

PFOS:
A REPRODUCTION STUDY WITH THE
NORTHERN BOBWHITE

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-108

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 20, 2003

Submitted to

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Environmental Laboratory
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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 Northern Bobwhite

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-108

STUDY COMPLETION: August 20, 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:

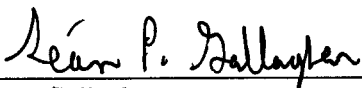
Complete signed and dated reports for blood and tissue analyses conducted by Exygen Research and 3M Corporation are not included in the report. Summary tables are included as appendices and full reports will be submitted separately.

The study was conducted under multiple protocols. The in-life and 3M analytical portions were conducted under one protocol (Wildlife International, Ltd. study number 454-108), 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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3/23/04

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John L. Newsted

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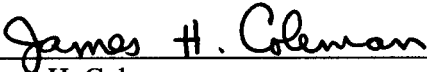
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QUALITY ASSURANCE STATEMENT

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:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO:	
		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
Feed Consumption	February 6, 2001	February 7, 2001	February 8, 2001
Body Weights, Environmental Conditions	February 6, 2001	February 7, 2001	February 9, 2001
Candling	June 12, 2001	June 12, 2001	June 13, 2001
Tissue Collection	June 19, 2001	June 20, 2001	October 1, 2001
14-Day Old Body Weights	June 25, 2001	June 25, 2001	July 5, 2001
Egg Shell Thickness Measurements	June 28, 2001	June 28, 2001	July 2, 2001
Hatchling Body Weights	July 2, 2001	July 2, 2001	July 5, 2001
Data Entry Check	August 10-13, 2001	August 13, 2001	August 14, 2001
Analytical Data, Draft Report	October 29-November 2 and November 5, 2001	November 5, 2001	November 9, 2001
Raw Data, Draft Report	November 27-30 and December 3-5, 2001	December 5, 2001	February 5, 2002
Final Report	August 20, 2003	August 20, 2003	August 20, 2003


James H. Coleman
Quality Assurance Representative


DATE

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REPORT APPROVAL

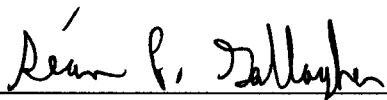
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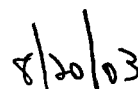
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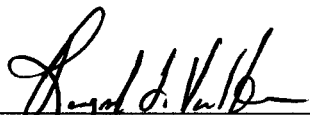
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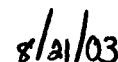
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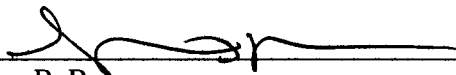

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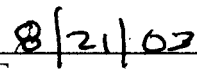
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SUMMARY

STUDY: PFOS: A Reproduction Study with the Northern Bobwhite

SPONSOR: 3M Corporation

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER 454-108

TEST DATES: Study Initiation – January 22, 2001
Experimental Start – January 23, 2001
Photostimulation – March 13, 2001
First Eggs Set – April 12, 2001
Analytical Termination – April 15, 2002
Adult Termination – June 19-20, 2001
Biological Termination – July 23, 2001
Experimental Termination – April 15, 2002

TEST ANIMALS: Northern bobwhite (*Colinus virginianus*)

AGE TEST ANIMALS: 24 weeks of age at the initiation of the test

SOURCE TEST ANIMALS: Morris Quail Farm, Inc.
18370 S.W. 232 Street
Goulds, Florida 33170-5399
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 body weight and feed consumption at the 50 and 150 ppm a.i. test concentrations. The 50 and 150 ppm a.i. test concentrations were terminated prior to the reproductive phase of the test. There were no apparent treatment-related effects upon body weight or feed consumption at the 10 ppm a.i. test concentration. There were overt signs of toxicity observed in adults at the 10 ppm a.i. test concentration. Additionally, there were reductions in testes size and treatment-related effects upon overall reproductive performance at the 10 ppm a.i. test concentration. Based on the effects observed at the 10 ppm a.i. test concentration, the no-observed-effect concentration for northern bobwhite 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 23, 2001. Raw data generated at Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454-108 in archives located on the Wildlife International, Ltd. site. Biological specimens are stored at 3M Corporation, St. Paul, Minnesota 55133.

OBJECTIVES

The objective of this study was to evaluate the effects upon the adult northern bobwhite (*Colinus virginianus*) 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 to their offspring.

EXPERIMENTAL DESIGN

Northern bobwhite (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 454-104) and additional toxicity information provided by the Sponsor.

PFOS Treatment Groups

Group	Nominal Concentration (ppm a.i.)	Pens per Group	Extra Pens - Hematology	<u>Birds per Pen</u>	
				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

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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 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 after Week 4 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. test concentration. Adults in the 50 ppm a.i. test concentrations were euthanized during 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 8, 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 21 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 Northern Bobwhite". 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

One hundred and seventy-seven (90 males and 87 females) pen-reared northern bobwhite were purchased from Morris Quail Farm, Inc., 18370 S.W. 232 Street, Goulds, Florida 33170-5399, U.S.A. At the start of acclimation, the bobwhite 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 177 to 250 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. Except eggs laid by birds maintained for blood collection, all eggs laid during the study were marked with the pen number using a permanent ink marking pen for identification. Hatchlings were identified by leg 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, Table 1). The basal ration contained at least 27% protein and 2.5% fat, and no more than 5% fiber.

The basal diet contained approximately 1.1% calcium, derived from feedstuffs and the 0.9% limestone used in the formulation of the basal diet by Agway. While this level of calcium is sufficient for growth and maintenance rations, additional calcium is required in the ration of breeding birds for egg shell formation. Therefore, an additional 5% (w/w) of limestone (approximately 38.5% Ca) was added to the basal diet for the adults. This raised the calcium level in the diet for the breeding birds to approximately 3%, slightly above the minimum recommended for quail (2.3%) and mallard (2.75%) (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. All offspring received a water-soluble vitamin and electrolyte mix in their water (Appendix II, Table 2). Neither the adults nor offspring received any form of medication in the feed during the test. Feed and water were analyzed periodically in accordance with Wildlife International, Ltd. Standard Operating Procedures.

Diet Preparation

Test diets were prepared by mixing PFOS into a premix that was used for weekly preparation of the final diet. Control diet and each treated diet were prepared weekly beginning on 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. The following changes were made.

- (1) High performance liquid chromatography with triple quadrupole mass detection (HPLC/MS/MS) was used rather than single quadrupole mass detection to reduce potential matrix interferences.
- (2) The internal standardization procedure was eliminated because an appropriate internal standard could not be identified.
- (3) A shorter analytical chromatography column (50 mm vs. 100 mm) was used 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 C₁₈ analytical column (50 mm × 2 mm I.D., 3-µm particle size)

fitted with a Keystone Javelin C18 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 $1.00 \mu\text{g a.i./L} = 1.00 \text{ pg a.i./}\mu\text{L}$. 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%,

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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 - 8 weeks.
2. Pre-photostimulation - 7 weeks.
3. Pre-egg laying (with photostimulation) - 3 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 Georgia Quail Farm Manufacturing (GQFM Model No. 0330), measuring approximately 25 X 51 cm. The pens had sloping floors that resulted in ceiling height ranging from 20 to 26 cm. The pens were constructed of galvanized wire mesh and galvanized sheeting. A diagram of the test layout is presented in Appendix V.

Each pen was equipped with feed and water troughs. 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 troughs were changed and water added as necessary to provide potable water (generally every 2-3 days).

Only birds associated with this study were maintained in the study room in order to avoid excessive disturbances. The average temperature in the adult northern bobwhite study room during the course of the test was $21.4^{\circ}\text{C} \pm 1.3^{\circ}\text{C}$ (SD) with an average relative humidity of $42\% \pm 18\%$ (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.

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The photoperiod in the adult northern bobwhite room was maintained by a time clock. The photoperiod during acclimation and the first seven weeks of the test was eight hours of light per day. The photoperiod was increased to 17 hours of light per day at the beginning of Week 8 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 199 lux (~ 18 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 8 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). An attempt was made to minimize feed wastage by the birds by using externally mounted feeders designed with a "feed-saver" lip. 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

An additional four pairs of adult birds in each test level were 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

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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 the 150 ppm a.i. treatment group were euthanized after 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 with carbon dioxide gas, necropsied, and disposed of by incineration. At the time of necropsy, tissues were collected for histopathological examination and analyses. When available, samples of gall bladder, liver, proventriculus, kidneys, brain, gonads, bursa of Fabricius and adipose tissue were fixed in 10% buffered formalin. Histology samples for the control group and 10 ppm a.i. treatment group were 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 test pens, cleaned and stored in a cold room until incubation or disposal (for those pens used for blood collection). The cold room was maintained at a mean temperature of $13.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ (SD) with a mean relative humidity of approximately $69\% \pm 6\%$ (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 to be incubated were fumigated with formaldehyde gas in an airtight cabinet with a circulating fan for approximately two hours, to reduce the possibility of pathogen contamination prior to incubation. Formaldehyde gas was generated by

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combining 26 g of potassium permanganate and 25 ml of 37% commercial grade formalin in a porcelain bowl at the base of the airtight cabinet.

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 $37.5^{\circ}\text{C} \pm 0.0^{\circ}\text{C}$ (SD) with an average wet bulb temperature of $30.5^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$ (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 21 of incubation. Eggs were candled on Day 11 or 12 of incubation to determine embryo viability and on Day 21 to determine embryo survival.

Hatching and Brooding

On Day 21 of incubation, the eggs were placed in a Petersime Hatcher (Model No. S-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 $37.2^{\circ}\text{C} \pm 0.0^{\circ}\text{C}$ (SD), and the average wet bulb temperature was raised to $33.3^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ (SD) (relative humidity of approximately 77%).

All hatchlings, unhatched eggs, and egg shells were removed from the hatcher on Day 25 or 26 of incubation. The group body weight of the surviving hatchlings by pen was determined. Hatchlings were leg 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 chicks was determined. The chicks were euthanized with carbon dioxide and disposed of by incineration.

Hatchlings were housed in batteries of brooding pens manufactured by Beacon Steel Company (Model B735Q). Each pen measured approximately 72 X 90 X 23 cm high. The external walls and

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ceilings of each pen were constructed of galvanized wire mesh and galvanized sheeting. Floors were of galvanized 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 14 days of age. The average ambient room temperature was 26.0°C ± 1.9°C (SD) with an average relative humidity of 59% ± 13% (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 14-day old offspring (Lot J), blood samples were collected from 10 offspring in both the control group and the 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 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 analyses. Fecal samples were also collected from droppings pans below the floor of brooders housing Lot J offspring and stored frozen for possible analyses. A single composite sample was collected each for the control group and 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 odd numbered pens during odd numbered weeks (1,3,5, etc.) and from each of 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 and shipped to the Sponsor for possible analysis. Eggs collected during weeks 3-9 were opened at the waist, and their contents were removed. The egg contents were separated into albumen and yolk, stored frozen and shipped to the Sponsor for possible analysis. The shells were thoroughly rinsed with water and then 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). Statistical analysis of the data was performed using Avian Reproduction Data System (ARDS) Software; a validated software package developed by Wildlife International, Ltd. (8). 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 11-12 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 by pen.
9. Hatchlings of Eggs Set - The number of hatchlings was divided by the number of eggs set by pen.

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10. 14-Day Old Survivors of Eggs Set - The number of 14-day old survivors was divided by the number of eggs set 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 mean 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 northern bobwhite received PFOS at nominal dietary concentrations of 10, 50, 150 ppm a.i. Due to overt signs of toxicity 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 after Week 4 of the test due to the limited recovery of birds at this level. Adults in the 50 ppm a.i. test concentrations were euthanized during Week 7 of the test due to treatment-related mortality and overt signs of toxicity 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

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2.93%, respectively. These values represented 98%, 100%, and 98% of nominal concentrations (Appendix IV, Table 4).

Samples collected during 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 108%, 100% and 103% 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

There were no treatment-related mortalities in the 10 ppm a.i. treatment group. However, there were three incidental mortalities in both the control group and 10 ppm a.i. treatment group during the course of the study. Control group mortalities occurred during Weeks 14, 19 and 21 of the test. Mortalities in the 10 ppm a.i. test group occurred during Weeks 12, 20 and 21 of the test. All mortalities in the control group and 10 ppm a.i. treatment group were noted with head lesions and/or neck injuries that suggest mortality was caused by incidental injury. Due to the nature of the lesions observed at necropsy, none of the mortalities in the 10 ppm a.i. treatment group were considered to be related to treatment.

Five mortalities occurred in the 50 ppm a.i. treatment group and three mortalities occurred in the 150 ppm a.i. treatment groups during the test. Two additional birds in the 150 ppm a.i. group were euthanized due to their debilitated conditions. All mortalities in the 50 and 150 ppm a.i. test concentrations exhibited overt signs of toxicity prior to death and were considered treatment-related. All remaining birds at the 150 ppm a.i. test concentration were euthanized at the beginning of Week 5 of the test. Necropsies were not performed on the birds euthanized from the 150 ppm a.i. treatment group. All remaining birds at the 50 ppm a.i. test concentration were euthanized and necropsied during Week 7 of the test. Necropsy findings are summarized in Table 2.

Clinical Observations

Clinical signs of toxicity were observed at all concentrations tested. At the 10 ppm a.i. test concentration, signs of toxicity were first observed during Week 5 of the test and continued intermittently until adult termination. Signs of toxicity at the 10 ppm a.i. test concentration included reduced reaction to external stimuli (sound and movement), ruffled appearance and lethargy.

Signs of toxicity at the 50 ppm a.i. test concentration were first noted during Week 4 of the test, when several birds were noted as ruffled in appearance and lethargic. During body weight measurements at the end of Week 4, several birds in the 50 ppm a.i. exhibited muscle spasms and a loss of coordination following handling. At the 150 ppm a.i. test concentration, signs of toxicity were first noted during Week 2 of the test when one individual was noted as ruffled in appearance and lethargic. All birds in the 150 ppm a.i. treatment group exhibited signs of toxicity induced by the stress of handling during body weight measurement at the end of Week 2. In several cases, birds convulsed and became rigid after being captured for body weight measurements. Signs of toxicity in the 50 and 150 ppm a.i. treatment groups continued until euthanasia and included reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, loss of righting reflex, lower limb rigidity, convulsions, shallow and rapid respiration, ruffled appearance, lower limb weakness, lethargy, gaping, prostrate posture and spasms.

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, concentration-responsive, treatment-related reductions in mean body weight for both males and females at the 50 and 150 ppm a.i. test concentrations during the Week 2 body weight interval. Reductions in mean body weight continued for both males and females in the 50 ppm a.i. treatment group through the Week 6 body weight interval. Following reduction of dietary concentrations to 20 ppm a.i. during Week 3 of the test, males in the 150 ppm a.i. test concentrations made slight weight gains at the Week 4 interval, while females in the 150 ppm a.i. treatment group maintained mean body weight. With the exception of mean female body weight at the 50 ppm a.i. test concentration during Week 2, all differences from the control group were statistically significant at $p < 0.05$ or 0.01 . 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 ppm a.i. test concentration. No statistically significant differences between the control group and 10 ppm a.i. treatment group were observed at any of the feed consumption intervals. However, treatment-related reductions in feed consumption were observed at the 50 ppm a.i. test concentration during Weeks 1-6 and at the 150 ppm a.i. test concentration during Week 1 and 2 of the test. All of the reductions were statistically significant at $p < 0.01$. The dietary concentration for the 150 ppm a.i. treatment group was reduced to 20 ppm a.i. at the beginning of Week 3 of the test. Following the reduction of the dietary concentration to 20 ppm a.i., feed consumption for the group continued to be significantly lower than the control group during Week 3 of the test. Feed consumption at the 20 ppm a.i. test concentration was comparable to the control group during Week 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 10 ppm a.i. test concentration were subjected to gross necropsy following adult termination. When compared to the control group, there was an increase in the number of males with small testes in the 10 ppm a.i. treatment group that was considered treatment related. One male in the control group was noted with small testes, compared to seven males in the 10 ppm a.i. treatment 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 apparent treatment-related effects upon the mean weights of male livers at the 10 ppm a.i. test concentration. Differences between male liver weights for the 10 ppm a.i. treatment group and the control group were slight and not statistically significant. There was a statistically significant ($p < 0.01$) increase in mean female liver weight at the 10 ppm a.i. test concentration, when compared to the control group. 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 liver, kidney, brain, and proventriculus of adult and offspring males and females, bursa of Fabricius in offspring males and females, adipose tissue of adult males and females, ovaries of adult and offspring female, and testes

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of male offspring. The increase in the number of adult males in the 10 ppm a.i. treated group with reduced testicular size, not accompanied by any morphological change in spermatogenesis, 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 or adipose tissue in male and female offspring could not be determined since none (bursa of Fabricius) or only one (adipose tissue) sample was submitted for evaluation. In addition, any potential test-article effects on the gallbladder from treated adult males and females 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 bobwhite 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 mallard study run concurrently with the same test concentrations. Summary results of blood analyses performed for this study are presented in Exygen report Tables XXVI through XLI 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 E01-1245. "Analytical Phase Report for PFOS: A Reproduction Study with the Northern Bobwhite".

Reproductive Results

When compared to the control group, there were no apparent treatment-related effects upon egg production at the 10 ppm a.i. test concentration. However, while not statistically significant, there were slight reductions in the number of viable embryos as a percentage of eggs set, hatchlings as a percentage

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of live 3-week embryos and in 14-day old survivors as a percentage of hatchlings. The slight reductions in fertility, hatchability and offspring survival also resulted in reductions in the numbers of hatchlings and 14-day old survivors as percentages of both the number of eggs set and the maximum number of eggs set. While slight, the reduction in the number of 14-day old survivors as a percentage of eggs set was statistically significant at $p < 0.05$. 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.

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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. 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, 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 E01-1245. "Analytical Phase Report for PFOS: A Reproduction Study with the Northern Bobwhite".

CONCLUSION

There were no apparent treatment-related effects upon adult body weight or feed consumption at the 10 ppm a.i. test concentration. However, minor overt signs of toxicity were observed in adults at the 10 ppm a.i. concentration. Additionally, there were reductions in testes size, and slight, but treatment-related, effects upon fertility, hatchability and offspring survival at the 10 ppm a.i. test concentration.

There were treatment-related mortalities, overt signs of toxicity and treatment-related effects upon body weight and feed consumption at the 50 and 150 ppm a.i. test concentrations. The 50 and 150 ppm a.i. test concentrations were terminated prior to the reproductive phase of the test. Based on the effects observed at the 10 ppm a.i. test concentration, the no-observed-effect concentration for northern bobwhite 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 Northern Bobwhite 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	108 ⁴	103	101	97	109	101	101	99
50	Mean									
	Measured % Nominal	100 ³	100 ⁴	97	104	104	-	-	-	-
150	Mean									
	Measured % Nominal	98 ³	103 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 <2.00 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 Northern Bobwhite Reproduction Study with PFOS
Adult Birds Found Dead/Euthanized during the Test

	Males		Females			
	50 ¹ ppm a.i.	150 ² ppm a.i.	Control (0 ppm a.i.)	10 ppm a.i.	50 ¹ ppm a.i.	150 ² ppm a.i.
Number of birds	16	3	3	3	16	2
Feathers wet around beak	0	0	1	0	0	0
Feather loss	5	0	0	0	0	0
Extensive head/neck lesions	0	0	1	2	0	0
Vent - pasty	0	0	0	0	1	0
Thin	1	0	0	0	0	0
Emaciated	1	1	1	1	2	1
Loss of muscle mass	2	1	1	1	2	1
Prominent keel	1	1	1	1	2	1
Intra-cranial bleeding	0	1	0	0	0	0
Neck - mid-length fracture	0	0	1	0	0	0
Neck - hematoma	0	0	1	1	1	0
Heart - no coronary fat	1	0	0	1	1	0
Spleen - small and/or pale	3	1	1	2	1	1
Abdominal cavity - autolysis	1	1	1	1	2	1
Abdominal cavity - small amount of loose blood	0	0	0	1	0	0
Crop - empty	1	0	1	1	0	0
Gizzard contents - bile-stained	0	0	0	1	0	0
Cecal contents - pasty	0	1	1	0	0	0
Kidneys - pale	0	0	0	1	0	0
Ovary - developing	-	-	0	1	0	0
Ovary - regressing	-	-	1	0	0	0
Not remarkable	7	1	0	0	13	0

¹ Remaining birds in the 50 ppm a.i. test concentration were euthanized during Week 7 of the test. Gross necropsy observations for birds maintained for collection of blood samples 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 Northern Bobwhite Reproduction Study with PFOS¹

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	208	6	214	-1	213	1	215	-1	214	1	215	7
	Female	210	4	214	-1	213	1	214	4	218	32	247	39
10	Male	208	2	211	-1	210	1	211	-2	209	-1	208	-1
	Female	206	4	209	-1	208	1	209	-1	209	40	249	42
50	Male	205	-8	197**	-13	185**	-8	179**	-	-	-	-	-
	Female	210	-7	202	-7	195*	-11	185**	-	-	-	-	-
150	Male	206	-34	172**	13	187**	-	-	-	-	-	-	-
	Female	212	-41	172**	3	172**	-	-	-	-	-	-	-

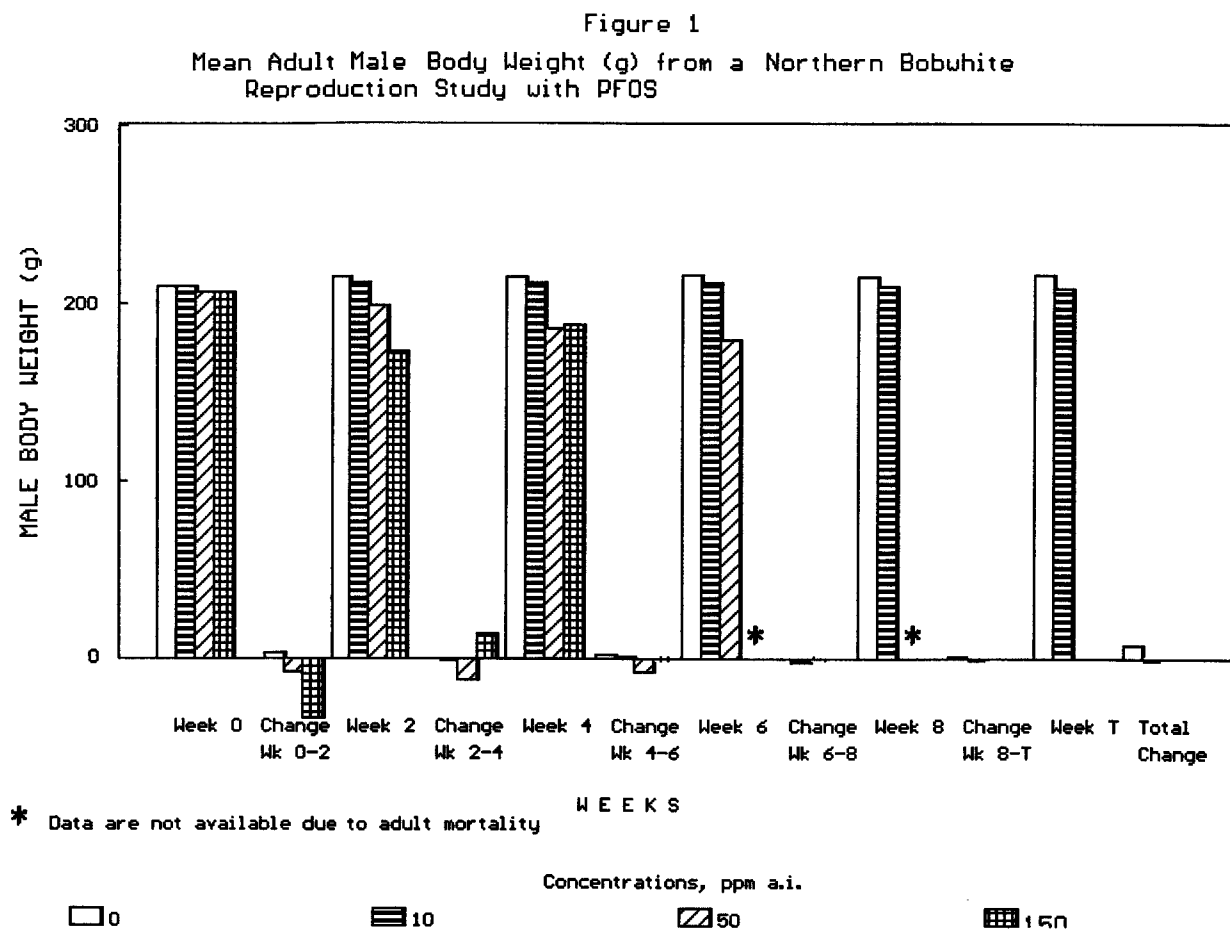
The means for body weights and body weight changes are calculated and rounded separately.

- Data are not available due to adult mortality.

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

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

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



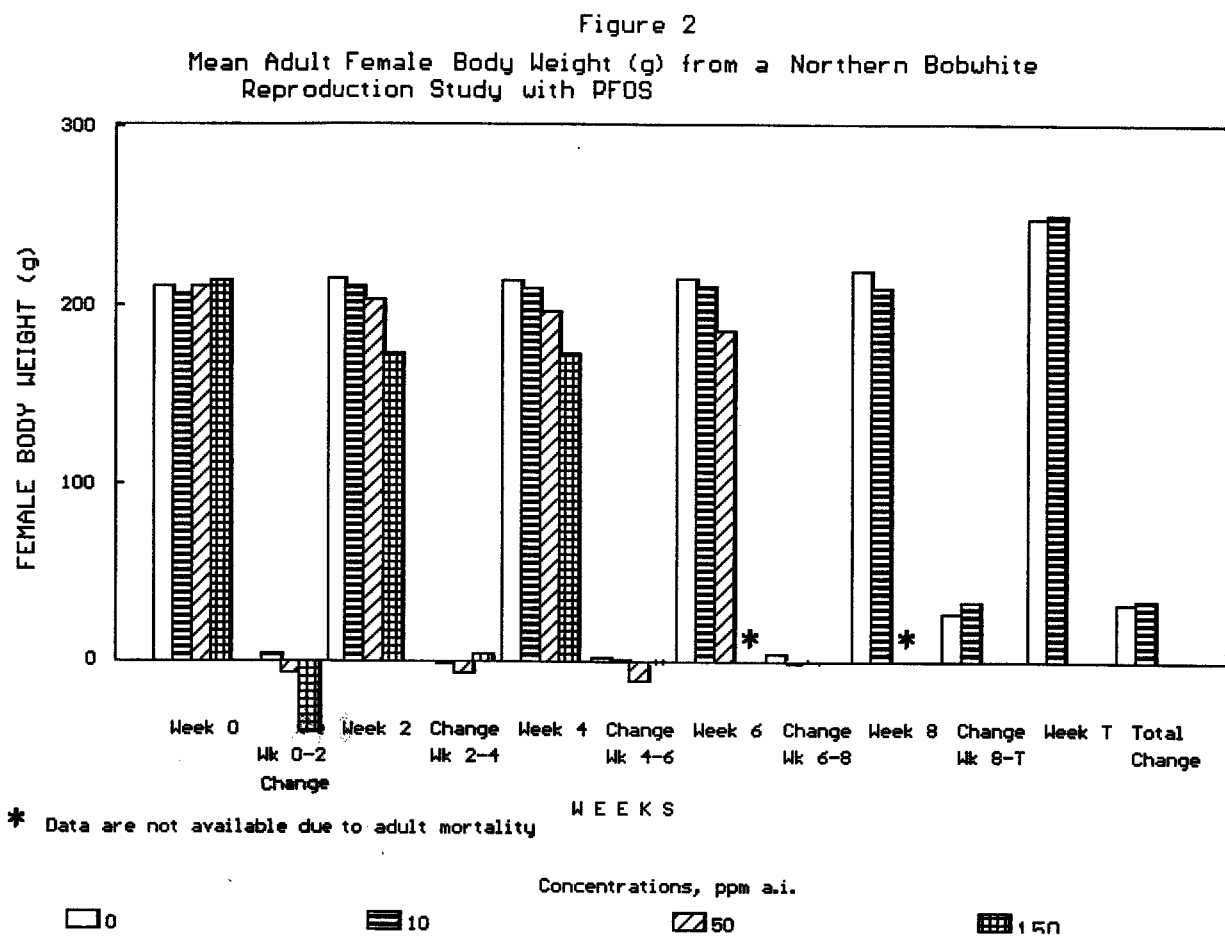


Table 4

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

Experimental Group (ppm a.i.)	W E E K S																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	12	14	13	13	13	12	12	15	14	14	15	17	18	20	19	20	19	19	19	20	19
10	12	13	13	13	13	12	12	14	13	13	14	16	18	20	20	19	20	20	20	21	19
50	11**	11**	10**	10**	9**	9**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
150	7**	6**	8**	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality

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

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

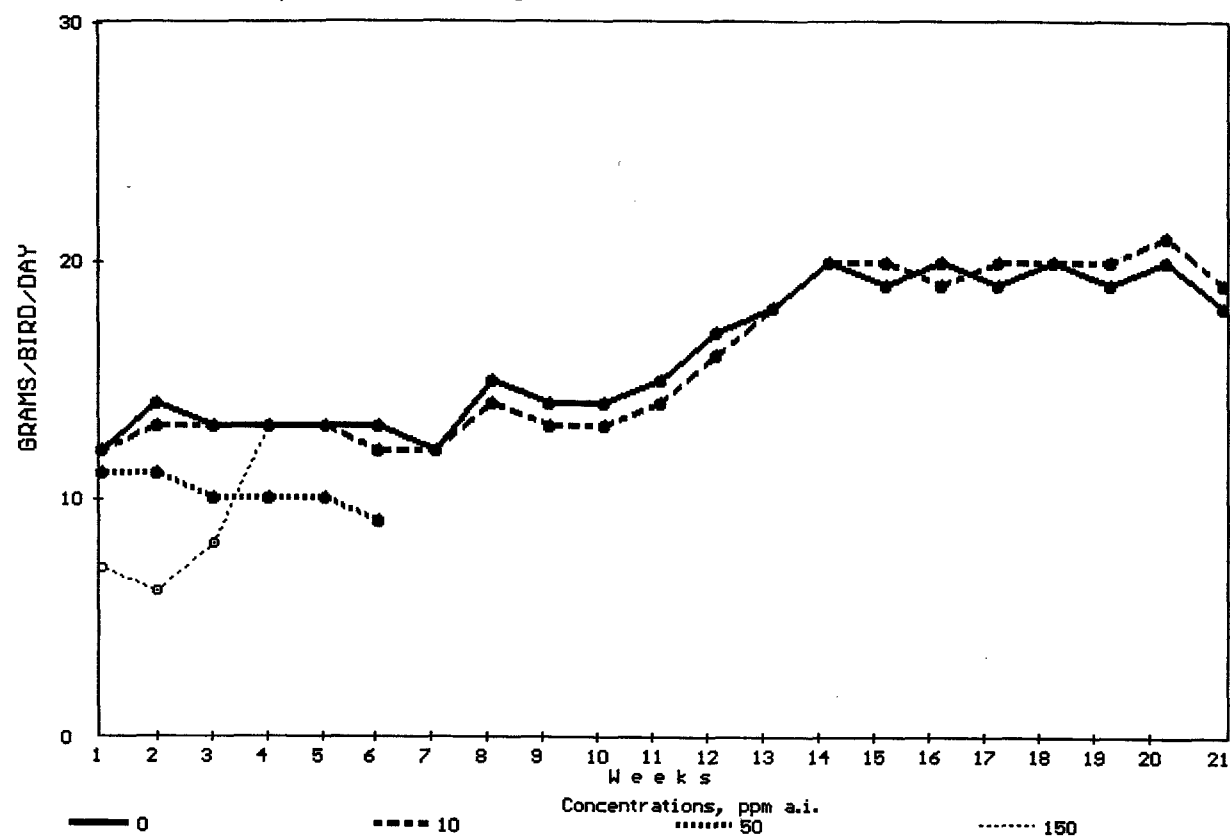


Table 5
Summary of Gross Pathological Observations
from a Northern Bobwhite Reproduction Study with PFOS
Adult Birds Euthanized at Test Termination¹

	Males		Females	
	Control (0 ppm a.i.)	10 ppm a.i.	Control (0 ppm a.i.)	10 ppm a.i.
Number of birds	16	16	13	13
Feather loss	3	5	6	9
Head lesions	0	0	0	1
Foot/leg lesions	0	0	1	0
Back/vent lesions	0	0	2	0
Liver - pale and mottled	0	0	0	1
Abdominal cavity - slight egg yolk peritonitis	-	-	0	1
Intestinal tract - area of hyperemia	7	8	0	1
Ceca - round worms found	0	3	3	0
Testes - one or both small (~1.0-1.5 cm)	1	6	-	-
Not remarkable	7	3	6	3

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

Table 6
Mean Liver Weights¹ (g)
from a Northern Bobwhite 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.	4.427 ±	0.496	8.662 ±	1.113	0.842 ±	0.154	0.850 ±	0.087
10 ppm a.i.	4.481 ±	0.545	10.884* ±	1.596	0.853 ±	0.172	0.882 ±	0.184
50 ppm a.i.	-	-	-	-	-	-	-	-
150 ppm a.i.	-	-	-	-	-	-	-	-

¹Mean ± standard deviation.

- Data not available due to mortality.

* Statistically different from the control group at p < 0.01.

Table 7

**Summary of Reproductive Performance from a Northern Bobwhite Reproduction Study with
PFOS**

Reproductive Parameter	Experimental Group (ppm a.i.)			
	Control	10	50	150
Number of Replicates	13	13	-	-
Total Eggs Laid	605	592	-	-
Eggs Cracked	23	3	-	-
Eggs Set	521	526	-	-
Viable Embryos	494	470	-	-
Live 3-Week Embryos	490	466	-	-
Hatchlings	473	414	-	-
14-Day Old Survivors	457	386	-	-
Eggs Laid/Hen	47	46	-	-
Eggs Laid/Hen/Day ¹	0.50	0.49	-	-
14-Day Old Survivors/Hen	35	30	-	-

¹ Based on 93 days of eggs production.

- Data are not available due to adult mortality

Figure 4
Mean Reproductive Performance from a Northern Bobwhite
Reproduction Study with PFOS

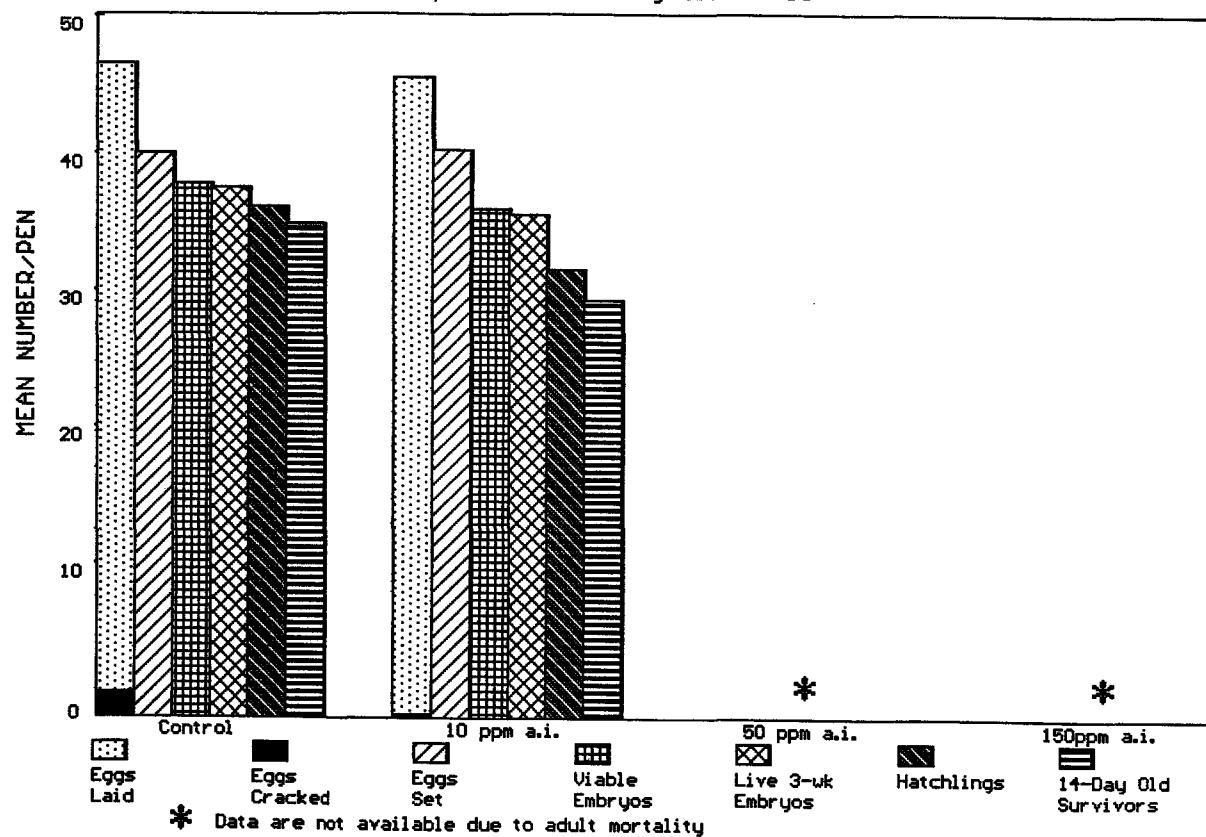


Table 8
Summary of Reproductive Performance, Normalized as Percentages (%)
from a Northern Bobwhite Reproduction Study with PFOS¹

Reproductive Parameter	Experimental Group (ppm a.i.)			
	Control	10	50	150
Number of Replicates	13	13	N/A	N/A
Total Eggs Laid ²	605	592	N/A	N/A
Eggs Laid/Maximum Laid (%)	74	72	N/A	N/A
Eggs Cracked/Eggs Laid (%)	3	0	N/A	N/A
Viable Embryos/Eggs Set (%)	94	89	N/A	N/A
Live 3-Week Embryos/Viable Embryos (%)	99	99	N/A	N/A
Hatchlings/Live 3-Week Embryos (%)	97	89	N/A	N/A
14-Day Old Survivors/Hatchlings (%)	97	93	N/A	N/A
Hatchlings/Eggs Set (%)	90	78	N/A	N/A
14-Day Old Survivors/Eggs Set (%)	87	72*	N/A	N/A
Hatchlings/Maximum Set (%)	64	56	N/A	N/A
14-Day Old Survivors/Maximum Set (%)	62	52	N/A	N/A

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

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

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 Northern Bobwhite Reproduction Study with PFOS

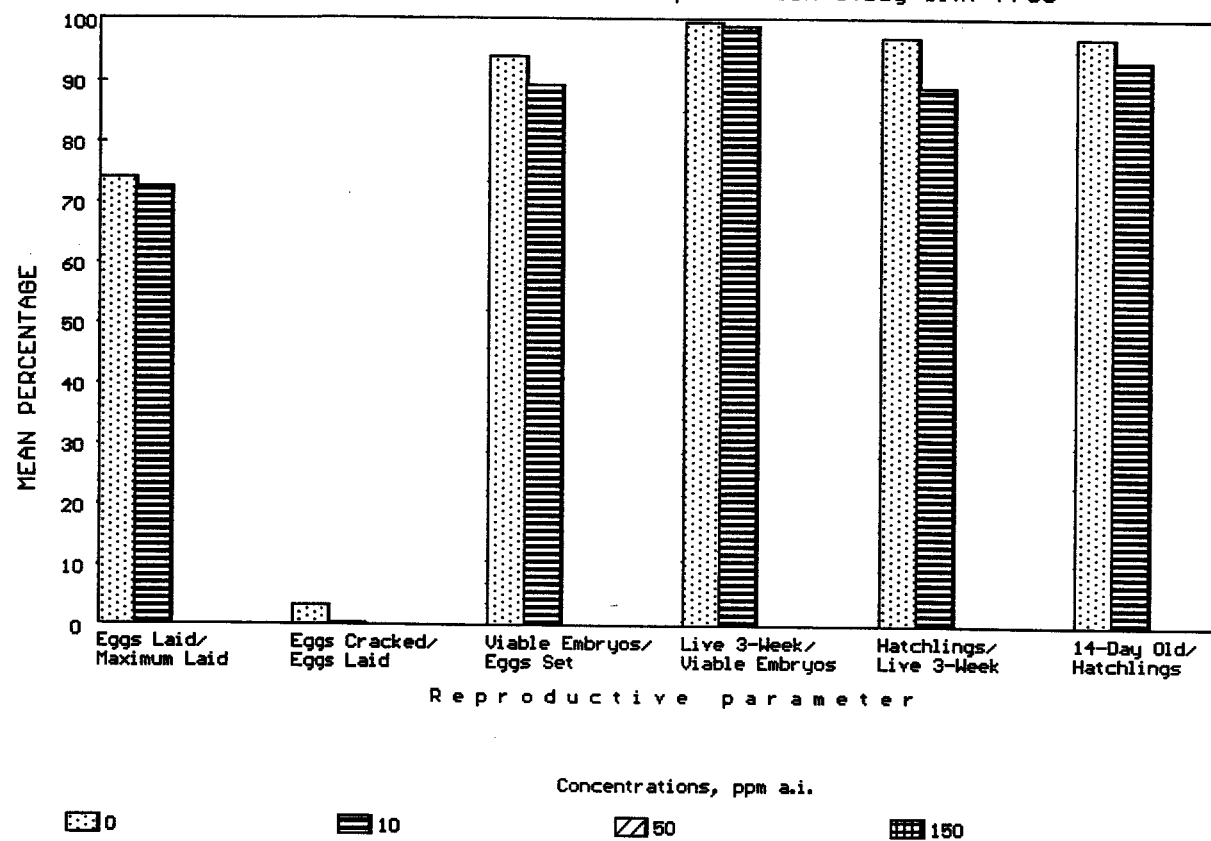


Table 9
Mean Eggshell Thickness Measurements (mm)
from a Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)	No. Eggs Measured	Shell Thickness Mean ($\pm$ SD)
Control	42	0.229 $\pm$ 0.021
10	45	0.227 $\pm$ 0.010
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 Northern Bobwhite Reproduction Study with PFOS*

Experimental Group (PPM A.I.)	Hatchlings		14-Day Old Survivors	
	Number	Mean (± SD)	Number	Mean (± SD)
Control	472	6 ± 0	457	29 ± 2
10	412	6 ± 1	386	30 ± 3
50	-		-	
150	-		-	

- Data are not available due to adult mortality

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 $\leq 10^{\circ}\text{C}$

Re-assessment Date: 08/31/06

¹Purity = 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%

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

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

⁴Sulfur 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
PFFA	Pentafluoropropanoic acid

⁶Theoretical value calculations based on the empirical formula, $\text{C}_2\text{F}_{17}\text{SO}_3\text{K}^+$ (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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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

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:

Charles Simons

Scientist, Centre Analytical Laboratories

10/11/01
Date

Reviewed By:

John Flaherty

Laboratory Manager, Centre Analytical Laboratories

10/11/01
Date

Appendix II
Diet and Supplement Formulations

Table 1
Wildlife International, Ltd. Game Bird Ration¹

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	2,500,000	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 HCl	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 D₃), 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 HCl, 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

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Table 2**Calculations**

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

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

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:

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

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

Fortification Recoveries

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

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

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 (454-108-)	Type	Interval	Fortified ¹	Measured ^{1,2}		
MAB-1	Matrix Blank	Week 1, Day 0	0.00	< 2.00	-	102
MAS-1	Matrix Fortification	Week 1, Day 0	5.00	4.85	97	
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	-	94
MAS-4	Matrix Fortification	Week 2, Day 0	5.00	4.55	91	
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	-	105
MAS-7	Matrix Fortification	Week 3, Day 0	5.00	5.50	110	
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	-	103
MAS-10	Matrix Fortification	Week 4, Day 0	5.00	5.20	104	
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 (454-108-)	Type	Interval	Fortified ¹	Measured ^{1,2}		
MAB-5	Matrix Blank	Week 8, Day 0	0.00	< 2.00	-	109
MAS-13	Matrix Fortification	Week 8, Day 0	5.00	5.40	108	
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	-	101
MAS-15	Matrix Fortification	Week 12, Day 0	5.00	5.21	104	
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	-	97
MAS-19	Matrix Fortification	Week 20, Day 0	5.00	4.86	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 (454-108-)	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	$\bar{x} = 9.75$ ppm a.i. SD = 0.313 ppm a.i. CV = 3.21%	98
	3	Top Right	9.47		
	4	Middle Left	9.87		
	5	Middle Right	10.2		
	6	Bottom Left	9.35		
	7	Bottom Right	9.93		
50	8	Top Left	49.9	$\bar{x} = 50.1$ ppm a.i. SD = 0.981 ppm a.i. CV = 1.96%	100
	9	Top Right	48.9		
	10	Middle Left	51.7		
	11	Middle Right	50.4		
	12	Bottom Left	50.3		
	13	Bottom Right	49.3		
150	14	Top Left	151	$\bar{x} = 147$ ppm a.i. SD = 4.31 ppm a.i. CV = 2.93%	98
	15	Top Right	153		
	16	Middle Left	143		
	17	Middle Right	143		
	18	Bottom Left	144		
	19	Bottom Right	147		

APPENDIX IV

Table 5

Verification of PFOS Concentrations in Avian Diet

Nominal Concentration (ppm a.i.)	Sample I.D. Number (454-108-)	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	$\bar{x}$ = 10.2 ppm a.i. SD = 0.553 ppm a.i. CV = 5.44%	110	102
	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		106	
	57	8	11.2		112	
	61	12	10.4		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 (454-108-)	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	$\bar{x}$ = 20.8 ppm a.i. SD = 2.14 ppm a.i. CV = 10.3%	93	104
	47	3	19.8		99	
	53	4	23.6		118	
	54	4	21.1		106	
50	37	2	48.7	$\bar{x}$ = 50.9 ppm a.i. SD = 2.04 ppm a.i. CV = 4.01%	97	102
	38	2	48.4		97	
	44	3	51.1		102	
	45	3	53.1		106	
	51	4	51.1		102	
	52	4	53.1		106	
150	39	2	172	$\bar{x}$ = 161 ppm a.i. SD = 15.6 ppm a.i. CV = 9.66%	115	108
	40	2	150		100	

APPENDIX IV

Table 6

Ambient Stability of PFOS in Avian Diet During the Northern Bobwhite Reproduction Study

Nominal Concentration (ppm a.i.)	PFOS Concentration						
	Week 1, Day 0 ¹			Week 1, Day 7			
	Sample Number (454-108-)	Mean Measured (ppm a.i.)	Mean Percent of Nominal	Sample Number (454-108-)	Measured ² (ppm a.i.)	Mean Measured (ppm a.i.)	Mean Percent of Day 0
0	1	<2.00	-	20	< 2.00	<2.00	-
10	2-7	9.75	98	21 22	9.74 11.2	10.5	108
50	8-13	50.1	100	23 24	50.1 50.5	50.3	100
150	14-19	147	98	25 26	148 154	151	103

¹Day 0 values are from homogeneity samples presented in Table 4.

²Less 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

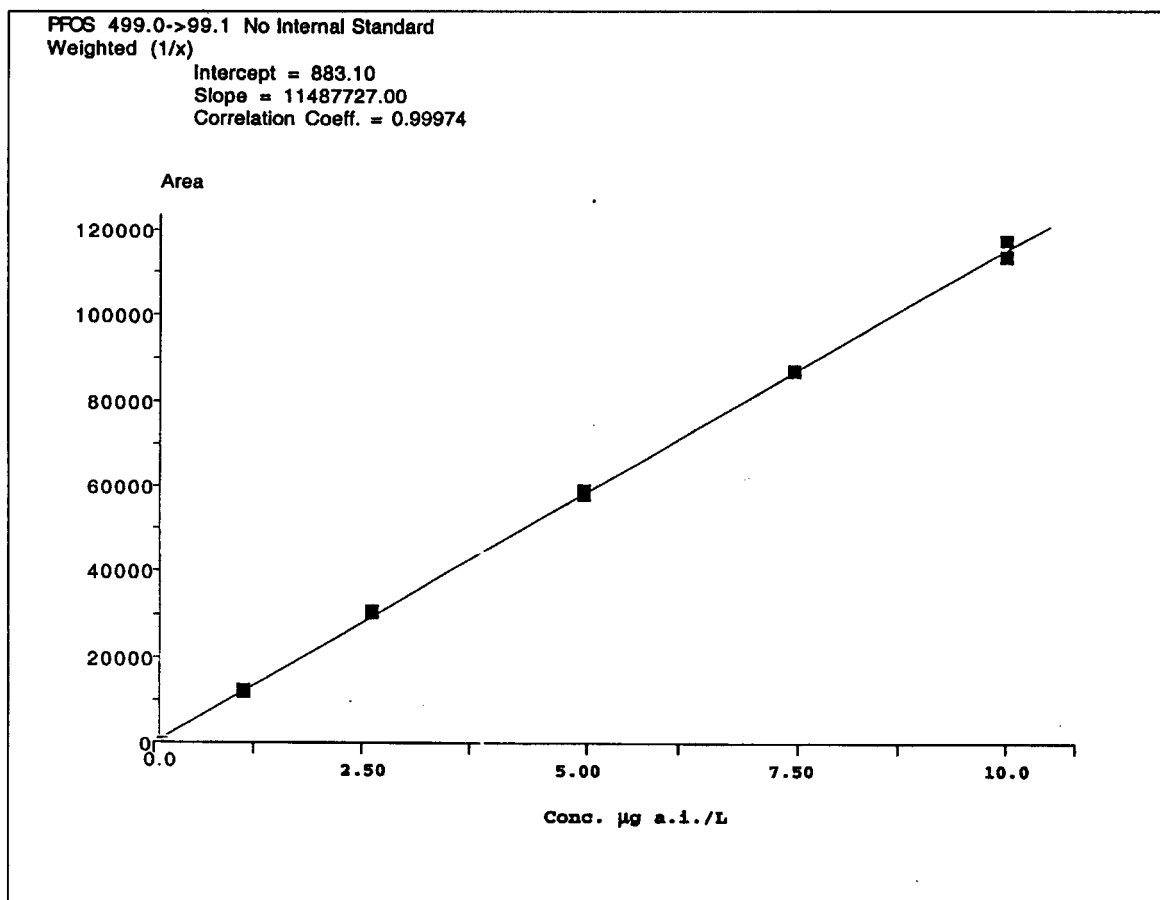


Figure 2. Typical calibration curve for PFOS. Slope = 11487727; Intercept = 883.10; $r = 0.99974$. Curve is weighted (1/x).

APPENDIX IV

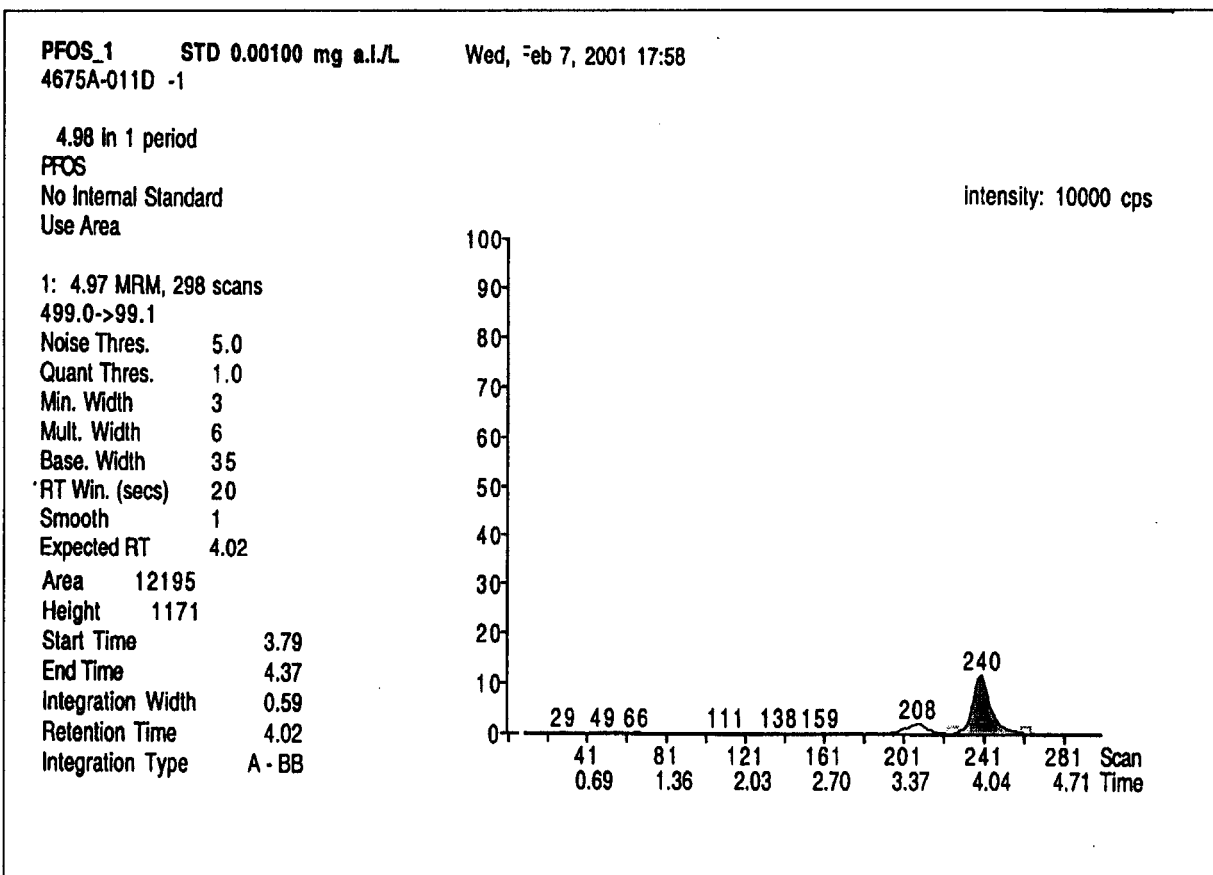


Figure 3. Typical ion chromatogram of a low PFOS calibration standard, 0.00100 mg a.i./L.
(Monitored mass 499.0 → 99.1).

APPENDIX IV

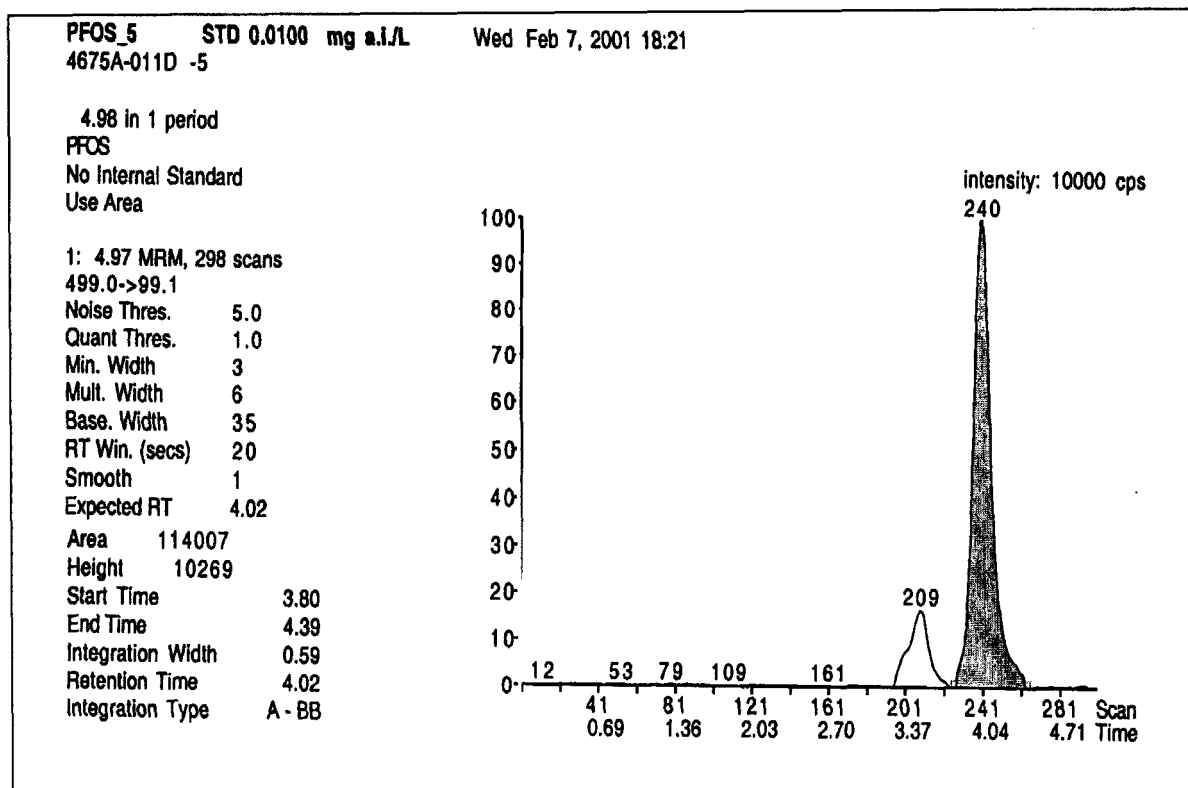


Figure 4. Typical ion chromatogram of a high PFOS calibration standard, 0.0100 mg a.i./L.
(Monitored mass 499.0 → 99.1).

APPENDIX IV

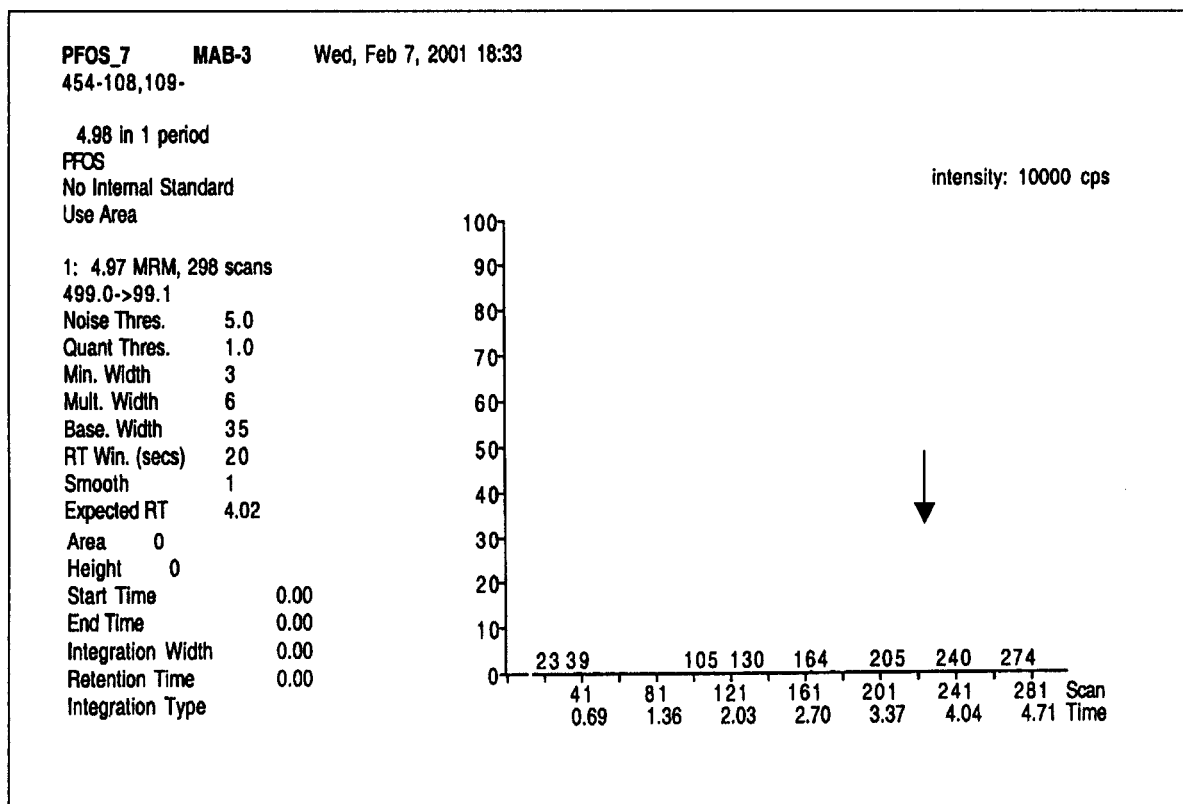


Figure 5. Typical ion chromatogram of a matrix blank sample (454-108-MAB-3). The arrow indicates the approximate retention time of PFOS. (Monitored mass 499.0 → 99.1).

APPENDIX IV

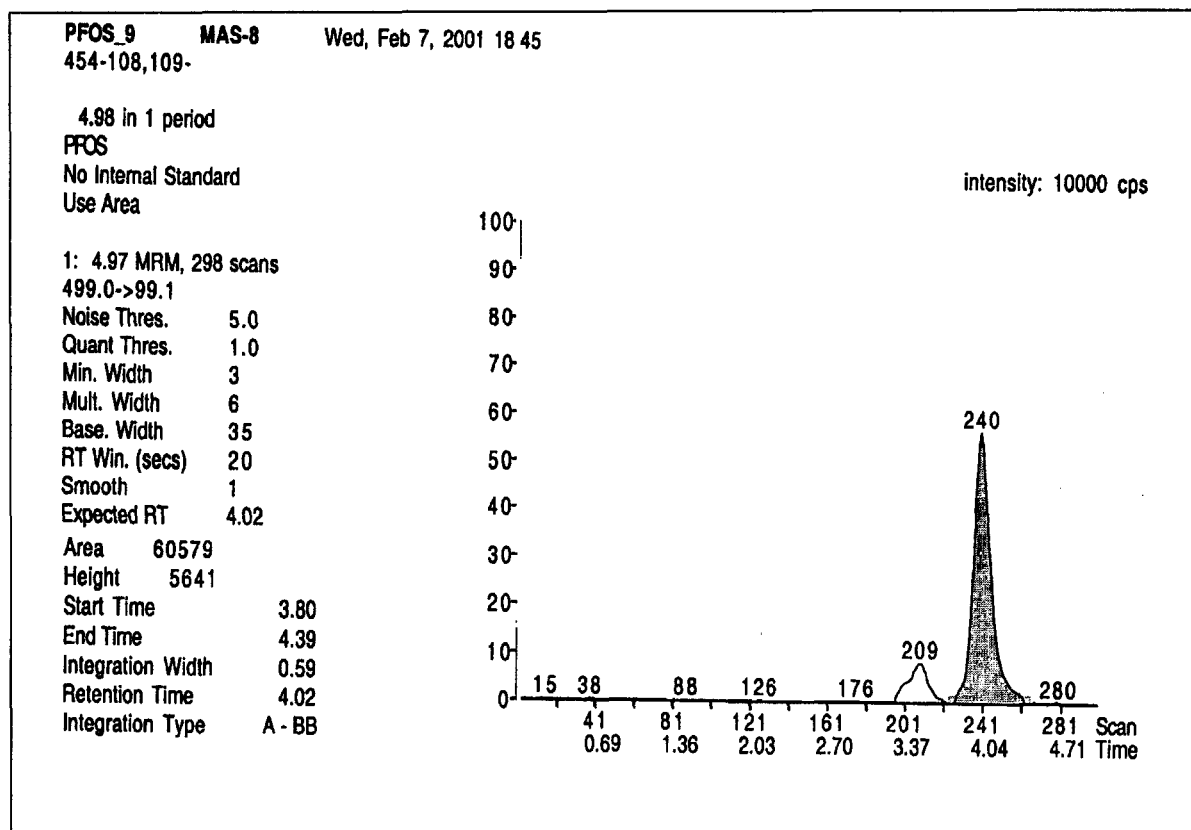


Figure 6. Typical ion chromatogram of a matrix fortification sample (454-108-MAS-8, 50.0 ppm a.i.) (Monitored mass 499.0 → 99.1).

APPENDIX IV

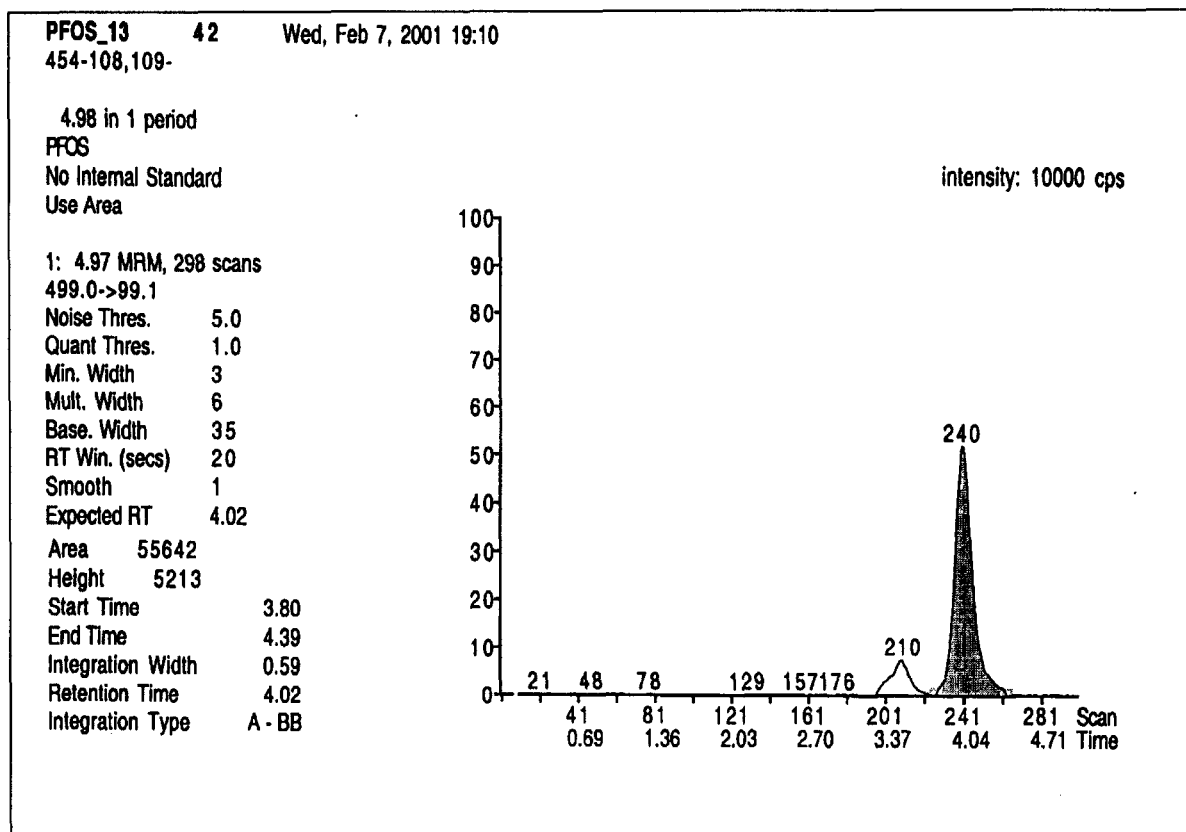
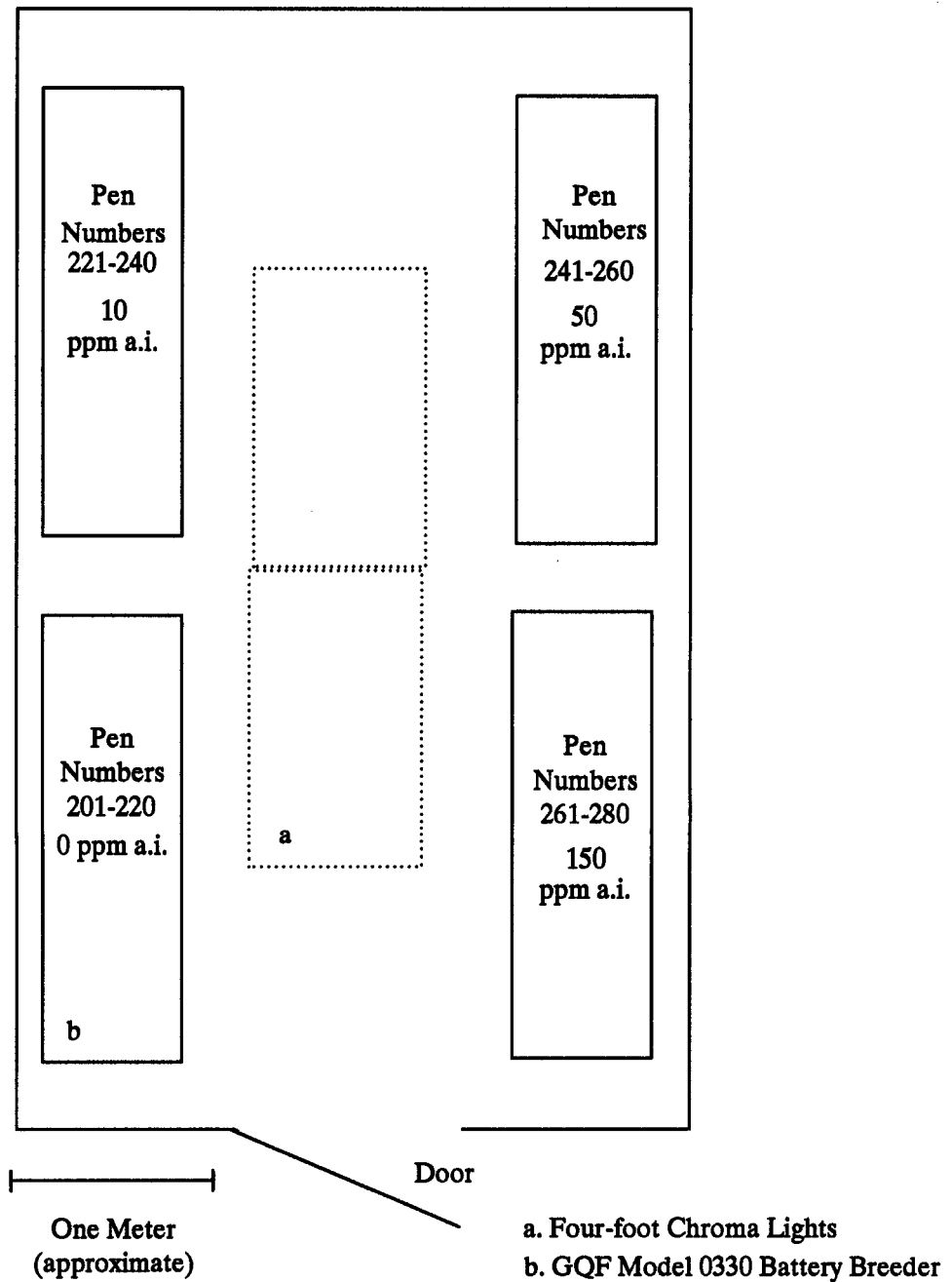


Figure 7. Typical ion chromatogram of an avian diet sample on Day 0 (454-108-42, 10.0 ppm a.i.).
Dilution factor = 2000X.
(Monitored mass 499.0 → 99.1).

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

Room 45



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 10-12 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 25 or 26 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, chicks will stick to their shells.
- Temperature: If too hot and humid, chicks 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 0 ppm a.i.	1	201	201	329	330
	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 10 ppm a.i.	1	217	221	369	370
	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 Northern Bobwhite 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	210	-2	208	0	208	0	208	1	209	5	214	4
202	214	1	215	-1	214	1	215	0	215	-15	200	-14
203	218	6	224	0	224	1	225	-1	224	-11	213	-5
204	206	5	211	-4	207	1	208	4	212	8	220	14
205	206	5	211	-2	209	3	212	3	215	1	216	10
206	184	6	190	-3	187	1	188	-3	185	14	199	15
207	182	4	186	4	190	3	193	-3	190	-3	187	5
208	205	8	213	1	214	2	216	1	217	3	220	15
209	209	4	213	-2	211	4	215	-1	214	5	219	10
210	207	7	214	2	216	-1	215	-18	197	21	218	11
211	213	7	220	0	220	-1	219	-4	215	3	218	5
212	212	5	217	-1	216	-1	215	1	216	-8	208	-4
213	195	2	197	-1	196	3	199	7	206	8	214	19
214	196	13	209	-2	207	2	209	-3	206	-5	201	5
215	232	9	241	-1	240	-2	238	4	242	-1	241	9
216	244	10	254	2	256	5	261	2	263	-9	254	10
Mean	208	6	214	-1	213	1	215	-1	214	1	215	7
SD	16	4	17	2	17	2	17	6	19	9	16	9

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

Appendix VII - Table 2

Adult Body Weight (g) from a Northern Bobwhite 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	191	3	194	-2	192	1	193	0	193	24	217	26
202	211	-1	210	2	212	2	214	6	220	28	248	37
203	220	3	223	-3	220	2	222	7	229	-	-	-
204	205	5	210	0	210	1	211	3	214	21	235	30
205	201	6	207	0	207	3	210	0	210	19	229	28
206	205	7	212	-4	208	0	208	-3	205	24	229	24
207	224	4	228	0	228	-3	225	11	236	-	-	-
208	220	2	222	-2	220	0	220	-4	216	48	264	44
209	191	6	197	0	197	-1	196	5	201	26	227	36
210	187	4	191	-2	189	2	191	25	216	19	235	48
211	197	4	201	-1	200	-1	199	3	202	57	259	62
212	211	7	218	2	220	1	221	4	225	-	-	-
213	217	1	218	-2	216	6	222	4	226	24	250	33
214	226	7	233	-1	232	1	233	-3	230	51	281	55
215	218	3	221	-1	220	5	225	5	230	29	259	41
216	234	2	236	-1	235	0	235	-3	232	44	276	42
Mean	210	4	214	-1	213	1	214	4	218	32	247	39
SD	14	2	14	2	14	2	14	7	13	13	20	11

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 3

Adult Body Weight (g) from a Northern Bobwhite 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	200	8	208	1	209	4	213	-4	209	0	209	9
218	225	1	226	1	227	2	229	-5	224	12	236	11
219	219	2	221	-2	219	0	219	-2	217	-6	211	-8
220	190	2	192	0	192	-1	191	6	197	3	200	10
221	198	3	201	-4	197	2	199	0	199	10	209	11
222	188	1	189	0	189	2	191	-1	190	4	194	6
223	209	2	211	3	214	-1	213	-4	209	-2	207	-2
224	205	9	214	-4	210	6	216	-4	212	-11	201	-4
225	198	2	200	-3	197	0	197	-6	191	-3	188	-10
226	217	4	221	-1	220	0	220	-6	214	-10	204	-13
227	209	2	211	-3	208	2	210	-3	207	1	208	-1
228	205	-8	197	7	204	-2	202	-1	201	10	211	6
229	214	5	219	-2	217	0	217	0	217	-1	216	2
230	211	3	214	2	216	0	216	-1	215	1	216	5
231	224	4	228	-3	225	0	225	-2	223	-7	216	-8
232	218	-1	217	0	217	-1	216	-3	213	-17	196	-22
Mean	208	2	211	-1	210	1	211	-2	209	-1	208	-1
SD	11	4	12	3	12	2	12	3	10	8	11	10

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 Northern Bobwhite 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	203	2	205	-3	202	-1	201	-1	200	58	258	55
218	220	5	225	-1	224	-1	223	-1	222	24	246	26
219	220	7	227	-4	223	1	224	3	227	20	247	27
220	178	2	180	-1	179	0	179	0	179	40	219	41
221	180	4	184	0	184	3	187	1	188	23	211	31
222	213	1	214	-2	212	2	214	-2	212	58	270	57
223	198	0	198	0	198	5	203	-3	200	23	223	25
224	197	8	205	-3	202	3	205	-5	200	-	-	-
225	194	2	196	1	197	3	200	2	202	35	237	43
226	219	7	226	1	227	1	228	3	231	-	-	-
227	235	-5	230	4	234	-1	233	1	234	35	269	34
228	199	7	206	-5	201	-2	199	0	199	45	244	45
229	214	5	219	-3	216	0	216	0	216	55	271	57
230	196	6	202	1	203	2	205	-2	203	49	252	56
231	236	4	240	-2	238	-4	234	1	235	52	287	51
232	189	3	192	1	193	2	195	-6	189	-	-	-
Mean	206	4	209	-1	208	1	209	-1	209	40	249	42
SD	18	3	17	2	17	2	16	3	17	14	22	12

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

- Data are not available due to adult mortality.

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

Appendix VII - Table 5

Adult Body Weight (g) from a Northern Bobwhite 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	216	0	216	-19	197	-4	193	-	-	-	-	-
234	207	-7	200	-9	191	-9	182	-	-	-	-	-
235	201	-12	189	-31	158	-13	145	-	-	-	-	-
236	198	-17	181	-29	152	-	-	-	-	-	-	-
237	206	-5	201	-7	194	1	195	-	-	-	-	-
238	209	-16	193	-18	175	-16	159	-	-	-	-	-
239	203	-2	201	-3	198	-8	190	-	-	-	-	-
240	204	-7	197	-9	188	-2	186	-	-	-	-	-
241	200	-9	191	-1	190	-27	163	-	-	-	-	-
242	223	-4	219	-11	208	-5	203	-	-	-	-	-
243	182	3	185	-10	175	1	176	-	-	-	-	-
244	202	-10	192	-15	177	0	177	-	-	-	-	-
245	207	-10	197	-10	187	-1	186	-	-	-	-	-
246	204	-9	195	-13	182	-4	178	-	-	-	-	-
247	214	-6	208	-5	203	6	209	-	-	-	-	-
248	203	-11	192	-11	181	-41	140	-	-	-	-	-
Mean	205	-8	197**	-13	185**	-8	179**	-	-	-	-	-
SD	9	5	10	8	15	12	20	-	-	-	-	-

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 Northern Bobwhite 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	199	-1	198	-1	197	2	199	-	-	-	-	-
234	206	-6	200	-5	195	-4	191	-	-	-	-	-
235	227	-5	222	-7	215	-3	212	-	-	-	-	-
236	211	-7	204	-3	201	-26	175	-	-	-	-	-
237	209	-4	205	-6	199	-24	175	-	-	-	-	-
238	196	-18	178	0	178	-3	175	-	-	-	-	-
239	199	-6	193	-5	188	-2	186	-	-	-	-	-
240	202	-6	196	-26	170	10	180	-	-	-	-	-
241	235	1	236	-1	235	-6	229	-	-	-	-	-
242	206	-8	198	-11	187	-13	174	-	-	-	-	-
243	204	-11	193	0	193	-3	190	-	-	-	-	-
244	223	-13	210	-30	180	-51	129	-	-	-	-	-
245	177	-2	175	-2	173	2	175	-	-	-	-	-
246	225	-17	208	-8	200	-31	169	-	-	-	-	-
247	217	-6	211	-13	198	-12	186	-	-	-	-	-
248	218	-7	211	1	212	-4	208	-	-	-	-	-
Mean	210	-7	202	-7	195*	-11	185**	-	-	-	-	-
SD	14	5	15	9	17	15	22	-	-	-	-	-

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 $p < 0.05$

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

Appendix VII - Table 7

Adult Body Weight (g) from a Northern Bobwhite 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	191	-22	169	7	176	-	-	-	-	-	-	-
250	213	-44	169	-	-	-	-	-	-	-	-	-
251	198	-15	183	15	198	-	-	-	-	-	-	-
252	218	-48	170	17	187	-	-	-	-	-	-	-
253	209	-34	175	-4	171	-	-	-	-	-	-	-
254	194	-55	139	-	-	-	-	-	-	-	-	-
255	199	-32	167	24	191	-	-	-	-	-	-	-
256	242	-35	207	18	225	-	-	-	-	-	-	-
257	195	-53	142	38	180	-	-	-	-	-	-	-
258	214	-28	186	4	190	-	-	-	-	-	-	-
259	212	-21	191	-8	183	-	-	-	-	-	-	-
260	194	-23	171	6	177	-	-	-	-	-	-	-
261	207	-25	182	-	-	-	-	-	-	-	-	-
262	211	-35	176	20	196	-	-	-	-	-	-	-
263	196	-47	149	31	180	-	-	-	-	-	-	-
264	197	-20	177	1	178	-	-	-	-	-	-	-
Mean	206	-34	172**	13	187**	-	-	-	-	-	-	-
SD	13	13	18	14	14	-	-	-	-	-	-	-

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 Northern Bobwhite 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	203	-44	159	21	180	-	-	-	-	-	-	-
250	250	-47	203	-	-	-	-	-	-	-	-	-
251	201	-36	165	10	175	-	-	-	-	-	-	-
252	234	-30	204	-70	134	-	-	-	-	-	-	-
253	214	-35	179	13	192	-	-	-	-	-	-	-
254	232	-26	206	17	223	-	-	-	-	-	-	-
255	206	-22	184	-34	150	-	-	-	-	-	-	-
256	236	-43	193	-4	189	-	-	-	-	-	-	-
257	178	-47	131	6	137	-	-	-	-	-	-	-
258	213	-40	173	18	191	-	-	-	-	-	-	-
259	181	-41	140	23	163	-	-	-	-	-	-	-
260	223	-62	161	29	190	-	-	-	-	-	-	-
261	219	-62	157	10	167	-	-	-	-	-	-	-
262	219	-17	202	15	217	-	-	-	-	-	-	-
263	182	-44	138	1	139	-	-	-	-	-	-	-
264	204	-55	149	-11	138	-	-	-	-	-	-	-
Mean	212	-41	172**	3	172**	-	-	-	-	-	-	-
SD	21	13	25	26	29	-	-	-	-	-	-	-

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 Northern Bobwhite Reproduction Study with PFOS
 (Control)*

Pen	W E E K S																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
201	11	13	12	12	12	12	12	13	12	13	17	18	19	22	18	21	20	21	21	22	17
202	11	12	12	13	13	12	12	14	12	12	14	16	16	18	18	12	14	16	18	16	15
203	13	16	14	13	13	13	12	16	16	15	17	18	20	20	13	13	12	14	14	14	13
204	12	13	12	13	13	12	12	14	13	12	13	15	16	18	17	17	19	20	18	20	17
205	12	14	12	12	12	12	12	14	13	13	14	14	14	16	16	17	18	20	20	22	19
206	13	14	14	13	13	13	13	14	14	14	13	14	16	18	18	18	18	20	19	21	20
207	12	13	13	13	13	12	12	15	15	14	12	21	20	24	24	23	19	12	11	16	16
208	11	12	13	13	12	12	12	13	13	13	16	17	17	19	20	21	20	21	19	19	19
209	12	14	13	13	13	13	12	16	14	15	15	16	17	20	21	22	21	20	19	21	20
210	12	13	13	13	12	12	12	15	13	13	14	16	18	20	20	20	20	20	20	20	19
211	12	13	13	13	12	12	11	14	13	14	15	16	19	21	20	20	21	21	21	22	20
212	12	15	13	13	12	12	12	15	14	14	17	18	19	22	21	21	21	21	21	21	18
213	13	14	14	14	14	14	14	16	16	17	17	17	19	21	20	21	21	21	21	22	22
214	14	14	13	13	13	13	12	13	14	14	17	17	19	20	20	22	21	22	20	19	19
215	13	14	13	13	13	13	12	16	14	15	15	18	22	23	23	22	23	23	22	23	20
216	14	16	14	15	14	14	13	16	14	16	17	17	21	21	21	22	22	21	22	23	21
Mean	12	14	13	13	13	12	12	15	14	14	15	17	18	20	19	20	19	19	19	20	19
SD	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	3	3	3	2

Appendix VIII - Table 2
Feed Consumption (g/bird/day) from a Northern Bobwhite Reproduction Study with PFOS
(10 PPM A.I.)

Pen	W E E K S																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
217	11	14	14	14	14	13	13	13	12	12	12	14	17	18	18	19	18	20	20	21	19
218	12	13	12	13	13	12	12	14	12	11	16	16	19	21	21	22	22	21	17	18	21
219	11	14	13	13	12	13	12	14	13	13	12	14	15	19	21	19	21	20	22	20	15
220	10	12	11	12	11	11	12	13	12	12	12	14	16	17	18	18	18	19	20	20	17
221	11	12	11	11	11	11	11	13	13	13	14	15	18	17	17	16	18	19	22	21	18
222	12	14	14	13	14	12	13	14	14	14	15	15	18	19	19	20	20	22	23	25	22
223	12	13	12	13	12	12	11	13	13	13	14	15	16	18	18	18	17	16	15	14	16
224	12	14	14	14	13	13	13	14	14	10	9	14	20	17	16	15	15	15	16	15	16
225	12	14	13	13	14	12	13	14	12	14	14	18	19	22	23	23	22	23	22	25	22
226	13	15	14	14	14	14	13	15	14	15	16	17	19	21	23	21	23	24	23	27	24
227	11	14	14	15	13	12	14	17	16	16	18	20	22	23	20	15	23	24	24	26	25
228	12	13	14	15	13	12	13	14	14	14	13	19	20	20	19	20	20	21	20	22	21
229	13	14	13	13	13	13	13	15	15	14	17	19	18	23	21	23	22	23	22	24	21
230	13	14	13	13	13	12	12	12	12	12	14	15	17	19	19	18	19	19	20	20	18
231	13	14	14	14	14	13	12	15	16	16	18	21	20	23	21	22	21	21	21	22	20
232	11	11	11	12	12	11	11	11	10	11	12	13	15	17	18	17	18	18	17	13	13
Mean	12	13	13	13	13	12	12	14	13	13	14	16	18	20	20	19	20	20	20	21	19
SD	1	1	1	1	1	1	1	1	2	2	3	2	2	2	2	3	2	3	3	4	3

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

Appendix VIII - Table 3
Feed Consumption (g/bird/day) from a Northern Bobwhite Reproduction Study with PFOS
(50 PPM A.I.)

Pen	W E E K S																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
233	12	9	10	10	12	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
234	10	12	9	9	8	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
235	11	10	9	9	10	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
236	11	10	9	9	8	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
237	11	12	10	12	10	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
238	11	8	9	9	10	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
239	13	12	11	12	11	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
240	11	10	9	9	10	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
241	14	12	13	12	11	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
242	11	11	9	9	8	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
243	11	13	12	12	12	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
244	11	9	10	8	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
245	10	10	10	10	11	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
246	11	10	10	10	7	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
247	13	11	11	11	8	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
248	11	10	10	11	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	11**	11**	10**	10**	9**	9**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SD	1	1	1	1	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Appendix VIII - Table 4
Feed Consumption (g/bird/day) from a Northern Bobwhite Reproduction Study with PFOS
(150 PPM A.I.)

Pen	W E E K S																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
249	8	8	9	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250	8	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
251	8	6	10	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
252	7	5	5	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
253	6	5	8	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
254	7	4	9	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
255	8	7	5	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
256	6	4	6	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
257	6	6	11	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
258	7	7	13	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
259	7	5	7	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
260	7	5	9	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
261	7	5	10	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
262	7	8	11	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
263	8	6	8	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
264	7	6	6	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	7**	6**	8**	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SD	1	1	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Appendix IX - Table 1
Individual Gross Pathological Observations
from a Northern Bobwhite 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
Feather loss	-	-	-	-	-	-	-	-	-	-	-	X	-	X	X	-
Intestinal tract - area of hyperemia	-	X	-	-	-	-	X	-	X	X	X	-	-	-	X	X
Testes - one or both small (~1.0-1.5 cm)	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-
Not remarkable	X	-	X	X	X	X	-	X	-	-	-	-	X	-	-	-

Females	Pens															
	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216
Feather loss	X	X	a	X	-	X	a	-	X	-	-	a	-	-	X	-
Foot/leg lesions	-	X	a	-	-	-	a	-	-	-	-	a	-	-	-	-
Back/vent lesions	-	-	a	-	-	X	a	-	X	-	-	a	-	-	-	-
Ceca - round worms found	X	-	a	X	-	-	a	-	-	-	-	a	-	-	-	X
Not remarkable	-	-	a	-	X	-	a	X	-	X	X	a	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 Northern Bobwhite 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
Feather loss	X	-	-	-	X	-	-	-	X	-	X	-	-	X	-	-
Intestinal tract - area of hyperemia	-	-	X	-	-	X	-	X	-	X	-	X	X	X	-	X
Ceca - round worms found	-	-	-	-	-	-	-	X	-	X	-	X	-	-	-	-
Testes - one or both small (~1.0-1.5 cm)	-	X	X	-	-	-	-	-	-	X	X	X	X	-	-	-
Not remarkable	-	-	-	X	-	-	X	-	-	-	-	-	-	-	X	-

Females	Pens															
	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232
Feather loss	-	-	-	X	X	X	X	a	X	a	X	-	X	X	X	a
Head lesions	-	-	X	-	-	-	-	a	-	a	-	-	-	-	-	a
Liver - pale and mottled	-	-	-	-	X	-	-	a	-	a	-	-	-	-	-	a
Abdominal cavity - slight egg yolk peritonitis	-	-	-	-	-	-	-	a	X	a	-	-	-	-	-	a
Intestinal tract - area of hyperemia	-	-	-	-	-	-	-	a	-	a	X	-	-	-	-	a
Not remarkable	X	X	-	-	-	-	-	a	-	a	-	X	-	-	-	a

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

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

Pen	Male	Female
	Liver	Liver
201	5.026	6.806
202	3.391	10.843
203	4.399	-
204	3.975	7.132
205	4.545	8.891
206	5.259	7.999
207	3.984	-
208	3.775	8.925
209	4.846	7.670
210	4.259	8.245
211	4.608	9.807
212	4.358	-
213	4.831	9.693
214	4.085	8.835
215	4.818	9.100
216	4.667	8.654
Mean	4.427	8.662
SD	0.496	1.113
- Data not reported due to mortality.		

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

Pen	Male	Female
	Liver	Liver
217	4.278	9.152
218	4.838	10.659
219	4.119	10.238
220	4.118	9.360
221	4.994	13.377
222	4.532	13.525
223	3.932	8.112
224	4.436	-
225	3.931	11.013
226	4.019	-
227	4.819	12.334
228	5.042	11.429
229	4.233	11.352
230	4.085	9.792
231	5.989	11.155
232	4.329	-
Mean	4.481	10.884 *
SD	0.545	1.596

- Data not reported due to mortality.
 * Statistically different from the control group at $p < 0.01$.

Appendix XI

Histopathology Report



EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

WILDLIFE INTERNATIONAL, LTD.
STUDY NUMBER 454-108
EPL PROJECT NUMBER 212-027

PFOS: A REPRODUCTION STUDY WITH THE
NORTHERN BOBWHITE

PATHOLOGY REPORT

Submitted by:

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

Submitted to:

Wildlife International, Ltd.
Easton, MD 21601

December 6, 2001

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PATHOLOGY SUMMARY

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

WILDLIFE INTERNATIONAL, LTD.
STUDY NUMBER 454-108
EPL PROJECT NUMBER 212-027

PFOS: A REPRODUCTION STUDY WITH
THE NORTHERN BOBWHITE

PATHOLOGY SUMMARY

Light microscopic examination was performed on sections of selected tissues from adult male and female Northern bobwhite quail (*Colinus virginianus*) 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-old offspring of the adult bobwhite were also examined by light microscopy. The objective of this study is to evaluate the effects upon adult bobwhite (*Colinus virginianus*) 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	6	4
10 ppm a.i.	20	19	5	5

^aAdults were euthanized after receiving the test substance in the diet for approximately 21 weeks.

^bOffspring did not receive the test substance in the diet. All offspring were euthanized on Day 14 post-hatching.



EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

Wildlife International, Ltd. Study Number 454-108

MATERIALS AND METHODS

At the completion of the study interval, all adult bobwhite were euthanized and necropsies were performed by Wildlife International, Ltd. Selected offspring were also euthanized at 14 days of age. 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 embedded in paraffin and made into hematoxylin and eosin-stained sections on glass microscope slides. The following tissues from adult and offspring 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 bobwhite 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 entries in these tables were transcribed from the gross necropsy observations provided by Wildlife International, Ltd.



EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

Wildlife International, Ltd. Study Number 454-108

RESULTS

Except for three control (334, 342, 352) and two 10 ppm (384, 388) adult females, all adults and offspring survived to the end of their respective study intervals. No effects considered possibly related to test article (PFOS) administration were noted in liver, kidney, brain, and proventriculus of adult and offspring males and females, bursa of Fabricius in offspring males and females, adipose tissue of adult 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 or adipose tissue in male and female offspring could not be determined since none (bursa of Fabricius) or only one (adipose tissue) sample was submitted for evaluation. The bursa of Fabricius typically regresses prior to sexual maturity. In addition, any potential test-article effects on the gallbladder from treated adult males and females could not be definitively determined due to the extensive autolysis that precluded any accurate diagnosis. There were no apparent test-article related effects noted on the gallbladders from the offspring.

Testes of all adult males exhibited normal spermatogenesis, characterized by the presence of numerous maturing or mature spermatozoa in the seminiferous tubules. In one (1) control and seven (7) treated males, the testes were observed macroscopically as being smaller in size (< 1.5 cm) when compared to other birds. In these birds, spermatogenesis was evident microscopically and there was no definitive difference in the morphology of the testes when compared to their unaffected cohorts. Therefore, no definitive microscopic changes could be associated with the size reduction. However, the reduced size of the testes of these birds was consistent with early post-reproductive phase regression, a normal physiological phenomenon. The increased incidence of reduced size of the testes in 10 ppm a.i. males when



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compared to controls, suggests that treatment may have accelerated the normal post-reproductive phase regression.

Testes of all offspring were listed as immature because they lacked all evidence of spermatogenesis, had small seminiferous tubules with absent or narrow lumens, and germinal epithelium with decreased cell layers (compared to adult testes). This morphology is consistent with normal physiological immaturity expected in 14 day-old birds. Because of the smaller size of the seminiferous tubules in the offspring testes, the melanin pigment-bearing cells normally present in the testicular interstitium were, in effect, more closely packed in a smaller area rather than being widely separated by larger seminiferous tubules (thus the microscopic diagnosis of "pigment concentration" in the testis).

Ovaries of adult females exhibited normal oogenesis, characterized by numerous ova in various stages of follicular development. 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.

Several additional microscopic findings (described below) were noted in adult and/or offspring bobwhite quail from control and treated groups. Generally, in both adult and offspring males and females, the incidence of these lesions 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 10 ppm a.i. treated birds when compared to controls. For these reasons, these findings were considered incidental and unrelated to test article (PFOS) administration.



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Wildlife International, Ltd. Study Number 454-108

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. Mineralization in the kidneys of both control and treated adult males was characterized by the presence of basophilic material in the cortex. In most cases this material appeared to be associated with the glomerulus.

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 and females, when compared to controls, there was no significant difference in the mean severity between the groups. Since this finding was also 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 birds was considered to be due to biological and/or distributional variability.

CONCLUSIONS AND SUMMARY

No lesions considered possibly related to test article (PFOS) administration were noted in liver, kidney, brain, and proventriculus of adult and offspring males and females, bursa of Fabricius in offspring males and females, adipose tissue of adult males and females, ovaries of adult and offspring

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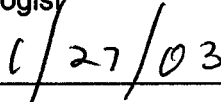
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Wildlife International, Ltd. Study Number 454-108

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, not accompanied by any morphological change in spermatogenesis, 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 or adipose tissue in male and female offspring could not be determined since none (bursa of Fabricius) or only one (adipose tissue) sample was submitted for evaluation. In addition, any potential test-article effects on the gallbladder from treated adult males and females 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 bobwhite from control and 10 ppm a.i. treated groups were considered incidental and unrelated to test article (PFOS) administration.



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



Date

FAR/lcp

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COMPLIANCE STATEMENT

Client Name Wildlife International, Ltd. EPL Project Coordinator Dr. Margarita M. Gruebbel

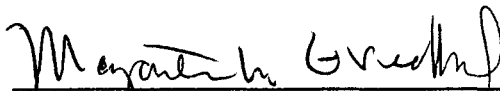
Client Study 454-108 EPL Pathologist Dr. Frederick A. Ruecker

Species Northern Bobwhite EPL Project Number 212-027

Study Title PFOS: A Reproduction Study with the Northern Bobwhite

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.


EPL Project Coordinator

Jan 28, 2003

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: PFOS: A Reproduction Study with the Northern Bobwhite

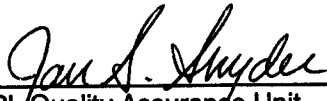
Client Study: 454-108 EPL Project Coordinator: Dr. Margarita M. Gruebbel

EPL Project Number: 212-027 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	10/11,15/01	10/11,15/01
Project Setup	10/12/01	10/12/01
Histology Setup	10/19/01	10/19/01
Data Review	11/1,2,5/01	11/1,2,5/01
Draft Report	11/29/01	11/29/01
Final Report	1/28/03	1/28/03

Date reported to Study Director/Management 1/28/03Date of last quarterly facility inspection 11/02


 EPL Quality Assurance Unit

1/28/03
 Date

SUMMARY INCIDENCE TABLES
ADULTS

Male Northern Bobwhite

SUMMARY INCIDENCE TABLE

[illegible]

E P L

Experimental Pathology Laboratories, Inc.

454-108

Adult Sacrifice

Female Northern Bobwhite

SUMMARY INCIDENCE TABLE

[illegible]

EPL

Experimental Pathology Laboratories, Inc.

HISTOPATHOLOGY INCIDENCE TABLES
ADULTS

HISTOPATHOLOGY INCIDENCE TABLE

454-108
Adult Sacrifice
Male Northern Bobwhite

[illegible]

II-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

HISTOPATHOLOGY INCIDENCE TABLE

		GROUP 10-ppmai																				
454-108 Adult Sacrifice Male Northern Bobwhite		A N I M A L	3 6 9	3 7 1	3 7 3	3 7 5	3 7 7	3 7 9	3 8 1	3 8 3	3 8 5	3 8 7	3 8 9	3 9 1	3 9 3	3 9 5	3 9 7	3 9 9	4 0 1	4 0 3	4 0 5	4 0 7
ADIPOSE TISSUE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Inflammation, Chronic																						
BRAIN			X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Inflammation, Meninges,																						
Granulomatous																						
Mineralizing Granuloma,																						
Meninges					P			P														
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	X	A	A	X	A
Autolysis Precludes Diagnosis			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P		P	P		P
KIDNEY			X	X		X	X	X		X	X	X		X						X	X	X
Infiltrate, Mononuclear Cell					1				1				1	1		1						
Inflammation, Chronic																						
Mineralization								1									1	1	3			
LIVER																						
Degeneration, Hepatocellular,																						
Centrilobular																						
Infiltrate, Mononuclear Cell								1	1					1	1		1	1		2		
Necrosis, Hepatocellular																						
Vacuolization, Hepatocellular			1	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	2	2	1
PROVENTRICULUS			X	X	X	X	X	X	X		X	X	X		X	X	X	X	X	X	X	X
Infiltrate, Mononuclear Cell											1				1							
Inflammation, Serosa																						
TESTIS			Xm	Xm	Xm	Xm	Xm	Xm	Xm	Xm	Xm	Xm	Xm	Xm	m	Xm	Xm	Xm	Xm	Xm	Xm	m
Infiltrate, Mononuclear Cell																						1
Reduced Spermatogenesis																3						

II-2

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

454-108
Adult Sacrifice
Female Northern Bobwhite

[illegible]

II-3

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

[illegible]

EPL

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

**CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
ADULTS**

454-108

Adult Sacrifice

Species: Northern Bobwhite

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Sex: Males

Group Identification: CONTROL - Sacrificed

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
345	TESTIS	Right testis approximately 1.5 cm	No Correlating Lesion

III-1

Adult Sacrifice

Sex: Males

Group Identification: 10-ppmai - Sacrificed

[illegible]

Adult Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Northern Bobwhite

Sex: Females

Group Identification: CONTROL - Unscheduled Sac./Death

[illegible]

454-108

Adult Sacrifice

Species: Northern Bobwhite

Sex: Females

Group Identification: 10-ppmai - Sacrificed

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
378	LIVER	Pale, mottled	Vacuolization, Hepatocellular
408	LIVER	Pale, mottled	Vacuolization, Hepatocellular

Adult Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Northern Bobwhite

Sex: Females

Group Identification: 10-ppmai - Unscheduled Sac./Death

[illegible]

**SUMMARY INCIDENCE TABLES
OFFSPRING**

454-108
Offspring Sacrifice
Male Northern Bobwhite

SUMMARY INCIDENCE TABLE

[illegible]

EPL

Experimental Pathology Laboratories, Inc.

IV-1

454-108
Offspring Sacrifice
Female Northern Bobwhite

SUMMARY INCIDENCE TABLE

[illegible]

EPL

Experimental Pathology Laboratories, Inc.

IV-2

HISTOPATHOLOGY INCIDENCE TABLES
OFFSPRING

HISTOPATHOLOGY INCIDENCE TABLE

[illegible]

V-1

E P L

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

454-108
Offspring Sacrifice
Female Northern Bobwhite

[illegible]

V-2

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 Red Blood Cells and Sera Collected through Week 15

Exygen Study No.: 023-066

Table XXVI. Summary of PFOS in Quail 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)
454-108-361-Wk5	0107077	051801A	5/18/01	5/18-19/01	NQ
454-108-361-Wk5*	0107077	051801A	5/18/01	5/18-19/01	NQ
454-108-362-Wk5	0107078	051801A	5/18/01	5/18-19/01	NQ
454-108-362-Wk5*	0107078	051801A	5/18/01	5/18-19/01	NQ
454-108-363-Wk5	0107079	051801A	5/18/01	5/18-19/01	NQ
454-108-363-Wk5*	0107079	051801A	5/18/01	5/18-19/01	NQ
454-108-364-Wk5	0107080	051801A	5/18/01	5/18-19/01	NQ
454-108-364-Wk5*	0107080	051801A	5/18/01	5/18-19/01	NQ
454-108-365-Wk5	0107081	051801A	5/18/01	5/18-19/01	NQ
454-108-365-Wk5*	0107081	051801A	5/18/01	5/18-19/01	NQ
454-108-366-Wk5	0107082	051801A	5/18/01	5/18-19/01	0.00292
454-108-366-Wk5*	0107082	051801A	5/18/01	5/18-19/01	0.00264
454-108-368-Wk5	0107083	051801A	5/18/01	5/18-19/01	NQ
454-108-368-Wk5*	0107083	051801A	5/18/01	5/18-19/01	NQ
AVERAGE:					0.000440
STANDARD DEVIATION:					0.000993

* 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 XXVII. Summary of PFOS in Quail 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)
454-108-401-Wk5	0107084	051801DR	5/18/01	5/21-22/01	113
454-108-401-Wk5*	0107084	051801DR	5/18/01	5/21-22/01	107
454-108-402-Wk5	0107085	051801DRD	5/18/01	5/22/01	353
454-108-402-Wk5*	0107085	051801DRD	5/18/01	5/22/01	351
454-108-403-Wk5	0107086	051801DR	5/18/01	5/21-22/01	30.5
454-108-403-Wk5*	0107086	051801DR	5/18/01	5/21-22/01	30.4
454-108-404-Wk5	0107087	051801DR	5/18/01	5/21-22/01	26.9
454-108-404-Wk5*	0107087	051801DR	5/18/01	5/21-22/01	25.8
454-108-405-Wk5	0107088	051801DRD	5/18/01	5/22/01	415
454-108-405-Wk5*	0107088	051801DRD	5/18/01	5/22/01	447
454-108-406-Wk5	0107089	051801DRD	5/18/01	5/22/01	1050
454-108-406-Wk5*	0107089	051801DRD	5/18/01	5/22/01	1040
454-108-407-Wk5	0107090	051801DRD	5/18/01	5/22/01	495
454-108-407-Wk5*	0107090	051801DRD	5/18/01	5/22/01	501
454-108-408-Wk5	0107091	051801DRD	5/18/01	5/22/01	451
454-108-408-Wk5*	0107091	051801DRD	5/18/01	5/22/01	471
AVERAGE:					369
STANDARD DEVIATION:					324

* Duplicate injection

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

Exygen Study No.: 023-066

Table XXVIII. Summary of PFOS in Quail 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)
454-108-441-Wk5	0107092	052201CD	5/22/01	5/24/01	166
454-108-441-Wk5*	0107092	052201CD	5/22/01	5/24/01	163
454-108-442-Wk5	0107093	052201CD	5/22/01	5/24/01	108
454-108-442-Wk5*	0107093	052201CD	5/22/01	5/24/01	103
AVERAGE:					135
STANDARD DEVIATION:					34

Table XXIX. Summary of PFOS in Quail 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)
454-108-361-Wk10	0107100	051801B	5/18/01	5/19/01	NQ
454-108-361-Wk10*	0107100	051801B	5/18/01	5/19/01	NQ
454-108-362-Wk10	0107101	051801B	5/18/01	5/19/01	NQ
454-108-362-Wk10*	0107101	051801B	5/18/01	5/19/01	NQ
454-108-363-Wk10	0107102	051801B	5/18/01	5/19/01	NQ
454-108-363-Wk10*	0107102	051801B	5/18/01	5/19/01	NQ
454-108-364-Wk10	0107103	051801B	5/18/01	5/19/01	NQ
454-108-364-Wk10*	0107103	051801B	5/18/01	5/19/01	NQ
454-108-365-Wk10	0107104	051801B	5/18/01	5/19/01	NQ
454-108-365-Wk10*	0107104	051801B	5/18/01	5/19/01	NQ
454-108-366-Wk10	0107105	051801B	5/18/01	5/19/01	NQ
454-108-366-Wk10*	0107105	051801B	5/18/01	5/19/01	NQ
454-108-367-Wk10	0107106	051801B	5/18/01	5/19/01	NQ
454-108-367-Wk10*	0107106	051801B	5/18/01	5/19/01	NQ
454-108-368-Wk10	0107107	051801B	5/18/01	5/19/01	NQ
454-108-368-Wk10*	0107107	051801B	5/18/01	5/19/01	NQ
AVERAGE:					NQ
STANDARD DEVIATION:					NQ

* 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 XXX. Summary of PFOS in Quail 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)
454-108-401-Wk10	0107108	051801DRD	5/18/01	5/22/01	672
454-108-401-Wk10*	0107108	051801DRD	5/18/01	5/22/01	671
454-108-402-Wk10	0107109	051801DRD	5/18/01	5/22/01	203
454-108-402-Wk10*	0107109	051801DRD	5/18/01	5/22/01	190
454-108-403-Wk10	0107110	051801DR	5/18/01	5/21-22/01	23.0
454-108-403-Wk10*	0107110	051801DR	5/18/01	5/21-22/01	20.7
454-108-404-Wk10	0107111	051801DR	5/18/01	5/21-22/01	19.0
454-108-404-Wk10*	0107111	051801DR	5/18/01	5/21-22/01	21.2
454-108-405-Wk10	0107112	051801DR	5/18/01	5/21-22/01	18.0
454-108-405-Wk10*	0107112	051801DR	5/18/01	5/21-22/01	20.0
454-108-406-Wk10	0107113	051801DR	5/18/01	5/21-22/01	48.9
454-108-406-Wk10*	0107113	051801DR	5/18/01	5/21-22/01	50.5
454-108-407-Wk10	0107114	051801DR	5/18/01	5/21-22/01	22.8
454-108-407-Wk10*	0107114	051801DR	5/18/01	5/21-22/01	22.3
454-108-408-Wk10	0107115	051801DR	5/18/01	5/21-22/01	30.4
454-108-408-Wk10*	0107115	051801DR	5/18/01	5/21-22/01	30.2
AVERAGE:					129
STANDARD DEVIATION:					220

* Duplicate injection

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

Exygen Study No.: 023-066

Table XXXI. Summary of PFOS in Quail 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)
454-108-361-Wk15	0107116	052101A	5/21/01	5/22/01	NQ
454-108-361-Wk15*	0107116	052101A	5/21/01	5/22/01	NQ
454-108-362-Wk15	0107117	052101A	5/21/01	5/22/01	NQ
454-108-362-Wk15*	0107117	052101A	5/21/01	5/22/01	NQ
454-108-363-Wk15	0107118	052101A	5/21/01	5/22/01	0.00226
454-108-363-Wk15*	0107118	052101A	5/21/01	5/22/01	0.00328
454-108-364-Wk15	0107119	052101A	5/21/01	5/22/01	0.00127
454-108-364-Wk15*	0107119	052101A	5/21/01	5/22/01	NQ
454-108-365-Wk15	0107120	052101A	5/21/01	5/22/01	0.00705
454-108-365-Wk15*	0107120	052101A	5/21/01	5/22/01	0.00391
454-108-366-Wk15	0107121	052101A	5/21/01	5/22/01	NQ
454-108-366-Wk15*	0107121	052101A	5/21/01	5/22/01	NQ
454-108-367-Wk15	0107122	052101A	5/21/01	5/22/01	0.00795
454-108-367-Wk15*	0107122	052101A	5/21/01	5/22/01	0.00816
454-108-368-Wk15	0107123	052101A	5/21/01	5/22/01	NQ
454-108-368-Wk15*	0107123	052101A	5/21/01	5/22/01	NQ
454-108-333-Wk15	0107124	052101A	5/21/01	5/22/01	0.00130
454-108-333-Wk15*	0107124	052101A	5/21/01	5/22/01	NQ
AVERAGE:					0.00198
STANDARD DEVIATION:					0.00304

* 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 XXXII. Summary of PFOS in Quail 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)
454-108-401-Wk15	0107125	052101AD	5/21/01	5/22-23/01	72.2
454-108-401-Wk15*	0107125	052101AD	5/21/01	5/22-23/01	69.1
454-108-402-Wk15	0107126	052101AD	5/21/01	5/22-23/01	2.36
454-108-402-Wk15*	0107126	052101AD	5/21/01	5/22-23/01	1.65
454-108-403-Wk15	0107127	052101AD	5/21/01	5/22-23/01	21.2
454-108-403-Wk15*	0107127	052101AD	5/21/01	5/22-23/01	18.8
454-108-404-Wk15	0107128	052101AD	5/21/01	5/22-23/01	1.30
454-108-404-Wk15*	0107128	052101AD	5/21/01	5/22-23/01	1.22
454-108-405-Wk15	0107129	052101AD	5/21/01	5/22-23/01	15.8
454-108-405-Wk15*	0107129	052101AD	5/21/01	5/22-23/01	17.9
454-108-406-Wk15	0107130	052101AD	5/21/01	5/22-23/01	3.61
454-108-406-Wk15*	0107130	052101AD	5/21/01	5/22-23/01	3.23
454-108-407-Wk15	0107131	052101AD	5/21/01	5/22-23/01	13.3
454-108-407-Wk15*	0107131	052101AD	5/21/01	5/22-23/01	9.60
454-108-408-Wk15	0107132	052201CD	5/21/01	5/24/01	1.24
454-108-408-Wk15*	0107132	052201CD	5/21/01	5/24/01	1.55
454-108-383-Wk15	0107133	052101AD	5/21/01	5/22-23/01	56.7
454-108-383-Wk15*	0107133	052101AD	5/21/01	5/22-23/01	54.1
AVERAGE:					20.3
STANDARD DEVIATION:					24.8

Table XXXIII. Summary of PFOS in Quail 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)
454-108-409-TERM	0107134	052201CD	5/22/01	5/24/01	361
454-108-409-TERM*	0107134	052201CD	5/22/01	5/24/01	356
454-108-410-TERM	0107135	052201CD	5/22/01	5/24/01	106
454-108-410-TERM*	0107135	052201CD	5/22/01	5/24/01	114
AVERAGE:					234
STANDARD DEVIATION:					144

* Duplicate injection

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

Exygen Study No.: 023-066

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

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk5	0107154	072301AR	7/23/01	7/25/01	0.0282
454-108-361-Wk5*	0107154	072301AR	7/23/01	7/25/01	0.0295
454-108-362-Wk5	0107155	072301AR	7/23/01	7/25/01	0.0149
454-108-362-Wk5*	0107155	072301AR	7/23/01	7/25/01	0.0134
454-108-363-Wk5	0107156	072301AR	7/23/01	7/25/01	0.0198
454-108-363-Wk5*	0107156	072301AR	7/23/01	7/25/01	0.0166
454-108-364-Wk5	0107157	072301AR	7/23/01	7/25/01	0.00510
454-108-364-Wk5*	0107157	072301AR	7/23/01	7/25/01	0.00535
454-108-366-Wk5	0107159	072301AR	7/23/01	7/25/01	0.0135
454-108-366-Wk5*	0107159	072301AR	7/23/01	7/25/01	0.0120
454-108-368-Wk5	0107160	072301AR	7/23/01	7/25/01	0.0138
454-108-368-Wk5*	0107160	072301AR	7/23/01	7/25/01	0.0156
AVERAGE:					0.0156
STANDARD DEVIATION:					0.00746

* Duplicate injection

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

Exygen Study No.: 023-066

**Table XXXV. Summary of PFOS in Quail Serum Samples at 10 ppm
a.i. for Week 5**

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-402-Wk5	0107162	072301AD	7/23/01	7/26/01	45.8
454-108-402-Wk5*	0107162	072301AD	7/23/01	7/26/01	45.6
454-108-403-Wk5	0107163	072301AD	7/23/01	7/26/01	115
454-108-403-Wk5*	0107163	072301AD	7/23/01	7/26/01	128
454-108-404-Wk5	0107164	072301AD	7/23/01	7/26/01	104
454-108-404-Wk5*	0107164	072301AD	7/23/01	7/26/01	98.3
454-108-405-Wk5	0107165	072301AD	7/23/01	7/26/01	138
454-108-405-Wk5*	0107165	072301AD	7/23/01	7/26/01	129
454-108-406-Wk5	0107166	072301AD	7/23/01	7/26/01	106
454-108-406-Wk5*	0107166	072301AD	7/23/01	7/26/01	107
454-108-407-Wk5	0107167	072301AD	7/23/01	7/26/01	42.4
454-108-407-Wk5*	0107167	072301AD	7/23/01	7/26/01	36.3
454-108-408-Wk5	0107168	072301AD	7/23/01	7/26/01	77.6
454-108-408-Wk5*	0107168	072301AD	7/23/01	7/26/01	84.4
AVERAGE:					89.8
STANDARD DEVIATION:					35.0

**Table XXXVI. Summary of PFOS in Quail Serum Samples at 50 ppm
a.i. for Week 5**

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-441-Wk5	0107092	072301AD	7/23/01	7/26/01	551
454-108-441-Wk5*	0107092	072301AD	7/23/01	7/26/01	562
454-108-442-Wk5	0107093	072301AD	7/23/01	7/26/01	529
454-108-442-Wk5*	0107093	072301AD	7/23/01	7/26/01	520
AVERAGE:					541
STANDARD DEVIATION:					19.4

* Duplicate injection

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

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

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk10	0107177	072301B	7/23/01	7/24/01	0.00483
454-108-361-Wk10*	0107177	072301B	7/23/01	7/24/01	0.00489
454-108-362-Wk10	0107178	072301B	7/23/01	7/24/01	0.00295
454-108-362-Wk10*	0107178	072301B	7/23/01	7/24/01	0.00249
454-108-363-Wk10	0107179	072301B	7/23/01	7/24/01	0.00467
454-108-363-Wk10*	0107179	072301B	7/23/01	7/24/01	0.00521
454-108-364-Wk10	0107180	072301B	7/23/01	7/24/01	0.00390
454-108-364-Wk10*	0107180	072301B	7/23/01	7/24/01	0.00375
454-108-365-Wk10	0107181	072301B	7/23/01	7/24/01	0.00271
454-108-365-Wk10*	0107181	072301B	7/23/01	7/24/01	0.00292
454-108-366-Wk10	0107182	072301B	7/23/01	7/24/01	0.00439
454-108-366-Wk10*	0107182	072301B	7/23/01	7/24/01	0.00437
454-108-367-Wk10	0107183	072301B	7/23/01	7/24/01	0.00563
454-108-367-Wk10*	0107183	072301B	7/23/01	7/24/01	0.00542
454-108-368-Wk10	0107184	072301B	7/23/01	7/24/01	0.00537
454-108-368-Wk10*	0107184	072301B	7/23/01	7/24/01	0.00526
AVERAGE:					0.00430
STANDARD DEVIATION:					0.00105

* Duplicate injection

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

Exygen Study No.: 023-066

**Table XXXVIII. Summary of PFOS in Quail Serum Samples at 10 ppm
a.i. for Week 10**

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-401-Wk10	0107185	072301DD	7/23/01	7/27-28/01	213
454-108-401-Wk10*	0107185	072301DD	7/23/01	7/27-28/01	207
454-108-402-Wk10	0107186	072301DD	7/23/01	7/27-28/01	53.6
454-108-402-Wk10*	0107186	072301DD	7/23/01	7/27-28/01	53.7
454-108-403-Wk10	0107187	072301DD	7/23/01	7/27-28/01	148
454-108-403-Wk10*	0107187	072301DD	7/23/01	7/27-28/01	177
454-108-404-Wk10	0107188	072301D	7/23/01	7/27-28/01	19.4
454-108-404-Wk10*	0107188	072301D	7/23/01	7/27-28/01	20.3
454-108-405-Wk10	0107189	072301DD	7/23/01	7/27-28/01	157
454-108-405-Wk10*	0107189	072301DD	7/23/01	7/27-28/01	147
454-108-407-Wk10	0107190	072301DD	7/23/01	7/27-28/01	105
454-108-407-Wk10*	0107190	072301DD	7/23/01	7/27-28/01	110
454-108-408-Wk10	0107191	072301DD	7/23/01	7/27-28/01	97.0
454-108-408-Wk10*	0107191	072301DD	7/23/01	7/27-28/01	93.7
AVERAGE:					114
STANDARD DEVIATION:					63.4

* Duplicate injection

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

Exygen Study No.: 023-066

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

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk15	0107192	072301C	7/23/01	7/24-25/01	NQ
454-108-361-Wk15*	0107192	072301C	7/23/01	7/24-25/01	NQ
454-108-362-Wk15	0107193	072301C	7/23/01	7/24-25/01	NQ
454-108-362-Wk15*	0107193	072301C	7/23/01	7/24-25/01	NQ
454-108-363-Wk15	0107194	072301C	7/23/01	7/24-25/01	0.00154
454-108-363-Wk15*	0107194	072301C	7/23/01	7/24-25/01	0.00160
454-108-364-Wk15	0107195	072301C	7/23/01	7/24-25/01	NQ
454-108-364-Wk15*	0107195	072301C	7/23/01	7/24-25/01	NQ
454-108-365-Wk15	0107196	072301C	7/23/01	7/24-25/01	NQ
454-108-365-Wk15*	0107196	072301C	7/23/01	7/24-25/01	NQ
454-108-366-Wk15	0107197	072301C	7/23/01	7/24-25/01	NQ
454-108-366-Wk15*	0107197	072301C	7/23/01	7/24-25/01	NQ
454-108-367-Wk15	0107198	072301C	7/23/01	7/24-25/01	0.00227
454-108-367-Wk15*	0107198	072301C	7/23/01	7/24-25/01	0.00249
454-108-368-Wk15	0107199	072301C	7/23/01	7/24-25/01	0.00253
454-108-368-Wk15*	0107199	072301C	7/23/01	7/24-25/01	0.00255
454-108-333-Wk15	0107200	072301C	7/23/01	7/24-25/01	0.00162
454-108-333-Wk15*	0107200	072301C	7/23/01	7/24-25/01	0.00160
AVERAGE:					0.000928
STANDARD DEVIATION:					0.00105

* 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) and for ND, zero was used to calculate the average and standard deviation.

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

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-401-Wk15	0107201	072301DD	7/23/01	7/27-28/01	181
454-108-401-Wk15*	0107201	072301DD	7/23/01	7/27-28/01	180
454-108-402-Wk15	0107202	072301DD	7/23/01	7/27-28/01	8.21
454-108-402-Wk15*	0107202	072301DD	7/23/01	7/27-28/01	8.45
454-108-403-Wk15	0107203	072301DD	7/23/01	7/27-28/01	246
454-108-403-Wk15*	0107203	072301DD	7/23/01	7/27-28/01	239
454-108-404-Wk15	0107204	072301DD	7/23/01	7/27-28/01	11.1
454-108-404-Wk15*	0107204	072301DD	7/23/01	7/27-28/01	10.7
454-108-405-Wk15	0107205	072301DD	7/23/01	7/27-28/01	141
454-108-405-Wk15*	0107205	072301DD	7/23/01	7/27-28/01	156
454-108-406-Wk15	0107206	072301DD	7/23/01	7/27-28/01	7.04
454-108-406-Wk15*	0107206	072301DD	7/23/01	7/27-28/01	6.98
454-108-407-Wk15	0107207	072301DD	7/23/01	7/27-28/01	107
454-108-407-Wk15*	0107207	072301DD	7/23/01	7/27-28/01	114
454-108-408-Wk15	0107208	072301DD	7/23/01	7/27-28/01	10.2
454-108-408-Wk15*	0107208	072301DD	7/23/01	7/27-28/01	8.91
454-108-383-Wk15	0107209	072301DD	7/23/01	7/27-28/01	131
454-108-383-Wk15*	0107209	072301DD	7/23/01	7/27-28/01	143
AVERAGE:					95.0
STANDARD DEVIATION:					86.6

Table XLI. Summary of PFOS in Quail Serum Samples at 50 ppm a.i. for TERM

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-410-TERM	0107211	072301DD	7/23/01	7/27-28/01	476
454-108-410-TERM*	0107211	072301DD	7/23/01	7/27-28/01	514
AVERAGE:					495
STANDARD DEVIATION:					26.9

* 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 Samples Collected at Termination

Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

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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 bobwhite quail and their offspring in both a control group and a 10 ppm, dosed group from Wildlife feeding study #454-108. The following tables summarize the $C_8F_{17}SO_3^-$ concentrations found in the liver and sera of study samples. These results only relate to the samples tested. Matrix-extracted PFOS standards were used to establish the HPLC-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	19	NA	<400 ng/mL (19 of 19)	20	<50.2 ng/g*	<50.2 ng/g (19 of 20)* 208 ng/g (1 of 20)*
Female Control Group	17	NA	<400 ng/mL (16 of 17) 2110 ng/mL (1 of 17)	20	<50.2 ng/g*	<50.2 ng/g (20 of 20)*
Male 10ppm Group	20	141000 ng/mL	94400 - 226000 ng/mL	20	88500 ng/g*	50100 - 147000 ng/g*
Female 10ppm Group	16	9990 ng/mL	4710 - 31900 ng/mL	20	4900 ng/g*	3290 - 7550 ng/g*
Male Offspring Control Group	6	NA	<400 ng/mL (6 of 6)	6	<20.0 ng/g*	<20.0 ng/g (6 of 6)*
Female Offspring Control Group	4	NA	<400 ng/mL (4 of 4)	4	<20.0 ng/g*	<20.0 ng/g (4 of 4)*
Male Offspring 10ppm Group	5	12600 ng/mL	8220 - 19200 ng/mL	5	7350 ng/g*	4940 - 13700 ng/g*
Female Offspring 10ppm Group	5	12400 ng/mL	7640 - 18500 ng/mL	5	7180 ng/g*	4590 - 13900 ng/g*

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

Accuracy: Sample results are accurate to 100% +/- 30%.

Sera QC samples met the acceptance criteria for the method.

* Liver QC samples did not meet the 1/2 of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

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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	19*	*	*	19	<50.2 ng/g**	<50.2 ng/g (19 of 19)**
Female Control Group	16	<400 ng/mL	<400 ng/mL (16 of 16)	20*	*	*
Male 10ppm Group	20*	*	*	20*	*	*
Female 10ppm Group	17	8700 ng/mL	4710 - 14700 ng/mL	20*	*	*
Male Offspring Control Group	6*	*	*	6*	*	*
Female Offspring Control Group	4*	*	*	4*	*	*
Male Offspring 10ppm Group	5*	*	*	4	5760 ng/g**	4940 - 6520 ng/g**
Female Offspring 10ppm Group	5*	*	*	4	5490 ng/g**	4590 - 7030 ng/g**

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

Accuracy: Sample results are accurate to 100% +/- 30%.

Sera QC samples met the acceptance criteria for the method.

** Liver QC samples did not meet the 1% of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

PURPOSE

The purpose of this analytical phase of the study is to quantify $C_8F_{17}SO_3^-$ in the liver and sera of both control and dosed Northern Bobwhite Quail and 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	88.9%	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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Table 10. $C_8F_{17}SO_3^-$ Data Summary, Female Quail Sera (Adult and Offspring)

3M Sample Number	Sample Description	PFOS Concentration (ppm)	Number of Sera	Conc. of $C_8F_{17}SO_3^-$ in Sample (µg/mL of Sera)	Average of 3 Samples
E01-1245-28444	454-108-330, Oppm female, adult, 01-31-02	0.4 0.4	1 1	<400 <400	<400
E01-1245-28446	454-108-332, Oppm female, adult, 01-31-02	0.05 0.05	1 1	<400 <400	<400
E01-1245-28449	454-108-336, Oppm female, adult, 01-31-02	0.3 0.3	1 1	<400 <400	<400
E01-1245-28451	454-108-338, Oppm female, adult, 01-31-02	0.3 0.3	1 1	<400 <400	<400
E01-1245-28453	454-108-340, Oppm female, adult, 01-31-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28456	454-108-344, Oppm female, adult, 01-31-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28458	454-108-348, Oppm female, adult, 02-01-02	0.1 0.1	1 1	<400 <400	<400
E01-1245-28460	454-108-348, Oppm female, adult, 02-01-02	0.3 0.3	1 1	<400 <400	<400
E01-1245-28462	454-108-350, Oppm female, adult, 02-01-02	0.4 0.4	1 1	<400 <400	<400
E01-1245-28464	454-108-352, Oppm female, adult, 02-01-02	0.4 0.4	1 1	<400 <400	<400
E01-1245-28466	454-108-354, Oppm female, adult, 02-01-02	0.3 0.3	1 1	<400 <400	<400
E01-1245-28468	454-108-356, Oppm female, adult, 02-01-02	0.3 0.3	1 1	<400 <400	<400
E01-1245-28470	454-108-358, Oppm female, adult, 02-01-02	0.3 0.3	1 1	<400 <400	<400
E01-1245-28472	454-108-360, Oppm female, adult, 02-01-02	0.3 0.3	1 1	2050 2180	2110 *
E01-1245-28474	454-108-362, Oppm female, adult, 02-01-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28477	454-108-366, Oppm female, adult, 02-01-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28479	454-108-368, Oppm female, adult, 02-01-02	0.3 0.3	1 1	<400 <400	<400
E01-1245-28481	454-108-370, 10ppm female, adult, 02-01-02	0.3	1	9170	
E01-1245-28483	454-108-372, 10ppm female, adult, 02-01-02	0.4	1	9140	
E01-1245-28485	454-108-374, 10ppm female, adult, 02-01-02	0.2	1	8400	
E01-1245-28487	454-108-376, 10ppm female, adult, 02-01-02	0.3	1	9280	
E01-1245-28489	454-108-378, 10ppm female, adult, 02-01-02	0.1	1	4980	
E01-1245-28491	454-108-380, 10ppm female, adult, 02-01-02	0.1	1	5790	

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U.S. Fish & Wildlife Service	Sample Description	Concentration (ppm)	Number of Samples	Concentration (ppm) C.F. 50.0 Sample (Average of Sample)	Average of Sample
E01-1245-28493	454-108-382, 10ppm female, adult, 02-01-02	0.3	10	31900 *	
E01-1245-28496	454-108-386, 10ppm female, adult, 02-01-02	0.4	1	14700	
E01-1245-28498	454-108-388, 10ppm female, adult, 02-01-02	0.3	1	11600	
E01-1245-28500	454-108-390, 10ppm female, adult, 02-01-02	0.2	1	8310	
E01-1245-28502	454-108-392, 10ppm female, adult, 02-01-02	0.3	1	9400	
E01-1245-28504	454-108-394, 10ppm female, adult, 02-01-02	0.3	1	11400	
E01-1245-28506	454-108-396, 10ppm female, adult, 02-01-02	0.09	1	4710	
E01-1245-28508	454-108-398, 10ppm female, adult, 02-01-02	0.1	1	10500	
E01-1245-28511	454-108-402, 10ppm female, adult, 02-01-02	0.4	1	6720	
E01-1245-28513	454-108-404, 10ppm female, adult, 02-01-02	0.1	1	9060	
E01-1245-28515	454-108-406, 10ppm female, adult, 02-01-02	0.2	1	8980	
E01-1245-28517	454-108-408, 10ppm female, adult, 02-01-02	0.2	1	7840	
E01-1245-28518	454-108-246, 0ppm female, offspring, 02-01-02	0.1 0.1	1 1	<400 <400	<400
E01-1245-28521	454-108-259, 0ppm female, offspring, 02-01-02	0.1 0.1	1 1	<400 <400	<400
E01-1245-28524	454-108-273, 0ppm female, offspring, 02-01-02	0.06 0.06	1 1	<400 <400	<400
E01-1245-28525	454-108-277, 0ppm female, offspring, 02-01-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28528	454-108-517, 10ppm female, offspring, 02-01-02	0.02 0.02	1 1	18500 16600	17600
E01-1245-28530	454-108-525, 10ppm female, offspring, 02-01-02	0.03 0.03	1 1	12800 12100	12500
E01-1245-28532	454-108-538, 10ppm female, offspring, 02-01-02	0.08 0.08	1 1	14800 12200	13400
E01-1245-28535	454-108-557, 10ppm female, offspring, 02-01-02	0.06 0.06	1 1	8330 7460	7900
E01-1245-28536	454-108-561, 10ppm female, offspring, 02-01-02	0.05 0.05	1 1	11500 10100	10800

* There are two results reported for these samples. Both values are included and averaged in the "Average of Dup Sample" column.

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

Accuracy: Sample results are accurate to 100% +/- 30%.

Sera QC samples met the acceptance criteria for the method.

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

Sample ID	Sample Description	Concentration (ng/L)	Number of Samples	Concentration $C_8F_{17}SO_3^-$ (ng/L)	Average of Samples
E01-1245-28443	454-108-329, Oppm male, adult, 01-31-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28445	454-108-331, Oppm male, adult, 01-31-02	0.4 0.4	1 1	<400 <400	<400
E01-1245-28447	454-108-333, Oppm male, adult, 01-31-02	0.4 0.4	1 1	<400 <400	<400
E01-1245-28448	454-108-335, Oppm male, adult, 01-31-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28450	454-108-337, Oppm male, adult, 01-31-02	0.1 0.1	1 1	<400 <400	<400
E01-1245-28452	454-108-339, Oppm male, adult, 01-31-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28454	454-108-341, Oppm male, adult, 01-31-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28455	454-108-343, Oppm male, adult, 01-31-02	0.1 0.1	1 1	<400 <400	<400
E01-1245-28457	454-108-345, Oppm male, adult, 01-31-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28459	454-108-347, Oppm male, adult, 02-01-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28461	454-108-349, Oppm male, adult, 02-01-02	0.02 0.02	1 1	<400 <400	<400
E01-1245-28465	454-108-353, Oppm male, adult, 02-01-02	0.3 0.3	1 1	<400 <400	<400
E01-1245-28467	454-108-355, Oppm male, adult, 02-01-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28469	454-108-357, Oppm male, adult, 02-01-02	0.07 0.07	1 1	<400 <400	<400
E01-1245-28471	454-108-359, Oppm male, adult, 02-01-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28473	454-108-361, Oppm male, adult, 02-01-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28475	454-108-363, Oppm male, adult, 02-01-02	0.1 0.1	1 1	<400 <400	<400
E01-1245-28476	454-108-365, Oppm male, adult, 02-01-02	0.4 0.4	1 1	<400 <400	<400
E01-1245-28478	454-108-367, Oppm male, adult, 02-01-02	0.1 0.1	1 1	<400 <400	<400

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Study Number	Sample Description	PFOS Concentration (ppm)	Number of Samples	Chemical Concentration (ppm) of Serum	Average of 10 Samples
E01-1245-28480	454-108-369, 10ppm male, adult, d100,02-01-02	0.2	100	107000	112000
		0.2	100	117000	
E01-1245-28482	454-108-371, 10ppm male, adult, d100,02-01-02	0.2	100	181000	180000
		0.2	100	179000	
E01-1245-28484	454-108-373, 10ppm male, adult, d100,02-01-02	0.1	100	144000	150000
		0.1	100	158000	
E01-1245-28486	454-108-375, 10ppm male, adult, d100,02-01-02	0.3	100	138000	138000
		0.3	100	138000	
E01-1245-28488	454-108-377, 10ppm male, adult, d100,02-01-02	0.07	100	94400	95300
		0.07	100	96100	
E01-1245-28490	454-108-379, 10ppm male, adult, d100,02-01-02	0.1	100	150000	158000
		0.1	100	166000	
E01-1245-28492	454-108-381, 10ppm male, adult, d100,02-01-02	0.1	100	141000	145000
		0.1	100	149000	
E01-1245-28494	454-108-383, 10ppm male, adult, d100,02-01-02	0.3	100	147000	151000
		0.3	100	156000	
E01-1245-28495	454-108-385, 10ppm male, adult, d100,02-01-02	0.1	100	119000	121000
		0.1	100	123000	
E01-1245-28497	454-108-387, 10ppm male, adult, d100,02-01-02	0.3	100	174000	185000
		0.3	100	196000	
E01-1245-28499	454-108-389, 10ppm male, adult, d100,02-01-02	0.1	100	129000	132000
		0.1	100	138000	
E01-1245-28501	454-108-391, 10ppm male, adult, d100,02-01-02	0.06	100	141000	144000
		0.06	100	147000	
E01-1245-28503	454-108-393, 10ppm male, adult, d100,02-01-02	0.2	100	160000	162000
		0.2	100	165000	
E01-1245-28505	454-108-395, 10ppm male, adult, d100,02-01-02	0.2	100	127000	131000
		0.2	100	136000	
E01-1245-28507	454-108-397, 10ppm male, adult, d100,02-01-02	0.3	100	99600	103000
		0.3	100	107000	
E01-1245-28509	454-108-399, 10ppm male, adult, d100,02-01-02	0.1	100	138000	148000
		0.1	100	159000	
E01-1245-28510	454-108-401, 10ppm male, adult, d100,02-01-02	0.1	100	203000	215000
		0.1	100	226000	
E01-1245-28512	454-108-403, 10ppm male, adult, d100,02-01-02	0.2	100	127000	132000
		0.2	100	138000	
E01-1245-28514	454-108-405, 10ppm male, adult, d100,02-01-02	0.1	100	108000	112000
		0.1	100	117000	
E01-1245-28516	454-108-407, 10ppm male, adult, d100,02-01-02	0.1	100	99200	105000
		0.1	100	112000	

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SILOPP Number	Sample Description	PFOS Concentration (ppm)	Volume Dilution	Concentration C,F,SO ₂ in Sample (µg/L or SER)	Average of Dup Samples
E01-1245-28519	454-108-253, 0ppm male, offspring, 02-01-02	0.02 0.02	1 1	<400 <400	<400
E01-1245-28520	454-108-256, 0ppm male, offspring, 02-01-02	0.02 0.02	1 1	<400 <400	<400
E01-1245-28522	454-108-266, 0ppm male, offspring, 02-01-02	0.07 0.07	1 1	<400 <400	<400
E01-1245-28523	454-108-270, 0ppm male, offspring, 02-01-02	0.2 0.2	1 1	<400 <400	<400
E01-1245-28526	454-108-283, 0ppm male, offspring, 02-01-02	0.02 0.02	1 1	<400 <400	<400
E01-1245-28527	454-108-291, 0ppm male, offspring, 02-01-02	0.05 0.05	1 1	<400 <400	<400
E01-1245-28529	454-108-522, 10ppm male, offspring, 02-01-02	0.07	10	19300	19300
E01-1245-28531	454-108-533, 10ppm male, offspring, 02-01-02	0.07 0.07	1 1	9310 8220	8760
E01-1245-28533	454-108-545, 10ppm male, offspring, 02-01-02	0.04 0.04	1 1	15500 13600	14800
E01-1245-28534	454-108-552, 10ppm male, offspring, 02-01-02	0.07 0.07	1 1	13600 11600	12500
E01-1245-28537	454-108-568, 10ppm male, offspring, 02-01-02	0.1 0.1	1 1	12100 10400	11200

* There are two results reported for these samples. Both values are included and averaged in the "Average of Dup Sample" column.

Accuracy: Sample results are accurate to 100% +/- 30%.

Sera QC samples met the acceptance criteria for the method.

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Table 12. $C_8F_{17}SO_2$ Data Summary, Female Quail Liver (Adult and Offspring)

QUAIL NUMBER	EXPOSURE CONCENTRATION	ADULT LIVER WEIGHT (G)	ADULT LIVER DILUTION	CONC. OF $C_8F_{17}SO_2$ B-UNIT (G/G OF LIVER)
E01-1342-30321	0 ppm a.i. 330 F Liver	1.0749	1	<50.2*
E01-1342-30339	0 ppm a.i. 332 F Liver	1.0469	1	<50.2*
E01-1342-30357	0 ppm a.i. 334 F Liver	1.0232	1	<50.2*
E01-1342-30375	0 ppm a.i. 336 F Liver	1.0188	1	<50.2*
E01-1342-30393	0 ppm a.i. 338 F Liver	1.0543	1	<50.2*
E01-1342-30411	0 ppm a.i. 340 F Liver	1.0182	1	<50.2*
E01-1342-30429	0 ppm a.i. 342 F Liver	1.0904	1	<50.2*
E01-1342-30447	0 ppm a.i. 344 F Liver	1.0087	1	<50.2*
E01-1342-30465	0 ppm a.i. 346 F Liver	1.0998	1	<50.2*
E01-1342-30483	0 ppm a.i. 348 F Liver	1.1038	1	<50.2*
E01-1342-30501	0 ppm a.i. 350 F Liver	1.0237	1	<50.2*
E01-1342-30519	0 ppm a.i. 352 F Liver	1.1509	1	<50.2*
E01-1342-30537	0 ppm a.i. 354 F Liver	1.0578	1	<50.2*
E01-1342-30555	0 ppm a.i. 356 F Liver	1.0312	1	<50.2*
E01-1342-30573	0 ppm a.i. 358 F Liver	1.1031	1	<50.2*
E01-1342-30591	0 ppm a.i. 360 F Liver	1.0586	1	<50.2*
E01-1342-30609	0 ppm a.i. 362 F Liver	1.0424	1	<50.2*
E01-1342-30627	0 ppm a.i. 364 F Liver	1.0364	1	<50.2*
E01-1342-30645	0 ppm a.i. 366 F Liver	1.0577	1	<50.2*
E01-1342-30663	0 ppm a.i. 368 F Liver	1.0920	1	<50.2*
E01-1342-30681	10 ppm a.i. 370 F Liver	1.0668	1	5920*
E01-1342-30699	10 ppm a.i. 372 F Liver	1.0748	1	4430*
E01-1342-30717	10 ppm a.i. 374 F Liver	1.0790	1	5430*
E01-1342-30735	10 ppm a.i. 376 F Liver	1.1036	1	3990*
E01-1342-30753	10 ppm a.i. 378 F Liver	1.0021	1	3750*
E01-1342-30771	10 ppm a.i. 380 F Liver	1.0825	1	3990*
E01-1342-30789	10 ppm a.i. 382 F Liver	1.0122	1	3290*
E01-1342-30807	10 ppm a.i. 384 F Liver	0.6864	1	4190*
E01-1342-30825	10 ppm a.i. 386 F Liver	1.1215	1	7550*
E01-1342-30843	10 ppm a.i. 388 F Liver	1.0878	1	3490*
E01-1342-30861	10 ppm a.i. 390 F Liver	1.0078	1	4870*
E01-1342-30879	10 ppm a.i. 392 F Liver	1.0060	1	5940*
E01-1342-30897	10 ppm a.i. 394 F Liver	1.0238	1	5030*
E01-1342-30915	10 ppm a.i. 396 F Liver	1.0370	1	4910*
E01-1342-30933	10 ppm a.i. 398 F Liver	1.0240	1	4400*
E01-1342-30951	10 ppm a.i. 400 F Liver	1.0181	1	5040*
E01-1342-30969	10 ppm a.i. 402 F Liver	1.0876	1	5900*
E01-1342-31000	10 ppm a.i. 404 F Liver	1.0173	1	5420*
E01-1342-31018	10 ppm a.i. 406 F Liver	1.0047	1	5870*
E01-1342-31036	10 ppm a.i. 408 F Liver	1.0044	1	4560*

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QALPHA E01-1245	Sample Description	RECOVERED (%)	REJECTION LEVEL (%)	CHLOROCYCLOPENTANE C ₁₀ F ₁₇ SO ₂ F SUMMARY (% OF LIVER)
E01-1365-31184	0 ppm a.i. 248 F Liver, Offspring	0.7302	1	<20.0*
E01-1365-31204	0 ppm a.i. 259 F Liver, Offspring	0.4434	1	<20.0*
E01-1365-31219	0 ppm a.i. 273 F Liver, Offspring	0.5004	1	<20.0*
E01-1365-31224	0 ppm a.i. 277 F Liver, Offspring	0.4326	1	<20.0*
E01-1365-31240	10 ppm a.i. 517 F Liver, Offspring	0.6570	1	5690*
E01-1365-31250	10 ppm a.i. 525 F Liver, Offspring	0.5720	1	7030*
E01-1365-31260	10 ppm a.i. 536 F Liver, Offspring	0.5211	10	13900*
E01-1365-31275	10 ppm a.i. 557 F Liver, Offspring	0.4654	1	4680*
E01-1365-31280	10 ppm a.i. 561 F Liver, Offspring	0.9417	1	4590*

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

Accuracy: Sample results are accurate to 100% +/- 30%.

* Liver QC samples did not meet the 1/2 of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

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Table 13. $C_8F_{17}SO_2$ Data Summary, Male Quail Liver (Adult and Offspring)

3M Sample Number	Sample Description	Weight of Liver (g)	Weight of Liver (g)	$C_8F_{17}SO_2$ in Sample (ng/g of liver)
E01-1342-30312	0 ppm a.i. 329 M Liver	1.0050	1	<50.2*
E01-1342-30330	0 ppm a.i. 331 M Liver	1.0530	1	<50.2*
E01-1342-30348	0 ppm a.i. 333 M Liver	1.0338	1	209 ±*
E01-1342-30366	0 ppm a.i. 335 M Liver	1.0062	1	<50.2*
E01-1342-30384	0 ppm a.i. 337 M Liver	1.0269	1	<50.2*
E01-1342-30402	0 ppm a.i. 339 M Liver	0.0886	1	<50.2*
E01-1342-30420	0 ppm a.i. 341 M Liver	1.0235	1	<50.2*
E01-1342-30438	0 ppm a.i. 343 M Liver	1.0090	1	<50.2*
E01-1342-30456	0 ppm a.i. 345 M Liver	1.1046	1	<50.2*
E01-1342-30474	0 ppm a.i. 347 M Liver	1.0586	1	<50.2*
E01-1342-30492	0 ppm a.i. 349 M Liver	1.0358	1	<50.2*
E01-1342-30510	0 ppm a.i. 351 M Liver	1.0583	1	<50.2*
E01-1342-30528	0 ppm a.i. 353 M Liver	1.0183	1	<50.2*
E01-1342-30546	0 ppm a.i. 355 M Liver	1.0899	1	<50.2*
E01-1342-30564	0 ppm a.i. 357 M Liver	1.0382	1	<50.2*
E01-1342-30582	0 ppm a.i. 359 M Liver	1.0941	1	<50.2*
E01-1342-30600	0 ppm a.i. 361 M Liver	1.0084	1	<50.2*
E01-1342-30618	0 ppm a.i. 363 M Liver	1.0618	1	<50.2*
E01-1342-30636	0 ppm a.i. 365 M Liver	1.0916	1	<50.2*
E01-1342-30654	0 ppm a.i. 367 M Liver	1.0327	1	<50.2*
E01-1342-30672	10 ppm a.i. 369 M Liver	1.0010	10	78600*
E01-1342-30690	10 ppm a.i. 371 M Liver	1.0856	10	78500*
E01-1342-30708	10 ppm a.i. 373 M Liver	1.0276	10	88300*
E01-1342-30726	10 ppm a.i. 375 M Liver	1.1008	10	70000*
E01-1342-30744	10 ppm a.i. 377 M Liver	1.0236	10	80300*
E01-1342-30762	10 ppm a.i. 379 M Liver	1.0312	10	90700*
E01-1342-30780	10 ppm a.i. 381 M Liver	1.0501	10	80000*
E01-1342-30798	10 ppm a.i. 383 M Liver	1.0110	10	73500*
E01-1342-30829	10 ppm a.i. 385 M Liver	1.0678	10	83400*
E01-1342-30847	10 ppm a.i. 387 M Liver	1.0315	10	79300*
E01-1342-30865	10 ppm a.i. 389 M Liver	1.0310	10	70300*
E01-1342-30883	10 ppm a.i. 391 M Liver	1.0947	10	70400*
E01-1342-30901	10 ppm a.i. 393 M Liver	1.0834	10	86800*
E01-1342-30919	10 ppm a.i. 395 M Liver	1.0489	10	65100*
E01-1342-30937	10 ppm a.i. 397 M Liver	1.0309	10	50100*
E01-1342-30955	10 ppm a.i. 399 M Liver	1.0276	10	63000*
E01-1342-30973	10 ppm a.i. 401 M Liver	1.0361	100	147000*
E01-1342-30991	10 ppm a.i. 403 M Liver	1.0019	100	146000*
E01-1342-31009	10 ppm a.i. 405 M Liver	1.0336	100	139000*
E01-1342-31027	10 ppm a.i. 407 M Liver	1.0956	100	132000*

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3M Sample Number	Sample Description	Weight of Liver (g)	Number of Samples	Conc. of $C_8F_{17}SO_3^-$ in Serum (ng/g of Liver)
E01-1365-31194	0 ppm a.i. 253 M Liver, Offspring	0.3806	1	<20.0*
E01-1365-31199	0 ppm a.i. 256 M Liver, Offspring	0.5947	1	<20.0*
E01-1365-31209	0 ppm a.i. 266 M Liver, Offspring	0.2673	1	<20.0*
E01-1365-31214	0 ppm a.i. 270 M Liver, Offspring	0.5900	1	<20.0*
E01-1365-31229	0 ppm a.i. 283 M Liver, Offspring	0.6666	1	<20.0*
E01-1365-31234	0 ppm a.i. 291 M Liver, Offspring	0.6072	1	<20.0*
E01-1365-31245	10 ppm a.i. 522 M Liver, Offspring	0.7325	10	13700 *
E01-1365-31255	10 ppm a.i. 533 M Liver, Offspring	0.6903	1	4940*
E01-1365-31265	10 ppm a.i. 545 M Liver, Offspring	0.4624	1	5580*
E01-1365-31270	10 ppm a.i. 552 M Liver, Offspring	0.5353	1	5990*
E01-1365-31285	10 ppm a.i. 568 M Liver, Offspring	0.7581	1	6520*

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

Attachment B contains data summary tables.

Accuracy: Sample results are accurate to 100% +/- 30%.

* Liver QC samples did not meet the 1/2 of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

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 19 of 19 quail (<400 ng/mL). The sera results from the female control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 16 of 16 birds (<400 ng/mL) (Table 15). Analytical results for twenty sera samples from the 10ppm dosed male group showed an average of 141000 ng/mL $C_8F_{17}SO_3^-$ (range 94400 - 226000 ng/mL, Table 14). Analytical results for seventeen sera samples from the 10ppm dosed female group showed an average of 8670 ng/mL $C_8F_{17}SO_3^-$ (range 4710 - 14700 ng/mL, Table 15).

Sera results from the male offspring control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 6 of 6 birds (<400 ng/mL). The sera results from the female offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 4 of 4 birds (<400 ng/mL, Table 14). Analytical results for five sera samples from the 10ppm dosed male offspring group showed an average of 12600 ng/mL (range 8220 - 19200 ng/mL, Table 14). Analytical results for five sera samples from the 10ppm dosed female offspring group showed an average of 12400 ng/mL (range 7640 - 18500 ng/mL, Table 14).

Liver results from the male control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 19 of 19 birds (<50.2 ng/g, Table 15) and quantifiable concentrations of $C_8F_{17}SO_3^-$ in 1 of 20 (209 ng/g, Table 14). The liver results from the female control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 20 of 20 birds (<50.2 ng/g, Table 14). Analytical results for twenty liver samples from the 10ppm dosed male group showed an average of 88500 ng/g $C_8F_{17}SO_3^-$ (range 50100 - 147000 ng/g, Table 14). Analytical results for twenty liver samples from the 10ppm dosed female group showed an average of 4900 ng/g $C_8F_{17}SO_3^-$ (range 3290 - 7550 ng/g, Table 14).

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Liver results from the male offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 6 of 6 birds (<50.2 ng/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 4 of 4 birds (<20.0 ng/g, Table 14). Analytical results for four liver samples from the 10ppm dosed male offspring group showed an average of 5760 ng/g (range 4940 – 6520 ng/g, Table 15). Analytical results for four liver samples from the 10ppm dosed female offspring group showed an average of 5490 ng/g (range 4590 - 7030 ng/g, Table 15).

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	19	NA	<400 ng/mL (19 of 19)	20	<50.2 ng/g*	<50.2 ng/g (19 of 20)* 209 ng/g (1 of 20)*
Female Control Group	17	NA	<400 ng/mL (16 of 17) 2110 ng/mL (1 of 17)	20	<50.2 ng/g*	<50.2 ng/g (20 of 20)*
Male 10ppm Group	20	141000 ng/mL	94400 - 226000 ng/mL	20	88500 ng/g*	50100 - 147000 ng/g*
Female 10ppm Group	18	9990 ng/mL	4710 - 31900 ng/mL	20	4900 ng/g*	3290 - 7550 ng/g*
Male Offspring Control Group	6	NA	<400 ng/mL (6 of 6)	6	<20.0 ng/g*	<20.0 ng/g (6 of 6)*
Female Offspring Control Group	4	NA	<400 ng/mL (4 of 4)	4	<20.0 ng/g*	<20.0 ng/g (4 of 4)*
Male Offspring 10ppm Group	5	12600 ng/mL	8220 - 19200 ng/mL	5	7350 ng/g*	4940 - 13700 ng/g*
Female Offspring 10ppm Group	5	12400 ng/mL	7640 - 18500 ng/mL	5	7180 ng/g*	4590 - 13900 ng/g*

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

Accuracy: Sample results are accurate to 100% +/- 30%.

Sera QC samples met the acceptance criteria for the method.

* Liver: QC samples did not meet the 1/2 of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

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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	19*	*	*	19	<50.2 ng/g**	<50.2 ng/g (19 of 19)**
Female Control Group	16	<400 ng/mL	<400 ng/mL (16 of 16)	20*	*	*
Male 10ppm Group	20*	*	*	20*	*	*
Female 10ppm Group	17	8700 ng/mL	4710 - 14700 ng/mL	20*	*	*
Male Offspring Control Group	6*	*	*	6*	*	*
Female Offspring Control Group	4*	*	*	4*	*	*
Male Offspring 10ppm Group	5*	*	*	4	5760 ng/g**	4940 - 6520 ng/g**
Female Offspring 10ppm Group	5*	*	*	4	5490 ng/g**	4590 - 7030 ng/g**

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

Accuracy: Sample results are accurate to 100% +/- 30%.

Sera QC samples met the acceptance criteria for the method.

** Liver QC samples did not meet the 1/2 of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

STATISTICAL METHODS

Statistical methods were limited to calculating means, standard deviations, % coefficient of variation and the Dixon's Q-Test. The Dixon's 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 which 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 measurement of accuracy available at this time is the quality control and matrix spike results. For sera data, the accuracy (the observed amount in the QC and MS samples, divided by the amount spiked, expressed as a percent) was found to range from 74.1% to 126%; while precision, expressed as the standard deviation of the recoveries, was found to range from 2.14% to 9.56%. For liver data, the accuracy was found to range from 72.6% to 141%; while precision was found to range from 2.11% to 5.51%.

Reproductive Performance by Pen

Appendix XIII - Table 1

Eggs Laid / Maximum Laid (%)

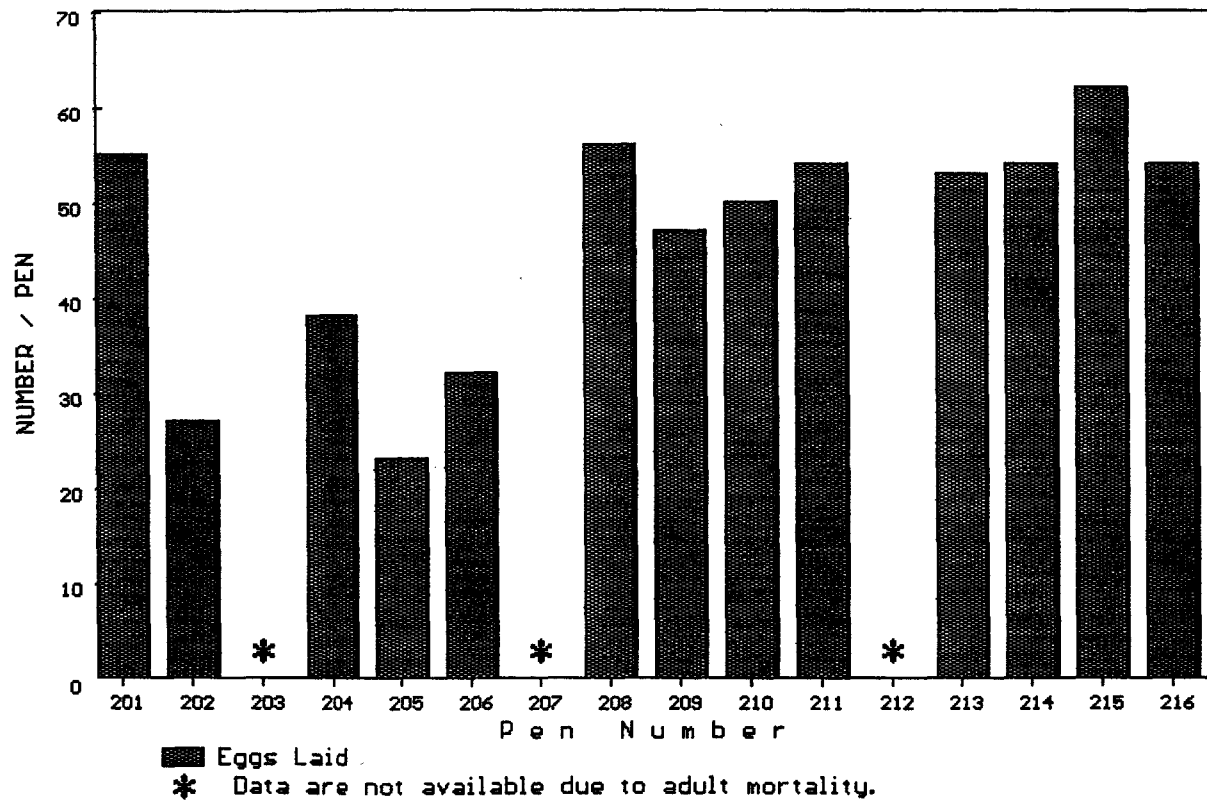
from a Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	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	55	63	87	69.12	34	63	54	47.28	-	-	-	-	-	-	-	-
2	27	63	43	40.89	46	63	73	58.70	-	-	-	-	-	-	-	-
3	-	-	-	-	43	63	68	55.71	-	-	-	-	-	-	-	-
4	38	63	60	50.95	42	63	67	54.74	-	-	-	-	-	-	-	-
5	23	63	37	37.17	47	63	75	59.74	-	-	-	-	-	-	-	-
6	32	63	51	45.45	53	63	84	66.52	-	-	-	-	-	-	-	-
7	-	-	-	-	21	63	33	35.26	-	-	-	-	-	-	-	-
8	56	63	89	70.53	-	-	-	-	-	-	-	-	-	-	-	-
9	47	63	75	59.74	54	63	86	67.79	-	-	-	-	-	-	-	-
10	50	63	79	62.98	-	-	-	-	-	-	-	-	-	-	-	-
11	54	63	86	67.79	44	63	70	56.69	-	-	-	-	-	-	-	-
12	-	-	-	-	44	63	70	56.69	-	-	-	-	-	-	-	-
13	53	63	84	66.52	63	63	100	90.00	-	-	-	-	-	-	-	-
14	54	63	86	67.79	41	63	65	53.78	-	-	-	-	-	-	-	-
15	62	63	98	82.76	60	63	95	77.40	-	-	-	-	-	-	-	-
16	54	63	86	67.79	-	-	-	-	-	-	-	-	-	-	-	-
Total	605	819			592	819			-	-	-	-	-	-	-	-
Mean	47	63	74	60.73	46	63	72	60.02	-	-	-	-	-	-	-	-
SD	12	0	20	13.29	11	0	17	13.50	-	-	-	-	-	-	-	-

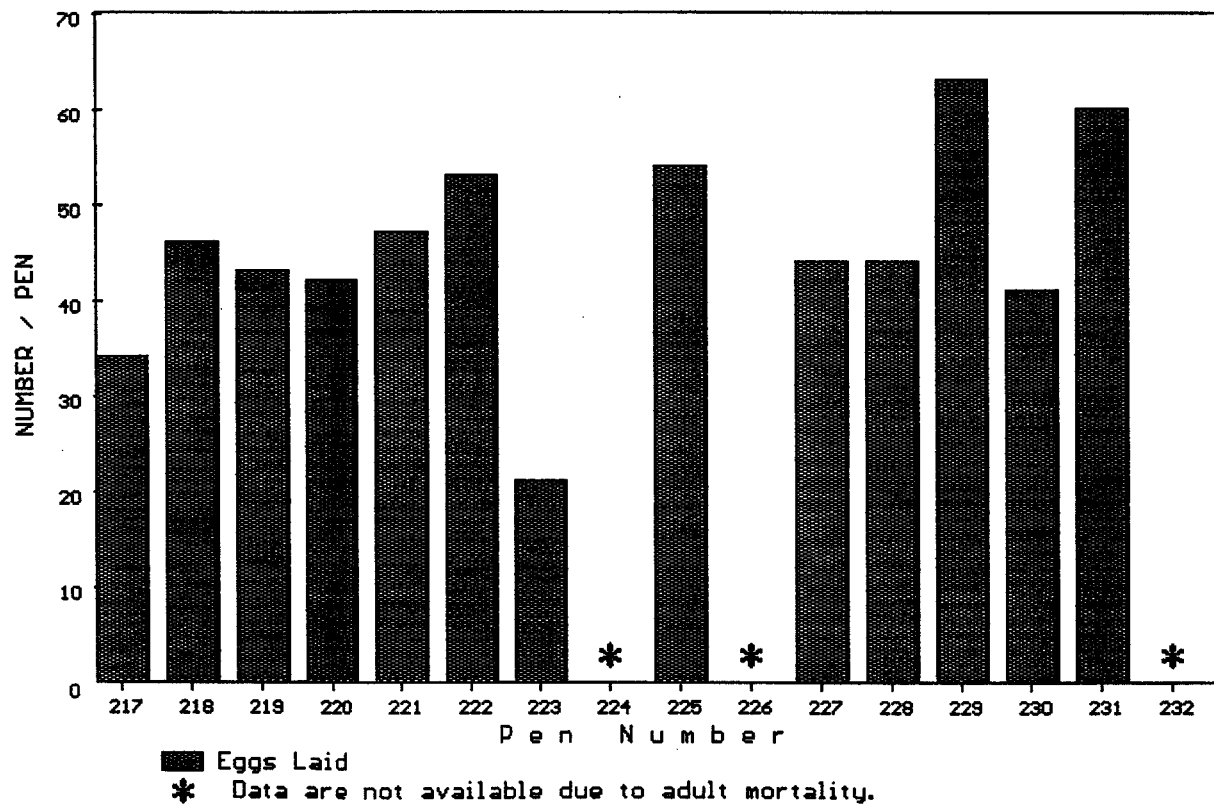
- Data are not available due to adult mortality.

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

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



Appendix XIII - Figure 1b
 Eggs Laid from a Northern Bobwhite
 Reproduction Study with PFOS - 10 PPM A.I.



Reproductive Performance by Pen

Appendix XIII - Table 2

Eggs Cracked / Eggs Laid (%)

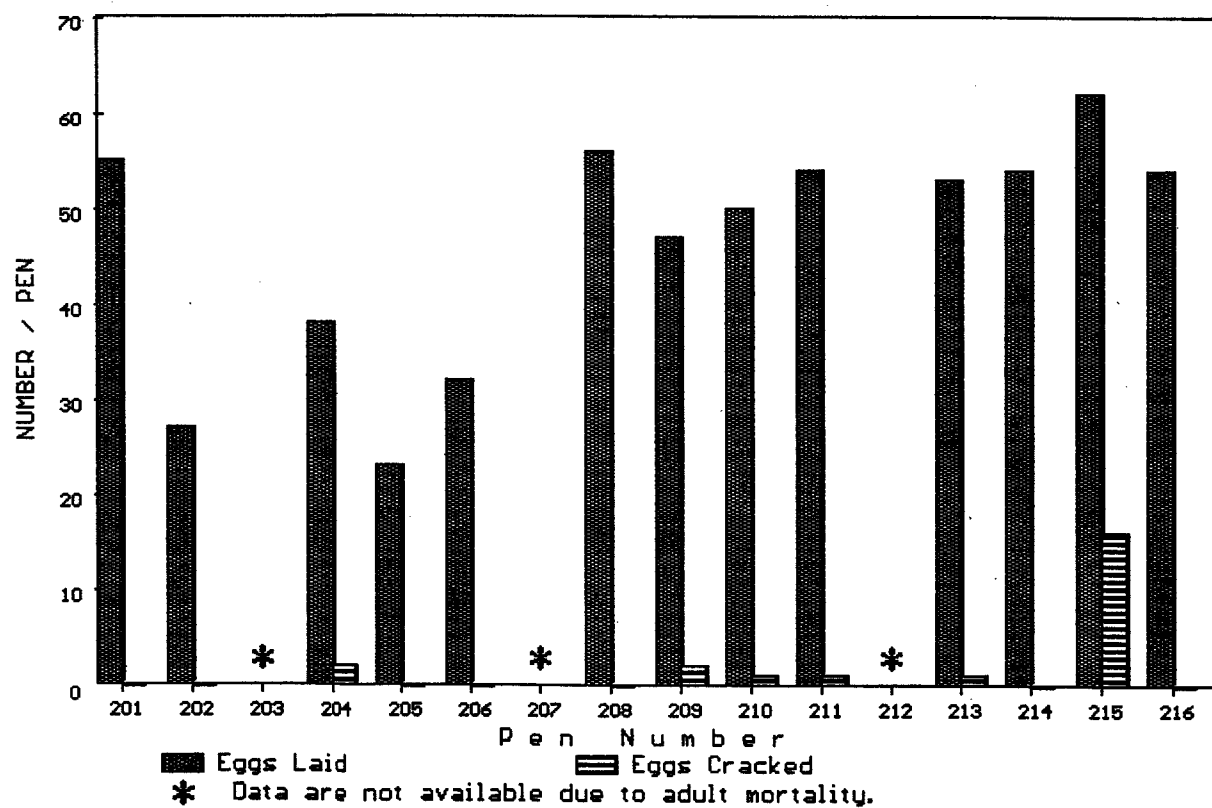
from a Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	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	0	55	0	0.00	0	34	0	0.00	-	-	-	-	-	-	-	-
2	0	27	0	0.00	1	46	2	8.48	-	-	-	-	-	-	-	-
3	-	-	-	-	0	43	0	0.00	-	-	-	-	-	-	-	-
4	2	38	5	13.26	0	42	0	0.00	-	-	-	-	-	-	-	-
5	0	23	0	0.00	0	47	0	0.00	-	-	-	-	-	-	-	-
6	0	32	0	0.00	0	53	0	0.00	-	-	-	-	-	-	-	-
7	-	-	-	-	0	21	0	0.00	-	-	-	-	-	-	-	-
8	0	56	0	0.00	-	-	-	-	-	-	-	-	-	-	-	-
9	2	47	4	11.90	0	54	0	0.00	-	-	-	-	-	-	-	-
10	1	50	2	8.13	-	-	-	-	-	-	-	-	-	-	-	-
11	1	54	2	7.82	0	44	0	0.00	-	-	-	-	-	-	-	-
12	-	-	-	-	0	44	0	0.00	-	-	-	-	-	-	-	-
13	1	53	2	7.90	0	63	0	0.00	-	-	-	-	-	-	-	-
14	0	54	0	0.00	0	41	0	0.00	-	-	-	-	-	-	-	-
15	16	62	26	30.53	2	60	3	10.52	-	-	-	-	-	-	-	-
16	0	54	0	0.00	-	-	-	-	-	-	-	-	-	-	-	-
Total	23	605			3	592			-	-			-	-		
Mean	2	47	3	6.12	0	46	0	1.46	-	-	-	-	-	-	-	-
SD	4	12	7	8.91	1	11	1	3.59	-	-	-	-	-	-	-	-

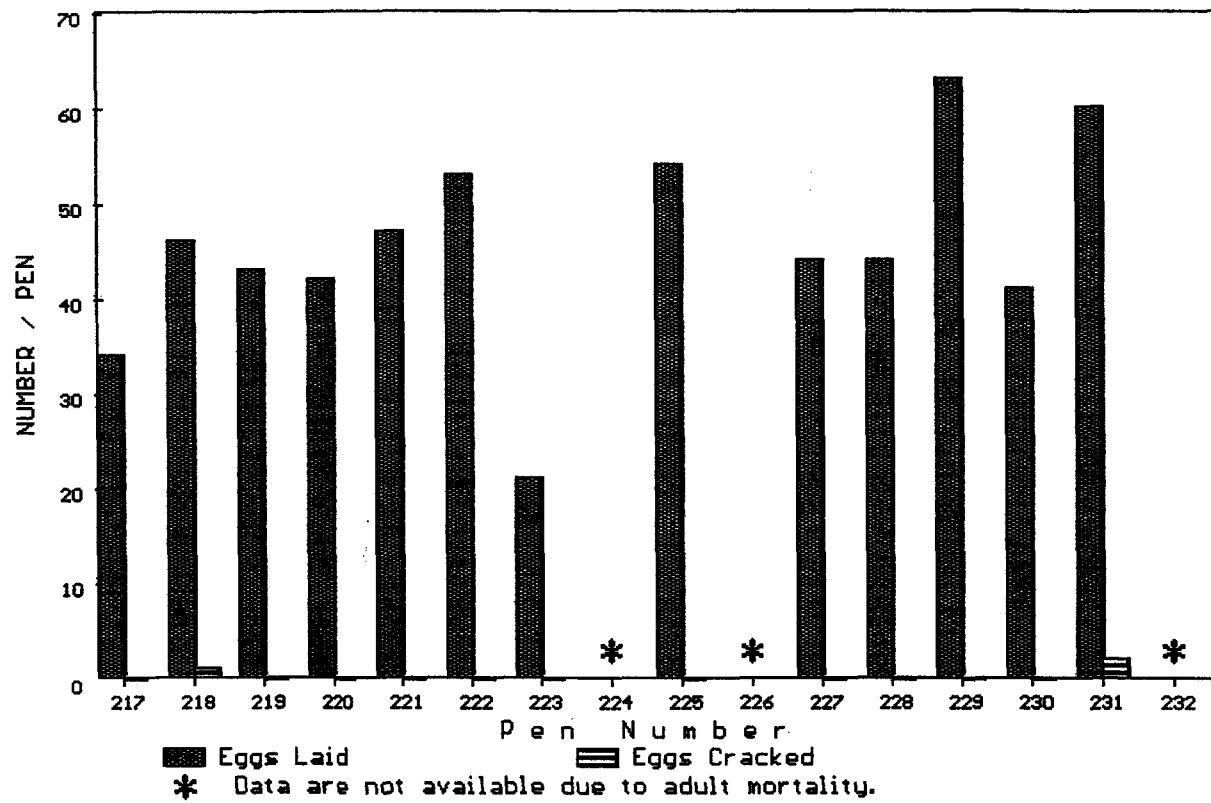
- Data are not available due to adult mortality.

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

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



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



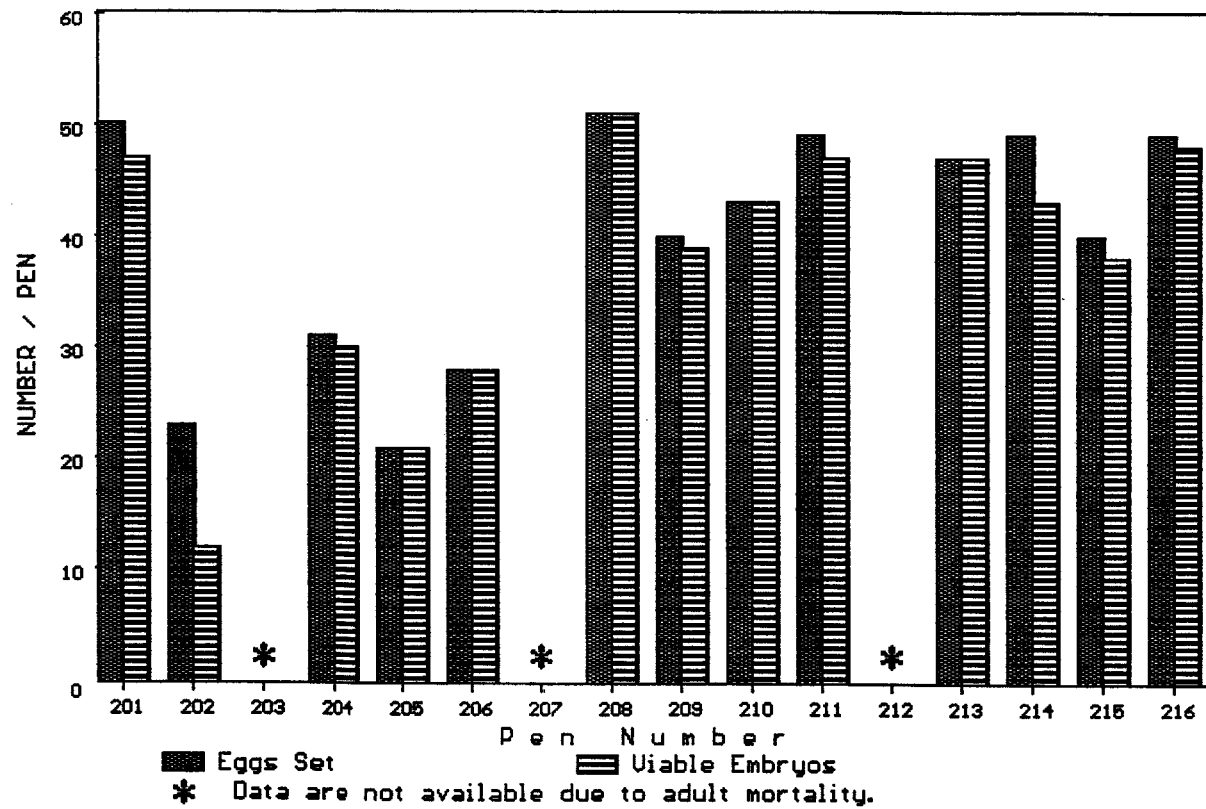
Reproductive Performance by Pen
Appendix XIII - Table 3
Viable Embryos / Eggs Set (%)
from a Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	Viable Eggs		Arcsin		Viable Eggs		Arcsin		Viable Eggs		Arcsin		Viable Eggs		Arcsin	
	Embr. Set	%	Trans.		Embr. Set	%	Trans.		Embr. Set	%	Trans.		Embr. Set	%	Trans.	
1	47	50	94	75.82	30	30	100	90.00	-	-	-	-	-	-	-	-
2	12	23	52	46.25	39	40	97	80.90	-	-	-	-	-	-	-	-
3	-	-	-	-	39	39	100	90.00	-	-	-	-	-	-	-	-
4	30	31	97	79.65	38	38	100	90.00	-	-	-	-	-	-	-	-
5	21	21	100	90.00	35	42	83	65.91	-	-	-	-	-	-	-	-
6	28	28	100	90.00	45	48	94	75.52	-	-	-	-	-	-	-	-
7	-	-	-	-	17	19	89	71.07	-	-	-	-	-	-	-	-
8	51	51	100	90.00	-	-	-	-	-	-	-	-	-	-	-	-
9	39	40	97	80.90	46	48	96	78.22	-	-	-	-	-	-	-	-
10	43	43	100	90.00	-	-	-	-	-	-	-	-	-	-	-	-
11	47	49	96	78.34	39	39	100	90.00	-	-	-	-	-	-	-	-
12	-	-	-	-	27	37	73	58.68	-	-	-	-	-	-	-	-
13	47	47	100	90.00	55	57	96	79.20	-	-	-	-	-	-	-	-
14	43	49	88	69.52	17	36	47	43.41	-	-	-	-	-	-	-	-
15	38	40	95	77.08	43	53	81	64.25	-	-	-	-	-	-	-	-
16	48	49	98	81.79	-	-	-	-	-	-	-	-	-	-	-	-
Total	494	521			470	526			-	-	-	-	-	-	-	-
Mean	38	40	94	79.95	36	40	89	75.17	-	-	-	-	-	-	-	-
SD	12	11	13	12.19	11	10	15	14.26	-	-	-	-	-	-	-	-

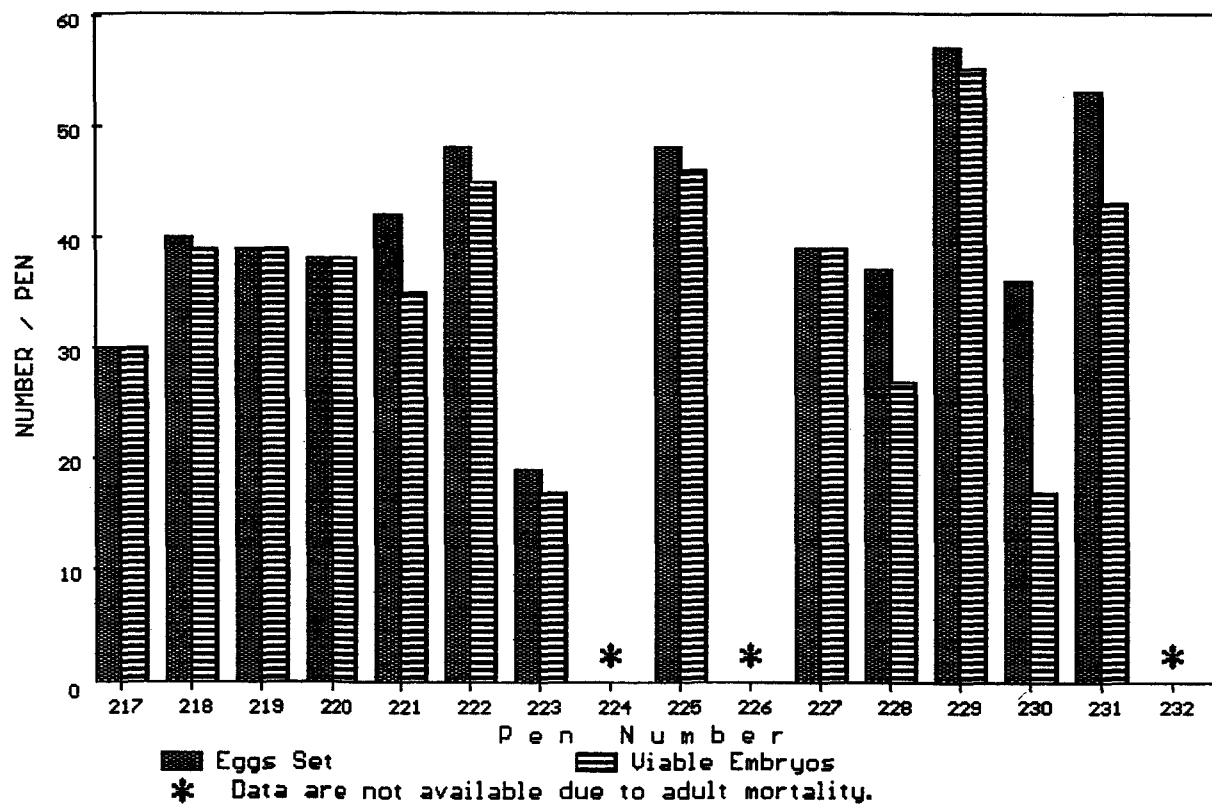
- Data are not available due to adult mortality.

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

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



Appendix XIII - Figure 3b
 Viable Embryos / Eggs Set from a Northern Bobwhite
 Reproduction Study with PFOS - 10 PPM A.I.



Reproductive Performance by Pen

Appendix XIII - Table 4

Live 3-Week Embryos / Viable Embryos (%)

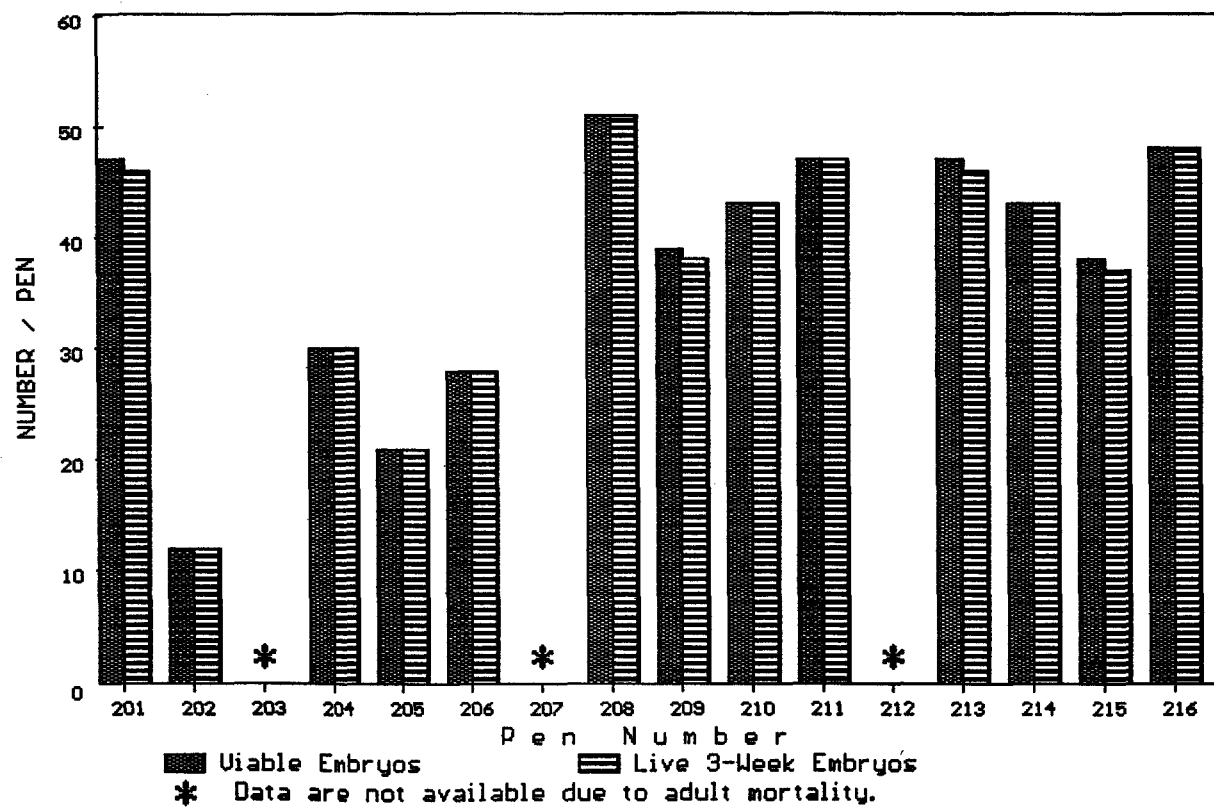
from a Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	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	46	47	98	81.61	29	30	97	79.48	-	-	-	-	-	-	-	-
2	12	12	100	90.00	39	39	100	90.00	-	-	-	-	-	-	-	-
3	-	-	-	-	39	39	100	90.00	-	-	-	-	-	-	-	-
4	30	30	100	90.00	38	38	100	90.00	-	-	-	-	-	-	-	-
5	21	21	100	90.00	34	35	97	80.27	-	-	-	-	-	-	-	-
6	28	28	100	90.00	45	45	100	90.00	-	-	-	-	-	-	-	-
7	-	-	-	-	16	17	94	75.96	-	-	-	-	-	-	-	-
8	51	51	100	90.00	-	-	-	-	-	-	-	-	-	-	-	-
9	38	39	97	80.79	46	46	100	90.00	-	-	-	-	-	-	-	-
10	43	43	100	90.00	-	-	-	-	-	-	-	-	-	-	-	-
11	47	47	100	90.00	39	39	100	90.00	-	-	-	-	-	-	-	-
12	-	-	-	-	27	27	100	90.00	-	-	-	-	-	-	-	-
13	46	47	98	81.61	55	55	100	90.00	-	-	-	-	-	-	-	-
14	43	43	100	90.00	16	17	94	75.96	-	-	-	-	-	-	-	-
15	37	38	97	80.66	43	43	100	90.00	-	-	-	-	-	-	-	-
16	48	48	100	90.00	-	-	-	-	-	-	-	-	-	-	-	-
Total	490	494			466	470			-	-	-	-	-	-	-	-
Mean	38	38	99	87.28	36	36	99	86.28	-	-	-	-	-	-	-	-
SD	12	12	1	4.25	11	11	2	5.91	-	-	-	-	-	-	-	-

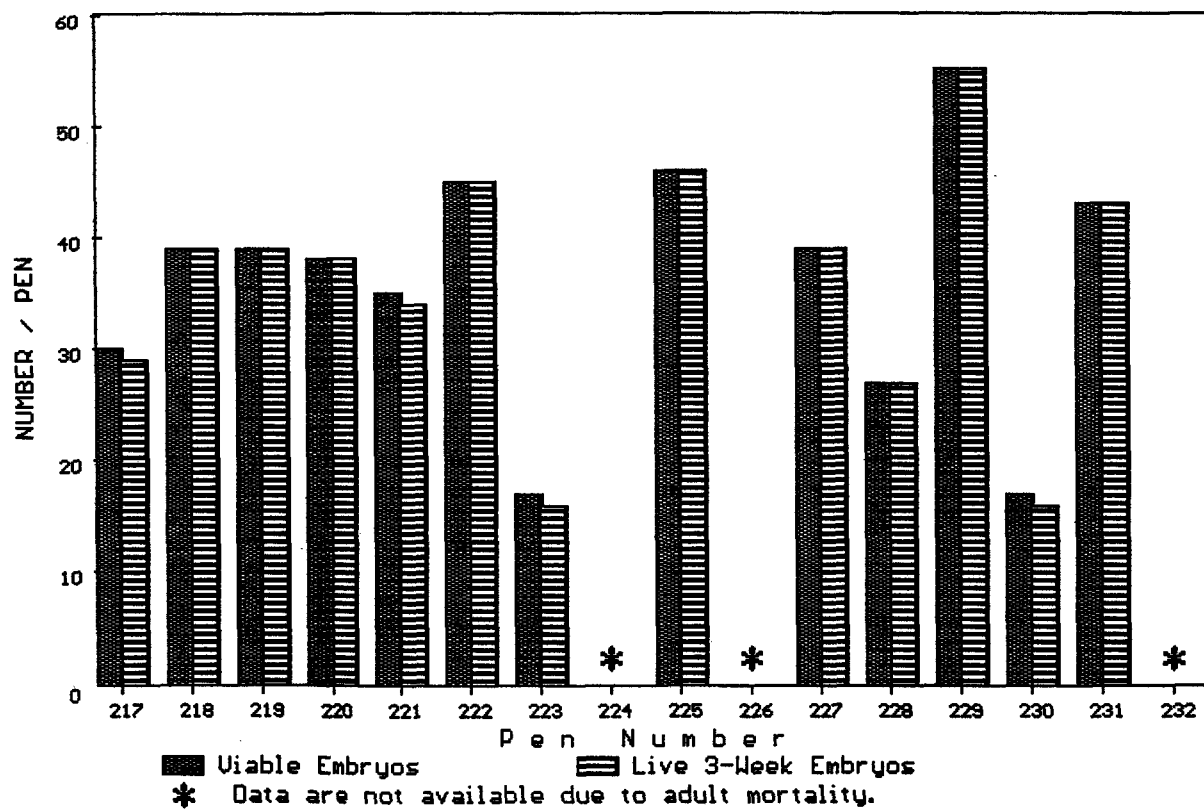
- Data are not available due to adult mortality.

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

Appendix XIII - Figure 4a
 Live 3-Week Embryos / Uiable Embryos from a Northern Bobwhite
 Reproduction Study with PFOS - Control



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



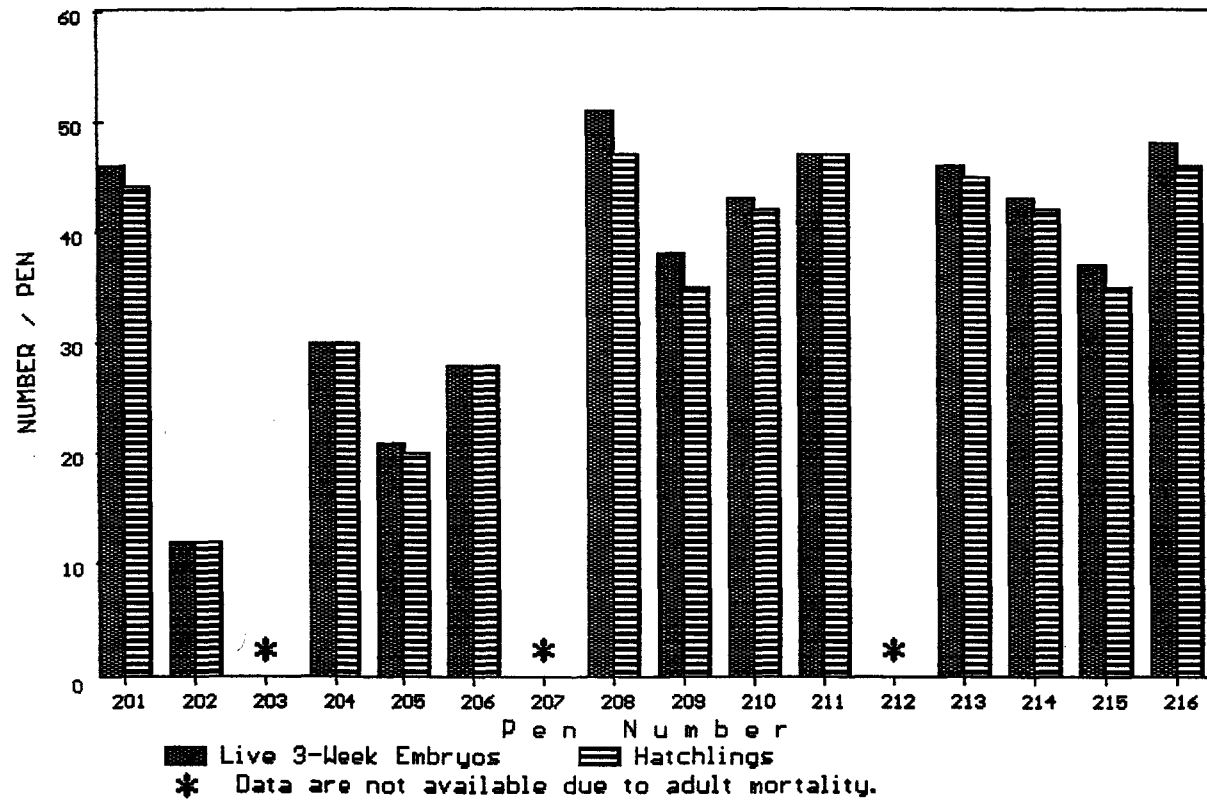
Reproductive Performance by Pen
Appendix XIII - Table 5
Hatchlings / Live 3-Week Embryos (%)
from a Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	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	44	46	96	77.96	20	29	69	56.15	-	-	-	-	-	-	-	-
2	12	12	100	90.00	36	39	92	73.90	-	-	-	-	-	-	-	-
3	-	-	-	-	38	39	97	80.79	-	-	-	-	-	-	-	-
4	30	30	100	90.00	32	38	84	66.59	-	-	-	-	-	-	-	-
5	20	21	95	77.40	31	34	91	72.72	-	-	-	-	-	-	-	-
6	28	28	100	90.00	39	45	87	68.58	-	-	-	-	-	-	-	-
7	-	-	-	-	13	16	81	64.34	-	-	-	-	-	-	-	-
8	47	51	92	73.74	-	-	-	-	-	-	-	-	-	-	-	-
9	35	38	92	73.68	42	46	91	72.85	-	-	-	-	-	-	-	-
10	42	43	98	81.23	-	-	-	-	-	-	-	-	-	-	-	-
11	47	47	100	90.00	39	39	100	90.00	-	-	-	-	-	-	-	-
12	-	-	-	-	27	27	100	90.00	-	-	-	-	-	-	-	-
13	45	46	98	81.52	55	55	100	90.00	-	-	-	-	-	-	-	-
14	42	43	98	81.23	16	16	100	90.00	-	-	-	-	-	-	-	-
15	35	37	95	76.56	26	43	60	51.04	-	-	-	-	-	-	-	-
16	46	48	96	78.22	-	-	-	-	-	-	-	-	-	-	-	-
Total	473	490			414	466			-	-	-	-	-	-	-	-
Mean	36	38	97	81.66	32	36	89	74.38	-	-	-	-	-	-	-	-
SD	11	12	3	6.29	12	11	13	13.21	-	-	-	-	-	-	-	-

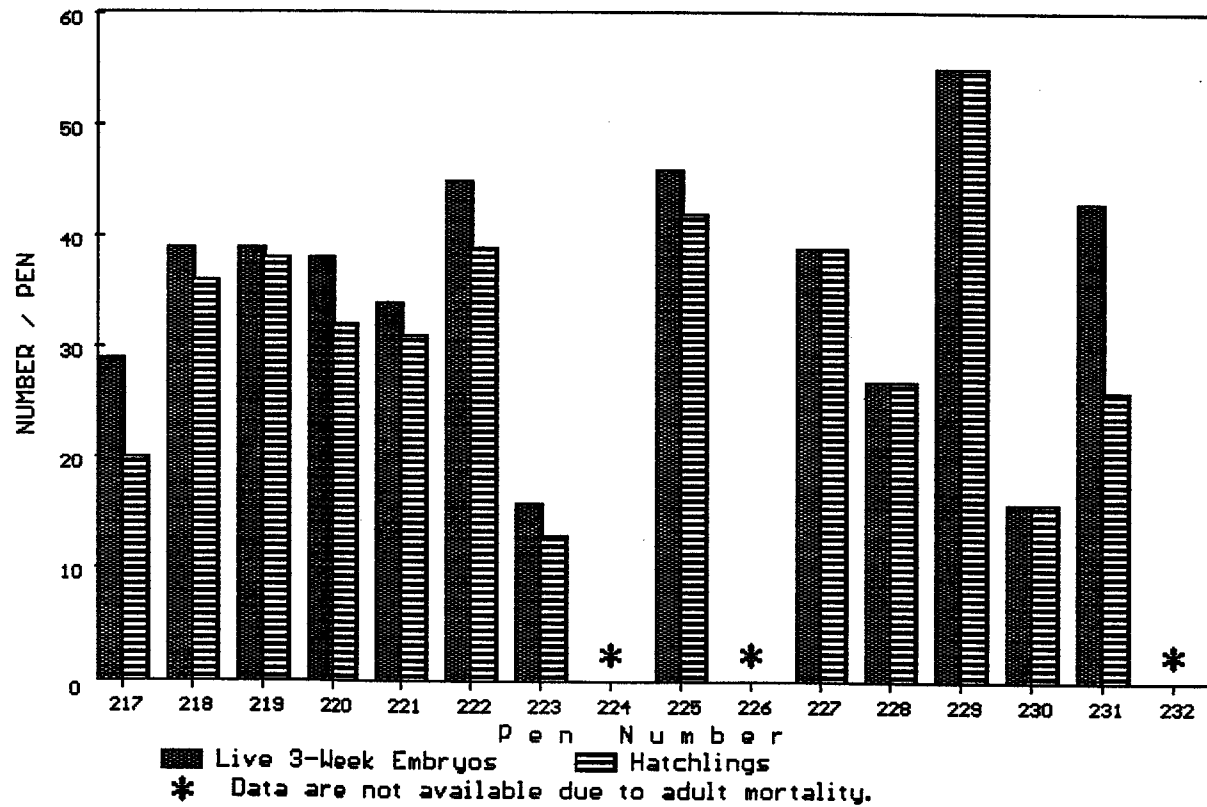
- Data are not available due to adult mortality.

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 Northern Bobwhite
Reproduction Study with PFOS - Control



Appendix XIII - Figure 5b
 Hatchlings / Live 3-Week Embryos from a Northern Bobwhite
 Reproduction Study with PFOS - 10 PPM A.I.



Reproductive Performance by Pen

Appendix XIII - Table 6

14-Day Old Survivors / Hatchlings (%)

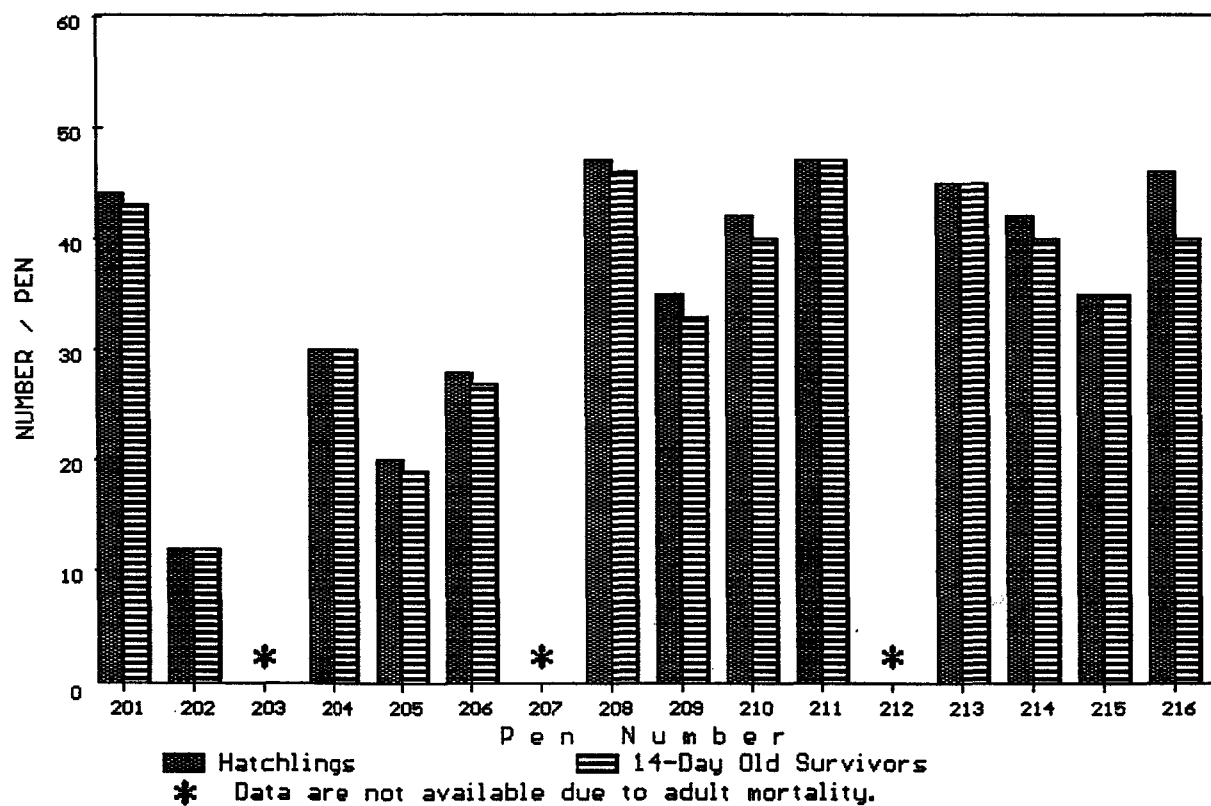
from a Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	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	43	44	98	81.33	20	20	100	90.00	-	-	-	-	-	-	-	-
2	12	12	100	90.00	36	36	100	90.00	-	-	-	-	-	-	-	-
3	-	-	-	-	29	38	76	60.88	-	-	-	-	-	-	-	-
4	30	30	100	90.00	30	32	94	75.52	-	-	-	-	-	-	-	-
5	19	20	95	77.08	30	31	97	79.65	-	-	-	-	-	-	-	-
6	27	28	96	79.11	38	39	97	80.79	-	-	-	-	-	-	-	-
7	-	-	-	-	10	13	77	61.29	-	-	-	-	-	-	-	-
8	46	47	98	81.61	-	-	-	-	-	-	-	-	-	-	-	-
9	33	35	94	76.17	37	42	88	69.82	-	-	-	-	-	-	-	-
10	40	42	95	77.40	-	-	-	-	-	-	-	-	-	-	-	-
11	47	47	100	90.00	37	39	95	76.91	-	-	-	-	-	-	-	-
12	-	-	-	-	27	27	100	90.00	-	-	-	-	-	-	-	-
13	45	45	100	90.00	53	55	96	79.01	-	-	-	-	-	-	-	-
14	40	42	95	77.40	16	16	100	90.00	-	-	-	-	-	-	-	-
15	35	35	100	90.00	23	26	88	70.14	-	-	-	-	-	-	-	-
16	40	46	87	68.83	-	-	-	-	-	-	-	-	-	-	-	-
Total	457	473			386	414			-	-	-	-	-	-	-	-
Mean	35	36	97	82.22	30	32	93	78.00	-	-	-	-	-	-	-	-
SD	11	11	4	7.09	11	12	8	10.39	-	-	-	-	-	-	-	-

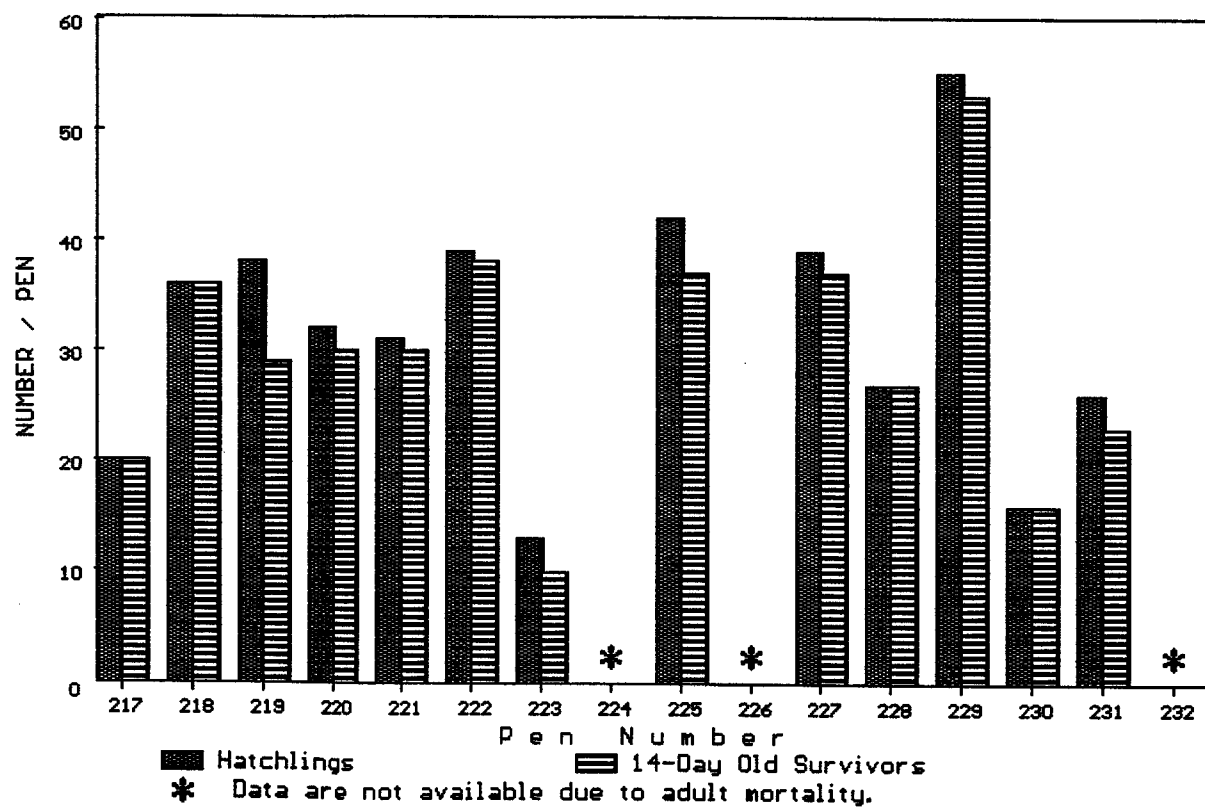
- Data are not available due to adult mortality.

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 Northern Bobwhite
 Reproduction Study with PFOS - Control



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



Reproductive Performance by Pen

Appendix XIII - Table 7

Hatchlings / Eggs Set (%)

from a Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	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	44	50	88	69.73	20	30	67	54.74	-	-	-	-	-	-	-	-
2	12	23	52	46.25	36	40	90	71.57	-	-	-	-	-	-	-	-
3	-	-	-	-	38	39	97	80.79	-	-	-	-	-	-	-	-
4	30	31	97	79.65	32	38	84	66.59	-	-	-	-	-	-	-	-
5	20	21	95	77.40	31	42	74	59.22	-	-	-	-	-	-	-	-
6	28	28	100	90.00	39	48	81	64.34	-	-	-	-	-	-	-	-
7	-	-	-	-	13	19	68	55.81	-	-	-	-	-	-	-	-
8	47	51	92	73.74	-	-	-	-	-	-	-	-	-	-	-	-
9	35	40	88	69.30	42	48	88	69.30	-	-	-	-	-	-	-	-
10	42	43	98	81.23	-	-	-	-	-	-	-	-	-	-	-	-
11	47	49	96	78.34	39	39	100	90.00	-	-	-	-	-	-	-	-
12	-	-	-	-	27	37	73	58.68	-	-	-	-	-	-	-	-
13	45	47	96	78.10	55	57	96	79.20	-	-	-	-	-	-	-	-
14	42	49	86	67.79	16	36	44	41.81	-	-	-	-	-	-	-	-
15	35	40	88	69.30	26	53	49	44.46	-	-	-	-	-	-	-	-
16	46	49	94	75.67	-	-	-	-	-	-	-	-	-	-	-	-
Total	473	521			414	526			-	-			-	-		
Mean	36	40	90	73.58	32	40	78	64.35	-	-	-	-	-	-	-	-
SD	11	11	12	10.24	12	10	18	14.02	-	-	-	-	-	-	-	-

- 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 Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	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	43	50	86	68.03	20	30	67	54.74	-	-	-	-	-	-	-	-
2	12	23	52	46.25	36	40	90	71.57	-	-	-	-	-	-	-	-
3	-	-	-	-	29	39	74	59.58	-	-	-	-	-	-	-	-
4	30	31	97	79.65	30	38	79	62.69	-	-	-	-	-	-	-	-
5	19	21	90	72.02	30	42	71	57.69	-	-	-	-	-	-	-	-
6	27	28	96	79.11	38	48	79	62.84	-	-	-	-	-	-	-	-
7	-	-	-	-	10	19	53	46.51	-	-	-	-	-	-	-	-
8	46	51	90	71.75	-	-	-	-	-	-	-	-	-	-	-	-
9	33	40	82	65.27	37	48	77	61.40	-	-	-	-	-	-	-	-
10	40	43	93	74.68	-	-	-	-	-	-	-	-	-	-	-	-
11	47	49	96	78.34	37	39	95	76.91	-	-	-	-	-	-	-	-
12	-	-	-	-	27	37	73	58.68	-	-	-	-	-	-	-	-
13	45	47	96	78.10	53	57	93	74.64	-	-	-	-	-	-	-	-
14	40	49	82	64.62	16	36	44	41.81	-	-	-	-	-	-	-	-
15	35	40	88	69.30	23	53	43	41.21	-	-	-	-	-	-	-	-
16	40	49	82	64.62	-	-	-	-	-	-	-	-	-	-	-	-
Total	457	521			386	526		*	-	-			-	-		
Mean	35	40	87	70.13	30	40	72	59.25	-	-	-	-	-	-	-	-
SD	11	11	12	9.08	11	10	17	11.35	-	-	-	-	-	-	-	-

- Data are not available due to adult mortality.

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

Reproductive Performance by Pen

Appendix XIII - Table 9

Hatchlings / Maximum Set (%)

from a Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	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	44	57	77	61.47	20	57	35	36.32	-	-	-	-	-	-	-	-
2	12	57	21	27.31	36	57	63	52.63	-	-	-	-	-	-	-	-
3	-	-	-	-	38	57	67	54.74	-	-	-	-	-	-	-	-
4	30	57	53	46.51	32	57	56	48.53	-	-	-	-	-	-	-	-
5	20	57	35	36.32	31	57	54	47.52	-	-	-	-	-	-	-	-
6	28	57	49	44.50	39	57	68	55.81	-	-	-	-	-	-	-	-
7	-	-	-	-	13	57	23	28.53	-	-	-	-	-	-	-	-
8	47	57	82	65.24	-	-	-	-	-	-	-	-	-	-	-	-
9	35	57	61	51.59	42	57	74	59.14	-	-	-	-	-	-	-	-
10	42	57	74	59.14	-	-	-	-	-	-	-	-	-	-	-	-
11	47	57	82	65.24	39	57	68	55.81	-	-	-	-	-	-	-	-
12	-	-	-	-	27	57	47	43.49	-	-	-	-	-	-	-	-
13	45	57	79	62.69	55	57	96	79.20	-	-	-	-	-	-	-	-
14	42	57	74	59.14	16	57	28	31.99	-	-	-	-	-	-	-	-
15	35	57	61	51.59	26	57	46	42.48	-	-	-	-	-	-	-	-
16	46	57	81	63.94	-	-	-	-	-	-	-	-	-	-	-	-
Total	473	741			414	741			-	-			-	-		
Mean	36	57	64	53.44	32	57	56	48.94	-	-	-	-	-	-	-	-
SD	11	0	20	11.95	12	0	20	13.22	-	-	-	-	-	-	-	-

- 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 Northern Bobwhite Reproduction Study with PFOS

Replicate	0 PPM A.I.				10 PPM A.I.				50 PPM A.I.				150 PPM A.I.			
	14-Day Max. Old	Set	%	Arcsin Trans.	14-Day Max. Old	Set	%	Arcsin Trans.	14-Day Max. Old	Set	%	Arcsin Trans.	14-Day Max. Old	Set	%	Arcsin Trans.
1	43	57	75	60.29	20	57	35	36.32	-	-	-	-	-	-	-	-
2	12	57	21	27.31	36	57	63	52.63	-	-	-	-	-	-	-	-
3	-	-	-	-	29	57	51	45.50	-	-	-	-	-	-	-	-
4	30	57	53	46.51	30	57	53	46.51	-	-	-	-	-	-	-	-
5	19	57	33	35.26	30	57	53	46.51	-	-	-	-	-	-	-	-
6	27	57	47	43.49	38	57	67	54.74	-	-	-	-	-	-	-	-
7	-	-	-	-	10	57	18	24.76	-	-	-	-	-	-	-	-
8	46	57	81	63.94	-	-	-	-	-	-	-	-	-	-	-	-
9	33	57	58	49.54	37	57	65	53.68	-	-	-	-	-	-	-	-
10	40	57	70	56.90	-	-	-	-	-	-	-	-	-	-	-	-
11	47	57	82	65.24	37	57	65	53.68	-	-	-	-	-	-	-	-
12	-	-	-	-	27	57	47	43.49	-	-	-	-	-	-	-	-
13	45	57	79	62.69	53	57	93	74.64	-	-	-	-	-	-	-	-
14	40	57	70	56.90	16	57	28	31.99	-	-	-	-	-	-	-	-
15	35	57	61	51.59	23	57	40	39.44	-	-	-	-	-	-	-	-
16	40	57	70	56.90	-	-	-	-	-	-	-	-	-	-	-	-
Total	457	741			386	741			-	-			-	-		
Mean	35	57	62	52.04	30	57	52	46.45	-	-	-	-	-	-	-	-
SD	11	0	19	11.44	11	0	19	12.41	-	-	-	-	-	-	-	-

- 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 Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
Control	LOT ¹																	
	A	2	0	-	0	0	0	-	1	0	0	0	-	2	0	3	1	9
	B	5	2	-	1	0	0	-	5	1	2	2	-	5	3	4	2	32
	C	5	5	-	2	0	1	-	4	5	3	4	-	5	4	6	5	49
	D	5	5	-	3	0	3	-	6	5	4	5	-	5	5	7	6	59
	E	6	6	-	5	0	3	-	6	7	6	7	-	6	6	7	6	71
	F	6	1	-	4	3	4	-	7	7	7	7	-	6	7	7	7	73
	G	7	0	-	5	3	5	-	7	7	8	8	-	6	7	7	7	77
	H	6	0	-	6	5	6	-	7	4	6	7	-	6	7	7	6	73
	I	6	4	-	6	5	5	-	7	6	7	6	-	7	7	7	7	80
J	7	4	-	6	7	5	-	6	5	7	8	-	5	8	7	7	82	
Totals		55	27	-	38	23	32	-	56	47	50	54	-	53	54	62	54	605

- Data are not available due to adult mortality

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

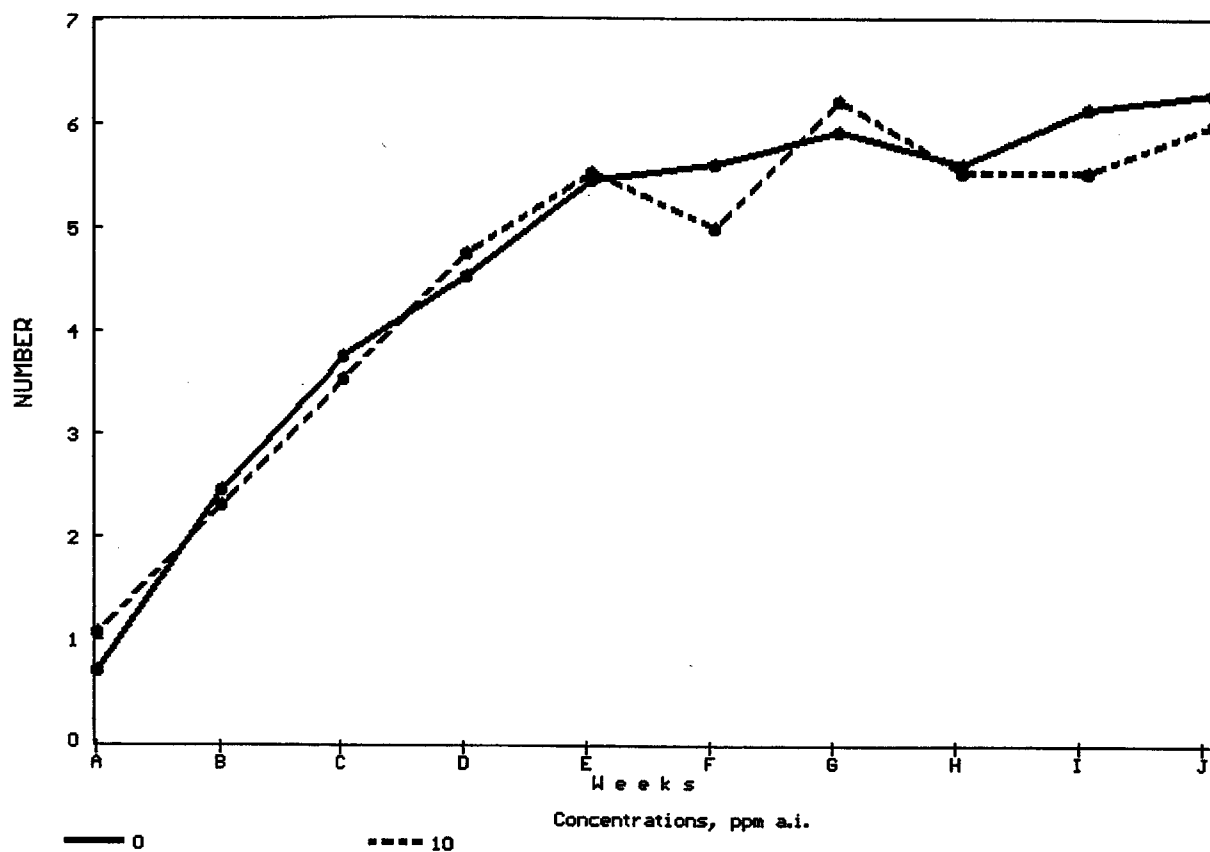
Reproductive Performance by Week and Pen
Appendix XIV - Table 1b
Eggs Laid by Week and Pen
from a Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
10	LOT ¹																	
	A	0	0	0	0	5	0	0	-	2	-	1	0	4	0	2	-	14
	B	0	1	0	0	4	3	1	-	2	-	4	3	5	2	5	-	30
	C	2	3	1	3	5	4	0	-	5	-	6	3	6	2	6	-	46
	D	2	5	4	4	6	6	4	-	5	-	5	4	6	5	6	-	62
	E	4	6	6	5	5	5	4	-	7	-	5	5	7	5	8	-	72
	F	4	7	5	5	4	7	4	-	6	-	0	5	7	5	6	-	65
	G	5	8	7	7	4	8	4	-	9	-	2	6	8	6	7	-	81
	H	6	6	6	5	4	6	3	-	6	-	7	5	6	5	7	-	72
	I	5	6	7	6	4	6	0	-	6	-	7	6	7	6	6	-	72
J	6	4	7	7	6	8	1	-	6	-	7	7	7	5	7	-	78	
Totals		34	46	43	42	47	53	21	-	54	-	44	44	63	41	60	-	592

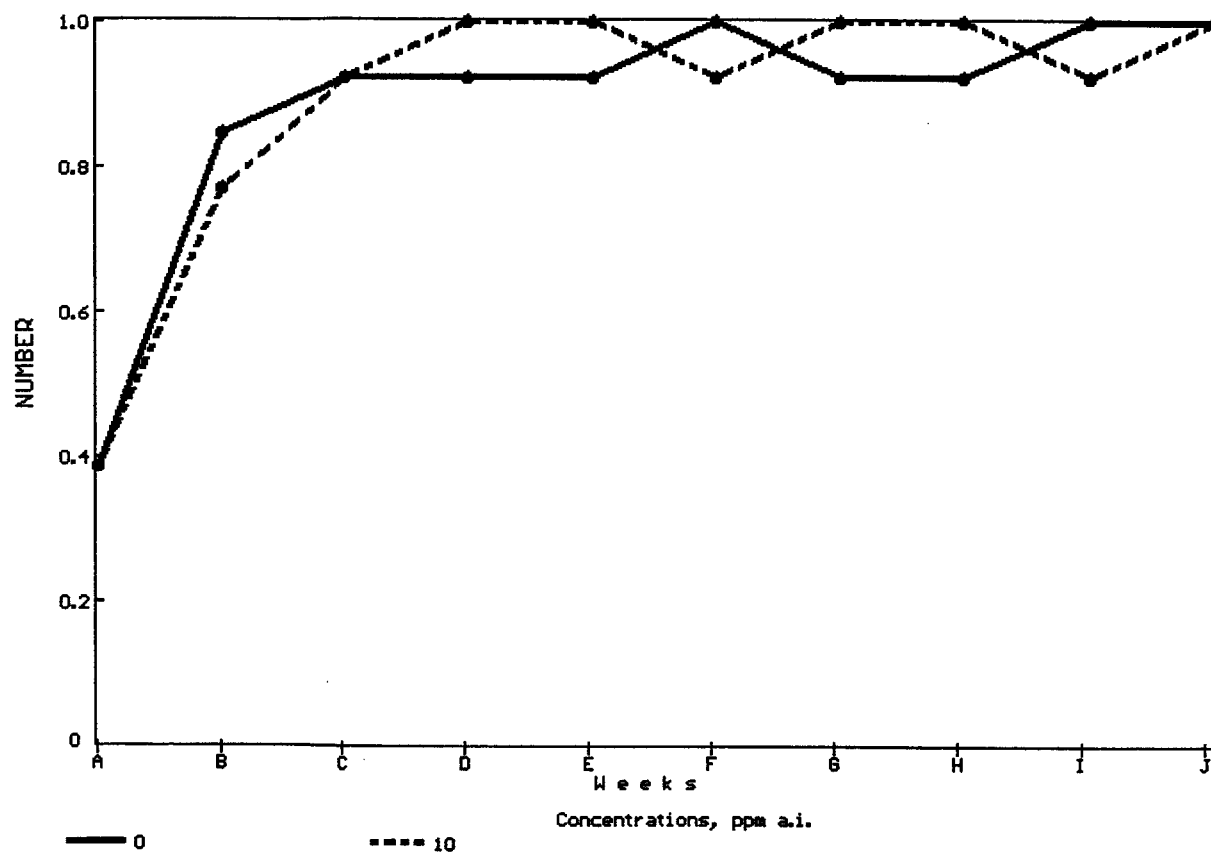
- Data are not available due to adult mortality

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

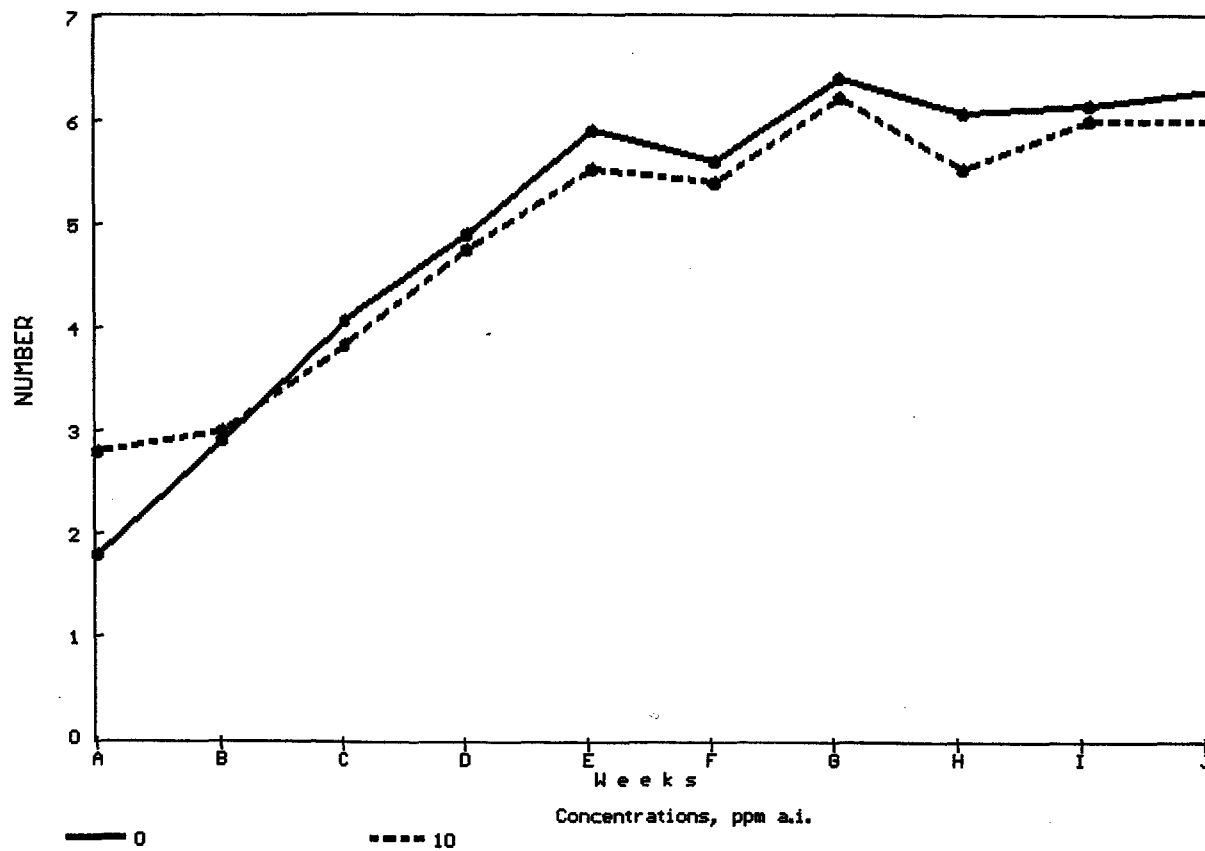
Appendix XIV - Figure 1
Eggs Laid per Hen from a Northern Bobwhite Reproduction Study with PFOS



Appendix XIV - Figure 2
Hens Laying per Pen from a Northern Bobwhite Reproduction Study with PFOS



Appendix XIV - Figure 3
Eggs per Laying Hen from a Northern Bobwhite Reproduction Study with PFOS



Reproductive Performance by Week and Pen
Appendix XIV - Table 2a
Eggs Cracked by Week and Pen
from a Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
Control	LOT ¹																	
	A	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	1	0	1
	C	0	0	-	0	0	0	-	0	2	0	0	-	0	0	1	0	3
	D	0	0	-	0	0	0	-	0	0	0	0	-	0	0	2	0	2
	E	0	0	-	0	0	0	-	0	0	1	0	-	0	0	1	0	2
	F	0	0	-	0	0	0	-	0	0	0	0	-	0	0	0	0	0
	G	0	0	-	0	0	0	-	0	0	0	0	-	1	0	5	0	6
	H	0	0	-	0	0	0	-	0	0	0	1	-	0	0	1	0	2
	I	0	0	-	0	0	0	-	0	0	0	0	-	0	0	2	0	2
J	0	0	-	2	0	0	-	0	0	0	0	-	0	0	3	0	5	
Totals		0	0	-	2	0	0	-	0	2	1	1	-	1	0	16	0	23

- 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 Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
10	LOT ¹																	
	A	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
	C	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	1	-	1
	E	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0	-	0
	F	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0	-	0
	G	0	0	0	0	0	0	0	-	0	-	0	0	0	0	1	-	1
	H	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0	-	0
	I	0	1	0	0	0	0	0	-	0	-	0	0	0	0	0	-	1
J	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0	0	-	0
Totals		0	1	0	0	0	0	0	-	0	-	0	0	0	0	2	-	3

- 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 3a
Eggs Set by Week and Pen
from a Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)	P E N S																Totals
	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	
LOT ¹																	
A	1	0	-	0	0	0	-	1	0	0	0	-	1	0	2	1	6
B	5	1	-	0	0	0	-	4	0	1	2	-	5	2	3	1	24
C	4	5	-	2	0	1	-	4	2	3	3	-	4	4	4	5	41
D	5	4	-	2	0	2	-	5	5	3	5	-	5	4	5	5	50
Control E	5	6	-	5	0	3	-	6	6	5	6	-	5	6	5	6	64
F	6	0	-	3	3	3	-	6	7	6	7	-	6	6	6	6	65
G	6	0	-	5	2	5	-	7	6	7	7	-	4	7	1	7	64
H	6	0	-	5	5	5	-	6	4	5	6	-	6	6	6	5	65
I	5	4	-	6	4	5	-	7	5	7	5	-	6	7	4	7	72
J	7	3	-	3	7	4	-	5	5	6	8	-	5	7	4	6	70
Totals	50	23	-	31	21	28	-	51	40	43	49	-	47	49	40	49	521

- 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 Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
10	LOT ¹																	
	A	0	0	0	0	4	0	0	-	1	-	0	0	3	0	1	-	9
	B	0	0	0	0	4	2	1	-	2	-	4	2	5	1	5	-	26
	C	1	3	0	3	4	4	0	-	4	-	5	3	5	2	5	-	39
	D	2	4	4	3	6	5	4	-	5	-	5	2	6	4	5	-	55
	E	3	6	5	5	4	5	3	-	6	-	4	5	6	5	7	-	64
	F	4	6	5	4	4	6	4	-	6	-	0	4	7	4	6	-	60
	G	4	8	6	7	3	8	3	-	7	-	1	6	6	6	5	-	70
	H	6	5	6	4	4	5	3	-	6	-	7	4	6	4	7	-	67
	I	4	5	6	6	3	6	0	-	5	-	6	6	6	6	5	-	64
J	6	3	7	6	6	7	1	-	6	-	7	5	7	4	7	-	72	
Totals		30	40	39	38	42	48	19	-	48	-	39	37	57	36	53	-	526

- 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 4a
Viable Embryos by Week and Pen
from a Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
Control	LOT ¹																	
	A	1	0	-	0	0	0	-	1	0	0	0	-	1	0	1	1	5
	B	5	1	-	0	0	0	-	4	0	1	2	-	5	0	3	1	22
	C	4	3	-	2	0	1	-	4	2	3	3	-	4	0	4	5	35
	D	5	0	-	2	0	2	-	5	5	3	5	-	5	4	5	4	45
	E	5	2	-	4	0	3	-	6	6	5	6	-	5	6	5	6	59
	F	5	0	-	3	3	3	-	6	7	6	5	-	6	6	5	6	61
	G	6	0	-	5	2	5	-	7	6	7	7	-	4	7	1	7	64
	H	4	0	-	5	5	5	-	6	4	5	6	-	6	6	6	5	63
	I	5	3	-	6	4	5	-	7	5	7	5	-	6	7	4	7	71
J	7	3	-	3	7	4	-	5	4	6	8	-	5	7	4	6	69	
Totals		47	12	-	30	21	28	-	51	39	43	47	-	47	43	38	48	494

- 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 Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	Totals
10	LOT ¹																	
	A	0	0	0	0	0	0	0	-	0	-	0	0	2	0	0	-	2
	B	0	0	0	0	1	2	1	-	2	-	4	0	5	0	4	-	19
	C	1	2	0	3	4	4	0	-	4	-	5	0	5	2	5	-	35
	D	2	4	4	3	6	5	4	-	5	-	5	0	6	1	5	-	50
	E	3	6	5	5	4	5	3	-	6	-	4	2	6	2	7	-	58
	F	4	6	5	4	4	5	3	-	6	-	0	4	6	1	3	-	51
	G	4	8	6	7	3	7	3	-	7	-	1	6	6	4	1	-	63
	H	6	5	6	4	4	5	2	-	6	-	7	4	6	1	7	-	63
	I	4	5	6	6	3	6	0	-	5	-	6	6	6	4	4	-	61
J	6	3	7	6	6	6	1	-	5	-	7	5	7	2	7	-	68	
Totals		30	39	39	38	35	45	17	-	46	-	39	27	55	17	43	-	470

- 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 5a
Live Three-Week Embryos by Week and Pen
from a Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
Control	LOT ¹																	
	A	0	0	-	0	0	0	-	1	0	0	0	-	0	0	1	1	3
	B	5	1	-	0	0	0	-	4	0	1	2	-	5	0	3	1	22
	C	4	3	-	2	0	1	-	4	2	3	3	-	4	0	4	5	35
	D	5	0	-	2	0	2	-	5	4	3	5	-	5	4	5	4	44
	E	5	2	-	4	0	3	-	6	6	5	6	-	5	6	5	6	59
	F	5	0	-	3	3	3	-	6	7	6	5	-	6	6	5	6	61
	G	6	0	-	5	2	5	-	7	6	7	7	-	4	7	1	7	64
	H	4	0	-	5	5	5	-	6	4	5	6	-	6	6	6	5	63
	I	5	3	-	6	4	5	-	7	5	7	5	-	6	7	4	7	71
J	7	3	-	3	7	4	-	5	4	6	8	-	5	7	3	6	68	
Totals		46	12	-	30	21	28	-	51	38	43	47	-	46	43	37	48	490

- 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 Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)	P E N S																Totals
	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	
LOT ¹																	
A	0	0	0	0	0	0	0	-	0	-	0	0	2	0	0	-	2
B	0	0	0	0	1	2	1	-	2	-	4	0	5	0	4	-	19
C	1	2	0	3	4	4	0	-	4	-	5	0	5	2	5	-	35
D	2	4	4	3	6	5	3	-	5	-	5	0	6	1	5	-	49
10 E	3	6	5	5	4	5	3	-	6	-	4	2	6	2	7	-	58
F	4	6	5	4	4	5	3	-	6	-	0	4	6	1	3	-	51
G	3	8	6	7	3	7	3	-	7	-	1	6	6	3	1	-	61
H	6	5	6	4	3	5	2	-	6	-	7	4	6	1	7	-	62
I	4	5	6	6	3	6	0	-	5	-	6	6	6	4	4	-	61
J	6	3	7	6	6	6	1	-	5	-	7	5	7	2	7	-	68
Totals	29	39	39	38	34	45	16	-	46	-	39	27	55	16	43	-	466

- 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 6a
Hatchlings by Week and Pen
from a Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)		P E N S																
		201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	Totals
Control	LOT ¹																	
	A	0	0	-	0	0	0	-	1	0	0	0	-	0	0	0	1	2
	B	5	1	-	0	0	0	-	4	0	1	2	-	5	0	3	1	22
	C	4	3	-	2	0	1	-	3	2	3	3	-	4	0	4	5	34
	D	4	0	-	2	0	2	-	5	4	3	5	-	5	3	5	4	42
	E	5	2	-	4	0	3	-	5	6	5	6	-	5	6	5	6	58
	F	5	0	-	3	3	3	-	6	7	6	5	-	6	6	5	5	60
	G	5	0	-	5	2	5	-	7	6	6	7	-	4	7	1	7	62
	H	4	0	-	5	4	5	-	6	2	5	6	-	5	6	5	4	57
	I	5	3	-	6	4	5	-	7	4	7	5	-	6	7	4	7	70
J	7	3	-	3	7	4	-	3	4	6	8	-	5	7	3	6	66	
Totals		44	12	-	30	20	28	-	47	35	42	47	-	45	42	35	46	473

- 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 Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)	P E N S																Totals
	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	
LOT ¹																	
A	0	0	0	0	0	0	0	-	0	-	0	0	2	0	0	-	2
B	0	0	0	0	1	0	0	-	2	-	4	0	5	0	3	-	15
C	0	2	0	1	3	3	0	-	3	-	5	0	5	2	3	-	27
D	1	4	4	1	6	3	3	-	5	-	5	0	6	1	4	-	43
10 E	3	6	5	5	3	5	2	-	5	-	4	2	6	2	3	-	51
F	3	5	5	3	4	5	3	-	5	-	0	4	6	1	3	-	47
G	2	7	6	7	3	6	2	-	7	-	1	6	6	3	0	-	56
H	2	4	5	4	2	5	2	-	5	-	7	4	6	1	4	-	51
I	4	5	6	5	3	6	0	-	5	-	6	6	6	4	3	-	59
J	5	3	7	6	6	6	1	-	5	-	7	5	7	2	3	-	63
Totals	20	36	38	32	31	39	13	-	42	-	39	27	55	16	26	-	414

- 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 7a
14-Day Old Survivors by Week and Pen
from a Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)	P E N S																Totals
	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	
LOT ¹																	
A	0	0	-	0	0	0	-	1	0	0	0	-	0	0	0	1	2
B	4	1	-	0	0	0	-	4	0	1	2	-	5	0	3	1	21
C	4	3	-	2	0	1	-	3	2	2	3	-	4	0	4	3	31
D	4	0	-	2	0	2	-	5	4	2	5	-	5	3	5	3	40
Control E	5	2	-	4	0	3	-	5	5	5	6	-	5	6	5	5	56
F	5	0	-	3	2	3	-	6	6	6	5	-	6	6	5	5	58
G	5	0	-	5	2	4	-	7	6	6	7	-	4	6	1	6	59
H	4	0	-	5	4	5	-	6	2	5	6	-	5	6	5	3	56
I	5	3	-	6	4	5	-	6	4	7	5	-	6	6	4	7	68
J	7	3	-	3	7	4	-	3	4	6	8	-	5	7	3	6	66
Totals	43	12	-	30	19	27	-	46	33	40	47	-	45	40	35	40	457

- 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 Northern Bobwhite Reproduction Study with PFOS

Experimental Group (ppm a.i.)	P E N S																Totals
	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	
LOT ¹																	
A	0	0	0	0	0	0	0	-	0	-	0	0	1	0	0	-	1
B	0	0	0	0	1	0	0	-	2	-	2	0	5	0	3	-	13
C	0	2	0	0	3	3	0	-	3	-	5	0	5	2	3	-	26
D	1	4	1	1	6	3	1	-	5	-	5	0	6	1	2	-	36
10 E	3	6	4	5	3	5	2	-	4	-	4	2	6	2	3	-	49
F	3	5	4	3	4	5	3	-	5	-	0	4	6	1	3	-	46
G	2	7	5	7	3	6	1	-	6	-	1	6	6	3	0	-	53
H	2	4	3	4	1	5	2	-	3	-	7	4	6	1	4	-	46
I	4	5	6	5	3	6	0	-	5	-	6	6	6	4	3	-	59
J	5	3	6	5	6	5	1	-	4	-	7	5	6	2	2	-	57
Totals	20	36	29	30	30	38	10	-	37	-	37	27	53	16	23	-	386

- Data are not available due to adult mortality

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 Northern Bobwhite Reproduction Study with PFOS
(Control)

Pen	Weeks										Mean	SD	[n]
	A	B	C	D	E	F	G	H	I	J			
201	-		0.232		0.219		0.221		0.223		0.224	0.006	4
202		-		0.231		0.229		-		-	0.230	0.002	2
203	-		-		-		-		-				
204		-		0.249		0.256		0.258		-	0.255	0.005	3
205	-		-		-		0.221		0.221		0.221	0.000	2
206		-		0.183		0.194		0.202		-	0.193	0.009	3
207	-		-		-		-		-				
208		-		0.230		0.239		0.244		-	0.238	0.007	3
209	-		0.194		0.196		0.184		0.169		0.186	0.012	4
210		-		0.238		0.230		0.261		-	0.243	0.016	3
211	-		0.228		0.220		0.219		0.228		0.224	0.005	4
212		-		-		-		-		-			
213	-		0.238		0.230		0.231		0.228		0.232	0.005	4
214		-		0.248		0.256		0.262		-	0.255	0.007	3
215	-		0.250		0.249		0.253		0.247		0.250	0.003	4
216		-		0.222		0.223		0.221		-	0.222	0.001	3
Mean and standard deviation of pen means:											Mean	SD	
											0.229	0.021	42
- No eggs available.													

Appendix XV - Table 2
Eggshell Thickness (mm) per Pen by Week
from a Northern Bobwhite Reproduction Study with PFOS
(10 PPM A.I.)

Pen	Weeks										Mean	SD	[n]
	A	B	C	D	E	F	G	H	I	J			
217	-		0.214		0.226		0.224		0.231		0.224	0.007	4
218		-		0.221		0.210		0.220		-	0.217	0.006	3
219	-		0.224		0.234		0.242		0.249		0.237	0.011	4
220		-		0.221		0.241		0.228		-	0.230	0.010	3
221	-		0.241		0.238		0.202		0.212		0.223	0.019	4
222		-		0.263		0.239		0.240		-	0.248	0.014	3
223	-		-		0.222		0.219		-		0.221	0.002	2
224		-		-		-		-		-			
225	-		0.222		0.231		0.228		0.221		0.226	0.005	4
226		-		-		-		-		-			
227	-		0.214		0.201		0.222		0.209		0.212	0.009	4
228		-		0.222		0.229		0.237		-	0.229	0.007	3
229	-		0.242		0.220		0.215		0.227		0.226	0.012	4
230		-		0.232		0.241		0.241		-	0.238	0.005	3
231	-		0.214		0.214		0.222		0.221		0.218	0.004	4
232		-		-		-		-		-			
Mean and standard deviation of pen means:											0.227	0.010	45

- 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 Northern Bobwhite Reproduction Study with PFOS
(Control)

Pen	Weeks [No. of Hatchlings]										Mean	SD	Total Hatch
	A [n]	B [n]	C [n]	D [n]	E [n]	F [n]	G [n]	H [n]	I [n]	J [n]			
201	[0]	6 [5]	7 [4]	7 [4]	7 [5]	7 [5]	7 [5]	7 [4]	6 [5]	7 [7]	7	0	44
202	[0]	5 [1]	6 [3]	[0]	6 [2]	[0]	[0]	[0]	6 [3]	6 [3]	6	0	12
203	-	-	-	-	-	-	-	-	-	-			
204	[0]	[0]	6 [2]	6 [2]	6 [4]	6 [3]	6 [5]	6 [5]	6 [6]	6 [3]	6	0	30
205	[0]	[0]	[0]	[0]	[0]	6 [3]	6 [2]	6 [4]	6 [4]	6 [7]	6	0	20
206	[0]	[0]	5 [1]	6 [2]	7 [3]	6 [3]	6 [4]	6 [5]	6 [5]	7 [4]	6	0	27
207	-	-	-	-	-	-	-	-	-	-			
208	5 [1]	6 [4]	6 [3]	6 [5]	6 [5]	6 [6]	5 [7]	6 [6]	6 [7]	7 [3]	6	0	47
209	[0]	[0]	6 [2]	6 [4]	6 [6]	6 [7]	6 [6]	6 [2]	6 [4]	6 [4]	6	0	35
210	[0]	6 [1]	6 [3]	6 [3]	6 [5]	6 [6]	6 [6]	6 [5]	6 [7]	6 [6]	6	0	42
211	[0]	6 [2]	6 [3]	6 [5]	6 [6]	7 [5]	6 [7]	7 [6]	7 [5]	7 [8]	6	0	47
212	-	-	-	-	-	-	-	-	-	-			
213	[0]	6 [5]	7 [4]	7 [5]	7 [5]	7 [6]	7 [4]	7 [5]	7 [6]	7 [5]	7	0	45
214	[0]	[0]	[0]	6 [3]	6 [6]	7 [6]	7 [7]	6 [6]	6 [7]	6 [7]	6	0	42
215	[0]	6 [3]	7 [4]	7 [5]	6 [5]	7 [5]	6 [1]	6 [5]	7 [4]	7 [3]	7	0	35
216	6 [1]	6 [1]	6 [5]	6 [4]	6 [6]	6 [5]	6 [7]	7 [4]	6 [7]	6 [6]	6	0	46
											Mean	SD	
											6	0	472

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 Northern Bobwhite Reproduction Study with PFOS
(10 PPM A.I.)

Pen	Weeks [No. of Hatchlings]										Mean	SD	Total Hatch
	A [n]	B [n]	C [n]	D [n]	E [n]	F [n]	G [n]	H [n]	I [n]	J [n]			
217	[0]	[0]	[0]	6 [1]	6 [3]	6 [3]	6 [2]	6 [2]	6 [4]	6 [5]	6	0	20
218	[0]	[0]	6 [2]	7 [4]	6 [6]	6 [5]	7 [7]	6 [4]	7 [5]	6 [3]	6	0	36
219	[0]	[0]	[0]	5 [3]	6 [5]	6 [5]	6 [6]	6 [5]	6 [6]	6 [7]	6	0	37
220	[0]	[0]	5 [1]	6 [1]	6 [5]	6 [3]	5 [7]	6 [4]	6 [5]	6 [6]	6	0	32
221	[0]	6 [1]	6 [3]	6 [6]	6 [3]	7 [4]	6 [3]	7 [2]	6 [3]	6 [6]	6	0	31
222	[0]	[0]	6 [3]	6 [3]	6 [5]	6 [5]	6 [6]	6 [5]	6 [6]	7 [6]	6	0	39
223	[0]	[0]	[0]	5 [3]	6 [2]	6 [3]	5 [2]	6 [2]	[0]	5 [1]	6	1	13
224	-	-	-	-	-	-	-	-	-	-			
225	[0]	5 [2]	5 [3]	6 [5]	5 [5]	5 [5]	5 [7]	5 [5]	6 [5]	6 [5]	5	0	42
226	-	-	-	-	-	-	-	-	-	-			
227	[0]	7 [4]	7 [5]	7 [5]	6 [4]	[0]	6 [1]	7 [7]	7 [6]	7 [7]	7	0	39
228	[0]	[0]	[0]	[0]	6 [2]	6 [4]	6 [6]	7 [4]	7 [6]	7 [5]	6	0	27
229	6 [1]	6 [5]	6 [5]	6 [6]	6 [6]	6 [6]	6 [6]	7 [6]	7 [6]	6 [7]	6	0	54
230	[0]	[0]	8 [2]	8 [1]	8 [2]	6 [1]	8 [3]	7 [1]	8 [4]	8 [2]	8	0	16
231	[0]	6 [3]	6 [3]	6 [4]	6 [3]	6 [3]	[0]	6 [4]	5 [3]	5 [3]	6	0	26
232	-	-	-	-	-	-	-	-	-	-			
											Mean	SD	
											6	1	412

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.

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 Northern Bobwhite Reproduction Study with PFOS
(Control)

Pen	Weeks [No. of 14-Day Old Survivors]										14-Day	
	A [n]	B [n]	C [n]	D [n]	E [n]	F [n]	G [n]	H [n]	I [n]	J [n]	Mean	SD Total
201	[0]	29[4]	29[4]	32[4]	27[5]	33[5]	29[5]	29[4]	34[5]	32[7]	30	2 43
202	[0]	22[1]	26[3]	[0]	26[2]	[0]	[0]	[0]	22[3]	25[3]	24	2 12
203	-	-	-	-	-	-	-	-	-	-		
204	[0]	[0]	29[2]	28[2]	32[4]	28[3]	29[5]	31[5]	32[6]	34[3]	31	2 30
205	[0]	[0]	[0]	[0]	[0]	28[2]	30[2]	30[4]	34[4]	31[7]	31	2 19
206	[0]	[0]	28[1]	31[2]	30[3]	31[3]	28[4]	32[5]	33[5]	29[4]	30	2 27
207	-	-	-	-	-	-	-	-	-	-		
208	26[1]	28[4]	32[3]	36[5]	32[5]	32[6]	33[7]	33[6]	40[6]	38[3]	34	3 46
209	[0]	[0]	24[2]	25[4]	22[5]	23[6]	27[6]	28[2]	32[4]	30[4]	26	3 33
210	[0]	22[1]	28[2]	23[2]	23[5]	25[6]	27[6]	27[5]	30[7]	32[6]	27	3 40
211	[0]	30[2]	26[3]	31[5]	29[6]	30[5]	30[7]	29[6]	30[5]	31[8]	30	1 47
212	-	-	-	-	-	-	-	-	-	-		
213	[0]	28[5]	25[4]	30[5]	32[5]	30[6]	28[4]	31[5]	31[6]	34[5]	30	2 45
214	[0]	[0]	[0]	29[3]	30[6]	27[6]	31[6]	28[6]	32[6]	32[7]	30	2 40
215	[0]	29[3]	23[4]	32[5]	30[5]	29[5]	27[1]	33[5]	37[4]	37[3]	31	4 35
216	24[1]	25[1]	34[3]	22[3]	30[5]	27[5]	30[6]	28[3]	32[7]	27[6]	29	3 40
											Mean	SD
											29	2 457

- 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 Northern Bobwhite Reproduction Study with PFOS
(10 PPM A.I.)

Pen	Weeks [No. of 14-Day Old Survivors]										14-Day	
	A [n]	B [n]	C [n]	D [n]	E [n]	F [n]	G [n]	H [n]	I [n]	J [n]	Mean	SD Total
217	[0]	[0]	[0]	13[1]	23[3]	29[3]	31[2]	20[2]	31[4]	31[5]	28	5 20
218	[0]	[0]	34[2]	34[4]	34[6]	32[5]	35[7]	30[4]	34[5]	32[3]	33	2 36
219	[0]	[0]	[0]	18[1]	28[4]	23[4]	26[5]	25[3]	26[6]	26[6]	26	2 29
220	[0]	[0]	[0]	31[1]	32[5]	33[3]	28[7]	25[4]	31[5]	31[5]	30	3 30
221	[0]	32[1]	27[3]	28[6]	28[3]	28[4]	29[3]	29[1]	32[3]	30[6]	29	1 30
222	[0]	[0]	28[3]	22[3]	30[5]	26[5]	27[6]	26[5]	30[6]	30[5]	28	2 38
223	[0]	[0]	[0]	24[1]	31[2]	33[3]	32[1]	22[2]	[0]	25[1]	29	5 10
224	-	-	-	-	-	-	-	-	-	-	-	-
225	[0]	26[2]	26[3]	27[5]	27[4]	24[5]	25[6]	27[3]	29[5]	28[4]	26	1 37
226	-	-	-	-	-	-	-	-	-	-	-	-
227	[0]	29[2]	35[5]	35[5]	31[4]	[0]	33[1]	33[7]	37[6]	37[7]	34	3 37
228	[0]	[0]	[0]	[0]	35[2]	30[4]	31[6]	32[4]	32[6]	34[5]	32	2 27
229	30[1]	36[5]	32[5]	29[6]	31[6]	33[6]	31[6]	35[6]	34[6]	34[6]	33	2 53
230	[0]	[0]	42[2]	36[1]	33[2]	31[1]	34[3]	36[1]	38[4]	36[2]	36	3 16
231	[0]	31[3]	33[3]	22[2]	33[3]	27[3]	[0]	30[4]	32[3]	30[2]	30	3 23
232	-	-	-	-	-	-	-	-	-	-	-	-
											Mean	SD
											30	3 386

- Data are not available due to adult mortality.

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 Northern Bobwhite Reproduction Study with PFOS

Control (0 ppm a.i.)

Bird	Male	Female		
	Liver	Liver		
1	0.672	0.950		
2	0.884	0.789		
3	0.658	0.767		
4	1.058	0.893		
5	0.922			
6	0.858			
Mean	0.842	0.850	Group	Mean 0.845
SD	0.154	0.087		SD 0.125

10 ppm a.i.

Bird	Male	Female		
	Liver	Liver		
1	1.022	1.017		
2	0.859	0.747		
3	0.637	0.682		
4	0.727	0.845		
5	1.019	1.121		
Mean	0.853	0.882	Group	Mean 0.868
SD	0.172	0.184		SD 0.169

The above differences from the control group were not statistically significant.

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 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 possible analysis. Eggs selected from Lots C through I were opened and the albumen and yolk were 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 delete the reference substance number from page two of the protocol.
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, and to add the 3M Environmental Laboratory Project Identification Number to be used for the study..
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. The temperature in Hatcher C, containing eggs for Lot J, was inadvertently not recorded on the afternoon of July 8, 2001.
16. The liver weight for an adult female (Band #428) in the 50 ppm a.i. treatment group was inadvertently not recorded.
17. Liver weights were recorded for adults and offspring selected for tissue collection, and data were compared statistically using Dunnett's t-test. Feather samples were also collected, when available, from birds from which tissue samples were collected. The protocol did not require weighing of livers or collection of these samples.
18. On May 28, 2001, the temperature in brooder level B35-4, housing offspring from the 10 ppm a.i. treatment group, was inadvertently not recorded. On June 29, 2001, the temperatures in brooder levels B04-3, 4 and 5, housing offspring from the 10 ppm a.i. treatment group, were inadvertently not recorded.
19. Dietary samples collected on Day 0 of Week 1 were not stored frozen for the entire time interval between collection and processing for analysis. These samples were removed from the freezer and stored at ambient temperature for one day prior to processing.
20. Week 15 blood samples were collected from one male in the control group and one male in the 10 ppm a.i. treatment group who were not designated for blood collection. The additional blood samples were collected from male birds in pens where the female had died.

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 of Avian Toxicology
- (3) Linda R. Mitchell, Manager of Ecotox Operations
- (4) Diana Temple, Laboratory Supervisor
- (5) Sean P. Gallagher, Senior Biologist

Analytical Chemistry

- (1) Willard B. Nixon, Ph.D., Director of Chemistry
- (2) Raymond L. Van Hoven, Ph.D., Scientist