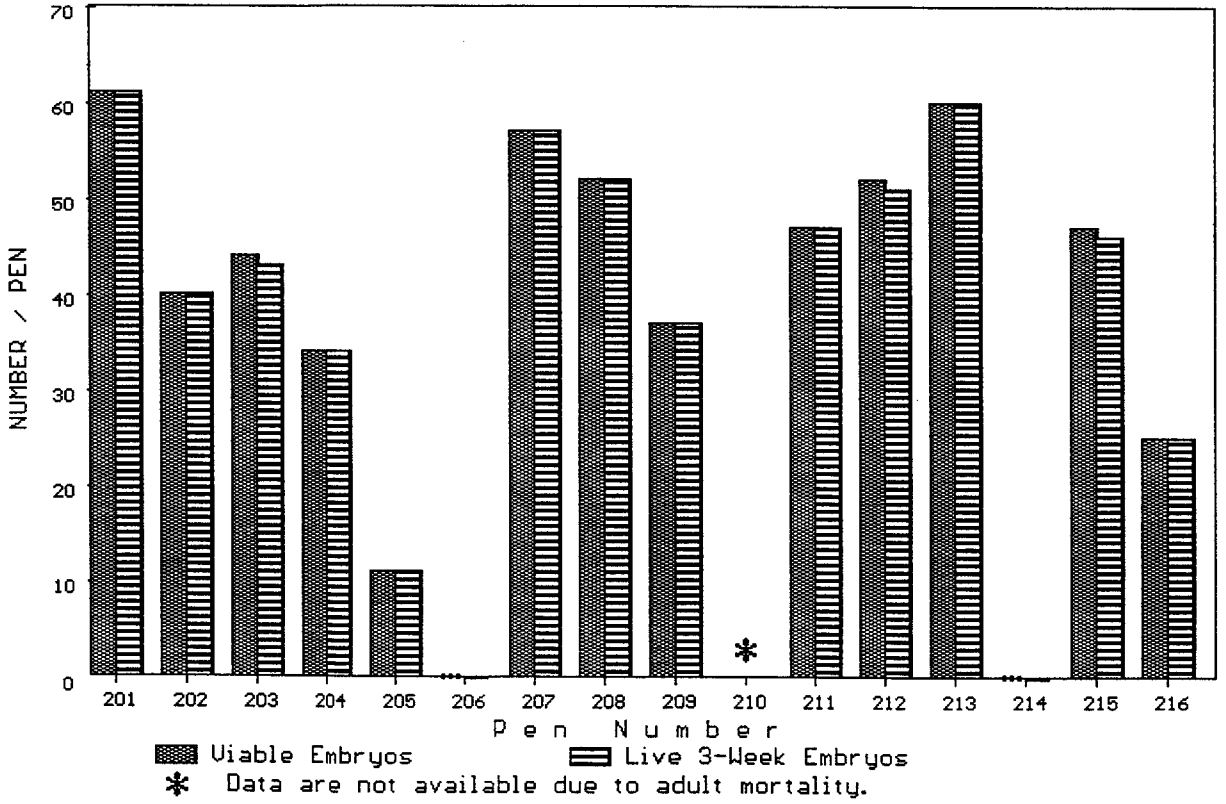
from a Mallard Reproduction Study with PFOS

	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	Live 3-Week	Viable Embr.	%	Arcsin Trans.	Live 3-Week	Viable Embr.	%	Arcsin Trans.	Live 3-Week	Viable Embr.	%	Arcsin Trans.	Live 3-Week	Viable Embr.	%	Arcsin Trans.
1	61	61	100	90.00	55	55	100	90.00	-	-	-	-	-	-	-	-
2	40	40	100	90.00	49	49	100	90.00	-	-	-	-	-	-	-	-
3	43	44	98	81.33	46	46	100	90.00	-	-	-	-	-	-	-	-
4	34	34	100	90.00	0	0			-	-	-	-	-	-	-	-
5	11	11	100	90.00	38	38	100	90.00	-	-	-	-	-	-	-	-
6	0	0			50	50	100	90.00	-	-	-	-	-	-	-	-
7	57	57	100	90.00	51	52	98	82.03	-	-	-	-	-	-	-	-
8	52	52	100	90.00	57	57	100	90.00	-	-	-	-	-	-	-	-
9	37	37	100	90.00	44	45	98	81.43	-	-	-	-	-	-	-	-
10	-	-	-	-	39	39	100	90.00	-	-	-	-	-	-	-	-
11	47	47	100	90.00	51	51	100	90.00	-	-	-	-	-	-	-	-
12	51	52	98	82.03	35	35	100	90.00	-	-	-	-	-	-	-	-
13	60	60	100	90.00	31	40	78	61.68	-	-	-	-	-	-	-	-
14	0	0			46	46	100	90.00	-	-	-	-	-	-	-	-
15	46	47	98	81.61	39	39	100	90.00	-	-	-	-	-	-	-	-
16	25	25	100	90.00	22	22	100	90.00	-	-	-	-	-	-	-	-
Total	564	567			653	664			-	-			-	-		
Mean	38	38	100	88.08	41	42	98	87.01	-	-	-	-	-	-	-	-
SD	20	20	1	3.66	14	14	6	7.58	-	-	-	-	-	-	-	-

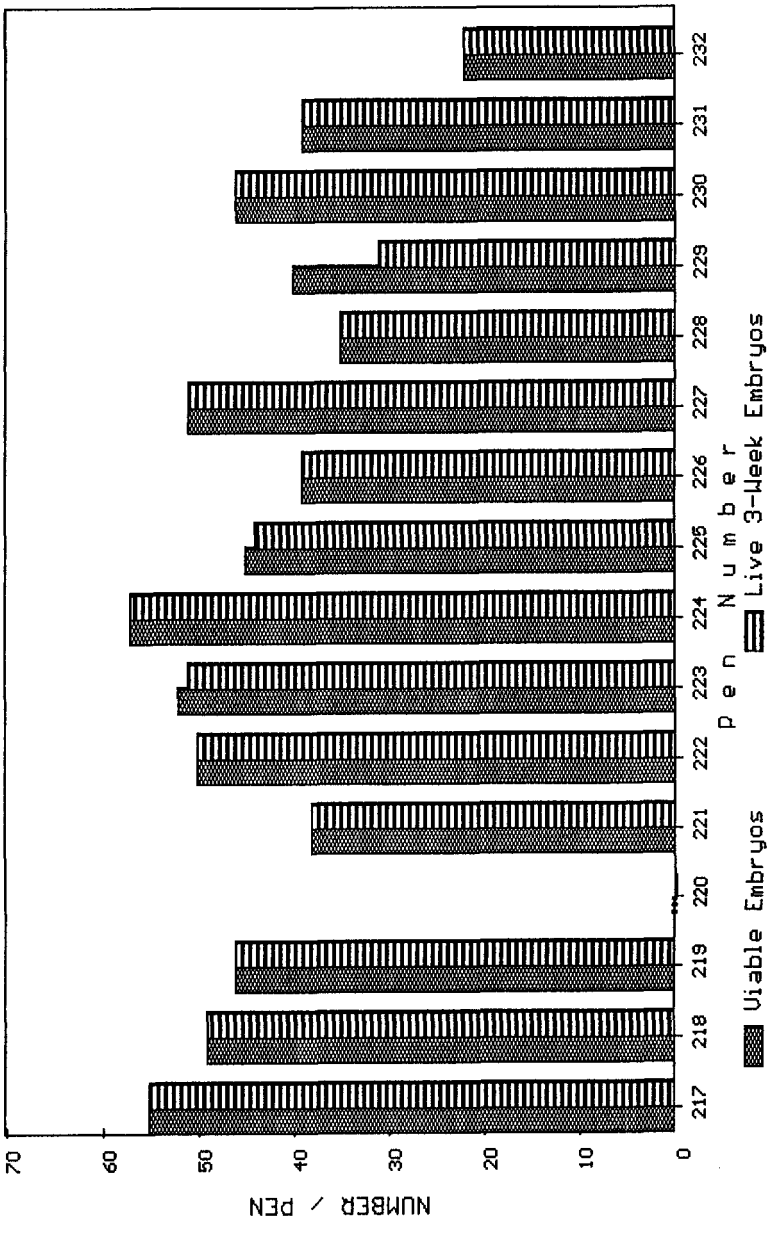
- Data are not available due to adult mortality.

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

Appendix XIII - Figure 4a
Live 3-Week Embryos / Viable Embryos from a Mallard Reproduction Study with PFOS - Control



Appendix XIII - Figure 4b
Live 3-Week Embryos / Viable Embryos from a Mallard
Reproduction Study with PFOS - 10 PPM A.I.



Reproductive Performance by Pen

Appendix XIII - Table 5

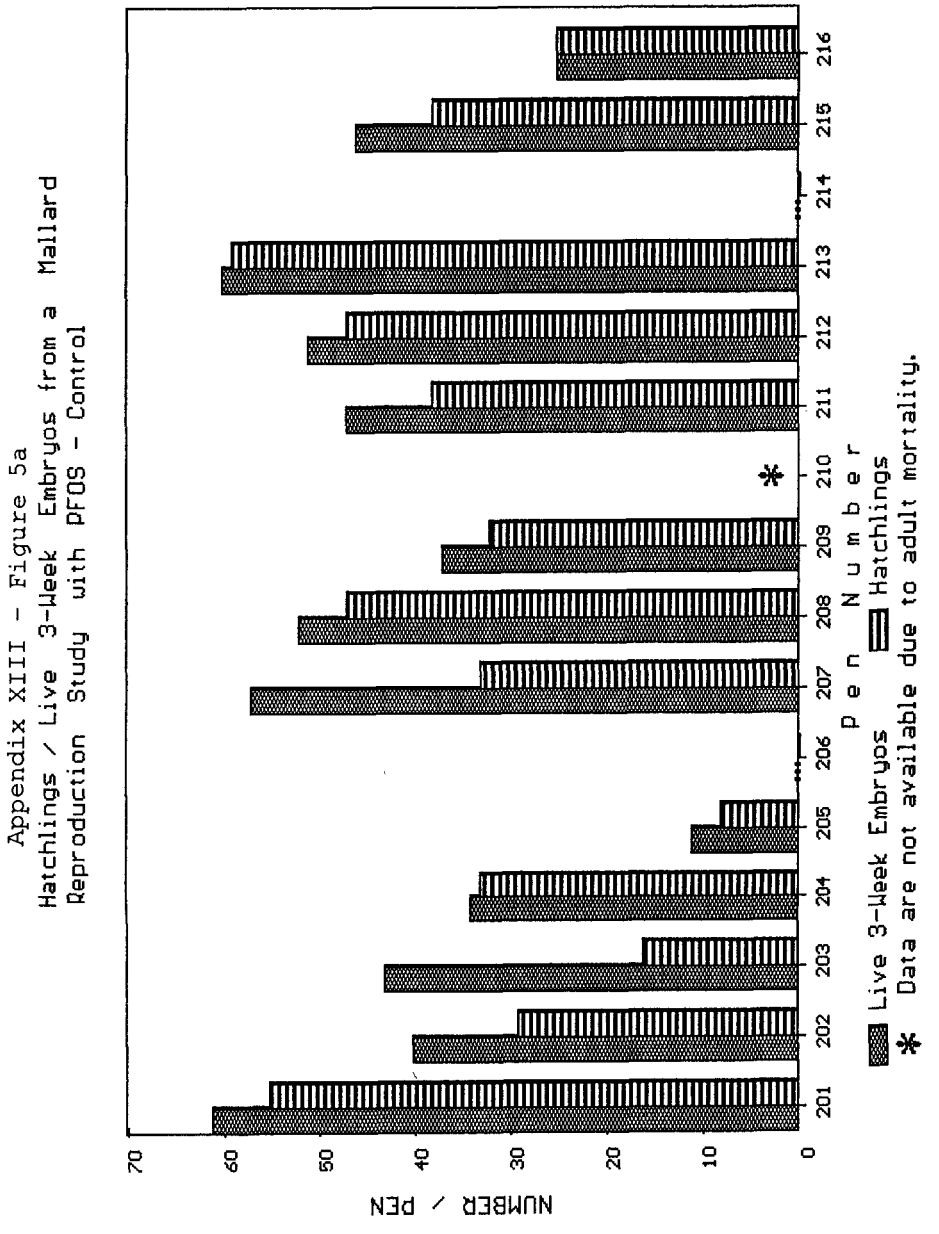
Hatchlings / Live 3-Week Embryos (%)

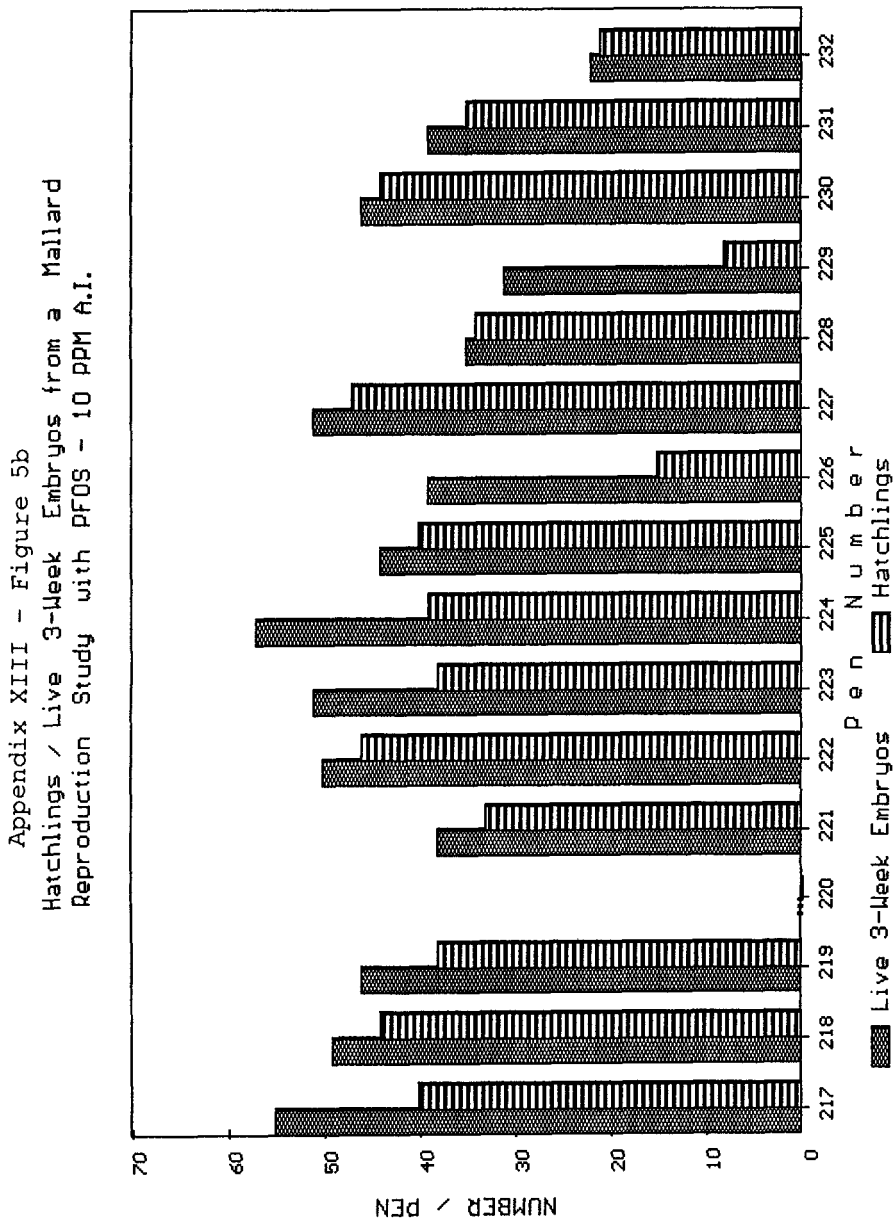
from a Mallard Reproduction Study with PFOS

	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	Number Hatch.	Live 3-Week	%	Arcsin Trans.	Number Hatch.	Live 3-Week	%	Arcsin Trans.	Number Hatch.	Live 3-Week	%	Arcsin Trans.	Number Hatch.	Live 3-Week	%	Arcsin Trans.
1	55	61	90	71.72	40	55	73	58.52	-	-	-	-	-	-	-	-
2	29	40	72	58.37	44	49	90	71.37	-	-	-	-	-	-	-	-
3	16	43	37	37.59	38	46	83	65.35	-	-	-	-	-	-	-	-
4	33	34	97	80.13	0	0			-	-	-	-	-	-	-	-
5	8	11	73	58.52	33	38	87	68.73	-	-	-	-	-	-	-	-
6	0	0			46	50	92	73.57	-	-	-	-	-	-	-	-
7	33	57	58	49.54	38	51	75	59.68	-	-	-	-	-	-	-	-
8	47	52	90	71.94	39	57	68	55.81	-	-	-	-	-	-	-	-
9	32	37	86	68.43	40	44	91	72.45	-	-	-	-	-	-	-	-
10	-	-	-	-	15	39	38	38.33	-	-	-	-	-	-	-	-
11	38	47	81	64.05	47	51	92	73.74	-	-	-	-	-	-	-	-
12	47	51	92	73.74	34	35	97	80.27	-	-	-	-	-	-	-	-
13	59	60	98	82.58	8	31	26	30.53	-	-	-	-	-	-	-	-
14	0	0			44	46	96	77.96	-	-	-	-	-	-	-	-
15	38	46	83	65.35	35	39	90	71.32	-	-	-	-	-	-	-	-
16	25	25	100	90.00	21	22	95	77.69	-	-	-	-	-	-	-	-
Total	460	564			522	653			-	-			-	-		
Mean	31	38	81	67.07	33	41	80	65.02	-	-	-	-	-	-	-	-
SD	18	20	18	14.04	14	14	21	14.43	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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


Appendix XIII - Figure 5a
Hatchlings / Live 3-Week Embryos from a Mallard
Reproduction Study with PFOS - Control
NUMBER / PEN
70
60
50
40
30
20
10
0
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
Pen Number
Live 3-Week Embryos
Hatchlings
* Data are not available due to adult mortality.


Appendix XIII - Figure 5b
Hatchlings / Live 3-Week Embryos from a Mallard
Reproduction Study with PFOS - 10 PPM A.I.
NUMBER / PEN
70
60
50
40
30
20
10
0
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
Pen Number
Live 3-Week Embryos
Hatchlings

Reproductive Performance by Pen

Appendix XIII - Table 6

14-Day Old Survivors / Hatchlings (%)

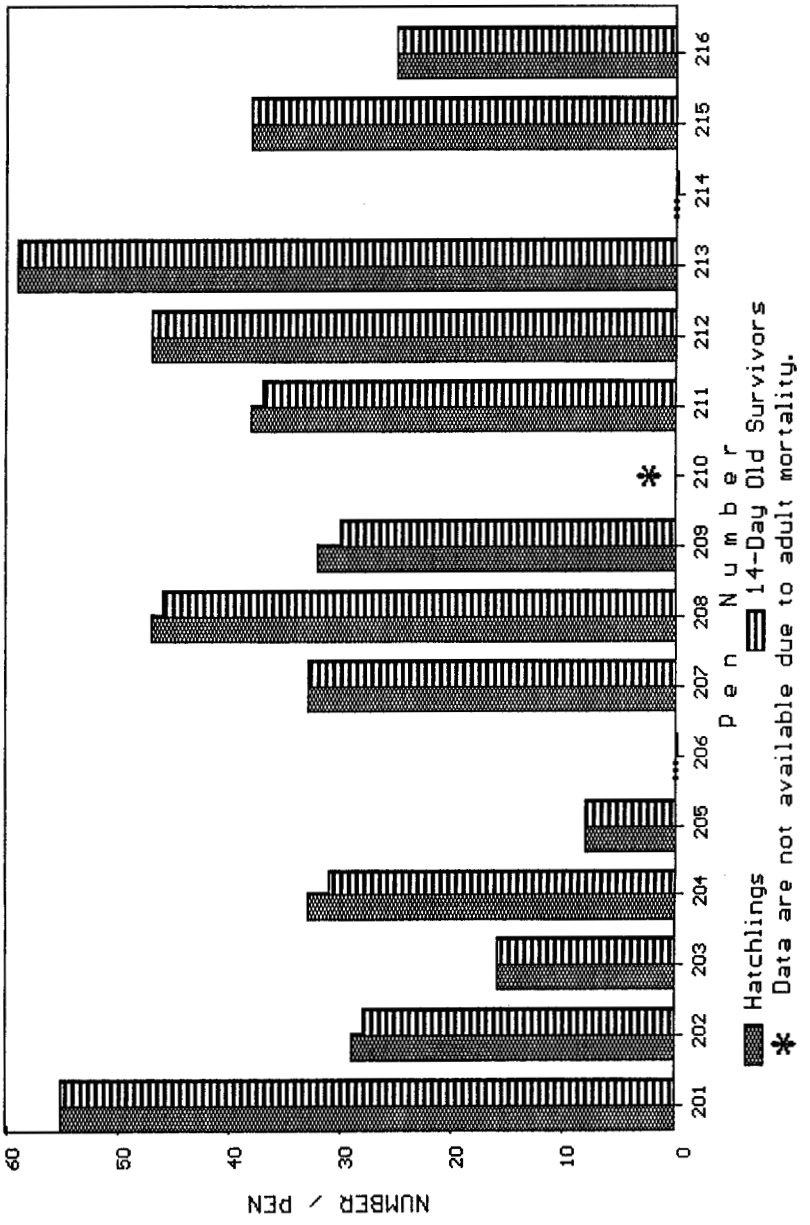
from a Mallard Reproduction Study with PFOS

	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	14-Day Old	Number Hatch.	%	Arcsin Trans.	14-Day Old	Number Hatch.	%	Arcsin Trans.	14-Day Old	Number Hatch.	%	Arcsin Trans.	14-Day Old	Number Hatch.	%	Arcsin Trans.
1	55	55	100	90.00	39	40	97	80.90	-	-	-	-	-	-	-	-
2	28	29	97	79.30	42	44	95	77.69	-	-	-	-	-	-	-	-
3	16	16	100	90.00	37	38	97	80.66	-	-	-	-	-	-	-	-
4	31	33	94	75.75	0	0			-	-	-	-	-	-	-	-
5	8	8	100	90.00	32	33	97	79.98	-	-	-	-	-	-	-	-
6	0	0			45	46	98	81.52	-	-	-	-	-	-	-	-
7	33	33	100	90.00	37	38	97	80.66	-	-	-	-	-	-	-	-
8	46	47	98	81.61	37	39	95	76.91	-	-	-	-	-	-	-	-
9	31	32	97	79.82	37	40	93	74.11	-	-	-	-	-	-	-	-
10	-	-	-	-	15	15	100	90.00	-	-	-	-	-	-	-	-
11	37	38	97	80.66	47	47	100	90.00	-	-	-	-	-	-	-	-
12	47	47	100	90.00	34	34	100	90.00	-	-	-	-	-	-	-	-
13	59	59	100	90.00	8	8	100	90.00	-	-	-	-	-	-	-	-
14	0	0			42	44	95	77.69	-	-	-	-	-	-	-	-
15	38	38	100	90.00	34	35	97	80.27	-	-	-	-	-	-	-	-
16	25	25	100	90.00	21	21	100	90.00	-	-	-	-	-	-	-	-
Total	454	460			507	522			-	-			-	-		
Mean	30	31	99	85.93	32	33	97	82.69	-	-	-	-	-	-	-	-
SD	18	18	2	5.51	14	14	2	5.67	-	-	-	-	-	-	-	-

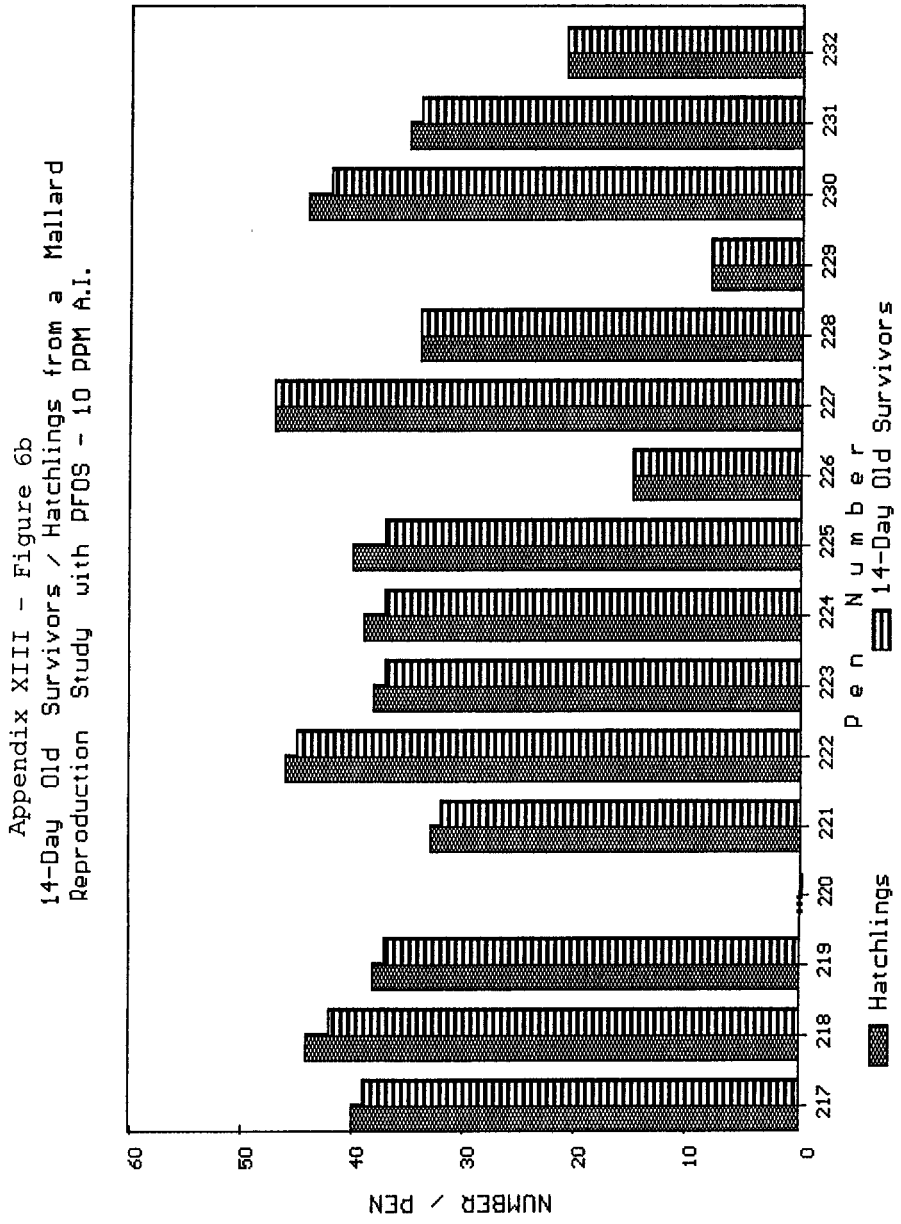
- Data are not available due to adult mortality.

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

Appendix XIII - Figure 6a
14-Day Old Survivors / Hatchlings from a Mallard
Reproduction Study with PFOS - Control



Appendix XIII - Figure 6b
14-Day Old Survivors / Hatchlings from a Mallard
Reproduction Study with PFOS - 10 PPM A.I.



Reproductive Performance by Pen

Appendix XIII - Table 7

Hatchlings / Eggs Set (%)

from a Mallard Reproduction Study with PFOS

	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	Number Hatch.	Eggs Set	%	Arcsin Trans.	Number Hatch.	Eggs Set	%	Arcsin Trans.	Number Hatch.	Eggs Set	%	Arcsin Trans.	Number Hatch.	Eggs Set	%	Arcsin Trans.
1	55	64	86	67.98	40	56	71	57.69	-	-	-	-	-	-	-	-
2	29	45	64	53.40	44	49	90	71.37	-	-	-	-	-	-	-	-
3	16	49	33	34.85	38	50	76	60.67	-	-	-	-	-	-	-	-
4	33	35	94	76.17	0	62	0	0.00	-	-	-	-	-	-	-	-
5	8	14	57	49.11	33	42	79	62.42	-	-	-	-	-	-	-	-
6	0	0			46	52	88	70.14	-	-	-	-	-	-	-	-
7	33	59	56	48.41	38	63	60	50.95	-	-	-	-	-	-	-	-
8	47	56	84	66.37	39	58	67	55.09	-	-	-	-	-	-	-	-
9	32	38	84	66.59	40	46	87	68.83	-	-	-	-	-	-	-	-
10	-	-	-	-	15	54	28	31.81	-	-	-	-	-	-	-	-
11	38	49	78	61.72	47	53	89	70.34	-	-	-	-	-	-	-	-
12	47	52	90	71.94	34	41	83	65.59	-	-	-	-	-	-	-	-
13	59	61	97	79.57	8	43	19	25.55	-	-	-	-	-	-	-	-
14	0	47	0	0.00	44	47	94	75.37	-	-	-	-	-	-	-	-
15	38	50	76	60.67	35	40	88	69.30	-	-	-	-	-	-	-	-
16	25	25	100	90.00	21	25	84	66.42	-	-	-	-	-	-	-	-
Total	460	644			522	781			-	-			-	-		
Mean	31	43	71	59.05	33	49	69	56.35	-	-	-	-	-	-	-	-
SD	18	18	28	22.11	14	10	28	20.49	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Reproductive Performance by Pen

Appendix XIII - Table 8

14-Day Old Survivors / Eggs Set (%)

from a Mallard Reproduction Study with PFOS

	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	14-Day Old	Eggs Set	%	Arcsin Trans.	14-Day Old	Eggs Set	%	Arcsin Trans.	14-Day Old	Eggs Set	%	Arcsin Trans.	14-Day Old	Eggs Set	%	Arcsin Trans.
1	55	64	86	67.98	39	56	70	56.57	-	-	-	-	-	-	-	-
2	28	45	62	52.07	42	49	86	67.79	-	-	-	-	-	-	-	-
3	16	49	33	34.85	37	50	74	59.34	-	-	-	-	-	-	-	-
4	31	35	89	70.24	0	62	0	0.00	-	-	-	-	-	-	-	-
5	8	14	57	49.11	32	42	76	60.79	-	-	-	-	-	-	-	-
6	0	0			45	52	87	68.48	-	-	-	-	-	-	-	-
7	33	59	56	48.41	37	63	59	50.03	-	-	-	-	-	-	-	-
8	46	56	82	65.00	37	58	64	53.01	-	-	-	-	-	-	-	-
9	31	38	82	64.58	37	46	80	63.75	-	-	-	-	-	-	-	-
10	-	-	-	-	15	54	28	31.81	-	-	-	-	-	-	-	-
11	37	49	76	60.34	47	53	89	70.34	-	-	-	-	-	-	-	-
12	47	52	90	71.94	34	41	83	65.59	-	-	-	-	-	-	-	-
13	59	61	97	79.57	8	43	19	25.55	-	-	-	-	-	-	-	-
14	0	47	0	0.00	42	47	89	70.96	-	-	-	-	-	-	-	-
15	38	50	76	60.67	34	40	85	67.21	-	-	-	-	-	-	-	-
16	25	25	100	90.00	21	25	84	66.42	-	-	-	-	-	-	-	-
Total	454	644			507	781			-	-			-	-		
Mean	30	43	70	58.20	32	49	67	54.85	-	-	-	-	-	-	-	-
SD	18	18	27	21.74	14	10	27	19.69	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Reproductive Performance by Pen

Appendix XIII - Table 9

Hatchlings / Maximum Set (%)

from a Mallard Reproduction Study with PFOS

	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	Number Hatch.	Max. Set	%	Arcsin Trans.	Number Hatch.	Max. Set	%	Arcsin Trans.	Number Hatch.	Max. Set	%	Arcsin Trans.	Number Hatch.	Max. Set	%	Arcsin Trans.
1	55	64	86	67.98	40	64	63	52.24	-	-	-	-	-	-	-	-
2	29	64	45	42.31	44	64	69	56.01	-	-	-	-	-	-	-	-
3	16	64	25	30.00	38	64	59	50.40	-	-	-	-	-	-	-	-
4	33	64	52	45.90	0	64	0	0.00	-	-	-	-	-	-	-	-
5	8	64	13	20.70	33	64	52	45.90	-	-	-	-	-	-	-	-
6	0	64	0	0.00	46	64	72	57.97	-	-	-	-	-	-	-	-
7	33	64	52	45.90	38	64	59	50.40	-	-	-	-	-	-	-	-
8	47	64	73	58.98	39	64	61	51.32	-	-	-	-	-	-	-	-
9	32	64	50	45.00	40	64	63	52.24	-	-	-	-	-	-	-	-
10	-	-	-	-	15	64	23	28.96	-	-	-	-	-	-	-	-
11	38	64	59	50.40	47	64	73	58.98	-	-	-	-	-	-	-	-
12	47	64	73	58.98	34	64	53	46.79	-	-	-	-	-	-	-	-
13	59	64	92	73.77	8	64	13	20.70	-	-	-	-	-	-	-	-
14	0	64	0	0.00	44	64	69	56.01	-	-	-	-	-	-	-	-
15	38	64	59	50.40	35	64	55	47.69	-	-	-	-	-	-	-	-
16	25	64	39	38.68	21	64	33	34.95	-	-	-	-	-	-	-	-
Total	460	960			522	1024			-	-			-	-		
Mean	31	64	48	41.93	33	64	51	44.41	-	-	-	-	-	-	-	-
SD	18	0	28	21.68	14	0	22	15.89	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Reproductive Performance by Pen

Appendix XIII - Table 10

14-Day Old Survivors / Maximum Set (%)

from a Mallard Reproduction Study with PFOS

	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
Replicate	14-Day Old	Max. Set	%	Arcsin Trans.	14-Day Old	Max. Set	%	Arcsin Trans.	14-Day Old	Max. Set	%	Arcsin Trans.	14-Day Old	Max. Set	%	Arcsin Trans.
1	55	64	86	67.98	39	64	61	51.32	-	-	-	-	-	-	-	-
2	28	64	44	41.41	42	64	66	54.10	-	-	-	-	-	-	-	-
3	16	64	25	30.00	37	64	58	49.49	-	-	-	-	-	-	-	-
4	31	64	48	44.10	0	64	0	0.00	-	-	-	-	-	-	-	-
5	8	64	13	20.70	32	64	50	45.00	-	-	-	-	-	-	-	-
6	0	64	0	0.00	45	64	70	56.98	-	-	-	-	-	-	-	-
7	33	64	52	45.90	37	64	58	49.49	-	-	-	-	-	-	-	-
8	46	64	72	57.97	37	64	58	49.49	-	-	-	-	-	-	-	-
9	31	64	48	44.10	37	64	58	49.49	-	-	-	-	-	-	-	-
10	-	-	-	-	15	64	23	28.96	-	-	-	-	-	-	-	-
11	37	64	58	49.49	47	64	73	58.98	-	-	-	-	-	-	-	-
12	47	64	73	58.98	34	64	53	46.79	-	-	-	-	-	-	-	-
13	59	64	92	73.77	8	64	13	20.70	-	-	-	-	-	-	-	-
14	0	64	0	0.00	42	64	66	54.10	-	-	-	-	-	-	-	-
15	38	64	59	50.40	34	64	53	46.79	-	-	-	-	-	-	-	-
16	25	64	39	38.68	21	64	33	34.95	-	-	-	-	-	-	-	-
Total	454	960			507	1024			-	-			-	-		
Mean	30	64	47	41.57	32	64	50	43.54	-	-	-	-	-	-	-	-
SD	18	0	28	21.57	14	0	21	15.43	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Reproductive Performance by Week and Pen

Appendix XIV – Table 1a

Eggs Laid by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
	LOT[1]																	
	A	8	0	0	0	1	0	3	0	0	-	0	0	4	0	0	0	16
	B	7	4	3	2	3	0	7	6	0	-	6	0	7	3	6	2	56
	C	7	8	5	7	0	0	7	7	6	-	7	7	7	7	7	5	87
	D	7	6	7	7	1	0	7	7	5	-	7	7	7	7	7	6	88
Control	E	7	2	7	6	4	0	7	7	7	-	7	7	7	7	7	5	87
	F	7	7	7	2	0	0	7	7	5	-	7	7	7	7	7	1	78
	G	7	6	7	7	4	0	7	7	5	-	6	7	7	7	6	5	88
	H	7	7	7	2	2	0	7	7	5	-	5	7	7	7	7	1	78
	I	6	5	3	7	2	0	7	7	5	-	6	7	7	6	6	2	76
	J	7	6	7	2	2	0	7	7	4	-	4	7	7	0	1	4	65
	Totals	70	51	53	42	19	0	66	62	42	-	55	56	67	51	54	31	719

- Data are not available due to adult mortality

[1] LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

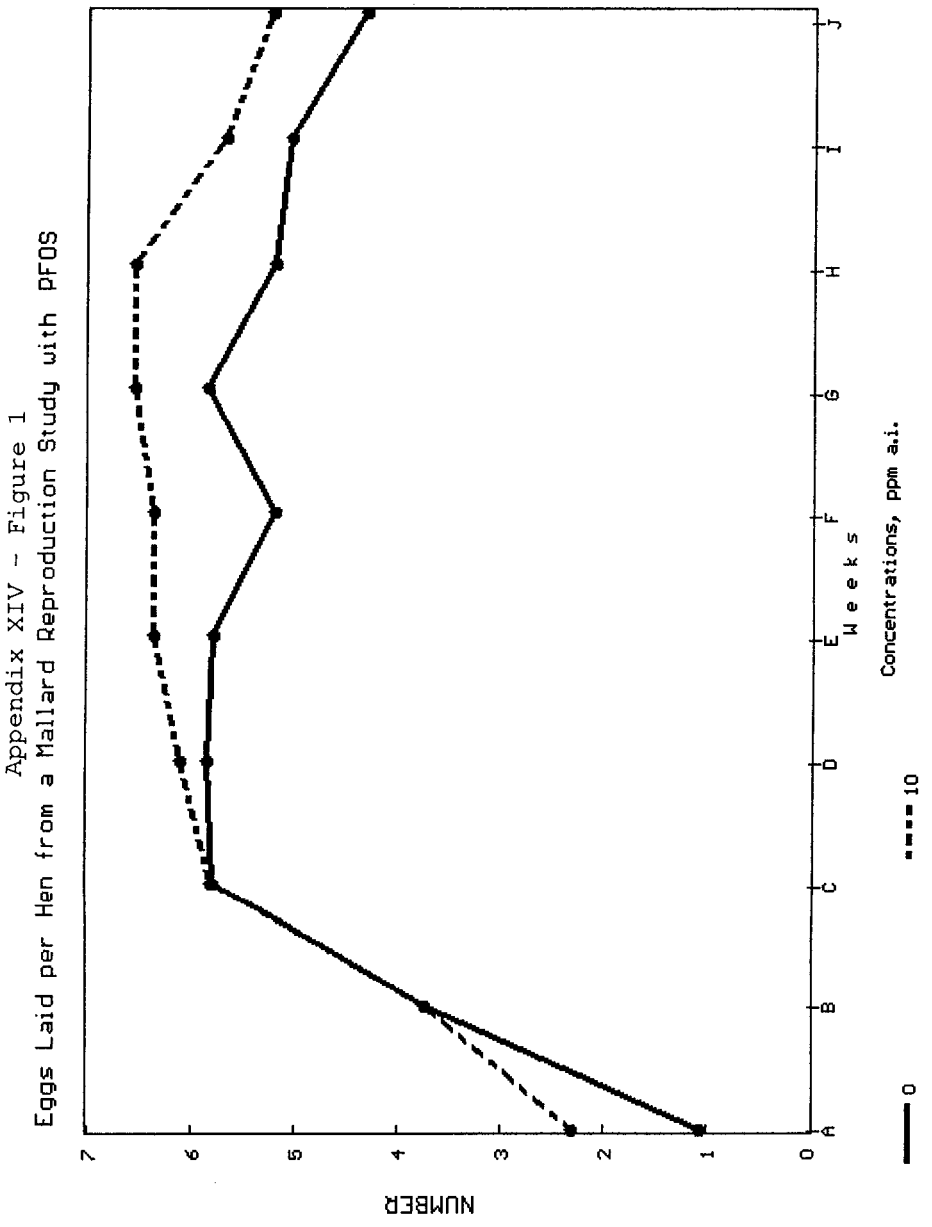
Appendix XIV - Table 1b

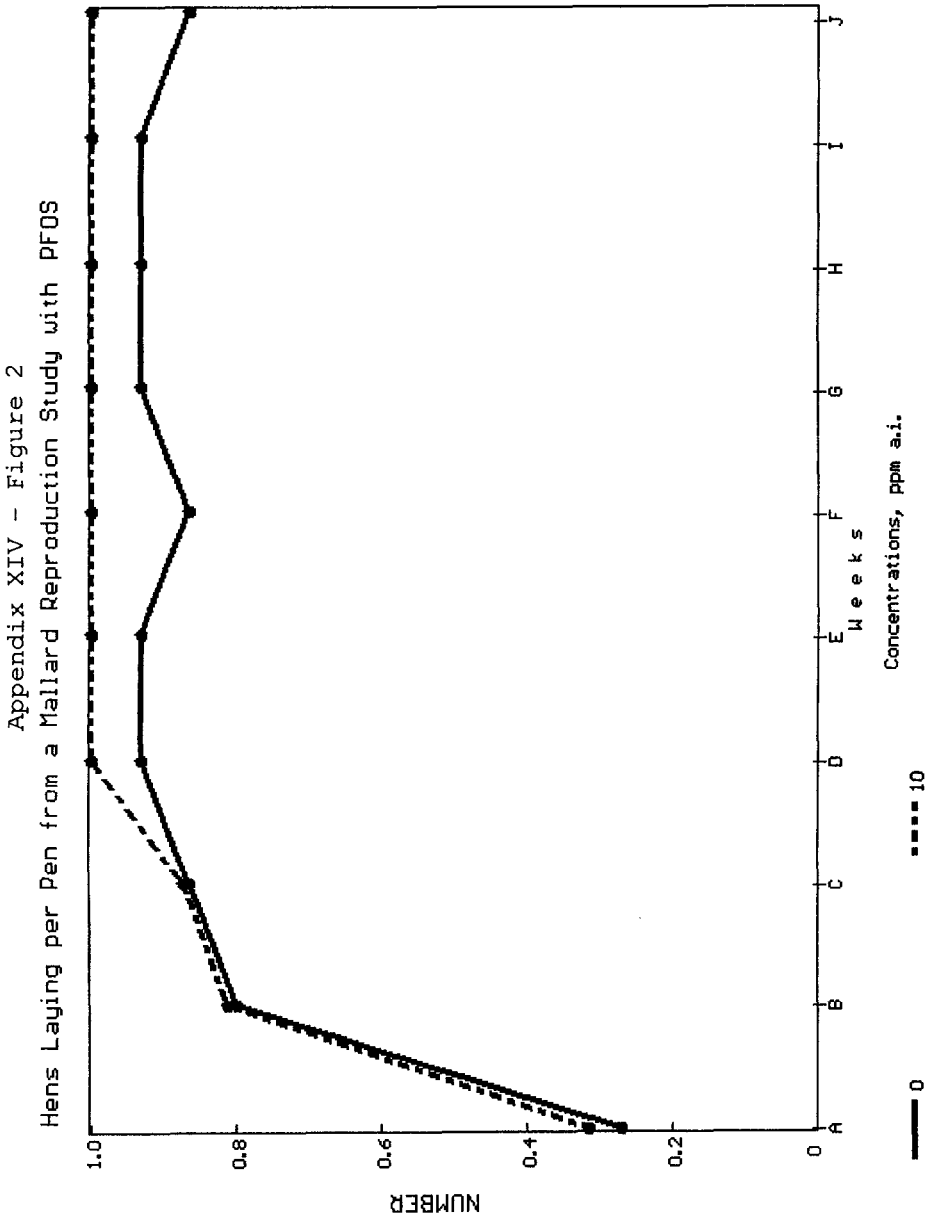
Eggs Laid by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
	LOT[1]	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
	A	9	0	0	9	0	0	9	4	0	6	0	0	0	0	0	0	37
	B	7	6	6	7	3	1	7	7	3	6	4	1	0	2	0	0	60
	C	7	7	7	7	7	7	7	7	7	7	7	7	0	7	0	2	93
	D	3	7	7	7	7	7	7	5	7	7	7	7	5	7	3	5	98
10	E	6	6	7	7	3	7	7	6	7	7	7	6	7	7	7	5	102
	F	7	7	5	7	5	7	7	7	7	6	7	6	7	7	7	3	102
	G	3	7	7	7	7	7	7	7	7	6	7	7	7	7	7	5	105
	H	7	7	6	7	7	7	7	8	7	6	7	4	7	7	7	4	105
	I	5	5	7	7	5	7	7	6	4	6	5	6	6	6	7	2	91
	J	7	6	2	7	2	7	7	6	7	2	6	3	7	6	5	4	84
	Totals	61	58	54	72	46	57	72	63	56	59	57	47	46	56	43	30	877

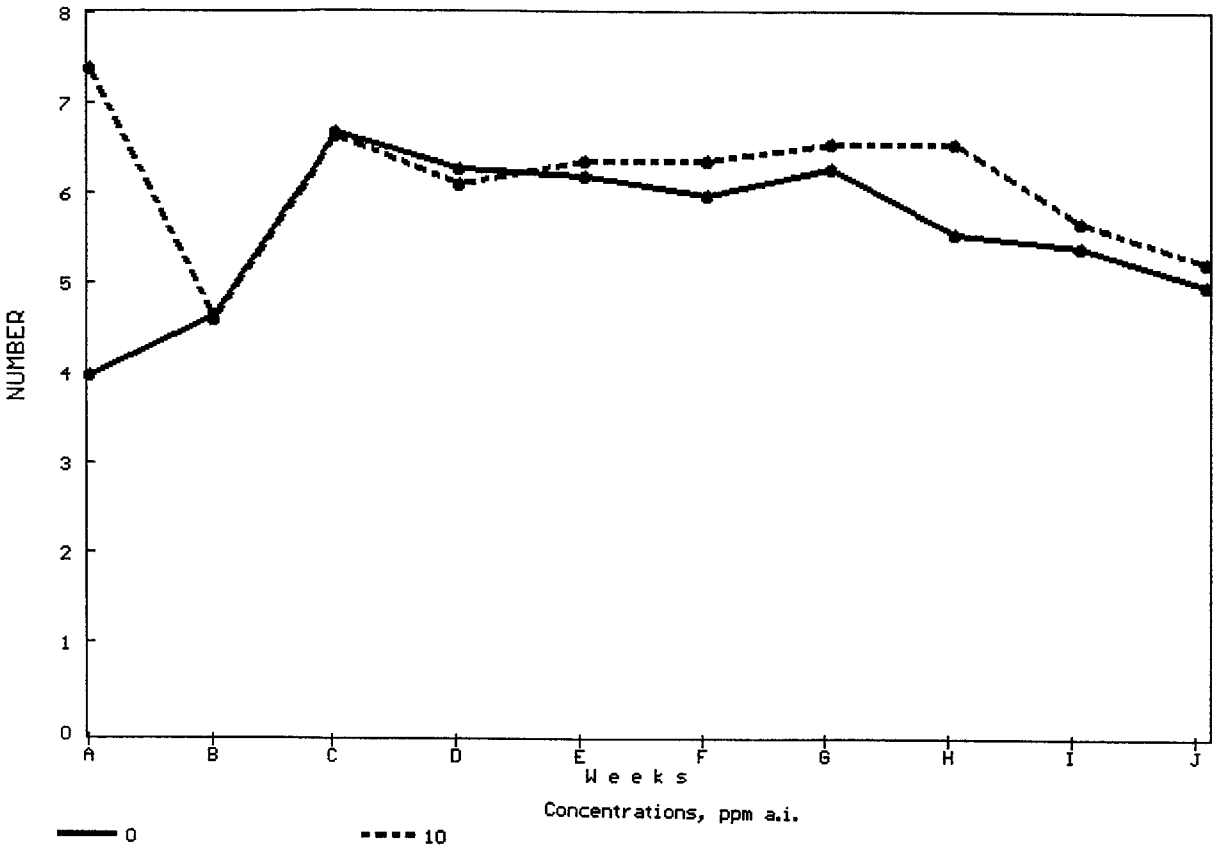
[1]LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.


Appendix XIV - Figure 1
Eggs Laid per Hen from a Mallard Reproduction Study with PFOS
NUMBER
0
1
2
3
4
5
6
7
A
B
C
D
E
F
G
H
I
J
Weeks
Concentrations, ppm a.i.
0
10


Appendix XIV - Figure 2
Hens Laying per Pen from a Mallard Reproduction Study with PFOS
NUMBER
1.0
0.8
0.6
0.4
0.2
0
A
B
C
D
E
F
G
H
I
J
Weeks
Concentrations, ppm a.i.
0
10

Appendix XIV - Figure 3

Eggs per Laying Hen from a Mallard Reproduction Study with PFOS



Reproductive Performance by Week and Pen

Appendix XIV - Table 2a

Eggs Cracked by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
	LOT[1]																	
	A	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
	B	1	0	0	0	0	0	0	0	0	-	1	0	0	0	0	1	3
	C	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
Control	E	0	1	0	0	0	0	0	0	0	-	0	0	0	0	0	0	1
	F	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
	G	0	0	0	1	0	0	0	0	0	-	0	0	0	0	0	0	1
	H	0	0	0	0	1	0	0	0	0	-	0	0	0	0	0	0	1
	I	0	0	0	1	0	0	0	1	0	-	1	0	1	0	0	0	4
	J	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
	Totals	1	1	0	2	1	0	0	1	0	-	2	0	1	0	0	1	10

- Data are not available due to adult mortality

[1]LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 2b

Eggs Cracked by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
	LOT[1]																	
	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
10	E	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	4
	F	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	G	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	J	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
	Totals	0	0	0	4	0	0	3	0	3	0	0	0	0	0	0	0	10

[1]LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 3a

Eggs Set by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
	LOT[1]																	
	A	7	0	0	0	0	0	2	0	0	-	0	0	3	0	0	0	12
	B	6	3	3	1	3	0	7	5	0	-	5	0	7	2	6	0	48
	C	6	8	4	7	0	0	5	7	5	-	6	7	6	7	6	5	79
	D	7	5	7	6	1	0	7	6	5	-	7	6	7	6	7	5	82
Control	E	6	1	6	6	3	0	6	7	6	-	6	7	6	7	6	5	78
	F	7	6	7	1	0	0	7	6	5	-	7	6	7	6	7	0	72
	G	6	6	6	6	3	0	5	7	4	-	5	7	6	7	5	5	78
	H	7	6	7	1	1	0	7	6	5	-	5	6	7	6	7	0	71
	I	5	5	2	6	1	0	6	6	4	-	4	7	5	6	5	2	64
	J	7	5	7	1	2	0	7	6	4	-	4	6	7	0	1	3	60
	Totals	64	45	49	35	14	0	59	56	38	-	49	52	61	47	50	25	644

- Data are not available due to adult mortality

[1] LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 3b

Eggs Set by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
	LOT[1]																	
	A	8	0	0	9	0	0	8	4	0	6	0	0	0	0	0	0	35
	B	7	5	6	6	3	0	7	6	3	5	4	0	0	1	0	0	53
	C	6	6	6	7	6	7	6	7	6	7	6	7	0	7	0	2	86
	D	3	6	7	6	7	6	7	4	6	6	7	6	5	6	3	3	88
10	E	5	6	6	5	2	7	4	6	6	7	6	6	6	6	6	5	89
	F	7	6	5	6	5	6	7	6	6	5	7	5	7	6	7	2	93
	G	2	7	6	6	6	7	5	7	5	6	6	7	6	6	6	5	93
	H	7	5	6	6	7	6	7	7	6	5	7	3	7	6	7	3	95
	I	4	4	6	7	4	7	6	6	2	6	4	5	5	5	6	2	79
	J	7	4	2	4	2	6	6	5	6	1	6	2	7	4	5	3	70
	Totals	56	49	50	62	42	52	63	58	46	54	53	41	43	47	40	25	781

[1] LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 4a

Viable Embryos by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
	LOT[1]																	
	A	5	0	0	0	0	0	1	0	0	-	0	0	3	0	0	0	9
	B	6	0	3	1	1	0	7	3	0	-	5	0	7	0	6	0	39
	C	5	6	2	6	0	0	5	7	4	-	6	7	6	0	5	5	64
	D	7	5	5	6	1	0	7	5	5	-	7	6	7	0	7	5	73
Control	E	6	1	6	6	2	0	6	7	6	-	6	7	6	0	5	5	69
	F	7	6	7	1	0	0	7	6	5	-	6	6	7	0	7	0	65
	G	6	6	6	6	3	0	5	7	4	-	4	7	6	0	5	5	70
	H	7	6	6	1	1	0	7	6	5	-	5	6	7	0	6	0	63
	I	5	5	2	6	1	0	6	6	4	-	4	7	5	0	5	2	58
	J	7	5	7	1	2	0	6	5	4	-	4	6	6	0	1	3	57
	Totals	61	40	44	34	11	0	57	52	37	-	47	52	60	0	47	25	567

- Data are not available due to adult mortality

[1] LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 4b

Viable Embryos by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
	LOT[1]	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
	A	8	0	0	0	0	0	8	4	0	0	0	0	0	0	0	0	20
	B	7	5	5	0	3	0	7	6	3	0	3	0	0	1	0	0	40
	C	6	6	6	0	5	7	6	7	6	3	6	3	0	7	0	2	70
	D	3	6	6	0	7	5	7	4	6	6	7	4	5	6	3	3	78
10	E	5	6	6	0	1	7	4	6	6	7	6	6	5	6	6	5	82
	F	7	6	5	0	4	6	2	6	6	5	6	5	7	6	7	2	80
	G	2	7	4	0	5	7	4	7	5	6	6	7	6	6	5	5	82
	H	7	5	6	0	7	6	7	7	5	5	7	3	5	6	7	3	86
	I	4	4	6	0	4	6	3	6	2	6	4	5	5	5	6	0	66
	J	6	4	2	0	2	6	4	4	6	1	6	2	7	3	5	2	60
	Totals	55	49	46	0	38	50	52	57	45	39	51	35	40	46	39	22	664

[1]LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 5a

Live Three-Week Embryos by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
	LOT[1]																	
	A	5	0	0	0	0	0	1	0	0	-	0	0	3	0	0	0	9
	B	6	0	3	1	1	0	7	3	0	-	5	0	7	0	6	0	39
	C	5	6	2	6	0	0	5	7	4	-	6	6	6	0	5	5	63
	D	7	5	5	6	1	0	7	5	5	-	7	6	7	0	7	5	73
Control	E	6	1	6	6	2	0	6	7	6	-	6	7	6	0	5	5	69
	F	7	6	7	1	0	0	7	6	5	-	6	6	7	0	6	0	64
	G	6	6	6	6	3	0	5	7	4	-	4	7	6	0	5	5	70
	H	7	6	6	1	1	0	7	6	5	-	5	6	7	0	6	0	63
	I	5	5	2	6	1	0	6	6	4	-	4	7	5	0	5	2	58
	J	7	5	6	1	2	0	6	5	4	-	4	6	6	0	1	3	56
	Totals	61	40	43	34	11	0	57	52	37	-	47	51	60	0	46	25	564

- Data are not available due to adult mortality

[1] LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 5b

Live Three-Week Embryos by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
	LOT[1]																	
	A	8	0	0	0	0	0	8	4	0	0	0	0	0	0	0	0	20
	B	7	5	5	0	3	0	7	6	3	0	3	0	0	1	0	0	40
	C	6	6	6	0	5	7	6	7	6	3	6	3	0	7	0	2	70
	D	3	6	6	0	7	5	7	4	6	6	7	4	1	6	3	3	74
10	E	5	6	6	0	1	7	4	6	5	7	6	6	4	6	6	5	80
	F	7	6	5	0	4	6	2	6	6	5	6	5	7	6	7	2	80
	G	2	7	4	0	5	7	4	7	5	6	6	7	3	6	5	5	79
	H	7	5	6	0	7	6	6	7	5	5	7	3	5	6	7	3	85
	I	4	4	6	0	4	6	3	6	2	6	4	5	5	5	6	0	66
	J	6	4	2	0	2	6	4	4	6	1	6	2	6	3	5	2	59
	Totals	55	49	46	0	38	50	51	57	44	39	51	35	31	46	39	22	653

[1]LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 6a

Hatchlings by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
	LOT[1]																	
	A	5	0	0	0	0	0	0	0	0	-	0	0	3	0	0	0	8
	B	5	0	0	1	1	0	6	2	0	-	4	0	7	0	6	0	32
	C	4	3	0	6	0	0	2	6	4	-	6	4	5	0	5	5	50
	D	7	3	0	6	1	0	3	4	5	-	5	6	7	0	1	5	53
Control	E	6	0	2	6	0	0	2	5	5	-	4	6	6	0	5	5	52
	F	6	5	3	1	0	0	3	6	4	-	5	6	7	0	4	0	50
	G	6	6	2	6	3	0	2	7	3	-	4	7	6	0	5	5	62
	H	7	6	4	1	0	0	3	6	4	-	3	6	7	0	6	0	53
	I	4	1	0	5	1	0	6	6	4	-	3	7	5	0	5	2	49
	J	5	5	5	1	2	0	6	5	3	-	4	5	6	0	1	3	51
	Totals	55	29	16	33	8	0	33	47	32	-	38	47	59	0	38	25	460

- Data are not available due to adult mortality

[1]LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV – Table 6b

Hatchlings by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
	LOT[1]																	
	A	7	0	0	0	0	0	8	1	0	0	0	0	0	0	0	0	16
	B	6	4	3	0	2	0	6	5	2	0	2	0	0	1	0	0	31
	C	5	6	4	0	3	5	3	5	4	1	6	3	0	6	0	1	52
	D	3	6	6	0	6	5	2	3	5	1	7	4	0	6	2	3	59
10	E	3	5	5	0	0	7	3	3	5	2	6	5	0	6	5	5	60
	F	3	4	4	0	4	6	2	3	6	1	6	5	2	6	7	2	61
	G	2	7	3	0	5	6	3	4	5	2	6	7	0	5	4	5	64
	H	4	5	5	0	7	6	5	7	5	5	7	3	1	6	6	3	75
	I	3	3	6	0	4	5	3	5	2	3	2	5	2	5	6	0	54
	J	4	4	2	0	2	6	3	3	6	0	5	2	3	3	5	2	50
	Totals	40	44	38	0	33	46	38	39	40	15	47	34	8	44	35	21	522

[1] LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 7a

14-Day Old Survivors by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
	LOT[1]																	
	A	5	0	0	0	0	0	0	0	0	-	0	0	3	0	0	0	8
	B	5	0	0	1	1	0	6	2	0	-	4	0	7	0	6	0	32
	C	4	2	0	6	0	0	2	6	4	-	6	4	5	0	5	5	49
	D	7	3	0	6	1	0	3	4	5	-	5	6	7	0	1	5	53
Control	E	6	0	2	6	0	0	2	5	5	-	4	6	6	0	5	5	52
	F	6	5	3	0	0	0	3	5	4	-	4	6	7	0	4	0	47
	G	6	6	2	6	3	0	2	7	2	-	4	7	6	0	5	5	61
	H	7	6	4	1	0	0	3	6	4	-	3	6	7	0	6	0	53
	I	4	1	0	4	1	0	6	6	4	-	3	7	5	0	5	2	48
	J	5	5	5	1	2	0	6	5	3	-	4	5	6	0	1	3	51
	Totals	55	28	16	31	8	0	33	46	31	-	37	47	59	0	38	25	454

- Data are not available due to adult mortality

[1]LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Reproductive Performance by Week and Pen

Appendix XIV - Table 7b

14-Day Old Survivors by Week and Pen

from a Mallard Reproduction Study with PFOS

Experimental Group (ppm a.i.)		PENS																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
	LOT[1]																	
	A	7	0	0	0	0	0	8	1	0	0	0	0	0	0	0	0	16
	B	6	3	2	0	1	0	6	4	0	0	2	0	0	0	0	0	24
	C	5	6	4	0	3	4	3	5	4	1	6	3	0	6	0	1	51
	D	3	6	6	0	6	5	2	3	5	1	7	4	0	6	1	3	58
10	E	3	5	5	0	0	7	3	3	5	2	6	5	0	6	5	5	60
	F	3	4	4	0	4	6	1	3	5	1	6	5	2	6	7	2	59
	G	2	7	3	0	5	6	3	3	5	2	6	7	0	5	4	5	63
	H	4	4	5	0	7	6	5	7	5	5	7	3	1	5	6	3	73
	I	3	3	6	0	4	5	3	5	2	3	2	5	2	5	6	0	54
	J	3	4	2	0	2	6	3	3	6	0	5	2	3	3	5	2	49
	Totals	39	42	37	0	32	45	37	37	37	15	47	34	8	42	34	21	507

[1] LOT A - Eggs Set During Week 12; LOT B - Eggs Set During Week 13; etc.

Appendix XV - Table 1

Eggshell Thickness (mm) per Pen by Week from a Mallard Reproduction Study with PFOS (Control)

	Weeks												
Pen	A	B	C	D	E	F	G	H	I	J	Mean	SD	[n]
201	-		0.387		0.397		0.391		0.393		0.392	0.004	4
202		-		0.398		0.382		0.381		-	0.387	0.010	3
203	-		0.437		0.428		0.440		0.455		0.440	0.011	4
204		-		0.353		0.357		0.347		-	0.352	0.005	3
205	-		-		0.378		0.368		0.374		0.373	0.005	3
206		-		-		-		-		-			
207	-		0.455		0.446		0.417		0.415		0.433	0.020	4
208		-		0.425		0.435		0.433		-	0.431	0.005	3
209	-		0.406		0.406		0.401		0.409		0.406	0.003	4
210		-		-		-		-		-			
211	-		0.366		0.375		0.352		0.344		0.359	0.014	4
212		-		0.401		0.386		0.408		-	0.399	0.011	3
213	-		0.447		0.426		0.438		0.425		0.434	0.010	4
214		-		0.385		0.384		0.395		-	0.388	0.006	3
215	-		0.349		0.369		0.383		0.384		0.371	0.017	4
216		-		0.363		0.332		0.289		-	0.328	0.037	3
											Mean	SD	
											0.392	0.034	49

Mean and standard deviation of pen means:

- No eggs available.

Appendix XV - Table 2

Eggshell Thickness (mm) per Pen by Week from a Mallard Reproduction Study with PFOS (10 PPM A.I.)

	Weeks												
Pen	A	B	C	D	E	F	G	H	I	J	Mean	SD	[n]
217	-		0.410		0.388		0.416		0.400		0.404	0.012	4
218		-		0.416		0.417		0.407		-	0.413	0.006	3
219	-		0.416		0.389		0.406		0.401		0.403	0.012	4
220		-		0.425		0.418		0.406		-	0.416	0.009	3
221	-		0.386		0.437		0.416		0.344		0.396	0.041	4
222		-		0.384		0.381		0.378		-	0.381	0.003	3
223	-		0.401		0.415		0.416		0.396		0.407	0.010	4
224		-		0.391		0.390		0.377		-	0.386	0.008	3
225	-		0.415		0.415		0.419		0.428		0.419	0.006	4
226		-		0.425		0.380		0.379		-	0.395	0.026	3
227	-		0.379		0.381		0.382		0.373		0.379	0.004	4
228		-		0.386		0.386		0.370		-	0.381	0.010	3
229	-		-		0.406		0.404		0.407		0.406	0.002	3
230		-		0.370		0.384		0.387		-	0.380	0.009	3
231	-		-		0.384		0.371		0.380		0.379	0.007	3
232		-		0.363		0.426		0.395		-	0.395	0.032	3
											Mean	SD	
							Mean and standard deviation of pen means:				0.396	0.014	54

- No eggs available.

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

Appendix XVI - Table 1

Mean Hatchling Body Weight (g) per Pen by Week from a Mallard Reproduction Study with PFOS (Control)

	Weeks [No. of Hatchlings]												
Pen	A[n]	B[n]	C[n]	D[n]	E[n]	F[n]	G[n]	H[n]	I[n]	J[n]	Mean	SD	Total Hatch
201	35[5]	35[5]	36[4]	37[7]	29[6]	33[6]	37[6]	34[7]	29[4]	33[5]	34	3	55
202	[0]	[0]	31[3]	35[3]	[0]	31[5]	34[6]	36[6]	23[1]	35[5]	33	3	29
203	[0]	[0]	[0]	[0]	30[2]	33[3]	36[2]	39[4]	[0]	34[5]	35	3	16
204	[0]	43[1]	42[6]	43[6]	34[6]	30[1]	43[6]	35[1]	28[4]	33[1]	38	6	32
205	[0]	30[1]	[0]	26[1]	[0]	[0]	30[3]	[0]	22[1]	31[2]	29	3	8
206	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]			
207	[0]	35[6]	39[2]	38[3]	34[2]	36[3]	40[2]	37[3]	34[6]	34[6]	35	2	33
208	[0]	38[2]	39[6]	41[4]	39[5]	34[6]	43[7]	42[6]	41[6]	40[5]	40	3	47
209	[0]	[0]	42[4]	44[5]	37[5]	41[4]	48[3]	45[4]	41[4]	43[3]	42	3	32
210	-	-	-	-	-	-	-	-	-	-			
211	[0]	36[4]	37[6]	39[5]	34[4]	32[5]	34[4]	37[3]	33[3]	34[4]	35	2	38
212	[0]	[0]	37[4]	37[6]	31[6]	31[6]	37[7]	36[6]	34[7]	33[5]	34	3	47
213	35[3]	35[7]	35[5]	40[7]	31[6]	34[7]	41[6]	38[7]	34[5]	35[6]	36	3	59
214	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]			
215	[0]	36[6]	36[5]	37[1]	34[5]	30[4]	39[5]	40[6]	35[5]	38[1]	36	3	38
216	[0]	[0]	32[5]	34[5]	33[5]	[0]	35[5]	[0]	30[2]	31[3]	33	2	25
											Mean	SD	
											35	3	459

The number of hatchlings weighed may differ from the total number of hatchlings since those hatchlings found dead were not weighed.
- Data are not available due to adult mortality.
SD = Standard deviation of mean body weight, by parental pen, by week.

Appendix XVI - Table 2

Mean Hatchling Body Weight (g) per Pen by Week from a Mallard Reproduction Study with PFOS (10 PPM A.I.)

	Weeks [No. of Hatchlings]												
Pen	A[n]	B[n]	C[n]	D[n]	E[n]	F[n]	G[n]	H[n]	I[n]	J[n]	Mean	SD	Total Hatch
217	39[7]	38[6]	37[5]	42[3]	37[3]	36[3]	42[2]	42[4]	39[3]	40[4]	39	2	40
218	[0]	35[4]	34[6]	35[6]	35[5]	28[4]	37[7]	35[5]	38[3]	36[4]	35	2	44
219	[0]	34[3]	33[4]	37[6]	35[5]	35[4]	40[3]	36[5]	35[6]	36[2]	36	2	38
220	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]			
221	[0]	35[2]	36[3]	34[6]	[0]	34[4]	41[5]	42[7]	36[4]	33[2]	37	4	33
222	[0]	[0]	38[5]	38[5]	37[7]	36[6]	38[6]	37[6]	32[5]	35[6]	36	2	46
223	37[8]	36[6]	37[3]	39[2]	36[3]	38[2]	47[3]	40[5]	45[3]	42[3]	39	4	38
224	32[1]	36[5]	34[5]	33[3]	35[3]	33[3]	36[4]	33[7]	33[5]	36[3]	34	1	39
225	[0]	34[2]	34[4]	35[5]	36[5]	31[5]	39[5]	36[5]	41[2]	35[6]	35	3	39
226	[0]	[0]	29[1]	33[1]	32[2]	30[1]	35[2]	33[5]	34[3]	[0]	33	2	15
227	[0]	36[2]	35[6]	35[7]	35[6]	35[6]	37[6]	36[7]	38[2]	37[5]	36	1	47
228	[0]	[0]	38[3]	41[4]	41[5]	45[5]	42[7]	39[3]	39[5]	40[2]	41	2	34
229	[0]	[0]	[0]	[0]	[0]	33[2]	[0]	35[1]	35[2]	39[3]	36	3	8
230	[0]	37[1]	32[6]	39[6]	37[6]	41[6]	37[5]	39[6]	41[5]	36[3]	38	3	44
231	[0]	[0]	[0]	34[2]	35[5]	41[7]	35[4]	39[6]	40[6]	36[5]	38	2	35
232	[0]	[0]	47[1]	42[3]	42[5]	43[2]	38[5]	42[3]	[0]	41[2]	41	2	21
											Mean	SD	
											37	2	521

The number of hatchlings weighed may differ from the total number of hatchlings since those hatchlings found dead were not weighed.
Differences between the control and this treatment group were not significant ($p > 0.05$).
SD = Standard deviation of mean body weight, by parental pen, by week.

Appendix XVII - Table 1

Mean 14-Day Old Survivors Body Weight (g) per Pen by Week from a Mallard Reproduction Study with PFOS (Control)

	Weeks [No. of 14-Day Old Survivors]												
Pen	A[n]	B[n]	C[n]	D[n]	E[n]	F[n]	G[n]	H[n]	I[n]	J[n]	Mean	SD	14-Day Total
201	308[5]	292[5]	306[4]	303[7]	289[6]	293[6]	271[6]	273[7]	272[4]	271[5]	288	14	55
202	[0]	[0]	232[2]	243[3]	[0]	254[5]	250[6]	252[6]	185[1]	247[5]	246	13	28
203	[0]	[0]	[0]	[0]	321[2]	295[3]	287[2]	309[4]	[0]	298[5]	302	10	16
204	[0]	320[1]	333[6]	320[6]	318[6]	[0]	323[6]	295[1]	321[4]	336[1]	323	8	31
205	[0]	224[1]	[0]	232[1]	[0]	[0]	241[3]	[0]	141[1]	265[2]	231	39	8
206	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]			
207	[0]	268[6]	286[2]	280[3]	295[2]	253[3]	258[2]	271[3]	261[6]	258[6]	267	12	33
208	[0]	310[2]	309[6]	279[4]	313[5]	298[5]	291[7]	324[6]	289[6]	299[5]	301	13	46
209	[0]	[0]	335[4]	311[5]	359[5]	329[4]	333[2]	337[4]	332[4]	335[3]	334	14	31
210	-	-	-	-	-	-	-	-	-	-			
211	[0]	286[4]	290[6]	295[5]	334[4]	301[4]	267[4]	327[3]	285[3]	258[4]	293	23	37
212	[0]	[0]	317[4]	280[6]	309[6]	307[6]	272[7]	305[6]	264[7]	278[5]	290	19	47
213	317[3]	276[7]	314[5]	304[7]	285[6]	297[7]	275[6]	306[7]	289[5]	277[6]	293	14	59
214	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]			
215	[0]	289[6]	302[5]	255[1]	298[5]	278[4]	271[5]	300[6]	287[5]	293[1]	289	12	38
216	[0]	[0]	289[5]	286[5]	278[5]	[0]	253[5]	[0]	270[2]	315[3]	281	18	25
											Mean	SD	
											287	28	454

- Data are not available due to adult mortality.

SD = Standard deviation of mean body weight, by parental pen, by week.

Appendix XVII - Table 2

Mean 14-Day Old Survivors Body Weight (g) per Pen by Week from a Mallard Reproduction Study with PFOS (10 PPM A.I.)

	Weeks [No. of 14-Day Old Survivors]												
Pen	A[n]	B[n]	C[n]	D[n]	E[n]	F[n]	G[n]	H[n]	I[n]	J[n]	Mean	SD	14-Day Total
217	271[7]	254[6]	266[5]	249[3]	243[3]	257[3]	242[2]	243[4]	239[3]	241[3]	254	11	39
218	[0]	303[3]	347[6]	277[6]	310[5]	303[4]	295[7]	328[4]	316[3]	281[4]	306	23	42
219	[0]	292[2]	287[4]	263[6]	289[5]	309[4]	292[3]	241[5]	244[6]	237[2]	271	25	37
220	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]			
221	[0]	243[1]	286[3]	268[6]	[0]	281[4]	290[5]	300[7]	277[4]	320[2]	285	16	32
222	[0]	[0]	318[4]	244[5]	272[7]	281[6]	261[6]	249[6]	278[5]	275[6]	271	20	45
223	291[8]	282[6]	296[3]	277[2]	300[3]	347[1]	301[3]	306[5]	278[3]	324[3]	296	15	37
224	270[1]	291[4]	287[5]	267[3]	290[3]	257[3]	299[3]	282[7]	256[5]	309[3]	281	16	37
225	[0]	[0]	300[4]	259[5]	267[5]	250[5]	273[5]	284[5]	293[2]	268[6]	272	15	37
226	[0]	[0]	226[1]	281[1]	265[2]	284[1]	255[2]	268[5]	274[3]	[0]	266	14	15
227	[0]	297[2]	309[6]	280[7]	309[6]	291[6]	288[6]	266[7]	279[2]	314[5]	292	16	47
228	[0]	[0]	316[3]	323[4]	334[5]	319[5]	304[7]	293[3]	313[5]	315[2]	315	12	34
229	[0]	[0]	[0]	[0]	[0]	210[2]	[0]	260[1]	206[2]	242[3]	227	22	8
230	[0]	[0]	266[6]	265[6]	284[6]	325[6]	317[5]	345[5]	309[5]	324[3]	302	28	42
231	[0]	[0]	[0]	241[1]	296[5]	305[7]	303[4]	303[6]	303[6]	317[5]	303	12	34
232	[0]	[0]	240[1]	237[3]	311[5]	273[2]	307[5]	313[3]	[0]	272[2]	289	30	21
											Mean	SD	
											282	23	507

Differences between the control and this treatment group were not significant ($p > 0.05$).
SD = Standard deviation of mean body weight, by parental pen, by week.

Appendix XVIII
Offspring Liver Weights (g)
from a Mallard Reproduction Study with PFOS

Control (0 ppm a.i.)

Bird	Male Liver	Female Liver		
1	19.845	18.179		
2	11.018	13.219		
3	12.740	11.862		
4		14.513		
5		15.982		
6		17.418		
7		13.485		
			Group	
Mean	14.534	14.951	Mean	14.826
SD	4.679	2.326	SD	2.917

10 ppm a.i.

Bird	Male Liver	Female Liver		
1	11.418	13.077		
2	10.819	13.409		
3	18.719	16.095		
4		11.881		
5		11.558		
6		17.125		
7		15.334		
			Group	
Mean	13.652	14.068	Mean	13.944
SD	4.398	2.143	SD	2.721

The above differences from the control group were not statistically significant (Student's t-test).

Appendix XIX

Changes To Study Protocol

This study was conducted in accordance with the study protocol signed on January 22, 2001 and the following amendments and deviations:

1. The protocol was amended to add four pairs of birds to each test group to be maintained for collection of blood samples during the test. Additionally, the protocol was amended to add collection of selected tissues for analyses and histopathological examination from all adult birds. When available, fecal samples were also to be collected from the lower digestive tract of test birds.

2. The protocol was amended to reduce the dietary concentration for the 150 ppm a.i. test concentration to 20 ppm a.i. at the beginning of Week 3 of the test. The concentration was reduced based upon treatment-related mortality and signs of toxicity at the 150 ppm a.i. concentration.

3. The protocol was amended to euthanize the 20 ppm a.i. treatment group as soon as practical after the end of Week 4 of the test. Week 4 body weight and observation data indicated birds had not recovered fully following reduction of the dietary concentration from 150 to 20 ppm a.i. Birds were euthanized without collection of samples.

4. The protocol was amended to euthanize the 50 ppm a.i. treatment group as soon as practical after the end of Week 6 of the test. The level was terminated due to treatment-related mortality and signs of toxicity at the 50 ppm a.i. level. Blood, liver, bile and fecal samples, when available, were collected from 10 pairs when the level was terminated.

5. The protocol was amended to add collection of samples from all eggs selected for egg shell thickness measurements. Eggs selected for Lots A, B and J were separated into shell, shell membrane, albumen and yolk and stored frozen for analysis. Eggs selected from Lots C through I will be opened and the yolk was separated and stored frozen for possible analysis.

6. The protocol was amended to add collection of blood, selected tissues and fecal samples, when available, from 10 offspring each from the control group and 10 ppm a.i. treatment group. Blood, tissues and feces were collected for possible analysis. Tissues were also taken for histopathological examination.

7. The protocol was amended to remove the reference substance number (4526) from page 2 of the protocol. The use of an internal standard was deemed unnecessary after refinements to the analytical method were made.

8. The protocol was amended to indicate samples collected for analysis would be shipped to 3M Corporation. Tissues collected for histopathological examination from birds in the 50 and 150 ppm a.i. test concentrations will not be shipped to EPL for examination, but will be disposed of.

9. The protocol was amended to add Attachment A, the analytical phase plan to be used by 3M Environmental Laboratory for analysis of samples.

10. The protocol was amended to update Attachment A, the analytical phase plan to be used by 3M Environmental Laboratory for analysis of samples.

11. The protocol was amended to change Attachment A, the 3M analytical phase plan, to redefine the peak area precision requirement and the method for calculating the calibration curve.

12. The protocol was amended to indicate the main 3M Environmental Laboratory Project Identification Number (LIMS) for the analytical phase plan results is E01-1245. Additional 3M tracking numbers associated with the study are U2723, E01-1342 and E01-1365. Additionally, the 3M Principal Analytical Investigator was changed from Harold Johnson to Lisa A. Stevenson.

13. The protocol was amended to change the Sponsor Representative from Rochelle R. Robideau to Susan A. Beach.

14. The protocol was amended to change the Sponsor Representative from Susan A. Beach to John L. Newsted.

15. Feather samples were collected, when available, from adult birds from which tissue samples were collected. The protocol did not require collection of these samples. Additionally, with the exception of liver samples, tissue samples collected for analysis were not weighed prior to being placed in sample containers.

16. On July 12, 2001, the temperature in brooder level S52-3, housing offspring from Lot J of the control group, was inadvertently not recorded.

17. Dietary samples collected on Day 0 of Week 1 of the test were not stored frozen between January 24 and January 25, 2001. Instead, samples were stored in the analytical chemistry laboratory prior to being processed for analysis on January 25, 2001. The protocol indicated that diet samples would be stored frozen until they were prepared for analysis.

18. Three adult birds in the 50 ppm a.i. test concentration, the hen in pen 243 and the pair in pen 246, were inadvertently euthanized at the beginning of Week 5 of the test during euthanasia of the 150 ppm a.i. test level.

Appendix XX

Personnel Involved in Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

Avian Toxicology

(1) Mark Jaber, Wildlife Toxicologist

(2) Joann B. Beavers, Director

(3) Linda R. Mitchell, Manager

(4) Diana Temple, Laboratory Supervisor

(5) Sean P. Gallagher, Senior Biologist

(6) Larry T. Frey, Senior Biologist

(7) Jennie Grimes, Senior Biologist

Analytical Chemistry

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

(2) Raymond L. VanHoven, Ph.D., Scientist