# PFOS:
# A PILOT REPRODUCTION STUDY WITH THE NORTHERN BOBWHITE

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-104

3M LAB REQUEST NO. U2723

FIFRA Guideline 71-4

AUTHORS: Sean P. Gallagher
Raymond L. VanHoven
Joann B. Beavers

STUDY INITIATION DATE: February 28, 2000

STUDY COMPLETION DATE: December 18, 2003

Submitted to

3M Corporation
Environmental Laboratory
935 Bush Avenue
St.Paul, Minnesota 55106

*Wildlife International, Ltd.*

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Pilot Reproduction Study with the Northern Bobwhite

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-104

STUDY COMPLETION: December 18, 2003

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

The study was conducted under multiple protocols. The in-life portion was conducted under one protocol (Wildlife International, Ltd. study number 454-104), and the analytical portions were conducted under two separate protocols (Exygen Research study numbers 023-041 and 023-063). Exygen Research study number 023-041 was initiated with a separate Study Director.

Results of analyses conducted by Exygen Research for study numbers 023-041 and 023-063 are reported separately.

STUDY DIRECTOR:

Sean P. Gallagher — 3/23/04

Sean P. Gallagher
Senior Biologist, Avian Toxicology

DATE

SPONSOR'S REPRESENTATIVE:

John L. Newsted — 3/24/04

John L. Newsted

DATE

## QUALITY ASSURANCE STATEMENT

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

| ACTIVITY | DATE CONDUCTED | DATE REPORTED TO: STUDY DIRECTOR | MANAGEMENT |
|---|---|---|---|
| Test Substance Preparation | February 28, 2000 | February 28, 2000 | March 1, 2000 |
| Sample Preparation | March 1, 2000 | March 1, 2000 | March 1, 2000 |
| Diet Preparation | March 7, 2000 | March 7, 2000 | March 7, 2000 |
| Analytical Data, Draft Report | January 16, 17, 28 and 29, 2003 | January 29, 2003 | January 31, 2003 |
| Biological Data, Draft Report | January 16, 17, 20, 28-31, 2003 | January 31, 2003 | February 19, 2003 |
| Statistics and Final Report | November 26, December 1-3, 2003 | December 3, 2003 | December 18, 2003 |

All inspections were study based unless otherwise noted.

Susan L. Coleman

Susan L. Coleman, B.A.
Senior Quality Assurance Representative

12-18-03

DATE

**REPORT APPROVAL**

SPONSOR: 3M Corporation

TITLE: PFOS: A Pilot Reproduction Study with the Northern Bobwhite

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3M LAB REQUEST NO.: U2723

STUDY DIRECTOR:

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

CHEMISTRY PRINCIPAL INVESTIGATOR:

Raymond L. Van Hoven, Ph.D. — DATE 12/19/03
Scientist, Analytical Chemistry

MANAGEMENT:

Joann B. Beavers — DATE 12/18/03
Director, Avian Toxicology

Willard B. Nixon, Ph.D. — DATE 12/18/03
Director of Chemistry

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## SUMMARY

STUDY: PFOS: A Pilot Reproduction Study with the Northern Bobwhite

SPONSOR: 3M Corporation

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-104

TEST DATES: Study Initiation – February 28, 2000
Experimental Start – February 29, 2000
Adult Termination – April 11 & July 13, 2000
Biological Termination – July 21, 2000

TEST ANIMALS: Northern bobwhite (*Colinus virginianus*)

AGE TEST ANIMALS: Approximately 30 weeks of age at the initiation of the test

SOURCE TEST ANIMALS: Trace Pheasantry, Inc.
288 Levengood Road
Douglassville, PA 19508
U.S.A.

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

RESULTS: Northern bobwhite were exposed to PFOS at dietary concentrations of 1.8, 6.2, and 17.6 ppm a.i. for 6 weeks. The control group and 17.6 ppm a.i. treatment groups were maintained on test diets for an additional 13 weeks. No treatment-related mortalities or overt signs of toxicity were observed at any of the test concentrations. There were no apparent treatment-related effects on body weight, feed consumption, or liver weight at the 1.8 and 6.2 ppm a.i. test concentrations. Additionally, there were no apparent treatment-related effects on female body weight or liver weight at the 17.6 ppm a.i. test concentration. There were no apparent treatment-related effects on any reproductive parameters measured during the study for any of the concentrations tested.

When compared to the control group, there was a significant reduction in mean male body weight in the 17.6 ppm a.i. treatment group. There was a significant treatment-related reduction in feed consumption for the 17.6 ppm a.i. treatment group when compared to the control group. There was also a significant reduction in mean liver weight for adult males at the 17.6 ppm a.i. treatment level that may have been related to the treatment. Histopathological examination of selected tissues also revealed regression of testicular tissue for two adult males in the 17.6 ppm a.i. test group that may have been related to treatment. Based upon the results of this study, the no-observed-effect concentration for northern bobwhite exposed to PFOS in the diet for 6 weeks was 6.2 ppm a.i.

## INTRODUCTION

This study was conducted by Wildlife International, Ltd. for 3M Corporation at the Wildlife International, Ltd. avian toxicology facility in Easton, Maryland 21601. The biological portion of the test was conducted from February 29, 2000 until July 21, 2000. Raw data generated at Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454-104 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 6 weeks or 19 weeks. Effects on adult health, body weight, and feed consumption were evaluated. In addition, the effects of adult exposure to PFOS on the number of eggs laid, fertility, embryo viability, hatchability and offspring survival were evaluated. Histopathological examination of selected tissues and analyses of blood and tissue samples were also used to evaluate the effects upon adults exposed to PFOS and to their offspring.

## EXPERIMENTAL DESIGN

Northern bobwhite (20 males and 20 females) were randomly distributed into one control group and three treatment groups. The test concentrations were selected in consultation with the Sponsor, based upon the results of a LC50 study (Wildlife International, Ltd. Project Number 454-103) and additional toxicity information provided by the Sponsor. The original test concentrations selected were 0, 2, 7 and 20 ppm a.i. Following experimental start, the test material was reanalyzed and the final purity was lower than originally reported. Therefore, the actual nominal test concentrations were 0, 1.8, 6.2 and 17.6 ppm a.i.

**PFOS Treatment Groups**

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

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

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

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

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

## MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "PFOS: A Pilot Reproduction Study with the 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 and the ASTM "Standard Practice for Conducting Reproductive Studies with Avian Species" (1,2).

### Test Substance and Internal Standard

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

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

### Test Organisms

Forty-eight (24 males and 24 females) pen-reared northern bobwhite were purchased from Trace Pheasantry, Inc., 288 Levengood Road, Douglassville, PA 19508, U.S.A. At the start of acclimation, the bobwhite appeared healthy and were phenotypically indistinguishable from wild type. The birds were from the same hatch, approaching their first breeding season and had not been used in any previous testing. At the start of acclimation, a random number generating function in a spreadsheet program was used to randomize pen assignment for each bird. Immediately prior to test initiation, all potential study

birds were examined for physical injuries and general health. Birds that did not appear healthy, either due to injury or inability to acclimate to laboratory conditions, or were outside the weight range for the test, were excluded from the study. All birds were approximately 30 weeks of age at test initiation (first day of exposure to test diet) and ranged in weight from 194 to 254 grams at test initiation. Sex of the birds was determined by a visual examination of the plumage.

**Identification**

Adult birds were identified by individual leg bands, each pen was identified with a unique number, and groups of pens were identified by project number and concentration. During the test, the number of eggs laid in each pen was recorded to evaluate egg production. All eggs collected for sampling or incubation were marked with the pen number using a permanent ink marking pen for identification. Hatchlings were 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%) (3). Offspring received basal diet without test substance and without the addition of 5% supplemental limestone.

Water was supplied by the town of Easton public water supply. 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 February 28, 2000 and presented to the birds on Tuesday of each week. Dietary concentrations were adjusted for purity of the test substance and are presented as parts per million active ingredient (ppm a.i.). Details of the weekly preparation of test and control diets are shown in Appendix III.

**Diet Sampling**

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

**Analytical Method**

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

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

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

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

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

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

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

**Housing and Environmental Conditions**

Housing and husbandry practices were conducted so as to adhere to the guidelines established by the National Research Council (4). The adult birds were housed indoors in batteries of pens manufactured by 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 22.3 ± 1.1°C (SD) with an average relative humidity of 50 ± 14% (SD). The air handling system in the study room was designed to vent up to 15 room air volumes every hour and replace them with fresh air.

The photoperiod in the adult northern bobwhite room was maintained by a time clock. The photoperiod during acclimation and the test was 17 hours of light per day to induce egg laying. Throughout the test, the birds received a mean of approximately 188 lux (~ 17.5 ft. candles) of illumination provided by fluorescent lights that closely approximated noon-day sunlight.

**Observations**

The test birds were acclimated to the facilities and study pens for approximately 6 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 approximately 12 weeks 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, 10, 11 and at adult termination. Feed consumption for each pen was measured weekly throughout the test. Feed consumption was determined by weighing the freshly filled feeder on Day 0, recording the amount of any additional diet added during the week, and weighing the feeder and remaining feed at the end of the feeding period (Day 7). 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. All remaining birds were euthanized two days after the beginning of Week 20. Therefore, no feed consumption estimate was calculated for Week 20.

**Adult Blood Collection**

At the time of adult termination (end of Week 6 for 1.8 and 6.2 ppm a.i. groups; beginning of Week 20 for control group and 17.6 ppm a.i. group), blood samples were collected from all surviving birds, when possible, prior to euthanasia. All blood samples were separated into serum and hemacytes/platelets, and serum was stored frozen and shipped to Centre Analytical Laboratories, Inc. for possible analysis.

**Adult Necropsy and Tissue Collection**

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

**Egg Collection and Storage**

Eggs laid during a seven day period beginning on the second day of Week 5 of the test were collected daily from test pens and stored in a cold room until incubation. The cold room was maintained at a mean temperature of 13.1°C ± 0.1°C (SD) with a mean relative humidity of approximately 72% ± 4% (SD). Groups of eggs were identified by an alphabetic lot code. All eggs laid during the week were considered as one lot.

**Candling and Incubation**

At the end of the weekly interval, all eggs were removed from the cold room, counted and candled with a Speed King (Model No. 32) egg-candling lamp to detect egg shell cracks or abnormal eggs. Cracked or abnormal eggs were recorded and discarded. All eggs 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 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 were placed in a Petersime Incubator (Model No. SP20). In the incubator the temperature was maintained at an average 37.5 ± 0.0°C (SD) with an average wet bulb temperature of 30.6 ± 0.1°C (SD) (relative humidity of approximately 60%). The incubator was equipped with a pulsator fan and blades that produced a mild breathing air movement designed to eliminate intracabinet temperature and humidity variation during incubation. In order to prevent adhesion of the embryo to the shell membrane, the incubator was also equipped with an automatic egg rotation device, designed to rotate the eggs from 50° off of vertical in one direction to 50° off of vertical in the opposite direction (total arc of rotation was 100°) every two hours through Day 21 of incubation. Eggs were candled on Day 11 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 ± 0.0°C (SD), and the average wet bulb temperature was raised to 33.3 ± 0.0°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 approximately 5 weeks of age. All hatchlings were moved to large flight pens, where they were housed approximately 7 weeks. The hatchlings were fed untreated diet without the addition of 5% supplemental limestone. At approximately 12 weeks of age, the average body weight by parental pen of all surviving offspring was determined, and the birds were euthanized with carbon dioxide and disposed of by incineration. Those offspring selected for blood and tissue sampling were stored frozen following necropsy and later disposed of by incineration.

Hatchlings were housed in batteries of brooding pens manufactured by Beacon Steel Company (Model B735Q). Each pen measured approximately 72 X 90 X 23 cm high. The external walls and 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 approximately 30 days of age. Brooding was discontinued once hatchlings were determined to be of sufficient size to thermoregulate. The average ambient room temperature in the room housing brooders was 28.6°C ± 1.0°C (SD) with an average relative humidity of 39% ± 8% (SD). All hatchlings were removed from brooding pens and transferred to flight pens at approximately 5 weeks of age. Each flight pen measured approximately 1.2 m X 1.2 m, with a ceiling height of approximately 1 m. External walls, floor and ceiling of each pen were constructed of wire mesh. 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 offspring, blood samples were collected from 10 offspring in each treatment group. All blood samples were separated into serum and hemacytes/platelets, stored frozen, and shipped to the Centre Analytical Laboratories, Inc. for possible analyses. Additionally, tissues from the 10 offspring in each group were collected for histopathological examination and analyses. When available, samples of gall bladder, liver, proventriculus, kidneys, brain, gonads, bursa of Fabricius and adipose tissue were fixed in 10% buffered formalin and shipped to EPL in Herndon, VA for histopathology. When available, samples of bile and liver tissue were stored frozen and shipped to Centre Analytical Laboratories, Inc. for possible analysis.

**Statistical Analyses**

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

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

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

## RESULTS AND DISCUSSION

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

### Analytical Results

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

6.0 ± 0.65 ppm a.i. and 17.6 ± 1.46 ppm a.i., respectively. The coefficients of variation were 7.2%, 11% and 8.3%, respectively. These values represented 100, 97 and 100% of nominal concentrations (Appendix IV, Table 4). Samples collected during Week 6, Day 0 of the test to verify test substance concentrations for the 1.8, 6.2 and 17.6 ppm a.i. diets had means and standard deviations of 2.0 ± 0.092 ppm a.i., 6.0 ± 0.67 ppm a.i. and 16.8 ± 0.608 ppm a.i., respectively. The coefficients of variation were 4.6%, 11% and 3.6%, respectively. These values represented 111, 97 and 95% of nominal concentrations (Appendix IV, Table 5). Analysis of diet samples collected from feeders after being held at ambient temperature for 7 days averaged 100%, 103% and 98% of the Day 0 values for the 1.8, 6.2 and 17.6 ppm a.i. test concentrations, respectively (Appendix IV, Table 6). A typical ion chromatogram of a test sample is shown in Appendix IV, Figure 7.

**Mortalities and Clinical Observations**

No adult mortalities occurred in the control group or in any of the treatment groups during the course of the test. Several birds were noted with head or foot lesions and feather loss as a result of pen wear and/or penmate aggression during the course of the test. An incidental clinical sign, lameness, was associated with the injuries. All other birds were normal in appearance and behavior for the duration of the test.

**Adult Body Weight**

When compared to the control group, there were no apparent treatment-related effects upon body weight at the 1.8 and 6.2 ppm a.i. test concentrations and any differences between the control group and those two treatment groups were not statistically significant at any of the body weight intervals. However, there were treatment-related reductions in mean body weight for males at the 17.6 ppm a.i. treatment group. With the exception of the Week 2 body weight interval, mean male body weight at the 17.6 ppm a.i. test concentration was statistically ($p<0.05$) different from the control group at all other intervals (Weeks 4, 6, 8, 10, 11, and 20). For female body weights, none of the differences observed between the treatment groups and the control group were statistically significant for any of the intervals monitored during the study. Mean body weight measurements are presented in Table 2 and individual body weight measurements are presented in Appendix VI.

**Feed Consumption**

Due to excessive wastage by some birds, feed consumption was variable between pens. However, when compared to the control group, there were no treatment-related effects upon feed consumption at the 1.8 or 6.2 ppm a.i. test concentrations. While statistically significant ($p<0.05$) differences between the control and the 1.8 ppm a.i. test group were observed during Week 5 and 6 of the study, these differences were not considered treatment-related due to the lack of a concentration-response relationship at the greater doses. At the 6.2 ppm a.i. test concentration, there was a statistically significant reduction in feed consumption during Week 4 and Week 6 when compared to the control group. However, these differences were not dose-responsive and the significance of the apparent reduction at Week 6 may have been a consequence of the fact that there was a relatively large increase in feed consumption in the control group at this time interval as compared to that observed at Week 5 or 7. Thus, at Week 6, all treatment group feed consumption rates were significantly reduced from the control level but were actually higher than observed at Week 5. As a result, the reductions in feed consumption at 6.2 ppm a.i. were not considered to be treatment-related. There was a treatment-related effect upon feed consumption at 17.6 ppm a.i. test concentration when compared to the control group. The reduction in feed consumption at 17.6 ppm a.i. test concentration was consistent throughout the study and statistically significant ($p<0.05$) from the control group at Weeks 2, 3, 4, 6, 8 and 15. Mean feed consumption measurements are shown in Table 3, and feed consumption measurements by pen are presented in Appendix VII.

**Gross Necropsy**

At the end of Week 6 (Day 42), all adult birds in the 1.8 and 6.2 ppm a.i. treatment groups were euthanized and subjected to gross necropsy. Adult birds in the control and 17.6 ppm a.i. treatment groups were maintained on the appropriate diets until the beginning of Week 20 and were then euthanized and subjected to gross necropsy. When compared to the control group, there was an increased incidence in the number of males with small testes in the 17.6 ppm a.i. group that was considered possibly treatment-related. All other findings observed were considered to be incidental to treatment. Necropsy findings are reported in Table 4 and Appendix VIII.

Study offspring were approximately 12 weeks of age at the time of euthanasia. A subsample of 10 juvenile birds for each test group were subjected to gross necropsy. Necropsy revealed one to three birds in each treatment level with some feather loss. Additionally, a single bird in the 6.2 ppm a.i.

treatment group was noted with abrasions on the rump. All findings observed were considered to be incidental to treatment.

**Histopathology**

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

Testes of two adult males at the 17.6 ppm a.i. test concentration exhibited decreased seminiferous tubule diameter most consistent with post-reproductive phase regression, a normal physiological phenomenon. The occurrence of testicular regression for adult males in the 17.6 ppm a.i. treatment group may be incidental to treatment, but a treatment-related effect could not be precluded. The full pathology report is provided in Appendix IX.

**Tissue Analysis**

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

**Reproductive Results**

There were no apparent treatment-related effects on egg production at any of the concentrations tested. While egg production was highly variable among hens, egg production at the 1.8, 6.2 or 17.6 ppm a.i. test concentrations was comparable to or exceeded the control group. Mean egg production by concentration is presented in Table 5. Individual egg production data are presented in Appendix X.

When compared to the control group, there were no apparent treatment-related effects on embryo viability, hatchability, hatchling health and survivability at the 1.8, 6.2 or 17.6 ppm a.i. levels. While there was a significant ($p<0.05$) reduction in the number of viable embryos at the 1.8 ppm a.i. test concentration, the reduction was due to fewer eggs being set for incubation at this level. Pen P207 of the 1.8 ppm a.i. level did not lay during the week that eggs were collected for incubation. As a result, there were fewer hatchlings and 14-day old survivors in the 1.8 ppm a.i. treatment group. However, when the reproductive endpoints were expressed as percentages, no statistically significant differences were observed at any of the concentrations tested. There was slight reduction in embryo viability at the 6.2 ppm a.i. test concentration when compared to the control. However, this reduction was not dose responsive and was not considered treatment-related. Reproductive data are summarized in Table 6. Reproductive data for individual pens is presented in Appendix XI.

**Offspring Body Weights**

There were no apparent treatment-related effects upon the body weights of hatchlings in any of the treatment groups. There were also no apparent treatment-related effects upon the body weight of juvenile birds in the 1.8, 6.2 or 17.6 ppm a.i. treatment groups. Offspring body weight data is presented in Table 7 and Appendix XII.

**Liver Weights**

When compared to the control group, there were no apparent treatment-related effects on male adult liver weight at the 1.8 or 6.2 ppm a.i. test concentrations, or on female adult liver weights at any of the concentrations tested. However, there was a statistically significant ($p<0.05$) reduction in mean male adult liver weight at the 17.6 ppm a.i. test concentration. Adult males in the control group had a mean liver weight of 3.402 grams compared to a mean liver weight of 2.527 grams for males at the 17.6 ppm a.i. test concentration. When compared to the control group, there were no apparent treatment-related effects on juvenile liver weight at any of the concentrations tested. Mean adult and offspring liver

weights are presented in Table 8. Individual adult liver weights are presented in Appendix XIII. Individual offspring liver weights are presented in Appendix XIV.

## CONCLUSION

Northern bobwhite were exposed to PFOS at dietary concentrations of 1.8, 6.2, and 17.6 ppm a.i. for 6 weeks. The control group and 17.6 ppm a.i. treatment groups were maintained on test diets for an additional 13 weeks. No treatment-related mortalities or overt signs of toxicity were observed at any of the test concentrations. There were no apparent treatment-related effects on body weight, feed consumption, or liver weight at the 1.8 and 6.2 ppm a.i. test concentrations. Additionally, there were no apparent treatment-related effects on female body weight or liver weight at the 17.6 ppm a.i. test concentration. There were no apparent treatment-related effects on any reproductive parameters measured during the study for any of the concentrations tested.

When compared to the control group, there was a significant reduction in mean male body weight in the 17.6 ppm a.i. treatment group. There was a significant treatment-related reduction in feed consumption for the 17.6 ppm a.i. treatment group when compared to the control group. There was also a significant reduction in mean liver weight for adult males at the 17.6 ppm a.i. treatment level that may have been related to the treatment. Histopathological examination of selected tissues also revealed regression of testicular tissue for two adult males in the 17.6 ppm a.i. test group that may have been related to treatment. Based upon the results of this study, the no-observed-effect concentration for northern bobwhite exposed to PFOS in the diet for 6 weeks was 6.2 ppm a.i.

## REFERENCES

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2 **American Society for Testing and Materials.** 1986. *Standard Practice for Conducting Reproductive Studies with Avian Species.* ASTM Standard E1062-86. Annual Book of ASTM Standards. Vol. 11.04. Philadelphia, PA. 15 pp.

3 **Merck & Co., Inc.** 1991. *The Merck Veterinary Manual.* Merck & Co. Rahway, NJ. 1832 pp.

4 **National Research Council.** 1996. *Guide for the Care and Use of Laboratory Animals.* Washington, DC. National Academy Press. 125 pp.

5 **Dunnett, C.W.** 1955. A Multiple Comparison's Procedure for Comparing Several Treatments with a Control. *Jour. Amer. Statis. Assoc.* 50: 1096-1121.

6 **Dunnett, C.W.** 1964. New Tables for Multiple Comparisons with a Control. *Biometrics* 20: 482-491.

Table 1

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

| Nominal Concentration[1] (ppm a.i.) | | Interval | | |
|---|---|---|---|---|
| | | Week 1 | | Week 6 |
| | | Day 0 | Day 7 | Day 0 |
| Control | Measured | < 0.879 [2] | < 1.41 | < 1.41 |
| 1.8 | Mean Measured | 1.8 | 1.8 | 2.0 |
| | % Nominal | 100 | 100 [3] | 111 |
| 6.2 | Mean Measured | 6.0 | 6.2 | 6.0 |
| | % Nominal | 97 | 103 [3] | 97 |
| 17.6 | Mean Measured | 17.6 | 17.2 | 16.8 |
| | % Nominal | 100 | 98 [3] | 95 |

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

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

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

Table 2

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

| Experimental Group | Sex | | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 | Change 6-8 | Week 8 | Change 8-10 | Week 10 | Change 10-11 | Week 11 | Change 11-20 | Week 20 | Change 0-20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | M | $\bar{X}$ | 214 | -1 | 213 | -1 | 212 | 2 | 213 | -1 | -1 | 212 | 3 | 215 | -3 | 212 | 4 | 216 | 2 |
| (0 ppm a.i.) | | SD | 5 | 3 | 6 | 2 | 6 | 1 | 7 | 5 | 2 | 6 | 2 | 5 | 2 | 5 | 2 | 7 | 4 |
| | F | $\bar{X}$ | 222 | 7 | 229 | -3 | 226 | 8 | 234 | 12 | 3 | 237 | -2 | 234 | -4 | 230 | 2 | 232 | 10 |
| | | SD | 14 | 3 | 11 | 4 | 14 | 7 | 12 | 6 | 5 | 9 | 13 | 17 | 6 | 12 | 16 | 21 | 10 |
| 1.8 | M | $\bar{X}$ | 206 | 0 | 207 | 4 | 211 | 1 | 212 | 6 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| ppm a.i. | | SD | 14 | 3 | 15 | 3 | 15 | 2 | 14 | 5 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | F | $\bar{X}$ | 221 | -1 | 221 | 1 | 222 | -10 | 211 | -10 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | | SD | 17 | 6 | 17 | 7 | 11 | 30 | 36 | 34 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 6.2 | M | $\bar{X}$ | 210 | -2 | 208 | -1 | 207 | 0 | 207 | -3 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| ppm a.i. | | SD | 13 | 2 | 13 | 2 | 12 | 2 | 11 | 4 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | F | $\bar{X}$ | 231 | 3 | 234 | 3 | 237 | -2 | 235 | 4 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | | SD | 17 | 7 | 17 | 6 | 23 | 8 | 23 | 7 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 17.6 | M | $\bar{X}$ | 208 | -10 * | 198 | -3 | 196 * | 0 | 196 * | -13 * | 0 | 196 * | -1 * | 195 * | -2 | 193 * | 4 | 197 * | -11 * |
| ppm a.i. | | SD | 11 | 4 | 9 | 3 | 7 | 2 | 6 | 5 | 2 | 7 | 2 | 6 | 2 | 7 | 1 | 7 | 5 |
| | F | $\bar{X}$ | 227 | 1 | 227 | 4 | 232 | 0 | 231 | 5 | 0 | 231 | 4 | 235 | -7 | 228 | -8 | 220 | -7 |
| | | SD | 16 | 4 | 16 | 4 | 14 | 6 | 14 | 3 | 4 | 15 | 2 | 16 | 5 | 16 | 19 | 33 | 24 |

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

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

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

Table 3

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

| Experimental Group | | Weeks 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Mean | 20 | 22 | 23 | 24 | 24 | 28 | 24 | 32 | 27 | 31 | 31 | 35 | 30 | 35 | 36 | 34 | 30 | 30 | 31 |
| 0 ppm a.i. | SD | 3 | 2 | 2 | 2 | 3 | 2 | 3 | 4 | 3 | 3 | 4 | 5 | 3 | 5 | 2 | 3 | 2 | 3 | 2 |
| 1.8 | Mean | 18 | 20 | 20 | 24 | 19 * | 22 * | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| ppm a.i. | SD | 2 | 3 | 2 | 2 | 4 | 2 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 6.2 | Mean | 18 | 20 | 20 | 21 * | 22 | 23 * | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| ppm a.i. | SD | 3 | 3 | 4 | 2 | 3 | 3 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 17.6 | Mean | 17 | 18 * | 20 * | 21 * | 22 | 23 * | 21 | 25 * | 26 | 28 | 29 | 31 | 26 | 27 | 28 * | 29 | 27 | 24 | 24 |
| ppm a.i. | SD | 1 | 1 | 2 | 2 | 2 | 2 | 3 | 4 | 6 | 6 | 5 | 5 | 4 | 5 | 6 | 6 | 5 | 9 | 6 |

[1] Mean ± standard deviation.

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

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

Table 4

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

Adult Birds Euthanized at 6 Weeks and Test Termination

| | Males - Treatment Group (ppm a.i.) | | | | Females - Treatment Group (ppm a.i.) | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1.8 | 6.2 | 17.6 | Control | 1.8 | 6.2 | 17.6 |
| Number of birds | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Feather loss | 0 | 0 | 0 | 0 | 4 | 1 | 3 | 4 |
| Foot lesions | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Right wing twisted | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Thin condition | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Right testis small, ~ 1.25 cm | 0 | 0 | 0 | 2 | - | - | - | - |
| Right testis small, ~ 1.5 cm | 0 | 0 | 1 | 0 | - | - | - | - |
| Both testes small, ~ 1.5 cm | 0 | 0 | 1 | 0 | - | - | - | - |
| Regressed ovary | - | - | - | - | 0 | 1 | 0 | 0 |
| Not remarkable | 5 | 5 | 3 | 3 | 1 | 3 | 2 | 1 |

Table 5

Mean Egg Production [1] (Eggs Laid/Hen and Eggs/Hen/Day)
From a Northern Bobwhite Pilot Reproduction Study with PFOS

| Experimental Group | | Weeks | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | Total | E/H/D[2] | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | Total | E/H/D[2] |
| Control | Total | 21 | 25 | 29 | 27 | 27 | 32 | 161 | | 30 | 33 | 33 | 28 | 28 | 26 | 32 | 30 | 34 | 32 | 30 | 30 | 31 | 8 | 566 | |
| 0 ppm a.i. | Mean | 4 | 5 | 6 | 5 | 5 | 6 | 32 | 0.77 | 6 | 7 | 7 | 6 | 6 | 5 | 6 | 6 | 7 | 6 | 6 | 6 | 6 | 2 | 113 | 0.84 |
| | SD | 2 | 1 | 1 | 1 | 1 | 1 | 6 | 0.14 | 2 | 1 | 1 | 3 | 3 | 3 | 1 | 1 | 0 | 1 | 2 | 2 | 2 | 1 | 12 | 0.09 |
| 1.8 | Total | 22 | 29 | 28 | 27 | 20 | 20 | 146 | | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | |
| ppm a.i. | Mean | 4 | 6 | 6 | 5 | 4 | 4 | 29 | 0.70 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | SD | 1 | 1 | 1 | 1 | 2 | 4 | 6 | 0.13 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 6.2 | Total | 20 | 24 | 27 | 27 | 27 | 33 | 158 | | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | |
| ppm a.i. | Mean | 4 | 5 | 5 | 5 | 5 | 7 | 32 | 0.75 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | SD | 2 | 2 | 2 | 1 | 2 | 1 | 7 | 0.17 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 17.6 | Total | 27 | 27 | 30 | 29 | 33 | 35 | 181 | | 32 | 34 | 35 | 34 | 34 | 34 | 33 | 31 | 28 | 30 | 28 | 25 | 21 | 5 | 585 | |
| ppm a.i. | Mean | 5 | 5 | 6 | 6 | 7 | 7 | 36 | 0.86 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 6 | 6 | 6 | 5 | 4 | 1 | 117 | 0.87 |
| | SD | 2 | 1 | 1 | 1 | 1 | 0 | 5 | 0.13 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 3 | 3 | 2 | 4 | 1 | 20 | 0.15 |

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

[2] Eggs per Hen per Day

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

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

Table 6

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

| Reproductive Parameter | Experimental Group | | | |
|---|---|---|---|---|
| | Control (0 ppm a.i.) | 1.8 ppm a.i. | 6.2 ppm a.i. | 17.6 ppm a.i. |
| Number of Replicates | 5 | 5 | 5 | 5 |
| Eggs Laid | 29 | 18 | 29 | 34 |
| Eggs Cracked | 0 | 0 | 0 | 0 |
| Eggs Set | 29 | 18 | 29 | 34 |
| Viable Embryos | 29 | 17 * | 25 | 34 |
| Live 3-Week Embryos | 28 | 17 | 25 | 33 |
| Hatchlings | 28 | 17 | 25 | 31 |
| Offspring Survivors | 27 | 17 | 20 | 25 |
| Eggs Laid/Hen | 6 | 4 | 6 | 7 |
| Offspring/Hen | 5 | 3 | 4 | 5 |
| Eggs Laid/Hen/Day | 0.83 | 0.64 | 0.83 | 0.97 |
| Eggs Cracked/Eggs Laid (%) | 0 | 0 | 0 | 0 |
| Viable Embryos/Eggs Set (%) | 100 | 95 | 86 | 100 |
| Live 3-Week Embryos/Viable (%) | 97 | 100 | 100 | 97 |
| Hatchlings/3-Week Embryos (%) | 100 | 100 | 100 | 94 |
| Offspring Survivors/Hatchlings (%) | 97 | 100 | 86 | 79 |
| Hatchlings/Eggs Set (%) | 97 | 95 | 86 | 91 |
| Offspring Survivors/Eggs Set (%) | 93 | 95 | 72 | 73 |
| Hatchlings/Hen/Day | 0.80 | 0.49 | 0.71 | 0.89 |
| Offspring Survivors/Hen/Day | 0.77 | 0.49 | 0.57 | 0.71 |

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

Table 7

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

| Experimental Group | Hatchlings | | Surviving Offspring[2] | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Control (0 ppm a.i.) | 6.1 | ± 0.5 | 175 | ± 10 |
| 1.8 ppm a.i. | 5.6 | ± 0.5 | 175 | ± 1 |
| 6.2 ppm a.i. | 5.7 | ± 0.6 | 179 | ± 5 |
| 17.6 ppm a.i. | 6.0 | ± 0.5 | 173 | ± 8 |

[1]Mean ± standard deviation.

[2]Offspring were approximately 12 weeks of age at final body weight interval.
Differences between the control group and the treatment groups were not statistically significant ($p>0.05$).

Table 8

Mean Liver Weight[1] (g) from a Northern Bobwhite
Pilot Reproduction Study with PFOS

| Experimental Group | Males Liver Mean | Males Liver SD | Females Liver Mean | Females Liver SD | Offspring[2] Liver Mean | Offspring[2] Liver SD |
|---|---|---|---|---|---|---|
| Control (0 ppm a.i.) | 3.402 | 0.446 | 7.645 | 1.295 | 3.746 | 0.339 |
| 1.8 ppm a.i. | 3.501 | 0.514 | 6.323 | 1.668 | 3.756 | 0.453 |
| 6.2 ppm a.i. | 3.684 | 0.686 | 5.623 | 0.505 | 4.985 | 1.367 |
| 17.6 ppm a.i. | 2.527 | 0.359 * | 6.851 | 1.880 | 3.429 | 0.495 |

[1] Mean ± standard deviation.

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

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

Appendix I

Certificate of Analysis

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

## *INTERIM CERTIFICATE OF ANALYSIS*

***Revision 3***

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

| Test Name | Specifications | Result |
|---|---|---|
| **Purity**[1] | | **86.9%** |
| Appearance | White Crystalline Powder | Conforms |
| Identification<br>NMR | | Positive |
| Metals (ICP/MS)<br>1. Calcium<br>2. Magnesium<br>3. Sodium<br>4. Potassium[2]<br>5. Nickel<br>6. Iron<br>7. Manganese | | 1. 0.005 wt./wt.%<br>2. 0.001 wt./wt.%<br>3. 1.439 wt./wt.%<br>4. 6.849 wt./wt.%<br>5. <0.001 wt./wt.%<br>6. 0.005 wt./wt.%<br>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)<br>1. Chloride<br>2. Fluoride<br>3. Bromide<br>4. Nitrate<br>5. Nitrite<br>6. Phosphate<br>7. Sulfate[4] | | 1. <0.015 wt./wt.%<br>2. 0.59 wt./wt.%<br>3. <0.040 wt./wt.%<br>4. <0.009 wt./wt.%<br>5. <0.006 wt./wt.%<br>6. <0.007 wt./wt.%<br>7. 8.76 wt./wt.% |
| Organic Acids [5] (IC)<br>1. TFA<br>2. PFPA<br>3. HFBA<br>4. NFPA | | 1. <0.1 wt./wt.%<br>2. <0.1 wt./wt.%<br>3. 0.10 wt./wt.%<br>4. 0.28 wt./wt.% |
| Elemental Analysis[6]:<br>1. Carbon<br>2. Hydrogen<br>3. Nitrogen<br>4. Sulfur<br>5. Fluorine | 1. Theoretical Value = 17.8%<br>2. Theoretical Value = 0%<br>3. Theoretical Value = 0%<br>4. Theoretical Value = 5.95%<br>5. Theoretical Value = 60% | 1. 12.48 wt./wt.%<br>2. 0.244 wt./wt.%<br>3. 1.74 wt./wt.%<br>4. 8.84 wt./wt.%<br>5. 54.1 wt./wt.% |

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Page 2

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**

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/06

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/06

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

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

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

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

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

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

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

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

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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: Chas Simons 10/11/01
Charles Simons Date
Scientist, Centre Analytical Laboratories

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

Appendix II
Diet and Supplement Formulations

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

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

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

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

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

Appendix II
Diet and Supplement Formulations

Table 2
Vitamins and Electrolytes Concentrate

| Water Soluble Powder | | |
|---|---|---|
| GUARANTEED ANALYSIS | Per 4 oz. | Per lb. |
| Vitamin A | 2,5000,000 | 10,000,000 IU |
| Vitamin D | 1,000,000 | 4,000,000 ICU |
| Vitamin E | 1,000 | 4,000 IU |
| Riboflavin | 750 | 3,000 Mg |
| d-Pantothenic Acid | 1,250 | 5,000 Mg |
| Niacin | 2,500 | 10,000 Mg |
| Vitamin B-12 | 2.5 | 10.0 Mg |
| MSBC | 1,000 | 4,000 Mg |
| Folic Acid | 65 | 260 Mg |
| Thiamine 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 $D_3$), Alpha Tocopheryl Acetate (source of Vitamin E). Riboflavin Supplement, d-Calcium Pantothenate, Niacin Supplement, Vitamin B-12 Supplement, Menadione Sodium Bisulfite (source of Vitamin K), Folic Acid, Thiamine 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 February 28, 2000; April 7, 2000; May 12, 2000; and June 19, 2000. Nominal preparation was as follows:

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

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

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

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

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

Appendix IV

The Analysis of PFOS in Avian Diet

APPENDIX IV

ANALYTICAL METHODS AND RESULTS

**TABLE 1**

Typical LC/MS Operational Parameters

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

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**TABLE 2**

CALCULATIONS

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

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

Determination of Sample Residues (PFOS)

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

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

Determination of Limit of Quantitation (LOQ)

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

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

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

Fortification Recoveries

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

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

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 (454-104-) | Type | Interval | Fortified[1] | Measured[1,2] | | |
| MAB-1 | Matrix Blank | Week 1, Day 0 | 0.00 | < 0.879 | - | |
| MAS-1 | Matrix Fortification | Week 1, Day 0 | 0.879 | 0.992 | 113 | 105 |
| MAS-2 | Matrix Fortification | Week 1, Day 0 | 8.79 | 8.89 | 101 | |
| MAS-3 | Matrix Fortification | Week 1, Day 0 | 22.0 | 22.0 | 100 | |
| MAB-2 | Matrix Blank | Week 1, Day 7 | 0.00 | < 1.41 | - | |
| MAS-4 | Matrix Fortification | Week 1, Day 7 | 1.76 | 1.97 | 112 | 104 |
| MAS-5 | Matrix Fortification | Week 1, Day 7 | 8.79 | 9.11 | 104 | |
| MAS-6 | Matrix Fortification | Week 1, Day 7 | 22.0 | 21.3 | 97.0 | |
| MAB-3 | Matrix Blank | Week 6, Day 0 | 0.00 | < 1.41 | - | |
| MAS-7 | Matrix Fortification | Week 6, Day 0 | 1.76 | 1.98 | 113 | 107 |
| MAS-8 | Matrix Fortification | Week 6, Day 0 | 8.79 | 9.40 | 107 | |
| MAS-9 | Matrix Fortification | Week 6, Day 0 | 22.0 | 22.0 | 100 | |
| MAB-4 | Matrix Blank | Re-extraction Set[4] | 0.00 | < 1.41 | - | |
| MAS-10 | Matrix Fortification | Re-extraction Set[4] | 1.76 | 1.84 | 105 | 102 |
| MAS-11 | Matrix Fortification | Re-extraction Set[4] | 8.79 | 8.86 | 101 | |
| MAS-12 | Matrix Fortification | Re-extraction Set[4] | 22.0 | 21.7 | 98.7 | |

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

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

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

[4] Re-extraction of: sample 454-104,105-13 from Week 1, Day 0; samples 454-104-23 and -26 from Week 1, Day 7 and sample 454-104,105-50 from Week 6, Day 0.

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**TABLE 4**

**Homogeneity of PFOS in Avian Diet**

| Nominal Concentration[1] (ppm a.i.) | Sample I.D. Number (S-454-104-) | Location Sampled in Mixing Vessel | Measured PFOS Concentration[1] (ppm a.i.) | Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV) | Mean Percent of Nominal |
|---|---|---|---|---|---|
| 1.8 | 3 | Top Left | 1.70 | | |
| | 4 | Top Right | 1.82 | | |
| | 5 | Middle Left | 1.92 | $\bar{x}$ = 1.8 ppm a.i | 100 |
| | 6 | Middle Right | 1.73 | SD = 0.13 ppm a.i. | |
| | 7 | Bottom Left | 1.77 | CV = 7.2% | |
| | 8 | Bottom Right | 2.05 | | |
| 6.2 | 9 | Top Left | 5.33 | | |
| | 10 | Top Right | 5.65 | | |
| | 11 | Middle Left | 6.09 | $\bar{x}$ = 6.0 ppm a.i. | 97 |
| | 12 | Middle Right | 5.81 | SD = 0.65 ppm a.i. | |
| | 13 | Bottom Left | 5.87[2] | CV = 11% | |
| | 14 | Bottom Right | 7.21 | | |
| 17.6 | 15 | Top Left | 16.5 | | |
| | 16 | Top Right | 17.7 | | |
| | 17 | Middle Left | 16.5 | $\bar{x}$ = 17.6 ppm a.i. | 100 |
| | 18 | Middle Right | 20.4 | SD = 1.46 ppm a.i. | |
| | 19 | Bottom Left | 17.0 | CV = 8.3% | |
| | 20 | Bottom Right | 17.6 | | |

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

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**TABLE 5**

**Verification of PFOS Concentrations in Avian Diet**

| Nominal Concentration[1] (ppm a.i.) | Sample I.D. Number (S-454-104-) | Interval (Day 0 of Week -) | Measured PFOS Concentration[1,2] (ppm a.i.) | Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV) | Mean Percent of Nominal |
|---|---|---|---|---|---|
| 0 | 1 | 1 | < 0.879 | N/A | - |
| | 2 | 1 | < 0.879 | | |
| | 43 | 6 | < 1.41 | N/A | - |
| | 44 | 6 | < 1.41 | | |
| 1.8 | 3-8 | 1 | - | (see Table 4) | 100 |
| | 45 | 6 | 1.94 | $\bar{x}$ =2.0 ppm a.i. | 111 |
| | 46 | 6 | 1.94 | SD = 0.092 ppm a.i. | |
| | 47 | 6 | 2.10 | CV = 4.6% | |
| 6.2 | 9-14 | 1 | - | (see Table 4) | 97 |
| | 48 | 6 | 6.31 | $\bar{x}$ = 6.0 ppm a.i. | 97 |
| | 49 | 6 | 6.52 | SD = 0.67 ppm a.i. | |
| | 50 | 6 | 5.27[3] | CV = 11% | |
| 17.6 | 15-20 | 1 | - | (see Table 4) | 100 |
| | 51 | 6 | 17.5 | $\bar{x}$ = 16.8 ppm a.i. | 95 |
| | 52 | 6 | 16.5 | SD = 0.608 ppm a.i. | |
| | 53 | 6 | 16.4 | CV = 3.6% | |

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

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

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

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**TABLE 6**

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

| | PFOS Concentration | | | | | | |
|---|---|---|---|---|---|---|---|
| | Week 1, Day 0[1] | | | Week 1, Day 7 | | | |
| Nominal Concentration[2] (ppm a.i.) | Sample Number (S-454-104-) | Mean Measured (ppm a.i.) | Mean Percent of Nominal | Sample Number (S-454-104-) | Measured[2,3] (ppm a.i.) | Mean Measured (ppm a.i.) | Mean Percent of Day 0 |
| 0 | 1-2 | < 0.879 | - | 21 | < 1.41 | - | - |
| | | | | 22 | < 1.41 | | |
| 1.8 | 3-8 | 1.8 | 100 | 23 | 1.97[4] | 1.8 | 100 |
| | | | | 24 | 1.65 | | |
| | | | | 25 | 1.72 | | |
| 6.2 | 9-14 | 6.0 | 97 | 26 | 5.71[4] | 6.2 | 103 |
| | | | | 27 | 7.03 | | |
| | | | | 28 | 5.82 | | |
| 17.6 | 15-20 | 17.6 | 100 | 29 | 18.1 | 17.2 | 98 |
| | | | | 30 | 17.2 | | |
| | | | | 31 | 16.2 | | |

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

APPENDIX IV

**METHOD OUTLINE FOR THE ANALYSIS OF PFOS IN AVIAN DIET**

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

↓

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

↓

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

↓

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

↓

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

↓

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

↓

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

↓

Ampulate and submit sample for LCMS analysis.

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

APPENDIX IV

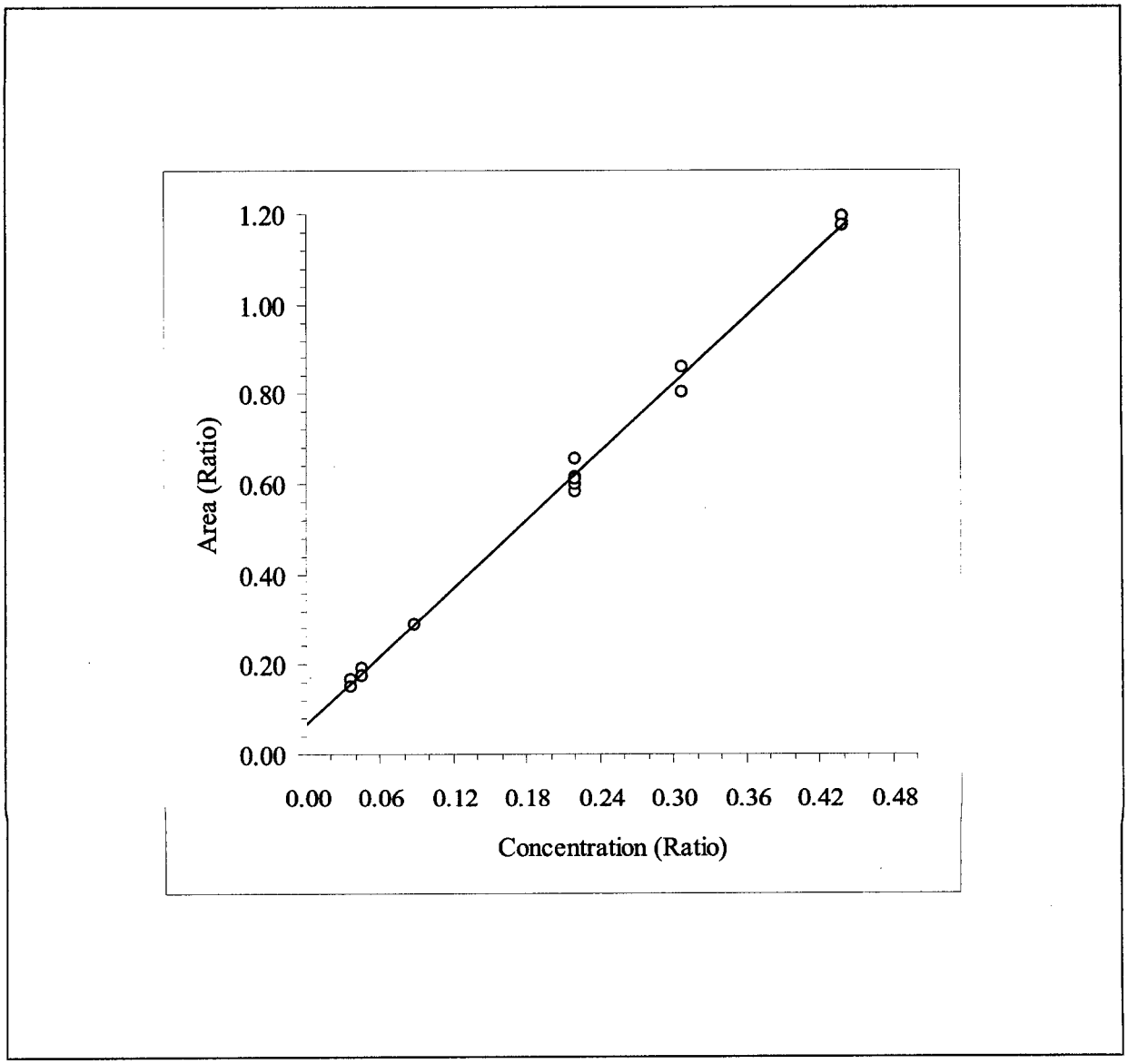


Figure 2. Typical calibration curve for PFOS. Slope = 2.48679; Intercept = 0.07396; r = 0.9985. Curve is weighted (1/x).

APPENDIX IV

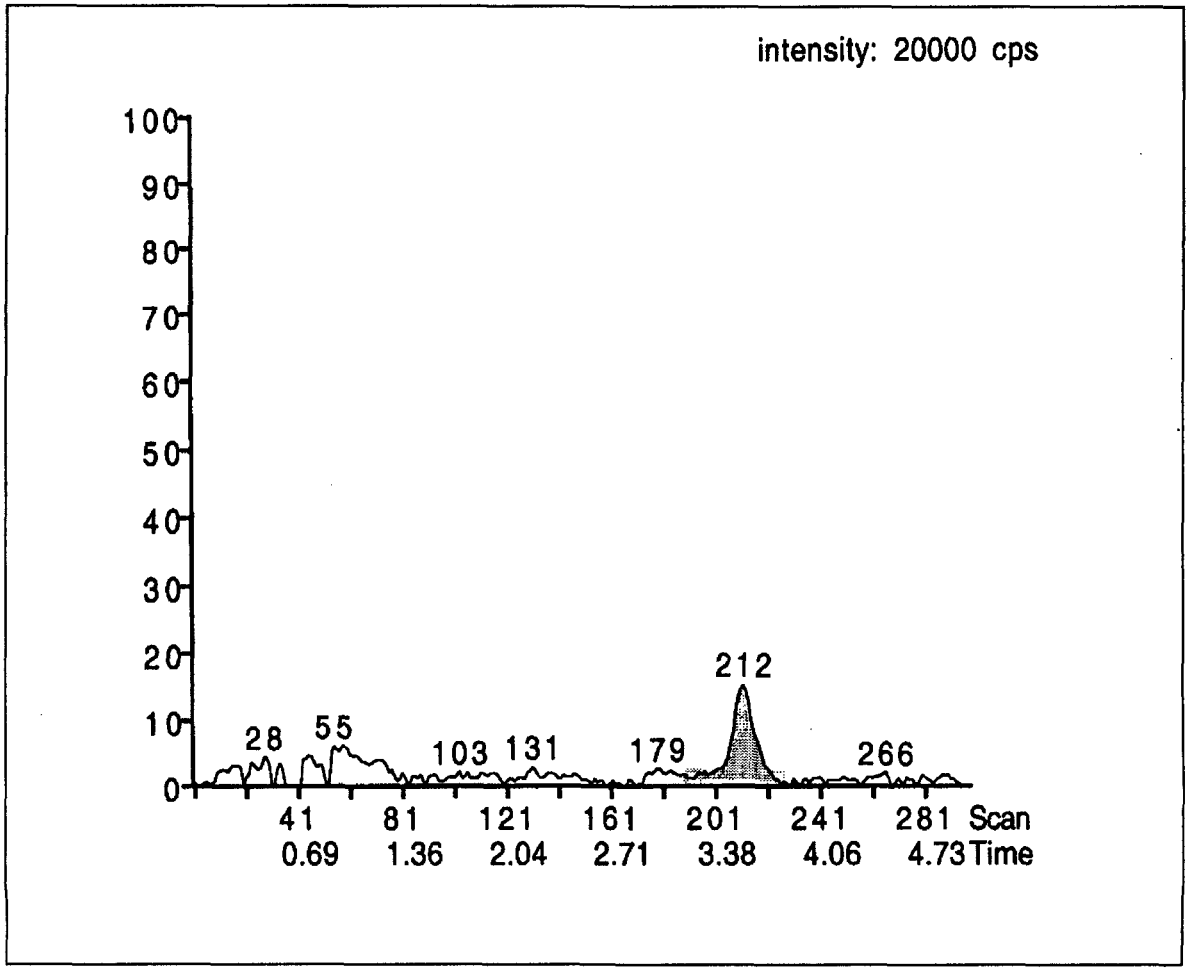


Figure 3. Typical ion chromatogram of a low-level PFOS standard, 0.000351 mg a.i./L.

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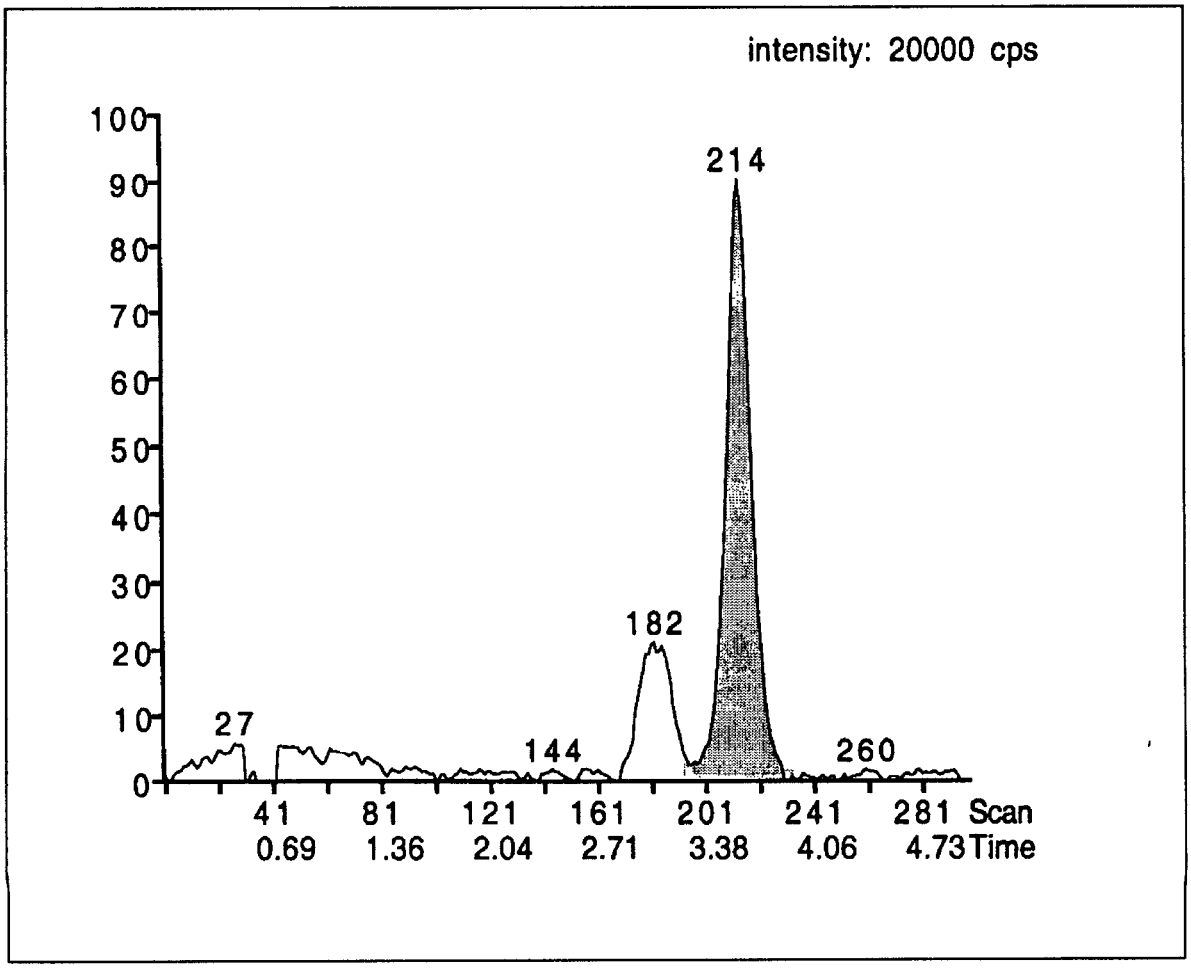


Figure 4. Typical ion chromatogram of a high-level PFOS standard, 0.00439 mg a.i./L.

APPENDIX IV

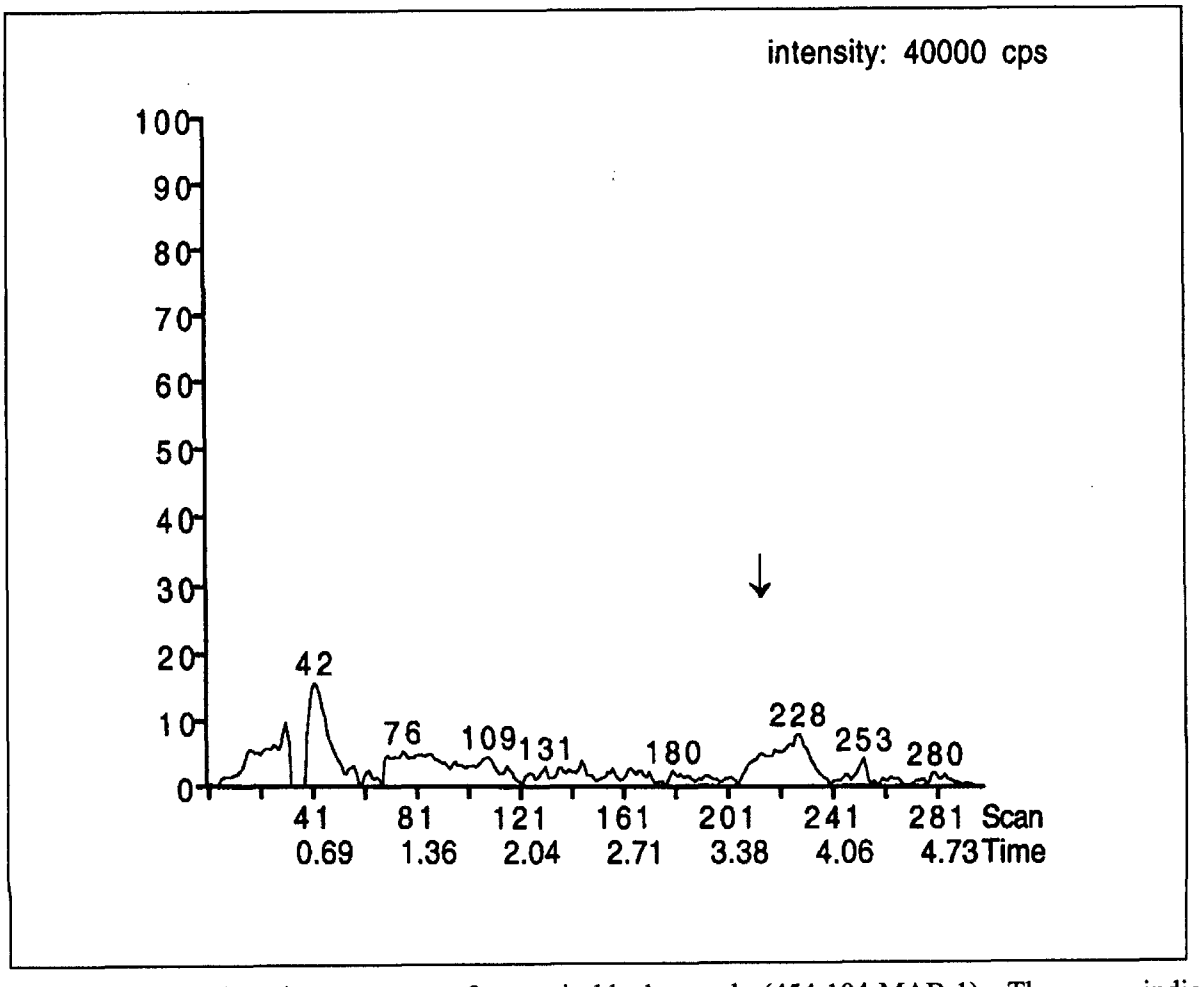


Figure 5. Typical ion chromatogram of a matrix blank sample (454-104-MAB-1). The arrow indicates approximate retention time of PFOS.

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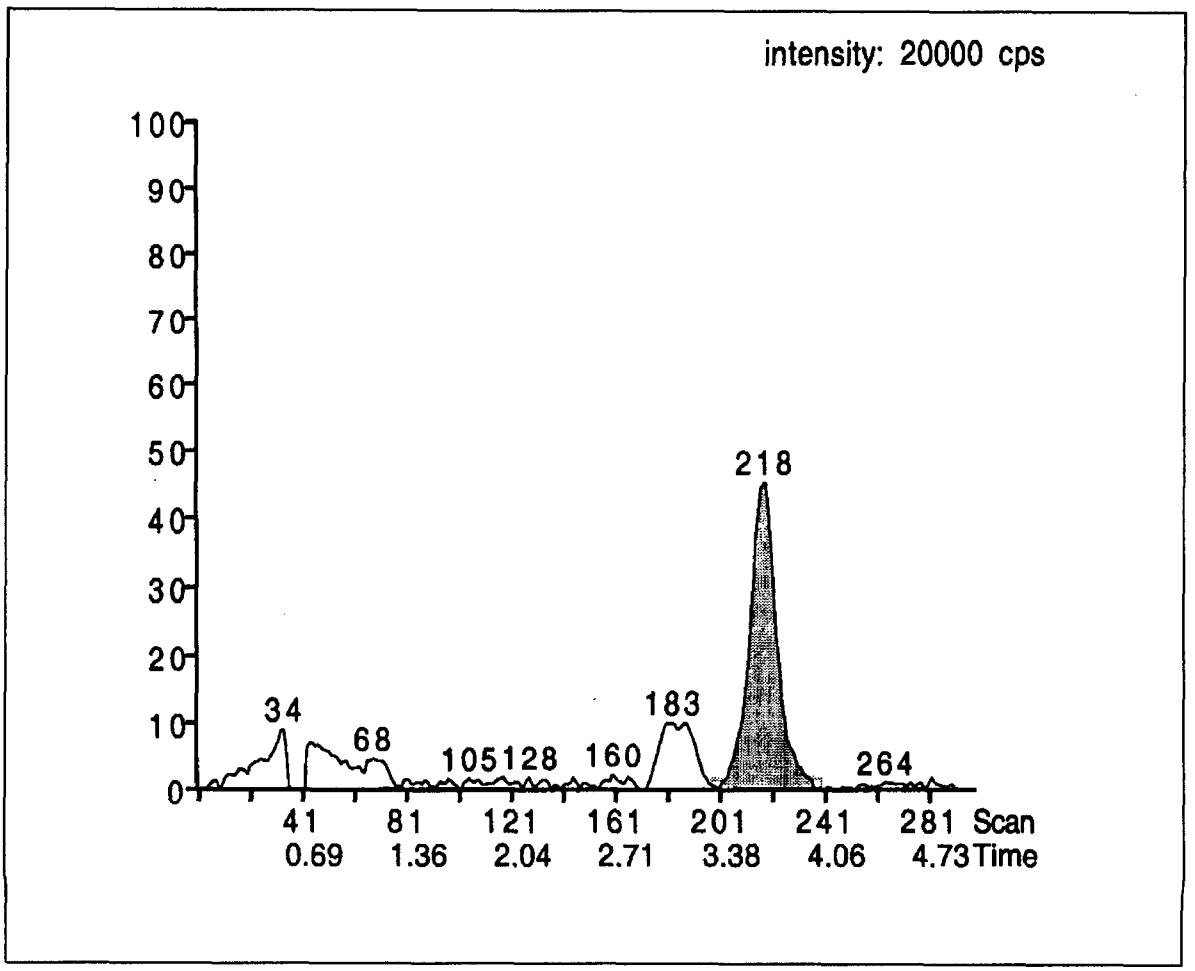



Figure 6. Typical ion chromatogram of a matrix fortification sample (454-104-MAS-9, 22.0 ppm a.i.).

APPENDIX IV

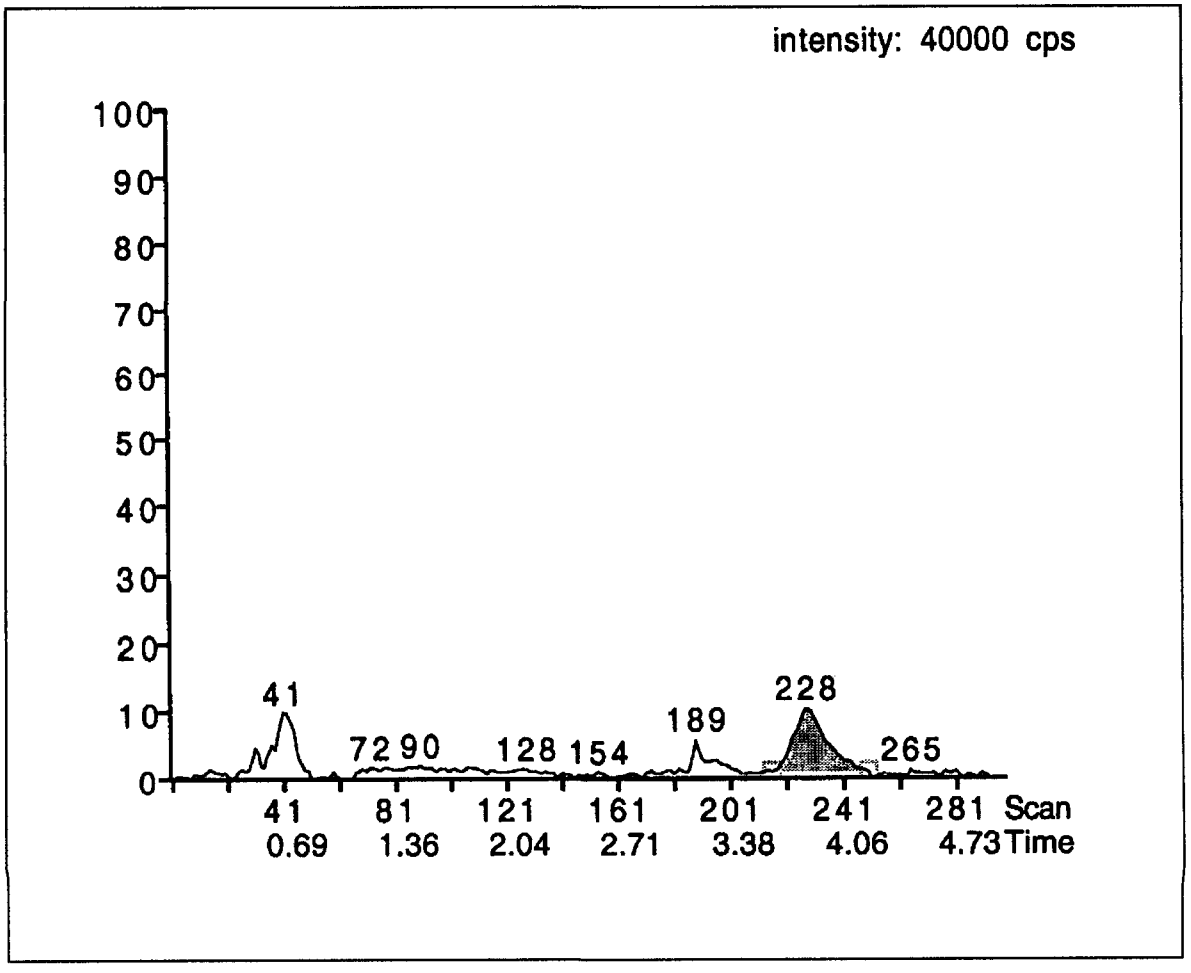
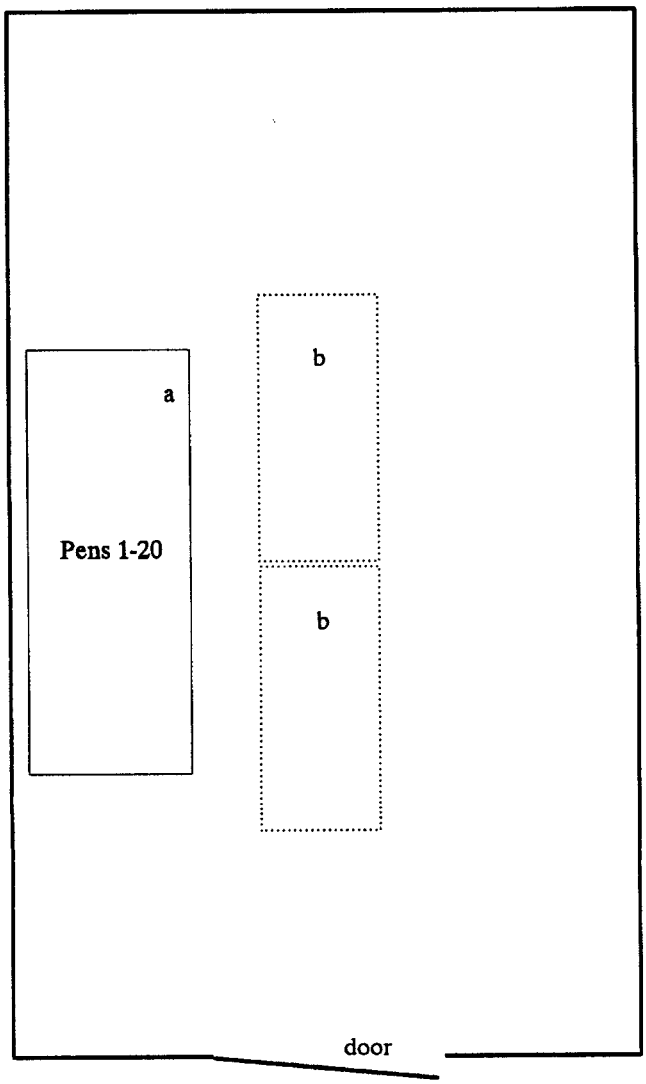



Figure 7. Typical ion chromatogram of an avian diet sample on Day 0 (S-454-104-3, 1.8 ppm a.i.).

Appendix V

Diagram of Test Layout




|——————| = approx. 1m a = GQF Battery Breeder Model No. 0330

b = Four X 4 ft. Chroma 50 light bulbs

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

Control (0 ppm a.i.) - Males

| Pen | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 | Change 6-8 | Week 8 | Change 8-10 | Week 10 | Change 10-11 | Week 11 | Change 11-20 | Week 20 | Change 0-20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 201 | 212 | 1 | 213 | 0 | 213 | 3 | 216 | 4 | -3 | 213 | 3 | 216 | -6 | 210 | 1 | 211 | -1 |
| 202 | 210 | -2 | 208 | -4 | 204 | 0 | 204 | -6 | 1 | 205 | 6 | 211 | -1 | 210 | 5 | 215 | 5 |
| 203 | 217 | -6 | 211 | 0 | 211 | 1 | 212 | -5 | -3 | 209 | 4 | 213 | -3 | 210 | 5 | 215 | -2 |
| 204 | 221 | 1 | 222 | -1 | 221 | 1 | 222 | 1 | -1 | 221 | 2 | 223 | -2 | 221 | 6 | 227 | 6 |
| 205 | 209 | 0 | 209 | 0 | 209 | 3 | 212 | 3 | -1 | 211 | 1 | 212 | -3 | 209 | 2 | 211 | 2 |
| Mean | 214 | -1 | 213 | -1 | 212 | 2 | 213 | -1 | -1 | 212 | 3 | 215 | -3 | 212 | 4 | 216 | 2 |
| SD | 5 | 3 | 6 | 2 | 6 | 1 | 7 | 5 | 2 | 6 | 2 | 5 | 2 | 5 | 2 | 7 | 4 |

Control (0 ppm a.i.) - Females

| Pen | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 | Change 6-8 | Week 8 | Change 8-10 | Week 10 | Change 10-11 | Week 11 | Change 11-20 | Week 20 | Change 0-20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 201 | 235 | 5 | 240 | -1 | 239 | 12 | 251 | 16 | -3 | 248 | 7 | 255 | -9 | 246 | 17 | 263 | 28 |
| 202 | 199 | 11 | 210 | -5 | 205 | 13 | 218 | 19 | 6 | 224 | 1 | 225 | 0 | 225 | -20 | 205 | 6 |
| 203 | 222 | 9 | 231 | -8 | 223 | 13 | 236 | 14 | 0 | 236 | 2 | 238 | -11 | 227 | 5 | 232 | 10 |
| 204 | 225 | 3 | 228 | -2 | 226 | 3 | 229 | 4 | 10 | 239 | 3 | 242 | -5 | 237 | -8 | 229 | 4 |
| 205 | 229 | 5 | 234 | 3 | 237 | -1 | 236 | 7 | 0 | 236 | -25 | 211 | 3 | 214 | 18 | 232 | 3 |
| Mean | 222 | 7 | 229 | -3 | 226 | 8 | 234 | 12 | 3 | 237 | -2 | 234 | -4 | 230 | 2 | 232 | 10 |
| SD | 14 | 3 | 11 | 4 | 14 | 7 | 12 | 6 | 5 | 9 | 13 | 17 | 6 | 12 | 16 | 21 | 10 |

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

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

1.8 ppm a.i. - Males

| Pen | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 |
|---|---|---|---|---|---|---|---|---|
| 206 | 194 | -1 | 193 | 7 | 200 | 4 | 204 | 10 |
| 207 | 210 | 6 | 216 | 2 | 218 | 2 | 220 | 10 |
| 208 | 228 | 0 | 228 | 4 | 232 | -2 | 230 | 2 |
| 209 | 204 | -1 | 203 | 6 | 209 | 2 | 211 | 7 |
| 210 | 196 | -2 | 194 | 1 | 195 | 0 | 195 | -1 |
| Mean | 206 | 0 | 207 | 4 | 211 | 1 | 212 | 6 |
| SD | 14 | 3 | 15 | 3 | 15 | 2 | 14 | 5 |

1.8 ppm a.i. - Females

| Pen | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 |
|---|---|---|---|---|---|---|---|---|
| 206 | 216 | 9 | 225 | 1 | 226 | 12 | 238 | 22 |
| 207 | 247 | -6 | 241 | -11 | 230 | -11 | 219 | -28 |
| 208 | 207 | -6 | 201 | 9 | 210 | 4 | 214 | 7 |
| 209 | 228 | 2 | 230 | 2 | 232 | 5 | 237 | 9 |
| 210 | 209 | -2 | 207 | 4 | 211 | -62 | 149 | -60 |
| Mean | 221 | -1 | 221 | 1 | 222 | -10 | 211 | -10 |
| SD | 17 | 6 | 17 | 7 | 11 | 30 | 36 | 34 |

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

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

6.2 ppm a.i. - Males

| Pen | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 |
|---|---|---|---|---|---|---|---|---|
| 211 | 194 | -2 | 192 | 1 | 193 | 3 | 196 | 2 |
| 212 | 205 | -3 | 202 | -1 | 201 | 0 | 201 | -4 |
| 213 | 222 | 1 | 223 | 0 | 223 | -3 | 220 | -2 |
| 214 | 204 | -1 | 203 | 1 | 204 | -1 | 203 | -1 |
| 215 | 225 | -4 | 221 | -5 | 216 | 1 | 217 | -8 |
| Mean | 210 | -2 | 208 | -1 | 207 | 0 | 207 | -3 |
| SD | 13 | 2 | 13 | 2 | 12 | 2 | 11 | 4 |

6.2 ppm a.i. - Females

| Pen | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 |
|---|---|---|---|---|---|---|---|---|
| 211 | 254 | 3 | 257 | 11 | 268 | -2 | 266 | 12 |
| 212 | 211 | 10 | 221 | -2 | 219 | -9 | 210 | -1 |
| 213 | 220 | -7 | 213 | -2 | 211 | 7 | 218 | -2 |
| 214 | 241 | -2 | 239 | 8 | 247 | 5 | 252 | 11 |
| 215 | 230 | 9 | 239 | 1 | 240 | -10 | 230 | 0 |
| Mean | 231 | 3 | 234 | 3 | 237 | -2 | 235 | 4 |
| SD | 17 | 7 | 17 | 6 | 23 | 8 | 23 | 7 |

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

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

17.6 ppm a.i. - Males

| Pen | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 | Change 6-8 | Week 8 | Change 8-10 | Week 10 | Change 10-11 | Week 11 | Change 11-20 | Week 20 | Change 0-20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 216 | 211 | -13 | 198 | -5 | 193 | 3 | 196 | -15 | 4 | 200 | -4 | 196 | 0 | 196 | 3 | 199 | -12 |
| 217 | 194 | -7 | 187 | 1 | 188 | 0 | 188 | -6 | -2 | 186 | 1 | 187 | -5 | 182 | 3 | 185 | -9 |
| 218 | 208 | -12 | 196 | 1 | 197 | -2 | 195 | -13 | 0 | 195 | 0 | 195 | -2 | 193 | 5 | 198 | -10 |
| 219 | 204 | -5 | 199 | -5 | 194 | 0 | 194 | -10 | 0 | 194 | 0 | 194 | -2 | 192 | 5 | 197 | -7 |
| 220 | 224 | -12 | 212 | -6 | 206 | -1 | 205 | -19 | 0 | 205 | -2 | 203 | -1 | 202 | 3 | 205 | -19 |
| Mean | 208 | -10 | 198 | -3 | 196 | 0 | 196 | -13 | 0 | 196 | -1 | 195 | -2 | 193 | 4 | 197 | -11 |
| SD | 11 | 4 | 9 | 3 | 7 | 2 | 6 | 5 | 2 | 7 | 2 | 6 | 2 | 7 | 1 | 7 | 5 |

17.6 ppm a.i. - Females

| Pen | Week 0 | Change 0-2 | Week 2 | Change 2-4 | Week 4 | Change 4-6 | Week 6 | Change 0-6 | Change 6-8 | Week 8 | Change 8-10 | Week 10 | Change 10-11 | Week 11 | Change 11-20 | Week 20 | Change 0-20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 216 | 214 | 5 | 219 | 7 | 226 | -9 | 217 | 3 | -1 | 216 | 1 | 217 | -8 | 209 | -25 | 184 | -30 |
| 217 | 220 | 1 | 221 | -1 | 220 | 8 | 228 | 8 | -7 | 221 | 6 | 227 | -6 | 221 | -33 | 188 | -32 |
| 218 | 248 | 3 | 251 | 2 | 253 | -2 | 251 | 3 | 1 | 252 | 6 | 258 | -4 | 254 | 6 | 260 | 12 |
| 219 | 213 | -2 | 211 | 9 | 220 | 1 | 221 | 8 | 4 | 225 | 5 | 230 | -2 | 228 | 5 | 233 | 20 |
| 220 | 239 | -4 | 235 | 4 | 239 | 1 | 240 | 1 | 1 | 241 | 4 | 245 | -16 | 229 | 7 | 236 | -3 |
| Mean | 227 | 1 | 227 | 4 | 232 | 0 | 231 | 5 | 0 | 231 | 4 | 235 | -7 | 228 | -8 | 220 | -7 |
| SD | 16 | 4 | 16 | 4 | 14 | 6 | 14 | 3 | 4 | 15 | 2 | 16 | 5 | 16 | 19 | 33 | 24 |

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

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

Control (0 ppm a.i.)

| | Weeks | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pen | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 201 | 18 | 20 | 20 | 23 | 22 | 25 | 22 | 27 | 25 | 27 | 27 | 28 | 25 | 29 | 34 | 30 | 27 | 24 | 28 |
| 202 | 23 | 24 | 25 | 28 | 28 | 30 | 28 | 37 | 31 | 34 | 34 | 37 | 33 | 37 | 38 | 34 | 32 | 31 | 31 |
| 203 | 19 | 21 | 21 | 24 | 21 | 27 | 23 | 32 | 27 | 29 | 28 | 31 | 29 | 36 | 36 | 35 | 30 | 29 | 31 |
| 204 | 17 | 22 | 26 | 24 | 25 | 27 | 20 | 36 | 28 | 33 | 37 | 40 | 31 | 43 | 39 | 39 | 31 | 33 | 32 |
| 205 | 22 | 24 | 24 | 23 | 26 | 29 | 26 | 31 | 25 | 31 | 28 | 37 | 30 | 32 | 33 | 34 | 30 | 30 | 32 |
| Mean | 20 | 22 | 23 | 24 | 24 | 28 | 24 | 32 | 27 | 31 | 31 | 35 | 30 | 35 | 36 | 34 | 30 | 30 | 31 |
| SD | 3 | 2 | 2 | 2 | 3 | 2 | 3 | 4 | 3 | 3 | 4 | 5 | 3 | 5 | 2 | 3 | 2 | 3 | 2 |

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

1.8 ppm a.i.

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Pen | 1 | 2 | 3 | 4 | 5 | 6 |
| 206 | 18 | 20 | 20 | 22 | 22 | 23 |
| 207 | 21 | 25 | 23 | 28 | 14 | 24 |
| 208 | 17 | 20 | 20 | 24 | 22 | 23 |
| 209 | 17 | 17 | 20 | 22 | 21 | 22 |
| 210 | 18 | 19 | 19 | 24 | 17 | 19 |
| Mean | 18 | 20 | 20 | 24 | 19 | 22 |
| SD | 2 | 3 | 2 | 2 | 4 | 2 |

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

6.2 ppm a.i.

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Pen | 1 | 2 | 3 | 4 | 5 | 6 |
| 211 | 20 | 19 | 18 | 22 | 21 | 22 |
| 212 | 14 | 15 | 15 | 17 | 19 | 19 |
| 213 | 18 | 23 | 25 | 24 | 24 | 26 |
| 214 | 18 | 20 | 21 | 20 | 21 | 23 |
| 215 | 22 | 22 | 21 | 21 | 25 | 26 |
| Mean | 18 | 20 | 20 | 21 | 22 | 23 |
| SD | 3 | 3 | 4 | 2 | 3 | 3 |

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

17.6 ppm a.i.

| | Weeks | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pen | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 216 | 18 | 19 | 20 | 23 | 22 | 24 | 23 | 27 | 27 | 31 | 30 | 34 | 30 | 24 | 23 | 32 | 28 | 22 | 22 |
| 217 | 16 | 17 | 18 | 22 | 20 | 21 | 20 | 22 | 22 | 23 | 25 | 28 | 25 | 26 | 25 | 27 | 22 | 13 | 17 |
| 218 | 18 | 19 | 22 | 20 | 24 | 27 | 25 | 32 | 34 | 36 | 36 | 38 | 31 | 35 | 38 | 35 | 33 | 35 | 33 |
| 219 | 16 | 17 | 18 | 17 | 19 | 21 | 18 | 21 | 20 | 23 | 23 | 24 | 21 | 22 | 22 | 20 | 22 | 19 | 20 |
| 220 | 18 | 19 | 21 | 22 | 22 | 22 | 21 | 24 | 25 | 26 | 29 | 31 | 22 | 30 | 31 | 29 | 28 | 30 | 27 |
| Mean | 17 | 18 | 20 | 21 | 22 | 23 | 21 | 25 | 26 | 28 | 29 | 31 | 26 | 27 | 28 | 29 | 27 | 24 | 24 |
| SD | 1 | 1 | 2 | 2 | 2 | 2 | 3 | 4 | 6 | 6 | 5 | 5 | 4 | 5 | 6 | 6 | 5 | 9 | 6 |

Appendix VIII
Table 1

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

Control (0 ppm a.i.)

| Males | Pens | | | | |
|---|---|---|---|---|---|
| | 201 | 202 | 203 | 204 | 205 |
| Not remarkable | X | X | X | X | X |

| Females | Pens | | | | |
|---|---|---|---|---|---|
| | 201 | 202 | 203 | 204 | 205 |
| Feather loss | - | X | X | X | X |
| Right wing twisted | - | - | - | - | X |
| Not remarkable | X | - | - | - | - |

Appendix VIII
Table 2

Individual Gross Pathological Observations
from a Northern Bobwhite Pilot Reproduction Study with PFOS
Birds Euthanized at 6 Weeks

1.8 ppm a.i.

| Males | Pens | | | | |
|---|---|---|---|---|---|
| | 206 | 207 | 208 | 209 | 210 |
| Not remarkable | X | X | X | X | X |

| Females | Pens | | | | |
|---|---|---|---|---|---|
| | 206 | 207 | 208 | 209 | 210 |
| Feather loss | - | X | - | - | - |
| Foot lesions | - | - | - | - | X |
| Thin condition | - | - | - | - | X |
| Regressed ovary | - | - | - | - | X |
| Not remarkable | X | - | X | X | - |

Appendix VIII
Table 3

Individual Gross Pathological Observations
from a Northern Bobwhite Pilot Reproduction Study with PFOS
Birds Euthanized at 6 Weeks

6.2 ppm a.i.

| Males | Pens | | | | |
|---|---|---|---|---|---|
| | 211 | 212 | 213 | 214 | 215 |
| Testes small, ~ 1.5 cm | - | - | X | - | - |
| Right testis small, ~ 1.5 cm | X | - | - | - | - |
| Not remarkable | - | X | - | X | X |

| Females | Pens | | | | |
|---|---|---|---|---|---|
| | 211 | 212 | 213 | 214 | 215 |
| Feather loss | X | X | - | X | - |
| Not remarkable | - | - | X | - | X |

Appendix VIII
Table 4

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

17.6 ppm a.i.

| Males | Pens | | | | |
|---|---|---|---|---|---|
| | 216 | 217 | 218 | 219 | 220 |
| Right testis small, ~ 1.25 cm | X | - | X | - | - |
| Not remarkable | - | X | - | X | X |

| Females | Pens | | | | |
|---|---|---|---|---|---|
| | 216 | 217 | 218 | 219 | 220 |
| Feather loss | X | X | - | X | X |
| Not remarkable | - | - | X | - | - |

Appendix IX

Histopathology Report

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

WILDLIFE INTERNATIONAL, LTD.
PROJECT NUMBER 454-104
EPL PROJECT NUMBER 212-024

# PFOS: A PILOT 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

January 25, 2001

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

TABLE OF CONTENTS

PATHOLOGY SUMMARY

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

WILDLIFE INTERNATIONAL, LTD.
STUDY NUMBER 454-104
EPL PROJECT NUMBER 212-024

PFOS: A PILOT 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 (*Colinus virginianus*) which were untreated or which received various concentrations of the test article (Perfluorooctanesulfonic acid, potassium salt [PFOS]) in the feed for six to 19 weeks. Selected tissues from untreated approximately 12-week-old offspring of the adult 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 a test substance over at least a six-week period. The experimental design was as follows:

| Group * | Adults | | Offspring ** | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| Control (0 ppm a. i.) | 5 | 5 | 5 | 5 |
| 1.8 ppm a. i. PFOS | 5 | 5 | 4 | 6 |
| 6.2 ppm a. i. PFOS | 5 | 5 | 5 | 5 |
| 17.6 ppm a. i. PFOS | 5 | 5 | 2 | 8 |

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

## MATERIALS AND METHODS

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

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

A tabulation of gross observations noted at necropsy with the corresponding microscopic change, if appropriate, is presented in the Correlation of Gross and Microscopic Findings tables. The entries in these tables were

transcribed from the Gross Necropsy records provided by Wildlife International, Ltd.

**RESULTS**

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

Testes of all adult males exhibited spermatogenesis characterized by the presence of numerous mature spermatozoa in the seminiferous tubules. In two 17.6 ppm a.i. males, seminiferous tubules had marginally decreased diameter (though spermatogenesis was still evident) compared to other control and 17.6 ppm a.i. testes, resulting in overall smaller testes which corresponded to grossly observed 1.25 cm right testes. The smaller testes of these birds were most consistent with early post-reproductive phase regression, a normal physiological phenomenon. The occurrence of such testicular regression only in the 17.6 ppm a.i. dose group may have been a fortuitous event accentuated by the small group sizes, but the possibility of a test article effect cannot be entirely ruled out.

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 had germinal epithelium with decreased cell layers (compared to adult testes). This morphology was consistent with normal physiological immaturity in young (approximately 12-week-old) birds rather than a pathological effect. The overall small diameter of offspring testes corresponded to measurements of less than 1.5 cm recorded at necropsy. Because of the

smaller size of the seminiferous tubules in 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 as in adult testes. This melanin pigment concentration corresponded to the grossly observed black offspring testes, but was considered a normal age-related finding and not a pathological effect.

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

Several additional microscopic findings (described below) were noted in adult and/or offspring bobwhite from control and treated groups. Incidences and mean severity were similar when groups from each generational cohort were compared with each other. Some of these lesions occurred only in a single bird. For these reasons, these findings were considered incidental and unrelated to test article (PFOS) administration.

Mononuclear cell infiltrates in various tissues consisted of discrete foci of small lymphocytes and plasma cells; a few heterophils were sometimes also present but were always a minor component. Chronic and chronic active inflammation of adipose tissue were characterized by infiltrates consisting exclusively or predominantly of macrophages, or mixed populations of heterophils and marophages, respectively. Scattered necrotic adipocytes were associated with chronic active inflammation in one 6.2 ppm a.i. offspring male. A small focus of cortical tubules exhibited cytoplasmic vacuolization in the kidney of one adult 17.6 ppm a.i. female; this change in a single bird was considered

incidental. Scattered clusters of coarsely to finely dark basophilic material in the renal cortex of a few adults and offspring denoted mineralization.

One control adult female exhibited foreign material and granulomatous inflammation in several tissues including the serosa of the ovary, liver, and gallbladder, and in the adipose tissue. Granulomatous inflammation characterized by small mononuclear cells, macrophages, and occasional multinucleated giant cells was associated with foci of foreign material (deep magenta globules and bright pink amorphous material consistent with extrafollicular egg yolk). Collectively, these findings were consistent with the common clinical condition usually referred to as "egg yolk peritonitis."

Liver pigment deposition consisted of dust-like to coarsely irregular, brown to golden-brown pigment granules present in hepatocyte cytoplasm, Kupffer cells, and/or periportal areas; the identity of the pigment was not determined, but may have been related to bile. Small single clusters of hyperplastic bile ducts were noted in one adult control female and one 1.8 ppm a.i. offspring female. A small focus of heterophils (acute inflammation) was noted in one 1.8 ppm a.i offspring male. The liver of an adult 17.6 ppm a.i. female exhibited amyloid deposition associated with secondary hepatocellular hypertrophy and necrosis. Liver amyloid deposits consisted of amorphous, pale bluish-violet material which filled and distended the hepatic sinusoids, and compressed adjacent hepatic cords.

Additional liver lesions included hepatocellular fatty change and vacuolization. Fatty change denoted variably sized, sharply demarcated, clear vacuoles (consistent with fat accumulation) in the hepatocellular cytoplasm. Vacuolization denoted multiple, usually small, confluent, poorly demarcated vacuoles which often resulted in a "lace-like" appearance of the hepatocellular cytoplasm. When present in most hepatocytes, these changes were designated

"diffuse," and when present in a few scattered areas, they were designated as "focal." When hepatocellular fatty change occurred predominantly in the areas adjacent to bile channels and associated blood vessels, it was designated as "periportal."

Hepatocellular fatty change was noted in the livers of adults and offspring, while hepatocellular vacuolization was seen only in offspring. This generational difference was considered to be due to normal age-related variation rather than being a treatment effect. When control and treated groups of each generational cohort were compared, combined incidences of diffuse, focal, and periportal fatty change in male and female adults and offspring were similar. Hepatocellular fatty change was considered incidental and unrelated to treatment. There was a relative increase in incidence of hepatocellular vacuolization in female offspring, but the magnitude was small, and the increase was not clearly dose-related. It is most likely that this finding was coincidental and not related to test article administration.

## CONCLUSIONS AND SUMMARY

No lesions considered possibly related to test article (PFOS) administration were noted in liver, kidney, adipose tissue, proventriculus, gallbladder, ovary, and brain of adult male and female bobwhite or their offspring, or in the testes of offspring bobwhite. Testes of two high dose (17.6 ppm a.i.) adult male bobwhite exhibited decreased seminiferous tubule diameter most consistent with early post-reproductive phase regression, a normal physiological phenomenon. The occurrence of testicular regression only in the 17.6 ppm a.i. dose group may have been a fortuitous event accentuated by the small group sizes, but the possibility of a test article effect cannot be entirely ruled out. The

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

Margarita M. Gruebbel

MARGARITA M. GRUEBBEL, DVM, PhD, Diplomate, ACVP
Veterinary Pathologist
1/25/01

MMG/lcp

EPL®

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE FINAL CERTIFICATION

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

Client Study: 454-104 EPL Project Coordinator: Dr. Margarita M. Gruebbel

EPL Project Number: 212-024 EPL Pathologist: Dr. Margarita M. Gruebbel

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

| Area Inspected | Dates | |
|---|---|---|
| | Inspection | Reporting |
| EPL Project Sheets | 9/11/00; 10/3/00 | 9/11/00; 10/3/00 |
| Project Setup | 9/27/00 | 9/28/00 |
| Histology Setup | 10/2/00 | 10/2/00 |
| Data Review | 11/13/00 | 11/13/00 |
| Draft Report | 12/12,27/00 | 12/13,27/00 |
| Final Report | 1/29/01 | 1/29/01 |

Date of last quarterly facility inspection 9/00

Patricia L. Runge 1/29/01

EPL Quality Assurance Unit Date

SUMMARY INCIDENCE TABLES

ADULT SACRIFICE

## SUMMARY INCIDENCE TABLE

454-104
Adult Sacrifice
Male Bobwhite

| | GROUP CONTROL | GROUP 17.6 | | | | |
|---|---|---|---|---|---|---|
| ADIPOSE TISSUE (NO. EXAMINED) | (5) | (5) | | | | |
| Foreign Material | | | | | | |
| Infiltrate, Mononuclear Cell | | | | | | |
| Inflammation, Chronic | | | | | | |
| Inflammation, Granulomatous | | | | | | |
| | | | | | | |
| BRAIN (NO. EXAMINED) | (5) | (5) | | | | |
| | | | | | | |
| BURSA OF FABRICIUS (NO. EXAMINED) | | | | | | |
| | | | | | | |
| CLOACA (NO. EXAMINED) | (2) | | | | | |
| | | | | | | |
| GALLBLADDER (NO. EXAMINED) | (5) | (4) | | | | |
| Infiltrate, Mononuclear Cell | 3 | 3 | | | | |
| Serosa, Foreign Material | | | | | | |
| Serosa, Inflammation, Granulomatous | | | | | | |
| | | | | | | |
| KIDNEY (NO. EXAMINED) | (5) | (5) | | | | |
| Infiltrate, Mononuclear Cell | | 2 | | | | |
| Tubules, Mineralization | 2 | 1 | | | | |
| Tubules, Vacuolization | | | | | | |
| | | | | | | |
| LIVER (NO. EXAMINED) | (5) | (5) | | | | |
| Amyloid Deposition | | | | | | |
| Bile Ducts, Hyperplasia | | | | | | |
| Hepatocytes, Fatty Change, Diffuse | 2 | 2 | | | | |
| Hepatocytes, Fatty Change, Focal | 1 | 2 | | | | |
| Hepatocytes, Fatty Change, Periportal | 1 | | | | | |
| Hepatocytes, Hypertrophy, Diffuse | | | | | | |
| Hepatocytes, Necrosis, Focal | | | | | | |
| Infiltrate, Mononuclear Cell | 5 | 3 | | | | |
| Pigment Deposition | 2 | 2 | | | | |
| Serosa, Foreign Material | | | | | | |
| Serosa, Inflammation, Granulomatous | | | | | | |
| | | | | | | |
| PROVENTRICULUS (NO. EXAMINED) | (5) | (5) | | | | |
| Infiltrate, Mononuclear Cell | 3 | 4 | | | | |
| | | | | | | |

## SUMMARY INCIDENCE TABLE

454-104
Adult Sacrifice
Male Bobwhite

| | GROUP CONTROL | GROUP 17.6 | | | | |
|---|---|---|---|---|---|---|
| TESTIS (NO. EXAMINED) | (5) | (5) | | | | |
| Infiltrate, Mononuclear Cell | | 1 | | | | |
| Seminiferous Tubules, | | | | | | |
| Decreased Diameter | | 2 | | | | |
| Spermatogenesis | 5 | 5 | | | | |

## SUMMARY INCIDENCE TABLE

454-104
Adult Sacrifice
Female Bobwhite

| | GROUP CONTROL | GROUP 17.6 | | | | |
|---|---|---|---|---|---|---|
| ADIPOSE TISSUE (NO. EXAMINED) | (4) | (4) | | | | |
| Foreign Material | 1 | | | | | |
| Infiltrate, Mononuclear Cell | | 2 | | | | |
| Inflammation, Chronic | | 1 | | | | |
| Inflammation, Granulomatous | 1 | | | | | |
| | | | | | | |
| BRAIN (NO. EXAMINED) | (5) | (5) | | | | |
| | | | | | | |
| BURSA OF FABRICIUS (NO. EXAMINED) | | | | | | |
| | | | | | | |
| CLOACA (NO. EXAMINED) | (2) | | | | | |
| | | | | | | |
| GALLBLADDER (NO. EXAMINED) | (5) | (5) | | | | |
| Infiltrate, Mononuclear Cell | 1 | 4 | | | | |
| Serosa, Foreign Material | 1 | | | | | |
| Serosa, Inflammation, Granulomatous | 1 | | | | | |
| | | | | | | |
| KIDNEY (NO. EXAMINED) | (5) | (5) | | | | |
| Infiltrate, Mononuclear Cell | 1 | 1 | | | | |
| Tubules, Mineralization | 1 | 2 | | | | |
| Tubules, Vacuolization | | 1 | | | | |
| | | | | | | |
| LIVER (NO. EXAMINED) | (5) | (5) | | | | |
| Amyloid Deposition | | 1 | | | | |
| Bile Ducts, Hyperplasia | 1 | | | | | |
| Hepatocytes, Fatty Change, Diffuse | | 3 | | | | |
| Hepatocytes, Fatty Change, Focal | | | | | | |
| Hepatocytes, Fatty Change, Periportal | 4 | 1 | | | | |
| Hepatocytes, Hypertrophy, Diffuse | | 1 | | | | |
| Hepatocytes, Necrosis, Focal | 2 | 2 | | | | |
| Infiltrate, Mononuclear Cell | 3 | 3 | | | | |
| Pigment Deposition | | | | | | |
| Serosa, Foreign Material | 1 | | | | | |
| Serosa, Inflammation, Granulomatous | 1 | | | | | |
| | | | | | | |
| OVARY (NO. EXAMINED) | (5) | (5) | | | | |
| Serosa, Foreign Material | 1 | | | | | |
| | | | | | | |

## SUMMARY INCIDENCE TABLE

454-104
Adult Sacrifice
Female Bobwhite

| | GROUP CONTROL | GROUP 17.6 | | | | |
|---|---|---|---|---|---|---|
| OVARY (CONTINUED) | | | | | | |
| Serosa, Inflammation, | | | | | | |
| Granulomatous | 1 | | | | | |
| | | | | | | |
| PROVENTRICULUS (NO. EXAMINED) | (5) | (5) | | | | |
| Infiltrate, Mononuclear Cell | 3 | 4 | | | | |

HISTOPATHOLOGY INCIDENCE TABLES

ADULT SACRIFICE

# HISTOPATHOLOGY INCIDENCE TABLE

454-104
Adult Sacrifice
Male Bobwhite

| | GROUP CONTROL | | | | | | GROUP 17.6 | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL | 45151 | 45153 | 45155 | 45157 | 45159 | | 48181 | 48183 | 48185 | 48187 | 48189 | | | | | | | | | | | |
| ADIPOSE TISSUE | X | X | X | X | X | | X | X | X | X | X | | | | | | | | | | | |
| Foreign Material | | | | | | | | | | | | | | | | | | | | | | |
| Infiltrate, Mononuclear Cell | | | | | | | | | | | | | | | | | | | | | | |
| Inflammation, Chronic | | | | | | | | | | | | | | | | | | | | | | |
| Inflammation, Granulomatous | | | | | | | | | | | | | | | | | | | | | | |
| BRAIN | X | X | X | X | X | | X | X | X | X | X | | | | | | | | | | | |
| BURSA OF FABRICIUS | N | N | N | N | N | | N | N | N | N | N | | | | | | | | | | | |
| CLOACA | X | X | | | | | | | | | | | | | | | | | | | | |
| GALLBLADDER | | | X | X | | | | N | | X | | | | | | | | | | | | |
| Infiltrate, Mononuclear Cell | 2 | 1 | | | 2 | | 1 | | 1 | | 2 | | | | | | | | | | | |
| Serosa, Foreign Material | | | | | | | | | | | | | | | | | | | | | | |
| Serosa, Inflammation, Granulomatous | | | | | | | | | | | | | | | | | | | | | | |
| KIDNEY | X | X | | X | | | | | X | X | | | | | | | | | | | | |
| Infiltrate, Mononuclear Cell | | | | | | | 1 | 1 | | | | | | | | | | | | | | |
| Tubules, Mineralization | | | 1 | | 1 | | | | | | 1 | | | | | | | | | | | |
| Tubules, Vacuolization | | | | | | | | | | | | | | | | | | | | | | |
| LIVER | | | | | | | | | X | | | | | | | | | | | | | |
| Amyloid Deposition | | | | | | | | | | | | | | | | | | | | | | |
| Bile Ducts, Hyperplasia | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocytes, Fatty Change, Diffuse | | 2 | 2 | | | | 2 | | | 2 | | | | | | | | | | | | |
| Hepatocytes, Fatty Change, Focal | 1 | | | | | | | 1 | | | 1 | | | | | | | | | | | |
| Hepatocytes, Fatty Change, Periportal | | | | 4 | | | | | | | | | | | | | | | | | | |
| Hepatocytes, Hypertrophy, Diffuse | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocytes, Necrosis, Focal | | | | | | | | | | | | | | | | | | | | | | |
| Infiltrate, Mononuclear Cell | 1 | 1 | 1 | 1 | 1 | | 1 | | | 1 | 1 | | | | | | | | | | | |
| Pigment Deposition | | | | 2 | 2 | | | 1 | | | 1 | | | | | | | | | | | |
| Serosa, Foreign Material | | | | | | | | | | | | | | | | | | | | | | |
| Serosa, Inflammation, Granulomatous | | | | | | | | | | | | | | | | | | | | | | |



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

# HISTOPATHOLOGY INCIDENCE TABLE

454-104
Adult Sacrifice
Male Bobwhite

| | GROUP CONTROL | | | | | | GROUP 17.6 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL | 45151 | 45353 | 45557 | 45759 | | 48181 | 48383 | 48585 | 48787 | 48989 |
| PROVENTRICULUS | | X | | | X | | | | | X | |
| Infiltrate, Mononuclear Cell | 1 | | 1 | 1 | | | 1 | 1 | 1 | | 1 |
| | | | | | | | | | | | |
| TESTIS | m | m | m | m | m | | m | m | m | m | m |
| Infiltrate, Mononuclear Cell | | | | | | | | 1 | | | |
| Seminiferous Tubules, | | | | | | | | | | | |
| Decreased Diameter | | | | | | | 1 | | 1 | | |
| Spermatogenesis | P | P | P | P | P | | P | P | P | P | P |

II-2



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

454-104
Adult Sacrifice
Female Bobwhite

| | GROUP CONTROL | | | | | | GROUP 17.6 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL | 45052 | 45454 | 45656 | 45858 | 46060 | | 48282 | 48484 | 48686 | 48888 | 49090 |
| ADIPOSE TISSUE | X | X | N | | X | | N | | X | | X |
| Foreign Material | | | | P | | | | | | | |
| Infiltrate, Mononuclear Cell | | | | | | | | 1 | | 1 | |
| Inflammation, Chronic | | | | | | | | 1 | | | |
| Inflammation, Granulomatous | | | | 1 | | | | | | | |
| BRAIN | X | X | X | X | X | | X | X | X | X | X |
| BURSA OF FABRICIUS | N | N | N | N | N | | N | N | N | N | N |
| CLOACA | X | | X | | | | | | | | |
| GALLBLADDER | X | X | | | X | | X | | | | |
| Infiltrate, Mononuclear Cell | | | 1 | | | | | 1 | 2 | 2 | 1 |
| Serosa, Foreign Material | | | | P | | | | | | | |
| Serosa, Inflammation, Granulomatous | | | | 2 | | | | | | | |
| KIDNEY | | | X | X | X | | | X | | X | |
| Infiltrate, Mononuclear Cell | 1 | | | | | | | | 1 | | |
| Tubules, Mineralization | | 1 | | | | | | | 1 | | 1 |
| Tubules, Vacuolization | | | | | | | 1 | | | | |
| LIVER | | | | | | | | | | | |
| Amyloid Deposition | | | | | | | 4 | | | | |
| Bile Ducts, Hyperplasia | | 1 | | | | | | | | | |
| Hepatocytes, Fatty Change, Diffuse | | | | | | | | 2 | 3 | | 3 |
| Hepatocytes, Fatty Change, Focal | | | | | | | | | | | |
| Hepatocytes, Fatty Change, Periportal | 3 | 3 | 3 | | 2 | | | | | 3 | |
| Hepatocytes, Hypertrophy, Diffuse | | | | | | | 3 | | | | |
| Hepatocytes, Necrosis, Focal | 1 | | 1 | | | | 2 | | | 1 | |
| Infiltrate, Mononuclear Cell | | 1 | 1 | | 1 | | 1 | 1 | | | 1 |
| Pigment Deposition | | | | | | | | | | | |
| Serosa, Foreign Material | | | | P | | | | | | | |
| Serosa, Inflammation, Granulomatous | | | | 1 | | | | | | | |



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

# HISTOPATHOLOGY INCIDENCE TABLE

454-104
Adult Sacrifice
Female Bobwhite

| | GROUP CONTROL | | | | | | GROUP 17.6 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL | 45452 | 45454 | 45456 | 45458 | 45460 | | 48482 | 48484 | 48486 | 48488 | 48490 |
| OVARY | X | X | X | | X | | X | X | X | X | X |
| Serosa, Foreign Material | | | | P | | | | | | | |
| Serosa, Inflammation, Granulomatous | | | | 1 | | | | | | | |
| PROVENTRICULUS | | X | | | X | | | X | | | |
| Infiltrate, Mononuclear Cell | 1 | | 1 | 1 | | | 1 | | 1 | 1 | 1 |



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

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

ADULT SACRIFICE

454-104
Adult Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 481 | TESTIS | Right 1.25 cm | Seminiferous Tubules, Decreased Diameter |
| 485 | TESTIS | Right ~1.25 cm | Seminiferous Tubules, Decreased Diameter |
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454-104
Adult Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 454 | EXTERNAL | Feather loss neck | No Comment Required |
| 456 | EXTERNAL | Feather loss neck | No Comment Required |
| 458 | EXTERNAL | Feather loss neck | No Comment Required |
| 460 | EXTERNAL | Right wing twisted | No Comment Required |
| | EXTERNAL | Feather loss neck and head | No Comment Required |
| | | | |
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## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 482 | EXTERNAL | Feather loss neck and back | No Comment Required |
| 484 | EXTERNAL | Feather loss neck and back | No Comment Required |
| 488 | EXTERNAL | Feather loss head and neck | No Comment Required |
| 490 | EXTERNAL | Feather loss neck and back | No Comment Required |
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SUMMARY INCIDENCE TABLES

OFFSPRING SACRIFICE

## SUMMARY INCIDENCE TABLE

454-104
Offspring Sacrifice
Male Bobwhite

| | GROUP CONTROL | GROUP 1.8 | GROUP 6.2 | GROUP 17.6 | | |
|---|---|---|---|---|---|---|
| ADIPOSE TISSUE (NO. EXAMINED) | (5) | (4) | (5) | (2) | | |
| Infiltrate, Mononuclear Cell | | 1 | | | | |
| Inflammation, Chronic | | | | | | |
| Inflammation, Chronic Active | | | 1 | | | |
| Mineralization | | | | | | |
| Necrosis | | | 1 | | | |
| | | | | | | |
| BRAIN (NO. EXAMINED) | (5) | (4) | (5) | (2) | | |
| | | | | | | |
| BURSA OF FABRICIUS (NO. EXAMINED) | (4) | (4) | (4) | (1) | | |
| | | | | | | |
| CLOACA (NO. EXAMINED) | | | (1) | (1) | | |
| | | | | | | |
| GALLBLADDER (NO. EXAMINED) | (5) | (4) | (5) | (2) | | |
| Infiltrate, Mononuclear Cell | 1 | | 1 | | | |
| | | | | | | |
| KIDNEY (NO. EXAMINED) | (5) | (4) | (5) | (2) | | |
| Infiltrate, Mononuclear Cell | | 1 | | | | |
| Tubules, Mineralization | 1 | 1 | 1 | | | |
| | | | | | | |
| LIVER (NO. EXAMINED) | (5) | (4) | (5) | (2) | | |
| Bile Ducts, Hyperplasia | | | | | | |
| Hepatocytes, Fatty Change, Diffuse | 3 | 1 | 3 | 1 | | |
| Hepatocytes, Fatty Change, Focal | 1 | | | 1 | | |
| Hepatocytes, Fatty Change, Periportal | | | | | | |
| Hepatocytes, Vacuolization, Diffuse | | 3 | 2 | | | |
| Infiltrate, Mononuclear Cell | 3 | 3 | 5 | | | |
| Inflammation, Acute | | 1 | | | | |
| | | | | | | |
| PROVENTRICULUS (NO. EXAMINED) | (5) | (4) | (5) | (2) | | |
| Infiltrate, Mononuclear Cell | 4 | 4 | 5 | 1 | | |
| | | | | | | |
| TESTIS (NO. EXAMINED) | (5) | (4) | (5) | (2) | | |
| Immature | 5 | 4 | 5 | 2 | | |
| Infiltrate, Mononuclear Cell | | 1 | 2 | | | |
| Pigment Concentration | 5 | 4 | 5 | 2 | | |
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## SUMMARY INCIDENCE TABLE

454-104
Offspring Sacrifice
Female Bobwhite

| | GROUP CONTROL | GROUP 1.8 | GROUP 6.2 | GROUP 17.6 | | |
|---|---|---|---|---|---|---|
| ADIPOSE TISSUE (NO. EXAMINED) | (4) | (6) | (5) | (8) | | |
| Infiltrate, Mononuclear Cell | | | | | | |
| Inflammation, Chronic | 2 | | 1 | 2 | | |
| Inflammation, Chronic Active | | | | | | |
| Mineralization | 1 | | | | | |
| Necrosis | | | | | | |
| | | | | | | |
| BRAIN (NO. EXAMINED) | (5) | (6) | (5) | (8) | | |
| | | | | | | |
| BURSA OF FABRICIUS (NO. | | | | | | |
| EXAMINED) | (4) | (6) | (3) | (8) | | |
| | | | | | | |
| CLOACA (NO. EXAMINED) | (1) | | | | | |
| | | | | | | |
| GALLBLADDER (NO. EXAMINED) | (5) | (6) | (3) | (8) | | |
| Infiltrate, Mononuclear Cell | 1 | 2 | 1 | 3 | | |
| | | | | | | |
| KIDNEY (NO. EXAMINED) | (5) | (6) | (5) | (8) | | |
| Infiltrate, Mononuclear Cell | 1 | 3 | 1 | 3 | | |
| Tubules, Mineralization | 2 | | | 1 | | |
| | | | | | | |
| LIVER (NO. EXAMINED) | (5) | (6) | (5) | (8) | | |
| Bile Ducts, Hyperplasia | | 1 | | | | |
| Hepatocytes, Fatty Change, | | | | | | |
| Diffuse | 1 | 1 | 1 | 1 | | |
| Hepatocytes, Fatty Change, | | | | | | |
| Focal | 2 | 3 | | 1 | | |
| Hepatocytes, Fatty Change, | | | | | | |
| Periportal | | | | 1 | | |
| Hepatocytes, Vacuolization, | | | | | | |
| Diffuse | 1 | 1 | 3 | 4 | | |
| Infiltrate, Mononuclear Cell | 2 | 3 | 2 | 4 | | |
| Inflammation, Acute | | | | | | |
| | | | | | | |
| OVARY (NO. EXAMINED) | (3) | (5) | (5) | (8) | | |
| Immature | 3 | 5 | 5 | 8 | | |
| | | | | | | |
| PROVENTRICULUS (NO. EXAMINED) | (5) | (6) | (5) | (8) | | |
| Infiltrate, Mononuclear Cell | 5 | 6 | 5 | 8 | | |
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HISTOPATHOLOGY INCIDENCE TABLES

OFFSPRING SACRIFICE

## HISTOPATHOLOGY INCIDENCE TABLE

454-104
Offspring Sacrifice
Male Bobwhite

| | GROUP CONTROL | | | | | | GROUP 1.8 | | | | | GROUP 6.2 | | | | | GROUP 17.6 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL | 95033 | 95038 | 95151 | 95202 | 95226 | | 95228 | 95229 | 95404 | 95444 | | 95449 | 95451 | 95552 | 95629 | 95633 | | 95818 | 95822 |
| ADIPOSE TISSUE | X | X | X | X | X | | X | X | | X | | X | X | X | X | | | X | X |
| Infiltrate, Mononuclear Cell | | | | | | | | | 1 | | | | | | | | | | |
| Inflammation, Chronic | | | | | | | | | | | | | | | | | | | |
| Inflammation, Chronic Active | | | | | | | | | | | | | | | | 2 | | | |
| Mineralization | | | | | | | | | | | | | | | | | | | |
| Necrosis | | | | | | | | | | | | | | | | 2 | | | |
| BRAIN | X | X | X | X | X | | X | X | X | X | | X | X | X | X | X | | X | X |
| BURSA OF FABRICIUS | N | X | X | X | X | | X | X | X | X | | N | X | X | X | X | | X | N |
| CLOACA | | | | | | | | | | | | X | | | | | | | X |
| GALLBLADDER | X | A | X | | X | | X | X | X | X | | X | X | X | X | | | X | X |
| Infiltrate, Mononuclear Cell | | | | 1 | | | | | | | | | | | | 2 | | | |
| KIDNEY | X | X | | X | X | | X | X | | X | | X | X | | X | X | | X | X |
| Infiltrate, Mononuclear Cell | | | | | | | | | 1 | | | | | | | | | | |
| Tubules, Mineralization | | | 1 | | | | | | 1 | | | | | 1 | | | | | |
| LIVER | | | | X | | | | | | | | | | | | | | | |
| Bile Ducts, Hyperplasia | | | | | | | | | | | | | | | | | | | |
| Hepatocytes, Fatty Change, Diffuse | | 2 | 1 | | 2 | | | 2 | | | | 2 | | 2 | | 3 | | 1 | |
| Hepatocytes, Fatty Change, Focal | 1 | | | | | | | | | | | | | | | | | | 2 |
| Hepatocytes, Fatty Change, Periportal | | | | | | | | | | | | | | | | | | | |
| Hepatocytes, Vacuolization, Diffuse | | | | | | | 2 | | 3 | 2 | | | 4 | | 3 | | | | |
| Infiltrate, Mononuclear Cell | 1 | | 1 | | 1 | | 1 | 1 | 1 | | | 1 | 1 | 1 | 1 | 1 | | | |
| Inflammation, Acute | | | | | | | | 1 | | | | | | | | | | | |
| PROVENTRICULUS | | | | | X | | | | | | | | | | | | | X | |
| Infiltrate, Mononuclear Cell | 1 | 1 | 1 | 2 | | | 1 | 1 | 1 | 2 | | 1 | 1 | 2 | 1 | 1 | | | 1 |
| TESTIS | | m | | | m | | | | | | | m | | | | | | | |
| Immature | P | P | P | P | P | | P | P | P | P | | P | P | P | P | P | | P | P |
| Infiltrate, Mononuclear Cell | | | | | | | | 1 | | | | | 3 | | | 2 | | | |
| Pigment Concentration | P | P | P | P | P | | P | P | P | P | | P | P | P | P | P | | P | P |



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

## HISTOPATHOLOGY INCIDENCE TABLE

454-104
Offspring Sacrifice
Female Bobwhite

| | GROUP CONTROL | | | | | | GROUP 1.8 | | | | | | GROUP 6.2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL | 95062 | 95128 | 95182 | 95225 | 95253 | | 95303 | 95331 | 95335 | 95341 | 95443 | | 95485 | 95561 | 95622 | 95644 | | | |
| ADIPOSE TISSUE | | X | | | N | | X | X | X | X | X | X | | X | | X | X | X | |
| Infiltrate, Mononuclear Cell | | | | | | | | | | | | | | | | | | | |
| Inflammation, Chronic | 1 | | 2 | | | | | | | | | | | | 1 | | | | |
| Inflammation, Chronic Active | | | | | | | | | | | | | | | | | | | |
| Mineralization | | | | 1 | | | | | | | | | | | | | | | |
| Necrosis | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| BRAIN | X | X | X | X | X | | X | X | X | X | X | X | | X | X | X | X | X | |
| | | | | | | | | | | | | | | | | | | | |
| BURSA OF FABRICIUS | X | X | N | X | X | | X | X | X | X | X | X | | N | N | X | X | X | |
| | | | | | | | | | | | | | | | | | | | |
| CLOACA | | | | X | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| GALLBLADDER | X | X | X | X | | | X | X | X | X | | | | X | | X | N | N | |
| Infiltrate, Mononuclear Cell | | | | | 1 | | | | | | 1 | 1 | | | 1 | | | | |
| | | | | | | | | | | | | | | | | | | | |
| KIDNEY | X | X | | | | | X | | | X | | X | | X | | X | X | X | |
| Infiltrate, Mononuclear Cell | | | | | 1 | | | 1 | 1 | | 1 | | | | 1 | | | | |
| Tubules, Mineralization | | | 1 | 2 | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| LIVER | X | | | | | | | | | | | | | | | | | X | |
| Bile Ducts, Hyperplasia | | | | | | | | 1 | | | | | | | | | | | |
| Hepatocytes, Fatty Change, | | | | | | | | | | | | | | | | | | | |
| Diffuse | | | 1 | | | | | | 1 | | | | | 3 | | | | | |
| Hepatocytes, Fatty Change, | | | | | | | | | | | | | | | | | | | |
| Focal | | 1 | | | 1 | | | 1 | | 1 | | 2 | | | | | | | |
| Hepatocytes, Fatty Change, | | | | | | | | | | | | | | | | | | | |
| Periportal | | | | | | | | | | | | | | | | | | | |
| Hepatocytes, Vacuolization, | | | | | | | | | | | | | | | | | | | |
| Diffuse | | | | 2 | | | 2 | | | | | | | | 3 | 3 | 4 | | |
| Infiltrate, Mononuclear Cell | | | | 1 | 1 | | | | 1 | 1 | 1 | | | 1 | | 1 | | | |
| Inflammation, Acute | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| OVARY | N | N | | | | | | | | | | N | | | | | | | |
| Immature | | | P | P | P | | P | P | P | P | P | | | P | P | P | P | P | |
| | | | | | | | | | | | | | | | | | | | |
| PROVENTRICULUS | | | | | | | | | | | | | | | | | | | |
| Infiltrate, Mononuclear Cell | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | |
| | | | | | | | | | | | | | | | | | | | |
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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

454-104
Offspring Sacrifice
Female Bobwhite

GROUP 17.6

| ANIMAL | 95567 | 95705 | 95759 | 95803 | 95834 | 95848 |
|---|---|---|---|---|---|---|
| ADIPOSE TISSUE | X | | X | X | X | X | X | X |
| Infiltrate, Mononuclear Cell | | | | | | | | |
| Inflammation, Chronic | | 1 | 1 | | | | | |
| Inflammation, Chronic Active | | | | | | | | |
| Mineralization | | | | | | | | |
| Necrosis | | | | | | | | |
| BRAIN | X | X | X | X | X | X | X | X |
| BURSA OF FABRICIUS | X | X | X | X | X | X | X | X |
| GALLBLADDER | | X | | X | X | X | | A |
| Infiltrate, Mononuclear Cell | 1 | | 1 | | | | 1 | |
| KIDNEY | | X | X | | X | | X | X |
| Infiltrate, Mononuclear Cell | 1 | | | 1 | | 2 | | |
| Tubules, Mineralization | | | | | | 1 | | |
| LIVER | X | | | | | | | |
| Bile Ducts, Hyperplasia | | | | | | | | |
| Hepatocytes, Fatty Change, Diffuse | | | | | 1 | | | |
| Hepatocytes, Fatty Change, Focal | | | | | | 1 | | |
| Hepatocytes, Fatty Change, Periportal | | | 2 | | | | | |
| Hepatocytes, Vacuolization, Diffuse | | 1 | | 2 | | | 3 | 3 |
| Infiltrate, Mononuclear Cell | | | 1 | 1 | | 1 | | 1 |
| Inflammation, Acute | | | | | | | | |
| OVARY | | | | | | | | |
| Immature | P | P | P | P | P | P | P | P |
| PROVENTRICULUS | | | | | | | | |
| Infiltrate, Mononuclear Cell | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 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

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

OFFSPRING SACRIFICE

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 9503 | TESTIS | Diffusely black, 0.4 - 0.6 x 0.2 cm (both present) (noted at gross trimming) | Immature; Pigment Concentration |
| 9508 | TESTIS | .5 x .3 cm black one present (noted at gross trimming) | Immature; Pigment Concentration |
| 9515 | TESTIS | .6 x .2 cm black both testes (noted at gross trimming) | Immature; Pigment Concentration |
| 9520 | TESTIS | One .5 x .3 cm, other .7 cm x .3 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
| 9526 | TESTIS | One present .2 x .2 cm black area, .2 cm x .2 cm white area (noted at gross trimming) | Immature; Pigment Concentration |
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## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 9528 | TESTIS | One testis .8 x .4 cm, other .5 x .4 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
| 9529 | TESTIS | One testis .4 x .2 cm, other .5 x .3 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
| 9540 | EXTERNAL | Feather loss on rump | No Comment Required |
| | TESTIS | Diffusely black, 0.4 x 0.2 cm, bilateral (noted at gross trimming) | Immature; Pigment Concentration |
| 9544 | TESTIS | One testis .5 x .2 cm, other .5 x .3 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
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454-104
Offspring Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 9549 | EXTERNAL | Feather loss on rump | No Comment Required |
| | TESTIS | One present fragmented, black, .7 cm x .3 cm (noted at gross trimming) | Immature; Pigment Concentration |
| 9551 | TESTIS | One testis .3 x .2 cm, other .6 x .2 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
| 9552 | EXTERNAL | Feather loss on rump | No Comment Required |
| | TESTIS | One testis .5 x .2 cm, other .4 x .2 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
| 9559 | TESTIS | One testis .4 x .2 cm, other .6 x .2 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
| 9563 | EXTERNAL | Feather loss abrasions on rump and head | No Comment Required |
| | TESTIS | One testis .6 x .3 cm, other .4 x .2 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
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454-104
Offspring Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 9581 | EXTERNAL | Feather loss on rump | No Comment Required |
| | TESTIS | One testis .7 x .4 cm, other .5 x .2 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
| 9582 | TESTIS | One testis .7 x .3 cm, other .5 x .2 cm, both black (noted at gross trimming) | Immature; Pigment Concentration |
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454-104
Offspring Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 9512 | EXTERNAL | Feather loss on rump | No Comment Required |
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454-104
Offspring Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 9531 | EXTERNAL | Feather loss on rump and back | No Comment Required |
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454-104
Offspring Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

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

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 9583 | EXTERNAL | Feather loss on rump | No Comment Required |
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Appendix X
Table 1
Egg Production (eggs laid/hen/week) from a Northern Bobwhite
Pilot Reproduction Study with PFOS

Control (0 ppm a.i.)

| | Weeks | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pen | 1 | 2 | 3 | 4 | 5 | 6 | Total | E/H/D[1] | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | Total | E/H/D[1] |
| 201 | 3 | 4 | 5 | 4 | 5 | 6 | 27 | 0.64 | 5 | 7 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 2 | 117 | 0.87 |
| 202 | 3 | 4 | 5 | 5 | 5 | 6 | 28 | 0.67 | 6 | 6 | 7 | 7 | 7 | 6 | 7 | 6 | 7 | 7 | 6 | 6 | 7 | 2 | 115 | 0.85 |
| 203 | 6 | 6 | 7 | 6 | 6 | 7 | 38 | 0.90 | 7 | 7 | 7 | 7 | 7 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 2 | 130 | 0.96 |
| 204 | 3 | 4 | 6 | 6 | 4 | 6 | 29 | 0.69 | 4 | 6 | 7 | 7 | 7 | 7 | 7 | 6 | 6 | 4 | 3 | 3 | 3 | 0 | 99 | 0.73 |
| 205 | 6 | 7 | 6 | 6 | 7 | 7 | 39 | 0.93 | 8 | 7 | 6 | 0 | 0 | 0 | 4 | 4 | 7 | 7 | 7 | 7 | 7 | 2 | 105 | 0.78 |
| Total | 21 | 25 | 29 | 27 | 27 | 32 | 161 | | 30 | 33 | 33 | 28 | 28 | 26 | 32 | 30 | 34 | 32 | 30 | 30 | 31 | 8 | 566 | |
| Mean | 4 | 5 | 6 | 5 | 5 | 6 | 32 | 0.77 | 6 | 7 | 7 | 6 | 6 | 5 | 6 | 6 | 7 | 6 | 6 | 6 | 6 | 2 | 113 | 0.84 |
| SD | 2 | 1 | 1 | 1 | 1 | 1 | 6 | 0.14 | 2 | 1 | 1 | 3 | 3 | 3 | 1 | 1 | 0 | 1 | 2 | 2 | 2 | 1 | 12 | 0.09 |

[1] Eggs laid per hen per day

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

1.8 ppm a.i.

| | Weeks | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Pen | 1 | 2 | 3 | 4 | 5 | 6 | Total | E/H/D[1] |
| 206 | 3 | 6 | 6 | 6 | 6 | 7 | 34 | 0.81 |
| 207 | 6 | 7 | 7 | 7 | 1 | 0 | 28 | 0.67 |
| 208 | 5 | 5 | 6 | 5 | 5 | 6 | 32 | 0.76 |
| 209 | 4 | 5 | 5 | 5 | 6 | 7 | 32 | 0.76 |
| 210 | 4 | 6 | 4 | 4 | 2 | 0 | 20 | 0.48 |
| Total | 22 | 29 | 28 | 27 | 20 | 20 | 146 | |
| Mean | 4 | 6 | 6 | 5 | 4 | 4 | 29 | 0.70 |
| SD | 1 | 1 | 1 | 1 | 2 | 4 | 6 | 0.13 |

[1] Eggs laid per hen per day

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

6.2 ppm a.i.

| | Weeks | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Pen | 1 | 2 | 3 | 4 | 5 | 6 | Total | E/H/D[1] |
| 211 | 5 | 7 | 6 | 7 | 7 | 7 | 39 | 0.93 |
| 212 | 0 | 2 | 3 | 4 | 5 | 6 | 20 | 0.48 |
| 213 | 6 | 5 | 7 | 6 | 3 | 7 | 34 | 0.81 |
| 214 | 5 | 5 | 5 | 6 | 6 | 7 | 34 | 0.81 |
| 215 | 4 | 5 | 6 | 4 | 6 | 6 | 31 | 0.74 |
| Total | 20 | 24 | 27 | 27 | 27 | 33 | 158 | |
| Mean | 4 | 5 | 5 | 5 | 5 | 7 | 32 | 0.75 |
| SD | 2 | 2 | 2 | 1 | 2 | 1 | 7 | 0.17 |

[1] Eggs laid per hen per day

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

17.6 ppm a.i.

| | Weeks | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pen | 1 | 2 | 3 | 4 | 5 | 6 | Total | E/H/D[1] | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | Total | E/H/D[1] |
| 216 | 5 | 5 | 5 | 5 | 7 | 7 | 34 | 0.81 | 5 | 6 | 7 | 6 | 6 | 6 | 6 | 4 | 0 | 0 | 1 | 3 | 0 | 0 | 84 | 0.62 |
| 217 | 5 | 6 | 7 | 6 | 7 | 7 | 38 | 0.90 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 7 | 7 | 7 | 7 | 2 | 0 | 0 | 116 | 0.86 |
| 218 | 6 | 7 | 7 | 6 | 7 | 7 | 40 | 0.95 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 2 | 133 | 0.99 |
| 219 | 3 | 4 | 4 | 5 | 5 | 7 | 28 | 0.67 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 9 | 6 | 6 | 7 | 1 | 120 | 0.89 |
| 220 | 8 | 5 | 7 | 7 | 7 | 7 | 41 | 0.98 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 7 | 7 | 7 | 7 | 7 | 2 | 132 | 0.98 |
| Total | 27 | 27 | 30 | 29 | 33 | 35 | 181 | | 32 | 34 | 35 | 34 | 34 | 34 | 33 | 31 | 28 | 30 | 28 | 25 | 21 | 5 | 585 | |
| Mean | 5 | 5 | 6 | 6 | 7 | 7 | 36 | 0.86 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 6 | 6 | 6 | 5 | 4 | 1 | 117 | 0.87 |
| SD | 2 | 1 | 1 | 1 | 1 | 0 | 5 | 0.13 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 3 | 3 | 2 | 4 | 1 | 20 | 0.15 |

[1] Eggs laid per hen per day

Appendix XI
Page 1
Reproductive Performance by Pen
from a Northern Bobwhite Pilot Reproduction Study with PFOS

Table 1
Reproductive Data (Count) By Pen

| Parameter | Control (0 ppm a.i.) | | | | | | 1.8 ppm a.i. | | | | | | 6.2 ppm a.i. | | | | | | 17.6 ppm a.i. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pens | 201 | 202 | 203 | 204 | 205 | Total | 206 | 207 | 208 | 209 | 210 | Total | 211 | 212 | 213 | 214 | 215 | Total | 216 | 217 | 218 | 219 | 220 | Total |
| Eggs Laid | 5 | 6 | 6 | 5 | 7 | 29 | 6 | 0 | 5 | 6 | 1 | 18 | 7 | 5 | 5 | 7 | 5 | 29 | 7 | 7 | 7 | 6 | 7 | 34 |
| Eggs Cracked | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eggs Set | 5 | 6 | 6 | 5 | 7 | 29 | 6 | 0 | 5 | 6 | 1 | 18 | 7 | 5 | 5 | 7 | 5 | 29 | 7 | 7 | 7 | 6 | 7 | 34 |
| Viable Embryos | 5 | 6 | 6 | 5 | 7 | 29 | 6 | 0 | 4 | 6 | 1 | 17 | 5 | 5 | 3 | 7 | 5 | 25 | 7 | 7 | 7 | 6 | 7 | 34 |
| Live 3-Wk Embryos | 5 | 5 | 6 | 5 | 7 | 28 | 6 | 0 | 4 | 6 | 1 | 17 | 5 | 5 | 3 | 7 | 5 | 25 | 7 | 6 | 7 | 6 | 7 | 33 |
| Hatchlings | 5 | 5 | 6 | 5 | 7 | 28 | 6 | 0 | 4 | 6 | 1 | 17 | 5 | 5 | 3 | 7 | 5 | 25 | 7 | 5 | 7 | 6 | 6 | 31 |
| Offspring Survivors | 5 | 5 | 5 | 5 | 7 | 27 | 6 | 0 | 4 | 6 | 1 | 17 | 5 | 5 | 3 | 2 | 5 | 20 | 7 | 3 | 6 | 3 | 6 | 25 |

Appendix XI
Page 2
Reproductive Performance by Pen
from a Northern Bobwhite Pilot Reproduction Study with PFOS

Table 2
Eggs Laid / Hen / Day

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Eggs Laid | Days | Eggs/ Hen/Day | Eggs Laid | Days | Eggs/ Hen/Day | Eggs Laid | Days | Eggs/ Hen/Day | Eggs Laid | Days | Eggs/ Hen/Day |
| 1 | 5 | 7 | 0.71 | 6 | 7 | 0.86 | 7 | 7 | 1.00 | 7 | 7 | 1.00 |
| 2 | 6 | 7 | 0.86 | 0 | 7 | | 5 | 7 | 0.71 | 7 | 7 | 1.00 |
| 3 | 6 | 7 | 0.86 | 5 | 7 | 0.71 | 5 | 7 | 0.71 | 7 | 7 | 1.00 |
| 4 | 5 | 7 | 0.71 | 6 | 7 | 0.86 | 7 | 7 | 1.00 | 6 | 7 | 0.86 |
| 5 | 7 | 7 | 1.00 | 1 | 7 | 0.14 | 5 | 7 | 0.71 | 7 | 7 | 1.00 |
| Total | 29 | | | 18 | | | 29 | | | 34 | | |
| Mean | 6 | | 0.83 | 4 | | 0.64 | 6 | | 0.83 | 7 | | 0.97 |
| SD | 1 | | 0.12 | 3 | | 0.34 | 1 | | 0.16 | 0 | | 0.06 |

Table 3
Eggs Cracked / Eggs Laid (%)

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Eggs Cracked | Eggs Laid | % | Eggs Cracked | Eggs Laid | % | Eggs Cracked | Eggs Laid | % | Eggs Cracked | Eggs Laid | % |
| 1 | 0 | 5 | 0 | 0 | 6 | 0 | 0 | 7 | 0 | 0 | 7 | 0 |
| 2 | 0 | 6 | 0 | 0 | 0 | | 0 | 5 | 0 | 0 | 7 | 0 |
| 3 | 0 | 6 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 7 | 0 |
| 4 | 0 | 5 | 0 | 0 | 6 | 0 | 0 | 7 | 0 | 0 | 6 | 0 |
| 5 | 0 | 7 | 0 | 0 | 1 | 0 | 0 | 5 | 0 | 0 | 7 | 0 |
| Total | 0 | 29 | | 0 | 18 | | 0 | 29 | | 0 | 34 | |
| Mean | 0 | 6 | 0 | 0 | 4 | 0 | 0 | 6 | 0 | 0 | 7 | 0 |
| SD | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

Appendix XI
Page 3
Reproductive Performance by Pen
from a Northern Bobwhite Pilot Reproduction Study with PFOS

Table 4
Viable Embryos / Eggs Set (%)

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Viable Embryos | Eggs Set | % | Viable Embryos | Eggs Set | % | Viable Embryos | Eggs Set | % | Viable Embryos | Eggs Set | % |
| 1 | 5 | 5 | 100 | 6 | 6 | 100 | 5 | 7 | 71 | 7 | 7 | 100 |
| 2 | 6 | 6 | 100 | 0 | 0 | | 5 | 5 | 100 | 7 | 7 | 100 |
| 3 | 6 | 6 | 100 | 4 | 5 | 80 | 3 | 5 | 60 | 7 | 7 | 100 |
| 4 | 5 | 5 | 100 | 6 | 6 | 100 | 7 | 7 | 100 | 6 | 6 | 100 |
| 5 | 7 | 7 | 100 | 1 | 1 | 100 | 5 | 5 | 100 | 7 | 7 | 100 |
| Total | 29 | 29 | | 17 | 18 | | 25 | 29 | | 34 | 34 | |
| Mean | 6 | 6 | 100 | 3 | 4 | 95 | 5 | 6 | 86 | 7 | 7 | 100 |
| SD | 1 | 1 | 0 | 3 | 3 | 10 | 1 | 1 | 19 | 0 | 0 | 0 |

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

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Live 3-Week | Viable | % | Live 3-Week | Viable | % | Live 3-Week | Viable | % | Live 3-Week | Viable | % |
| 1 | 5 | 5 | 100 | 6 | 6 | 100 | 5 | 5 | 100 | 7 | 7 | 100 |
| 2 | 5 | 6 | 83 | 0 | 0 | | 5 | 5 | 100 | 6 | 7 | 86 |
| 3 | 6 | 6 | 100 | 4 | 4 | 100 | 3 | 3 | 100 | 7 | 7 | 100 |
| 4 | 5 | 5 | 100 | 6 | 6 | 100 | 7 | 7 | 100 | 6 | 6 | 100 |
| 5 | 7 | 7 | 100 | 1 | 1 | 100 | 5 | 5 | 100 | 7 | 7 | 100 |
| Total | 28 | 29 | | 17 | 17 | | 25 | 25 | | 33 | 34 | |
| Mean | 6 | 6 | 97 | 3 | 3 | 100 | 5 | 5 | 100 | 7 | 7 | 97 |
| SD | 1 | 1 | 7 | 3 | 3 | 0 | 1 | 1 | 0 | 1 | 0 | 6 |

Reproductive Performance by Pen
from a Northern Bobwhite Pilot Reproduction Study with PFOS

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

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Live | | | Live | | | Live | | | Live | |
| Replicate | Hatch | 3-Week | % | Hatch | 3-Week | % | Hatch | 3-Week | % | Hatch | 3-Week | % |
| 1 | 5 | 5 | 100 | 6 | 6 | 100 | 5 | 5 | 100 | 7 | 7 | 100 |
| 2 | 5 | 5 | 100 | 0 | 0 | | 5 | 5 | 100 | 5 | 6 | 83 |
| 3 | 6 | 6 | 100 | 4 | 4 | 100 | 3 | 3 | 100 | 7 | 7 | 100 |
| 4 | 5 | 5 | 100 | 6 | 6 | 100 | 7 | 7 | 100 | 6 | 6 | 100 |
| 5 | 7 | 7 | 100 | 1 | 1 | 100 | 5 | 5 | 100 | 6 | 7 | 86 |
| Total | 28 | 28 | | 17 | 17 | | 25 | 25 | | 31 | 33 | |
| Mean | 6 | 6 | 100 | 3 | 3 | 100 | 5 | 5 | 100 | 6 | 7 | 94 |
| SD | 1 | 1 | 0 | 3 | 3 | 0 | 1 | 1 | 0 | 1 | 1 | 9 |

Table 7
Surviving Offspring / Hatchlings

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Offspring | | | Offspring | | | Offspring | | | Offspring | | |
| Replicate | Surv. | Hatch | % | Surv. | Hatch | % | Surv. | Hatch | % | Surv. | Hatch | % |
| 1 | 5 | 5 | 100 | 6 | 6 | 100 | 5 | 5 | 100 | 7 | 7 | 100 |
| 2 | 5 | 5 | 100 | 0 | 0 | | 5 | 5 | 100 | 3 | 5 | 60 |
| 3 | 5 | 6 | 83 | 4 | 4 | 100 | 3 | 3 | 100 | 6 | 7 | 86 |
| 4 | 5 | 5 | 100 | 6 | 6 | 100 | 2 | 7 | 29 | 3 | 6 | 50 |
| 5 | 7 | 7 | 100 | 1 | 1 | 100 | 5 | 5 | 100 | 6 | 6 | 100 |
| Total | 27 | 28 | | 17 | 17 | | 20 | 25 | | 25 | 31 | |
| Mean | 5 | 6 | 97 | 3 | 3 | 100 | 4 | 5 | 86 | 5 | 6 | 79 |
| SD | 1 | 1 | 7 | 3 | 3 | 0 | 1 | 1 | 32 | 2 | 1 | 23 |

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Page 5
Reproductive Performance by Pen
from a Northern Bobwhite Pilot Reproduction Study with PFOS

Table 8
Hatchlings / Eggs Set (%)

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Hatch | Eggs Set | % | Hatch | Eggs Set | % | Hatch | Eggs Set | % | Hatch | Eggs Set | % |
| 1 | 5 | 5 | 100 | 6 | 6 | 100 | 5 | 7 | 71 | 7 | 7 | 100 |
| 2 | 5 | 6 | 83 | 0 | 0 | | 5 | 5 | 100 | 5 | 7 | 71 |
| 3 | 6 | 6 | 100 | 4 | 5 | 80 | 3 | 5 | 60 | 7 | 7 | 100 |
| 4 | 5 | 5 | 100 | 6 | 6 | 100 | 7 | 7 | 100 | 6 | 6 | 100 |
| 5 | 7 | 7 | 100 | 1 | 1 | 100 | 5 | 5 | 100 | 6 | 7 | 86 |
| Total | 28 | 29 | | 17 | 18 | | 25 | 29 | | 31 | 34 | |
| Mean | 6 | 6 | 97 | 3 | 4 | 95 | 5 | 6 | 86 | 6 | 7 | 91 |
| SD | 1 | 1 | 7 | 3 | 3 | 10 | 1 | 1 | 19 | 1 | 0 | 13 |

Table 9
Surviving Offspring / Eggs Set (%)

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Offspring Surv | Eggs Set | % | Offspring Surv | Eggs Set | % | Offspring Surv | Eggs Set | % | Offspring Surv | Eggs Set | % |
| 1 | 5 | 5 | 100 | 6 | 6 | 100 | 5 | 7 | 71 | 7 | 7 | 100 |
| 2 | 5 | 6 | 83 | 0 | 0 | | 5 | 5 | 100 | 3 | 7 | 43 |
| 3 | 5 | 6 | 83 | 4 | 5 | 80 | 3 | 5 | 60 | 6 | 7 | 86 |
| 4 | 5 | 5 | 100 | 6 | 6 | 100 | 2 | 7 | 29 | 3 | 6 | 50 |
| 5 | 7 | 7 | 100 | 1 | 1 | 100 | 5 | 5 | 100 | 6 | 7 | 86 |
| Total | 27 | 29 | | 17 | 18 | | 20 | 29 | | 25 | 34 | |
| Mean | 5 | 6 | 93 | 3 | 4 | 95 | 4 | 6 | 72 | 5 | 7 | 73 |
| SD | 1 | 1 | 9 | 3 | 3 | 10 | 1 | 1 | 30 | 2 | 0 | 25 |

Appendix XI
Page 6
Reproductive Performance by Pen
from a Northern Bobwhite Pilot Reproduction Study with PFOS

Table 10
Hatchlings / Hen / Day

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Hatch | Days | Hatch/ Hen/Day | Hatch | Days | Hatch/ Hen/Day | Hatch | Days | Hatch/ Hen/Day | Hatch | Days | Hatch/ Hen/Day |
| 1 | 5 | 7 | 0.71 | 6 | 7 | 0.86 | 5 | 7 | 0.71 | 7 | 7 | 1.00 |
| 2 | 5 | 7 | 0.71 | 0 | 7 | 0.00 | 5 | 7 | 0.71 | 5 | 7 | 0.71 |
| 3 | 6 | 7 | 0.86 | 4 | 7 | 0.57 | 3 | 7 | 0.43 | 7 | 7 | 1.00 |
| 4 | 5 | 7 | 0.71 | 6 | 7 | 0.86 | 7 | 7 | 1.00 | 6 | 7 | 0.86 |
| 5 | 7 | 7 | 1.00 | 1 | 7 | 0.14 | 5 | 7 | 0.71 | 6 | 7 | 0.86 |
| Total | 28 | | | 17 | | | 25 | | | 31 | | |
| Mean | 6 | | 0.80 | 3 | | 0.49 | 5 | | 0.71 | 6 | | 0.89 |
| SD | 1 | | 0.13 | 3 | | 0.40 | 1 | | 0.20 | 1 | | 0.12 |

Table 11
Surviving Offspring / Hen / Day

| | Control (0 ppm a.i.) | | | 1.8 ppm a.i. | | | 6.2 ppm a.i. | | | 17.6 ppm a.i. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Offspring Surv | Days | Offspring/ Hen/Day | Offspring Surv | Days | Offspring/ Hen/Day | Offspring Surv | Days | Offspring/ Hen/Day | Offspring Surv | Days | Offspring/ Hen/Day |
| 1 | 5 | 7 | 0.71 | 6 | 7 | 0.86 | 5 | 7 | 0.71 | 7 | 7 | 1.00 |
| 2 | 5 | 7 | 0.71 | 0 | 7 | 0.00 | 5 | 7 | 0.71 | 3 | 7 | 0.43 |
| 3 | 5 | 7 | 0.71 | 4 | 7 | 0.57 | 3 | 7 | 0.43 | 6 | 7 | 0.86 |
| 4 | 5 | 7 | 0.71 | 6 | 7 | 0.86 | 2 | 7 | 0.29 | 3 | 7 | 0.43 |
| 5 | 7 | 7 | 1.00 | 1 | 7 | 0.14 | 5 | 7 | 0.71 | 6 | 7 | 0.86 |
| Total | 27 | | | 17 | | | 20 | | | 25 | | |
| Mean | 5 | | 0.77 | 3 | | 0.49 | 4 | | 0.57 | 5 | | 0.71 |
| SD | 1 | | 0.13 | 3 | | 0.40 | 1 | | 0.20 | 2 | | 0.27 |

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

Mean Hatchling Body Weight (g)

| Replicate | Control (0 ppm a.i.) | 1.8 ppm a.i. | 6.2 ppm a.i. | 17.6 ppm a.i. |
|---|---|---|---|---|
| 1 | 5.6 | 5.3 | 6.6 | 6.1 |
| 2 | 6.2 | -- | 5.8 | 5.6 |
| 3 | 6.3 | 6.0 | 5.7 | 6.7 |
| 4 | 6.6 | 6.0 | 4.9 | 6.2 |
| 5 | 5.6 | 5.0 | 5.8 | 5.3 |
| Mean | 6.1 | 5.6 | 5.7 | 6.0 |
| SD | 0.5 | 0.5 | 0.6 | 0.5 |

Mean Surviving Offspring[1] Body Weight (g)

| Replicate | Control (0 ppm a.i.) | 1.8 ppm a.i. | 6.2 ppm a.i. | 17.6 ppm a.i. |
|---|---|---|---|---|
| 1 | 185 | 175 | 181 | 164 |
| 2 | 171 | -- | 183 | 169 |
| 3 | 160 | 174 | 171 | 175 |
| 4 | 178 | 176 | 181 | 175 |
| 5 | 182 | 173 | 181 | 184 |
| Mean | 175 | 175 | 179 | 173 |
| SD | 10 | 1 | 5 | 8 |

[1] Offspring were approximately 12 weeks of age at final body weight interval.
-- No offspring available.

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

Control (0 ppm a.i.)

| | Male | Female |
|---|---|---|
| Pen | Liver | Liver |
| 201 | 3.260 | 9.210 |
| 202 | 3.156 | 7.606 |
| 203 | 3.373 | 7.908 |
| 204 | 4.170 | 5.612 |
| 205 | 3.051 | 7.889 |
| Mean | 3.402 | 7.645 |
| SD | 0.446 | 1.295 |

1.8 ppm a.i.

| | Male | Female |
|---|---|---|
| Pen | Liver | Liver |
| 206 | 3.161 | 7.020 |
| 207 | 3.750 | 8.556 |
| 208 | 4.211 | 5.744 |
| 209 | 3.496 | 6.278 |
| 210 | 2.887 | 4.016 |
| Mean | 3.501 | 6.323 |
| SD | 0.514 | 1.668 |

Appendix XIII
Adult Liver Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with PFOS
Page 2

6.2 ppm a.i.

| | Male | Female |
|---|---|---|
| Pen | Liver | Liver |
| 211 | 3.552 | 6.052 |
| 212 | 4.394 | 5.041 |
| 213 | 4.075 | 5.404 |
| 214 | 2.591 | 6.246 |
| 215 | 3.810 | 5.372 |
| Mean | 3.684 | 5.623 |
| SD | 0.686 | 0.505 |

17.6 ppm a.i.

| | Male | Female |
|---|---|---|
| Pen | Liver | Liver |
| 216 | 3.056 | 8.880 |
| 217 | 2.076 | 4.147 |
| 218 | 2.505 | 6.768 |
| 219 | 2.625 | 6.131 |
| 220 | 2.375 | 8.329 |
| Mean | 2.527 | 6.851 |
| SD | 0.359 | 1.880 |

Appendix XIV

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

| Control (0 ppm a.i.) | | 1.8 ppm a.i. | | 6.2 ppm a.i. | | 17.6 ppm a.i. | |
|---|---|---|---|---|---|---|---|
| Pen | Liver | Pen | Liver | Pen | Liver | Pen | Liver |
| 201 | 3.905 | 206 | 3.819 | 211 | 3.826 | 216 | 4.370 |
| 201 | 3.804 | 206 | 3.300 | 211 | 3.533 | 216 | 3.032 |
| 202 | 3.977 | 206 | 3.655 | 212 | 6.097 | 217 | 2.998 |
| 202 | 4.125 | 208 | 4.418 | 212 | 4.645 | 217 | 3.050 |
| 203 | 3.688 | 208 | 3.458 | 213 | 3.899 | 218 | 3.336 |
| 203 | 2.973 | 209 | 4.128 | 213 | 3.771 | 218 | 3.271 |
| 204 | 3.612 | 209 | 4.146 | 214 | 7.795 | 219 | 4.281 |
| 204 | 4.133 | 209 | 3.596 | 214 | 5.996 | 219 | 3.211 |
| 205 | 3.563 | 209 | 4.095 | 215 | 4.701 | 220 | 3.459 |
| 205 | 3.680 | 210 | 2.940 | 215 | 5.582 | 220 | 3.286 |
| Mean | 3.746 | Mean | 3.756 | Mean | 4.985 | Mean | 3.429 |
| SD | 0.339 | SD | 0.453 | SD | 1.367 | SD | 0.495 |

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

Appendix XV

Changes to Study Protocol

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

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

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

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

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

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

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

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

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

Appendix XV
Page 2

Changes to Study Protocol

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

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

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

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

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

Appendix XVI
Personnel Involved in the 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, Avian Toxicology

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

(4) Diana Temple, Laboratory Supervisor

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

Analytical Chemistry

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

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