DIETARY ACUTE NORTHERN BOBWHITE STUDY

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks field: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.2200, OECD 205, and FIFRA Subdivision E. Section 71-2
Type: Dietary acute
GLP: Yes
Year completed: 1999 (Test), 2000 (Report)
Species: *Colinus virginianus*
Supplier: Wildlife International Ltd. Production Flock, Easton, Maryland
Analytical monitoring: Test substance concentration in standards and samples were determined by reversed-phase HPLC and mass spectroscopy. PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.
Test phases: Acclimation – 10 days
Exposure - 5 days
Post-exposure observation – 3 or 17 days
Statistical methods: LC_{50} values calculated by probit analysis using the computer software of C.E. Stephan. Body weight data were compared by Dunnett's test using TOXSTAT software. No statistical analyses were applied to feed consumption data.
Test bird age: 10 days
Pretreatment: None

Test conditions:

Housing and environmental conditions: Indoors in batteries of thermostatically controlled brooding pens. Each pen's floor space measured approximately 72 X 90 cm. Ceiling height was approximately 23 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.
Identification: Each group of birds identified by pen number and test concentration. Individuals identified by leg bands.

Number of replicates: Six for controls, two for each treatment group
Number of bobwhite per replicate: five
Number of concentrations: seven plus a negative control
Feed and water: Game bird ration formulated as below, water from the town of Easton public water supply. Both provided ad libitum during acclimation and testing.

Game Bird Ration

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)[1]	0.25
Salt Iodized	0.10

[1]Fermentation by-products (source of unidentified growth factors)

Vitamin and Mineral Premix

Vitamin or mineral	Amount 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 grams

Prophylaxis: None
Brooding compartment mean temperature: 38 ± 2°C
Ambient room mean temperature: 27.3 ± 1.2°C
Average relative humidity: 31 ± 14%
Photoperiod: Sixteen hours light per day
Lighting: fluorescent lights which closely approximate noon-day sunlight; average of approximately 139 lux of illumination

Test diet preparation: Test substance mixed directly into the ration by means of a Hobart mixer. No carrier was used.

Diet sampling: Homogeneity of the test substance in the diet evaluated by collecting six samples from the 18.3 ppm concentration and six from the 1171 ppm concentration. Samples collected from the top, middle, and bottom of the left and right sections of the mixing vessel. These samples also served as the verification samples for these concentrations. Verification samples of the other treatment groups (two samples from each) and the control (one sample) were collected at preparation on Day 0. Stability samples were collected at the end of the exposure period (Day 5) from the control (one sample) and each treatment group (two samples each).

RESULTS

Nominal concentrations: Bk control, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm
Measured concentrations: <LOQ, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm
Element value: Dietary LC_{50} = 220 (164 – 289) ppm
No mortality concentration = 73.2 ppm
NOEC (body weight gain) = 73.2 ppm
All element values based on nominal concentrations

Analytical Methodology: Diet samples were extracted with methanol. Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 1.15 ppm in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7. Samples collected for determination of homogeneity in diet ranged from 102-107% of nominal. Samples collected for verification in diet had measured values from 92 to 119% of nominal. Measured values for

ambient stability samples taken at Day 5 ranged from 101-122% of nominal.

Summary of analytical chemistry data:

Homogeneity in Avian Diet

Nominal Test Concentration, ppm	Measured Values at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
18.3	18.5, 23.4, 18.3, 17.3, 19.4, 19.9,	19.5	107
1171	1239, 1221, 1118, 1301, 1163, 1133	1196	102

Verification in Avian Diet

Nominal Test Concentration, ppm	Measured Duplicate Concentrations at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
Negative Control	< LOQ	-	-
36.6	45.7, 34.6	40.2	110
73.2	77.8, 71.2	74.5	102
146	176, 172	174	119
293	274, 307	291	99
586	550, 523	537	92

Ambient Stability in Avian Diet

Day 0 Mean Measured Concentration, ppm	Measured Duplicate Concentrations at Day 5, ppm	Mean Measured Concentration, ppm	Mean Percent of Day 0
Negative Control	< LOQ	-	-
19.5	19.2, 19.9	19.6	101
40.2	44.4, 53.8	49.1	122
74.5	76.4, 77.9	77.2	104
174	177, 174	176	101
291	318, 315	317	109
537	560, 665	613	114
1196	1260, 1187	1224	102

Biological observations

Mortalities and clinical observations: One incidental mortality occurred in the control group as a result of a broken leg on the morning of Day 5. It was subsequently euthanized on Day 6. Two other birds in the control group were intermittently noted with foot lesions associated with cage mate aggression. Otherwise, all control birds were observed to be normal in appearance and behavior throughout the test.

The first treatment-related mortalities occurred on Day 3 in the 586 and 1171 ppm treatment groups. Mortality occurred through Day 8 in all dose groups $\geq$ 146 ppm with some of the deaths being during the post-exposure period. There were no treatment-related mortalities or overt signs of toxicity at concentrations $\leq$ 73.2 ppm.

There was 11% mortality in the 146 ppm treatment group, and two additional birds displayed clinical signs of toxicity (wing droop). All other birds in this test group displayed normal appearance and behavior for the duration of the test. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 80% mortality (occurring on Days 5,6, and 7) for birds in the 293 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, wing droop, loss of coordination, lower limb weakness and convulsions. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 100% mortality (occurring from Day 3 through Day 7) for birds in the 586 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, lower limb rigidity, prostrate posture, and convulsions.

There was 100% mortality (occurring from Day 2 (noted on Day 3 for Day 2 afternoon) through Day 4) for birds in the 1171 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, and lower limb rigidity.

Body weight gain: When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations ≤ 73.2 ppm. During Days 0-5, statistically significant reductions in body weight gain or body weight loss occurred in the 146, 293, and 586 ppm treatment groups. Body weight effects could not be determined for test organisms in the 1171 ppm group due to total mortality.

Feed Consumption: No apparent treatment related effects were noted for feed consumption for birds in concentrations ≤146 ppm. Reduced feed consumption was noted for birds in treatment groups ≥ 293 ppm from Days 0-5. No treatment-related effects on feed consumption in any of the surviving treatment groups during the Day 6-8 post-exposure period were observed.

Gross Necropsy: All birds that died during the study, half of those surviving at Day 8 and the rest at test termination were subjected to a gross necropsy. Necropsy results for birds found dead were similar, including thin condition, loss of muscle mass, altered spleen color, autolysis of tissues and pale organs. These necropsy findings were considered to be treatment related. The single bird euthanized from the 293 ppm treatment was found to have treatment related necropsy findings. Necropsy results for all other birds euthanized on Day 8 and Day 22 were unremarkable.

% Cumulative Mortality

Nominal Concentration, ppm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8*
Negative Control	0	0	0	0	0	0	0	0
18.3	0	0	0	0	0	0	0	0
36.6	0	0	0	0	0	0	0	0
73.2	0	0	0	0	0	0	0	0
146	0	0	10**	0	0	0	10	10
293	0	0	0	0	20	40	80	80
586	0	0	10	20	50	80	100	100
1171	0	0	30	100	100	100	100	100

*No mortalities occurred in any of the treatment levels from Day 8 to Day 22
** Bird euthanized on day 3 after sustaining a broken leg.

Body Weight (grams)

	Exposure Period		Recovery Period				
Nominal Concentration (ppm)	Mean Body Weight, Day 0	Mean Body Weight Change Day 0-5	Mean Body Weight Change Day 5-8	Mean Body Weight Change Day 8-15	Mean Body Weight Change Day 15-22	Mean Total Body Weight Change, Day 8-22	Mean Body Weight, Day 22
Negative Control	20	+10	+8	+23	+22	45	82
18.3	21	+11	+9	+24	+23	47	87
36.6	20	+11	+8	+26	+24	50	89
73.2	20	+9	+7	+24	+20	44	79
146	20	+7*	+6**	+24	+21	45	79
293	20	-2**	-1**	+14	+20	34	55
586	20	-4**	--	--	--	--	--
1171	20	--	--	--	--	--	-

Note: Numbers may not add manually due to rounding. Values for 293 ppm treatment group are impacted by the fact that only one bird remained in that treatment group after Day 8.
*Statistically different from the control group at p<0.05 (Dunnett's t-test).
**Statistically different from the control group at p=<0.01 Dunnett's t-test
(--) = No data available due to mortality.

Mean Average Feed Consumption

Nominal Concentration, ppm	Grams Feed/Bird/Day Days 0-5	Grams Feed/Bird/Day Days 6-8	Grams Feed/Bird/Day Days 8-15	Grams Feed/Bird/Day Days 15-22
Negative Control	9	10	9	13
18.3	9	11	10	12
36.6	8	12	14	15
73.2	10	13	13	15
146	9	10	11	14
293	5	9	8	9
586	6	19	--	--
1171	4	--	--	--

(--) = No data available due to mortality.

Gross Pathological Observations from Birds that Died in Study

Finding	Male, Female, and Undetermined (ppm)				
	Control N = 1	146 N = 2	293 N = 8	586 N = 10	1171 N = 10
Abdominal cavity, some autolysis	0	0	2	2	4
Abdominal cavity, autolysis throughout	0	0	0	1	1
Crop, empty	0	0	2	5	2
Emaciated	0	0	2	5	8
Fractured leg	1	1	0	0	0
G.I. Tract empty	0	0	1	1	0
Gizzard contents bile stained	0	0	2	5	1
Heart, anterior portion mottled white color	0	0	1	0	0
Heart, pale	0	0	0	2	1
Intestinal contents tar-like	0	0	0	2	0
Keel, prominent	0	0	1	3	10
Kidneys, pale	0	0	0	2	0
Liver, pale and mottled	0	1	0	0	0
Loss of muscle mass	0	0	4	7	9
Muscular-skeletal, pale	0	1	0	0	0
Small in stature	0	0	3	0	0
Spleen, black	0	0	0	1	0
Spleen, dark	0	0	0	0	2
Spleen, grey	0	0	0	1	0
Spleen, grey-brown	0	0	0	0	1
Spleen, pale	0	0	1	0	1
Spleen, small	0	0	0	0	1
Spleen, small and pale	0	0	0	3	0
Thin	0	0	0	4	2
Not remarkable	0	0	1	0	0

CONCLUSIONS

The dietary LC50 value for Northern Bobwhite exposed to perfluorooctanesulfonate was determined to be 220 ppm with a 95% confidence interval of 164 to 289 ppm. The slope of the concentration-response curve was 7.005 and the chi-square value was 0.023. The no mortality concentration was 73.2 ppm. Based upon treatment related mortality, signs of toxicity and effects upon body weight gain at the 146 ppm test concentration, the no observed effect concentration was 73.2 ppm.

Author and/or submitter: 3M Corporation, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 1.

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

OTHER

Last changed: 5/3/00

PFOS: A Dietary LC50 Study with the Northern Bobwhite

PFOS:
A DIETARY LC50 STUDY WITH THE NORTHERN BOBWHITE

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-103
3M LAB REQUEST NO.: U2723

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines (draft)
OPPTS Number 850.2200

FIFRA Subdivision E, Section 71-2

OECD Guideline 205

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STUDY INITIATION: April 21, 1999

STUDY COMPLETION: April 26, 2000

SUBMITTED TO

3M Corporation
Environmental Laboratory
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St. Paul, Minnesota 55144

Wildlife International, Ltd.

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Dietary LC50 Study with the Northern Bobwhite

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-103

3M LAB REQUEST NO.: U2723

STUDY COMPLETION: April 26, 2000

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, (OCDE/GD(92) 32, Environment Monograph No. 45, Paris, 1992); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984 with the following exception:

The test substance was not characterized in accordance with full GLP compliance; however, the characterization was performed according to 3M Standard Operating Procedures and Methods, and all raw data are being maintained in the 3M archives. The test substance is being recharacterized in accordance with GLP.

The stability of the test substance and reference standard under conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

Sean P. Gallagher DATE 4/26/00

Sean P. Gallagher
Senior Biologist

SPONSOR'S REPRESENTATIVE

Susan A. Beach DATE 4/27/00

Ms. Susan A. Beach

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 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, (OCDE/GD (92) 32, Environment Monograph No. 45, Paris, 1992); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all audits and inspections and the dates that 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 Prep. & Analytical Sampling	April 22, 1999	April 22, 1999	April 23, 1999
Matrix Fortification	April 22, 1999	April 22, 1999	April 23, 1999
Feed Consumption & Analytical Sampling	April 27, 1999	April 27, 1999	May 4, 1999
Analytical Data and Draft Report	July 7, 8, 9, 1999	July 9, 1999	July 16, 1999
Biology Data and Draft Report	August 26, 27, 30, 31, 1999	August 31, 1999	September 13, 1999
Final Report	April 17-18, 2000	April 18, 2000	April 19, 2000

Susan L. Coleman DATE 4-19-00

Susan L. Coleman
Senior Quality Assurance Representative

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A Dietary LC50 Study with the Northern Bobwhite

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-103

3M LAB REQUEST NO.: U2723

STUDY DIRECTOR:

Sean P. Gallagher — Date: 4/26/00
Senior Biologist, Avian Toxicology

CHEMISTRY PRINCIPAL INVESTIGATOR:

Raymond L. Van Hoven, Ph.D — Date: 4-26-00
Scientist, Analytical Chemistry

REPORT APPROVED BY:

Joann B. Beavers — Date: 4/26/00
Director, Avian Toxicology

Willard B. Nixon, Ph.D. — Date: 4/26/00
Manager, Analytical Chemistry

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SUMMARY

SPONSOR: 3M Corporation

TEST SUBSTANCE: PFOS

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-103

STUDY: PFOS: A Dietary LC50 Study with the Northern Bobwhite

RESULTS: The dietary LC50 value for northern bobwhite exposed to PFOS was determined to be 220 ppm a.i. with a 95% confidence interval of 164 ppm a.i. to 289 ppm a.i. The slope of the concentration-response curve was 7.005 and the chi-square value was 0.023. The no mortality concentration was 73.2 ppm a.i. Based upon treatment related mortality, signs of toxicity and effects upon body weight gain at the 146 ppm a.i. test concentration, the no-observed-effect concentration was 73.2 ppm a.i.

TEST DATES: Hatch – April 12, 1999
Acclimation – April 12-22, 1999
Experimental Start –April 22, 1999
Experimental Termination – May 14, 1999

NOMINAL TEST CONCENTRATIONS: 0, 18.3, 36.6, 73.2, 146, 293, 586 and 1171 ppm a.i.

TEST ANIMALS: Northern Bobwhite (*Colinus virginianus*)

AGE TEST ANIMALS: 10 days of age at test initiation

SOURCE TEST ANIMALS: Wildlife International Ltd. Production Flock
8598 Commerce Drive
Easton, Maryland 21601

STUDY COMPLETION: April 26, 2000

INTRODUCTION

This study was conducted by Wildlife International Ltd. for 3M Corporation at the Wildlife International Ltd. avian toxicology facility in Easton, Maryland. The in-life portion of the test was conducted from April 22, 1999 to May 14, 1999. Raw data generated at Wildlife International Ltd. and a copy of the final report are filed under Project Number 454-103 in archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of this study was to evaluate the toxicity of a test substance to the Northern Bobwhite (*Colinus virginianus*) administered through the diet for five days. An LC50 value will be calculated, if possible.

MATERIALS AND METHODS

The methods used in conducting this study are based upon procedures specified in the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.2200 (1), Section 71-2 of the Environmental Protection Agency's Registration Guidelines, Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms (2); OECD Guideline 205, Guideline for Testing of Chemicals, Avian Dietary Toxicity Test (3); and upon ASTM Standard E857-87, "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species" (4).

Test Substance

The test substance was received from 3M Corporation on October 29, 1998 and was assigned Wildlife International Ltd. Identification Number 4675 upon receipt. The test substance was white powder identified as: FC-95; Lot No.:217. The reported purity of the test substance was 98.9%, with an expiration date of 2008. Following test termination, the test material was reanalyzed. The results of reanalysis indicate a test substance purity of 90.49%. All test concentrations have been adjusted to reflect the purity reported on the new Certificate of Analysis (Appendix I). The test substance was stored under ambient conditions.

The internal standard was received from 3M Corporation on July 2, 1998 and was assigned Wildlife International Ltd. identification number 4526 upon receipt. The internal standard, a granular

material, was identified as: 1H, 1H, 2H, 2H Perfluorooctane Sulfonic Acid, Chemical Abstract Number: 27619-97-2. The standard was stored under ambient conditions.

Treatment Groups

The test consisted of a geometric series of seven test concentrations and a control group. Thirty northern bobwhite chicks were assigned to the control group and ten northern bobwhite chicks were assigned to each of the treatment groups. The birds were sorted by weight, then chosen indiscriminately from within each represented weight class for placement into control and treatment groups. The birds were housed in brooding pens containing five chicks each. Nominal dietary concentrations used in this study were 0, 18.3, 36.6, 73.2, 146, 293, 586 and 1171 parts per million active ingredient (ppm a.i.) of PFOS. The dietary concentrations were established based upon known toxicity data and information supplied by the Sponsor.

Each group was fed the appropriate test or control diet for five days. During the exposure period the control group received untreated feed. Following the five-day exposure period all groups were given untreated basal diet for three days. On Day 8, half of the surviving treatment and control birds were euthanized and liver tissue, blood, and bile samples were collected for analysis. The remaining birds were fed basal ration until Day 22. On Day 22, these birds were euthanized and also sampled for liver weight, blood, and bile.

Duration of the Test

The primary phases of this test and their durations were:

1. Acclimation - 10 days.
2. Exposure - 5 days.
3. Post-exposure observation - 3 or 17 days

Test Birds

All northern bobwhite (*Colinus virginianus*) were 10 days of age and appeared to be in good health at initiation of the test. The birds were obtained from Wildlife International Ltd. Production Flock, Easton, MD and were hatched on April 12, 1999. Birds ranged in weight from 18 to 23 grams at test initiation. The birds used in this study were immature and could not be differentiated by sex.

All birds were from the same hatch, pen-reared and phenotypically indistinguishable from wild birds. All birds were acclimated to the caging and facilities from the day of hatch until initiation of the test.

Animal Diet

Throughout acclimation and testing all test birds were fed a game bird ration formulated to Wildlife International Ltd.'s specifications (Appendix II). The chicks were given a vitamin supplement in their water from the day they were hatched until the initiation of the test. Water from the town of Easton public water supply, and feed were provided *ad libitum* during acclimation and testing. The birds received no form of antibiotic medication during acclimation or testing.

Diet Preparation

The test substance was mixed directly into the ration. Mixing was done with a Hobart mixer (Model Number AS200T). All dietary test concentrations were adjusted to 100% PFOS based upon the reported purity of the test substance. All dietary concentrations and the LC50 value are reported as ppm a.i. in the diet. Nominal dietary test concentrations used in this study were 18.3, 36.6, 73.2, 146, 293, 586, 1171 ppm a.i. (Appendix IV).

Diet Sampling

Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the 18.3 ppm a.i. a.i. test diet and six samples from the 1171 ppm a.i. test diet at preparation on Day 0. Homogeneity samples were collected from the top, middle and bottom of the left and right sections of the mixing vessel. The homogeneity samples also served as verification samples. One verification sample was collected from the control diet and two verification samples were collected from each remaining treatment group at preparation on Day 0. At the end of the exposure period (Day 5), one sample was collected from the control and two samples were collected from each treatment group to determine stability of the test substance in the diet under test conditions. The stability samples were collected from feed remaining in the feeders after being at ambient test pen conditions for five days. Samples were transferred immediately to Wildlife International Ltd. analytical chemistry.

Analytical Method

The method used for the analysis of the avian diet samples was based upon methodology developed at Wildlife International Ltd. and entitled "Method Outline for the Determination of PFOS in Avian Feed".

Avian diet samples were extracted with methanol. Methanol was added to a requisite quantity of feed contained in a French-square glass bottle. Bottles were capped and shaken on a shaker table. Samples were vacuum filtered using qualitative filter paper. The retained feed was rinsed three times with methanol into the filtrate. The filtrate was transferred to a volumetric flask and brought to volume with methanol. As appropriate, samples were further diluted with methanol. Each sample then was diluted with a 50% methanol : 50% NANOpure® water solution containing 0.100 mg 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 flowchart is provided in Appendix III, Figure 1.

Concentrations of PFOS in the standards and samples were determined by reversed-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer API 100LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. HPLC separations were achieved using a Keystone Betasil C_{18} analytical column (100 mm x 2 mm I.D., 3 µm particle size). The instrument parameters are summarized in Appendix III, Table 1.

Calibration standards of PFOS prepared in a 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v), ranging in concentration from 0.00229 to 0.0457 mg a.i./L 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. A typical calibration curve is presented in Appendix III, Figure 2. The concentration of PFOS in the samples was determined by substituting the peak area response ratios into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards are presented in Appendix III, Figures 3 and 4, respectively.

The method limit of quantitation (LOQ) for these analyses was set at 1.15 ppm a.i. calculated as the product of the lowest calibration standard analyzed (0.00229 mg a.i./L) and the dilution factor of the matrix blank samples (500).

Two matrix blank samples were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during sample analyses (Appendix III, Table 2). An interference in the feed appeared at approximately the same retention time as the peak of interest but it was well below the LOQ. A representative chromatogram of a matrix blank is presented in Appendix III, Figure 5.

Avian diet was fortified at 4.57, 183 and 1830 ppm a.i. and analyzed concurrently with the samples to determine the mean procedural recovery (Appendix III, Table 3). Sample concentrations were not corrected for the mean procedural recovery of 94.7%. A representative chromatogram of a matrix fortification is presented in Appendix III, Figure 6.

An example calculation is presented for sample number 454-103-2, nominal concentration of 18.3 ppm a.i. in avian diet.

Initial Weight: 10.0 g
Final Volume: 200 mL
Dilution Factor: 100 (intermediate dilution factor × final dilution factor)
PFOS Peak Area: 113568
Internal Standard Peak Area: 413160
Peak Area Ratio: 0.2749

Calibration curve equation.
Slope: 2.77397
Intercept: 0.01894
Curve is weighted (1/x).

$$\text{PFOS (mg a.i./L) at instrument} = \frac{(\text{Peak area ratio} - (\text{Y-intercept})) \times \text{I.S. Concentration}}{\text{Slope}}$$

$$= \frac{(0.2749 - 0.01894) \times 0.100 \text{ mg/L}}{2.77397}$$

$$= 0.00923 \text{ mg a.i./L}$$

Note: I.S. = internal standard.

$$\text{PFOS (ppm a.i.) in sample} = \frac{\text{PFOS (mg a.i./L) at instrument} \times \text{Final Volume (L)} \times \text{Dilution Factor}}{\text{Initial Weight (Kg)}}$$

$$= \frac{0.00923 \times 0.200 \times 100}{0.01}$$

$$= 18.5 \text{ ppm a.i.}$$

$$\text{Percent of Nominal Concentration} = \frac{\text{PFOS (ppm a.i.) in sample}}{\text{PFOS (ppm a.i.) nominal}} \times 100$$

$$= \frac{18.5}{18.3} \times 100 = 101\%$$

Housing and Environmental Conditions

During acclimation and testing, all birds were housed indoors in batteries of thermostatically controlled brooding pens manufactured by Beacon Steel Products Co. (Model No. B735Q). Each pen had floor space that measured approximately 72 X 90 cm. Ceiling height was approximately 23 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting. Birds were sorted by weight, then chosen indiscriminately from within each represented weight class for assignment to pens. Each group of birds was identified by pen number and test concentration. Individual birds were identified by leg bands.

During the test the average temperature in the brooding compartment of the pens was 38°C ± 2°C (SD). Average ambient room temperature for this study was 27.3°C ± 1.2°C (SD) with an average relative humidity of 31% ± 14% (SD). The photoperiod (maintained by a time clock) was sixteen hours of light per day during acclimation and throughout the test. The light source was fluorescent lights which closely approximate noon-day sunlight. The birds were exposed to an average of

approximately 139 lux of illumination. Housing and husbandry practices were based on guidelines established by the National Research Council (5).

Observations

During acclimation all birds were observed daily. Birds exhibiting abnormal behavior or physical injury were not used. Following test initiation and continuing until termination, all birds were typically observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behavior.

Animal Body Weights/Feed Consumption

Individual body weights were measured at the initiation of the test, on Day 5, Day 8, and on Days 15 and Day 22 for all remaining birds. Average feed consumption values during the exposure period (Days 0-5) and the post-exposure observation period (Days 6-8) were determined by pen for each treatment group and the control group. Additionally, feed consumption was determined for Days 8-15 and 15-22 for the remaining treatment and control birds. Feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time. The accuracy of feed consumption values may have been affected by the unavoidable wastage of feed by the birds.

Gross Necropsy

All test birds that died during the course of the test and all birds remaining at the termination were subjected to a gross necropsy. Additionally, livers were weighed and liver tissue, blood, and bile were collected from birds euthanized on Day 8 and 22, and when possible from those that died during the course of the study.

Statistical Analyses

Mortality data were analyzed using the computer program of C.E. Stephan (6). The program was designed to calculate the LC50 value and the 95% confidence interval by probit analysis, moving average method or the binomial probability method (7,8,9). In this study, the LC50 value was determined using the probit method. The slope of the concentration-response curve and results of the goodness of fit test are reported. Body weight data were compared by Dunnett's test using TOXSTAT software (10,11). No statistical analyses were applied to feed consumption data.

RESULTS

Diet Analysis

Avian diet samples were collected from the 18.3 and 1171 ppm a.i. test concentrations and analyzed to evaluate homogeneity of the test substance in the avian diet. The analysis of these samples also served as verification of test substance concentrations. Resulting mean measured concentrations, standard deviations and coefficients of variation (CV) for these test concentrations were 19.5 ± 2.13 ppm a.i. (CV = 10.9%) and 1196 ± 70.2 ppm a.i. (CV = 5.87%), respectively (Appendix III, Table 4). Control avian diet samples collected during the test showed no interferences above the LOQ. Samples collected during the test to verify the 36.6, 73.2, 146, 293 and 586 ppm a.i. test substance concentrations had mean measured concentrations of 40.2, 74.5, 174, 291 and 537 ppm a.i., respectively. These values represented 110, 102, 119, 99.3 and 91.6% of the nominal concentrations, respectively (Appendix III, Table 5). Analysis of avian diet samples collected from feeders after being held at ambient temperature for five days averaged 101, 122, 104, 101, 109, 114 and 102% of the Day 0 values for the 18.3, 36.6, 73.2, 146, 293, 586 and 1171 ppm a.i. test substance concentrations, respectively (Appendix III, Table 6). A representative chromatogram of a test sample is shown in Appendix III, Figure 7.

Mortalities and Clinical Observations

One incidental mortality occurred in the control group during the course of the study (Table 1 and Appendix V). On the morning of Day 5, one bird was noted with a broken leg and was subsequently euthanized on Day 6. Additionally, two birds in the control group were intermittently noted with foot lesions associated with cage mate aggression. Otherwise, all control birds were normal in appearance and behavior throughout the test.

No treatment related mortalities or overt signs of toxicity were observed in the 18.3, 36.6, or 73.2 ppm a.i. treatment groups. One bird in the 18.3 ppm a.i. treatment group was noted as lame from Day 6 through Day 8, and blood of undetermined origin was noted on the underside of one bird from the 73.2 ppm a.i. treatment group on Day 22. Otherwise, all birds in the 18.3, 36.6 and 73.2 ppm a.i. treatment groups were normal in appearance and behavior throughout the test period.

There was 11% (1 of 9) mortality in the 146 ppm a.i. treatment group, 80% (8 of 10) mortality in the 293 ppm a.i. treatment group and 100% (10 of 10) mortality in the 586 and 1171 ppm a.i. treatment groups. In the 146 ppm a.i. treatment group, one bird was euthanized on Day 3 after sustaining a broken leg. This incidental mortality was not used in the calculation of the LC50 value. Additionally, there was one treatment-related mortality in the 146 ppm a.i. treatment group, a bird found dead on the morning of Day 7. Clinical signs of toxicity were observed in this treatment group on Day 5, when two birds displayed wing droop. All other birds at this test concentration were normal in appearance and behavior for the duration of the test.

In the 293 ppm a.i. treatment group there were eight treatment-related mortalities, occurring on Days 5, 6 and 7. Signs of toxicity were first observed on the morning of Day 4 and continued to be exhibited through the morning of Day 8 for the single bird euthanized on Day 8, and through the afternoon of Day 8 for the single bird surviving until Day 22. Signs of toxicity included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, wing droop, loss of coordination, lower limb weakness and convulsions. The single remaining bird appeared to have recovered and was normal in appearance and behavior from the afternoon of Day 9 until test termination.

In the 586 ppm a.i. treatment group mortalities were first noted on Day 3 and continued to be observed through Day 7, at which point all birds had died. Overt signs of toxicity were first observed on the afternoon of Day 2 and continued through the morning of Day 7, when the final birds were found dead. Signs of toxicity observed among birds in the 586 ppm a.i. treatment group included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, lower limb rigidity, prostrate posture, and convulsions.

In the 1171 ppm a.i. treatment group 100% mortality had occurred by the morning of Day 4. Signs of toxicity in the 1171 ppm a.i. treatment group were first observed on the afternoon of Day 2, with the first mortalities noted on the morning of Day 3. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, loss of coordination, wing droop, and lower limb weakness and rigidity.

Body Weight and Feed Consumption

When compared to the control group, there were no apparent treatment related effects upon body weight among birds in the 18.3, 36.6 or 73.2 ppm a.i. treatment groups. However, there was a concentration responsive reduction in body weight gain or body weight loss in the 146, 293 and 586 ppm a.i. treatment groups during the exposure period (Days 0-5) (Table 2 and Appendix VI). Differences from the control group were statistically significant at $p < 0.05$ for the 146 ppm a.i. level and at $p<0.01$ for the 293 and 586 ppm a.i. levels. A statistically significant ($p<0.01$) reduction in body weight gain continued to be observed at the 146 ppm a.i. test concentration through Day 8 of the study. At the 293 ppm a.i. concentration a statistically significant ($p<0.01$) mean weight loss continued through Day 8 of the study and a marked reduction in weight gain was noted through Day 15 of the study. Due to total mortality, body weight effects could not be determined for the 1171 ppm a.i. level during the exposure period or for the 586 and 1171 ppm a.i. treatment groups for post-exposure period.

There were no apparent treatment related effects upon feed consumption at the 18.3, 36.6, 73.2 or 146 ppm a.i. test concentrations (Table 3 and Appendix VII). However, a reduction in feed consumption was noted at the 293, 586 and 1171 ppm a.i. treatment groups during the exposure period (Days 0-5). There were no treatment-related effects on feed consumption in any of the surviving treatment groups during the Day 6-8 post-exposure period. In the 293 ppm a.i. treatment group only one bird survived to Day 22. The reduction in feed consumption observed at the 293 ppm a.i. test concentration during both the Day 8-15 and 15-22 post-exposure periods was the result of having only one bird in the pen, and was not considered to be treatment related.

Gross Necropsy

During the course of the test, all birds that died were subjected to a gross necropsy. Necropsy results for birds found dead were similar. Common observations included thin condition, loss of muscle mass, altered spleen color, autolysis of tissues and pale organs. Details of the necropsy findings are presented in Table 4.

Half of the surviving birds were subjected to gross necropsy on Day 8 and the remaining birds were necropsied on Day 22, following test termination. On Day 8, one bird in the 73.2 ppm a.i. treatment group was noted with a slightly pale liver. Due to the isolated nature of this finding, it was

not considered to be related to treatment. The single bird euthanized from the 293 ppm a.i. treatment group was observed at necropsy to have with a lack of muscle mass and general thinness. Since these findings correlated with an impact upon body weight noted at this concentration, the findings were considered to be treatment related. Necropsy results were unremarkable for all other birds euthanized on Day 8. Similarly, Day 22 necropsy findings were unremarkable for all birds.

CONCLUSION

The dietary LC50 value for northern bobwhite exposed to PFOS was determined to be 220 ppm a.i. with a 95% confidence interval of 164 ppm a.i. to 289 ppm a.i. The slope of the concentration-response curve was 7.005 and the chi-square value was 0.023. The no mortality concentration was 73.2 ppm a.i. Based upon treatment related mortality, signs of toxicity and effects upon body weight gain at the 146 ppm a.i. a.i test concentration, the no-observed-effect concentration was 73.2 ppm a.i..

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TABLE 1

Cumulative Mortality from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS

Experimental Group	No. Dead Per No. Exposed Exposure Period						No. Dead Per No. Exposed Post-Exposure Period		
(ppm a.i.)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8 [1]
Control									
0	0/30	0/30	0/30	0/30	0/30	0/30	1/30	1/30	1/30
Treatment									
18.3	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
36.6	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
73.2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
146	0/10	0/10	0/10	1/10[2]	0/9	0/9	0/9	1/9	1/9
293	0/10	0/10	0/10	0/10	0/10	2/10	4/10	8/10	8/10
586	0/10	0/10	0/10	1/10	2/10	5/10	8/10	10/10	10/10
1171	0/10	0/10	0/10	3/10	10/10	10/10	10/10	10/10	10/10

The LC50 value was calculated to be 220 ppm a.i. with a 95% confidence interval of 164 ppm a.i. to 289 ppm a.i..
[1] – No mortalities occurred in any of the control or treatment groups from Day 8 to Day 22.
[2] – Bird euthanized on day 3 after sustaining a broken leg.

TABLE 2
Page 1

Mean Body Weight (g) from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS

Experimental Group (ppm a.i.)		Exposure Period			Post-Exposure Period		
		Day 0	Day 5	Change[1] Day 0-5	Day 8	Change[1] Day 5-8	Total Change[1]
Control							
0	Mean	20	30	10	38	8	18
	SD	1	4	3	5	2	4
Treatment							
18.3	Mean	21	31	11	40	9	20
	SD	1	4	3	5	2	3
36.6	Mean	20	31	11	39	8	19
	SD	2	3	2	3	1	2
73.2	Mean	20	30	9	37	7	16
	SD	1	2	1	3	1	2
146	Mean	20	27*	7*	33*	6**	13**
	SD	2	3	3	3	2	4
293	Mean	20	18**	-2**	18**	-2**	-1**
	SD	1	2	2	4	4	5
586	Mean	20	16**	-4**	-	-	-
	SD	1	2	2	-	-	-
1171	Mean	20	-	-	-	-	-
	SD	1	-	-	-	-	-

[1]Mean change is calculated separately from the mean body weights using individual body weights (See Appendix VI).
(-) = No data available due to mortality.
*Statistically different from the control group at $p < 0.05$ (Dunnett's t-test).
**Statistically different from the control group at $p < 0.01$ (Dunnett's t-test).

TABLE 2
Page 2

Mean Body Weight (g) from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS

Experimental Group (ppm a.i.)		Post-Exposure Period					
		Day 8	Day 15	Change[1] Day 8-15	Day 22	Change[1] Day 15-22	Total Change[1](8-22)
Control							
0	Mean	37	59	23	82	22	45
	SD	6	10	5	13	3	8
Treatment							
18.3	Mean	40	68	24	87	23	47
	SD	6	8	3	7	3	3
36.6	Mean	38	65	26	89	24	50
	SD	3	5	2	7	2	4
73.2	Mean	35	60	24	79	20	44
	SD	3	4	2	4	2	2
146	Mean	34*	58	24	79	21	45
	SD	3	3	1	2	2	1
293	Mean[2]	21	35	14	55	20	34
	SD	-	-	-	-	-	-
586	Mean	-	-	-	-	-	-
	SD	-	-	-	-	-	-
1171	Mean	-	-	-	-	-	-
	SD	-	-	-	-	-	-

[1]Mean change is calculated separately from the mean body weights using individual body weights (See Appendix VI).
[2]n=1, could not be evaluated statistically with Dunnett's t-test.
(-) = No data available due to mortality.
*Statistically different from the control group at $p < 0.05$)(Dunnett's t-test).

TABLE 3
Page 1

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

Experimental Group (ppm a.i.)		Exposure Period Day 0-5	Post-Exposure Period Day 6-8
Control			
0	Mean	9	10
	SD	2	2
Treatment			
18.3		9	11
36.6		8	12
73.2		10	13
146		9	10
293		5	9
586		6	19
1171		4	-

(-) = No data available due to mortality.

TABLE 3
Page 2

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

Experimental Group (ppm a.i.)		Post-Exposure Period	
		Day 8-15	Day 15-22
Control			
0	Mean	9	13
	SD	2	1
Treatment			
18.3		10	12
36.6		14	15
73.2		13	15
146		11	14
293		8	9
586		-	-
1171		-	-

(-) = No data available due to mortality.

TABLE 4
Group Gross Pathological Observations
from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS

Birds that died during the course of the study

Finding	Control	Male, Female, and Undetermined PPM A.I.			
		146	293	586	1171
	N=1	N =2	N =8	N =10	N =10
Abdominal cavity, some autolysis	0	0	2	2	4
Abdominal cavity, autolysis throughout	0	0	0	1	1
Crop, empty	0	0	2	5	2
Emaciated	0	0	2	5	8
Fractured leg	1	1	0	0	0
G.I. tract empty	0	0	1	1	0
Gizzard contents bile stained	0	0	2	5	1
Heart, anterior portion mottled white color	0	0	1	0	0
Heart, pale	0	0	0	2	1
Intestinal contents tar-like	0	0	0	2	0
Keel, prominent	0	0	1	3	10
Kidneys, pale	0	0	0	2	0
Liver, pale and mottled	0	1	0	0	0
Loss of muscle mass	0	0	4	7	9
Muscular-skeletal, pale	0	1	0	0	0
Small in stature	0	0	3	0	0
Spleen, black	0	0	0	1	0
Spleen, dark	0	0	0	0	2
Spleen, grey	0	0	0	1	0
Spleen, grey-brown	0	0	0	0	1
Spleen, pale	0	0	1	0	1
Spleen, small	0	0	0	0	1
Spleen, small and pale	0	0	0	3	0
Thin	0	0	0	4	2
Not Remarkable	0	0	1	0	0

Table 5
Cumulative Mortality (Estimated Cumulative Dose, mg/kg[1]) from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS

Experimental Group (ppm a.i.)	Pen	No. Dead Per No. Exposed (Cumulative Dose, mg/kg) Exposure Period						No. Dead Per No. Exposed Post-Exposure Period		
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8[2]
Control										
0	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0	3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0	4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0	5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	1/5
0	6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Treatment										
18.3	1	0/5	0/5 (7)	0/5 (14)	0/5 (21)	0/5 (28)	0/5 (35)	0/5	0/5	0/5
18.3	2	0/5	0/5 (6)	0/5 (12)	0/5 (18)	0/5 (24)	0/5 (30)	0/5	0/5	0/5
36.6	1	0/5	0/5 (13)	0/5 (26)	0/5 (39)	0/5 (52)	0/5 (65)	0/5	0/5	0/5
36.6	2	0/5	0/5 (11)	0/5 (22)	0/5 (33)	0/5 (44)	0/5 (55)	0/5	0/5	0/5
73.2	1	0/5	0/5 (33)	0/5 (66)	0/5 (99)	0/5 (132)	0/5 (165)	0/5	0/5	0/5
73.2	2	0/5	0/5 (32)	0/5 (64)	0/5 (96)	0/5 (128)	0/5 (160)	0/5	0/5	0/5
146	1	0/5	0/5 (46)	0/5 (92)	0/5 (138)	0/5 (184)	0/5 (230)	0/5	1/5	1/5
146	2	0/5	0/5 (49)	0/5 (98)	1/5 (147)	1/5 (196)	1/5 (245)	1/5	1/5	1/5
293	1	0/5	0/5 (66)	0/5 (132)	0/5 (198)	0/5 (264)	1/5 (330)	1/5	4/5	4/5
293	2	0/5	0/5 (101)	0/5 (202)	0/5 (303)	0/5 (404)	1/5 (505)	3/5	4/5	4/5
586	1	0/5	0/5 (213)	0/5 (429)	1/5 (639)	2/5 (852)	2/5 (1065)	4/5	5/5	5/5
586	2	0/5	0/5 (178)	0/5 (356)	0/5 (534)	0/5 (712)	3/5 (890)	4/5	5/5	5/5
1171	1	0/5	0/5 (256)	0/5 (512)	3/5 (768)	5/5	5/5	5/5	5/5	5/5
1171	2	0/5	0/5 (256)	0/5 (512)	0/5 (768)	5/5	5/5	5/5	5/5	5/5

The LC50 value was calculated to be approximately 220 ppm a.i. with a 95% confidence interval of 164 to 289 ppm a.i..

*- Bird was euthanized due to a broken leg.

[1]- Estimated cumulative dose is based upon the average body weight and feed consumption over the 5-day exposure period, and serves as a rough approximaton of the actual amount of test substance consumed.

[2]- No mortalities occurred in any of the treatment or control groups after Day 8.

APPENDIX I

Certificate Of Analysis

FC-95, Lot 217

March 9, 2000

Richard M. Payfer

This sample was analyzed using LC/MS, ^{1}H-NMR, ^{19}F-NMR, and elemental analyses techniques. The results of these tests show the sample to contain the following weight percent composition:

$C_2F_5SO_3^-K^+$	0.04 %
$C_3F_7SO_3^-K^+$	0.83 %
$C_4F_9SO_3^-K^+$	1.38 %
$C_5F_{11}SO_3^-K^+$	1.30 %
$C_6F_{13}SO_3^-K^+$	3.71 %
$C_7F_{15}SO_3^-K^+$	1.19 %
$C_8F_{17}SO_3^-K^+$	90.49 %
$C_9F_{19}SO_3^-K^+$	0.49 %
$C_{10}F_{21}SO_3^-K^+$	0.13 %
$C_{11}F_{23}SO_3^-K^+$	0.04 %
CF_3-$CO_2^-K^+$	0.05 %
$SF_5C_6F_{16}SO_3^-K^+$	0.35 %

Additionally, the isomer distribution of the sample was determined using ^{19}F-NMR techniques and found to contain the following mole percent composition:

$CF_3(CF_2)_x$-$SO_3^-K^+$ (Normal chain, where x is mainly 7)	70.5%
$CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$- $SO_3^-K^+$ (Internal monomethyl branch, where x+y is mainly 5, and $x \neq 0$, $y \neq 0$)	17.1%
$(CF_3)_2CF$-$(CF_2)_x$- $SO_3^-K^+$ (Isopropyl branch, where x is mainly 5)	10.3%
C_nF_{2n+1}-$CF(CF_3)$- $SO_3^-K^+$ (Alpha branch, where x is mainly 6)	1.6%
$(CF_3)_3C$-$(CF_2)_x$- $SO_3^-K^+$ (t-butyl branch, where x is mainly 4)	0.2%
CF_3-$(CF_2)_x$-$C(CF_3)_2$-$(CF_2)_y$- $SO_3^-K^+$ (Internal gem-dimethyl branch, where x+y is mainly 4, and $x \neq 0$)	0.2%

APPENDIX II

DIET FORMULATION
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	AMOUNT ADDED PER TON
Vitamin D_3	2,000,000 I.C.U.
Vitamin A	7,000,000 I.U.
Riboflavin	6 grams
Niacin	40 grams
Pantothenic Acid	10 grams
Vitamin B_{12}	8 mgs
Folic Acid	600 mgs
Biotin	64 mgs
Pyridoxine	1.2 grams
Thiamine	1.2 grams
Vitamin E	20,000 I.U.
Vitamin K (Menadione Dimethylpyrimidinol Bisulfite)	5.8 grams
Manganese	102 grams
Zinc	47 grams
Copper	6.8 grams
Iodine	1.5 grams
Iron	51 grams
Selenium	182 mgs

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

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

APPENDIX III

ANALYTICAL METHODS AND RESULTS

APPENDIX III

Table 1

Typical LC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer 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 (100 mm x 2 mm I.D., 3 µm particle size)
OVEN TEMPERATURE:	30°C
STOP TIME:	10.0 minutes
FLOW RATE:	0.220 mL/minute
MOBILE PHASE:	72.0% Methanol : 28.0% NANOpure® Water containing 0.1% Formic Acid
INJECTION VOLUME:	25.0 µL
PFOS RETENTION TIME:	Approximately 7.0 minutes
INTERNAL STANDARD RETENTION TIME:	Approximately 4.8 minutes
PFOS MONITORED MASS:	498.6 amu
INTERNAL STANDARD MONITORED MASS:	426.7 amu

APPENDIX III

Table 2

Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of PFOS[1] (ppm a.i.)
Number (454-103-)	Type	
MAB-1	Matrix Blank	< LOQ
MAB-2	Matrix Blank	< LOQ

[1] The limit of quantitation (LOQ) was 1.15 ppm a.i. based upon the product of the lowest calibration standard analyzed (0.00229 mg a.i./L) and the dilution factor of the matrix blank samples (500).

APPENDIX III

Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (454-103-)	Concentrations of PFOS (ppm a.i.)		Percent Recovered
	Fortified	Measured	
MAS-1A	4.57	4.54	99.2
MAS-4A	4.57	4.79	105
MAS-2	183	176	96.1
MAS-5	183	162	88.3
MAS-3	1830	1576	86.1
MAS-6	1830	1716	93.7
		Mean =	94.7
		Standard Deviation =	6.99
		CV =	7.38
		N =	6

Note: Results and corrections for new test substance purity were generated using MacQuan version 1.5 software and manual calculations. Values have been rounded for reporting purposes.

APPENDIX III

Table 4

Homogeneity of PFOS in Avian Diet

Nominal Concentration (ppm a.i.)	Sample Number (S-454-103-)	Location Sampled in Mixing Vessel	PFOS Measured Concentration (ppm a.i.)	Mean Measured Concentration ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)[1]	Mean Percent of Nominal
18.3	2	Top Left	18.5		
	3	Top Right	23.4	$\bar{x}$ = 19.5 ppm a.i.	
	4	Middle Left	18.3	SD = 2.13 ppm a.i.	107
	5	Middle Right	17.3	CV = 10.9%	
	6	Bottom Left	19.4		
	7	Bottom Right	19.9		
1171	18	Top Left	1239		
	19	Top Right	1221	$\bar{x}$ = 1196 ppm a.i	
	20	Middle Left	1118	SD = 70.2 ppm. a.i	102
	21	Middle Right	1301	CV = 5.87%	
	22	Bottom Left	1163		
	23	Bottom Right	1133		

Note: Results and corrections for new test substance purity were generated using MacQuan version 1.5 software and manual calculations. Values have been rounded for reporting purposes.

[1]Coefficient of variation was calculated using full precision of mean and standard deviation results.

APPENDIX III

Table 5

Verification of PFOS Concentrations in Avian Diet

Nominal Concentration (ppm a.i.)	Sample Number (S-454-103-)	Sampling Interval (Day)	PFOS Measured Concentration[1] (ppm a.i.)	Percent of Nominal	Mean Measured Concentration (ppm a.i.)	Mean Percent of Nominal
0.0	1	0	< LOQ	–	–	–
18.3	–	–	–	–	19.5[2]	107[2]
36.6	8	0	45.7	125	40.2	110
	9	0	34.6	94.5		
73.2	10	0	77.8	106	74.5	102
	11	0	71.2	97.3		
146	12	0	176	120	174	119
	13	0	172	117		
293	14	0	274	93.8	291	99.3
	15	0	307	105		
586	16	0	550	93.9	537	91.6
	17	0	523	89.4		
1171	–	–	–	–	1196[2]	102[2]

Note: Results and corrections for new test substance purity were generated using MacQuan version 1.5 software and manual calculations. Values have been rounded for reporting purposes.

[1]The limit of quantitation (LOQ) was 1.15 ppm a.i. based upon the product of the lowest calibration standard analyzed (0.00229 mg a.i./L) and the dilution factor of the matrix blank samples (500).

[2]Result obtained from Table 4.

APPENDIX III

Table 6

Ambient Stability of PFOS in Avian Diet During the Northern Bobwhite LC50 Study

Nominal Concentration (ppm a.i.)	Day 0[1]			Day 5			
	Sample Number (S-454-103-)	Mean Measured Concentration (ppm a.i.)	Mean Percent of Nominal	Sample Number (S-454-103-)	Measured Concentration[2] (ppm a.i.)	Mean Measured Concentration (ppm a.i.)	Mean Percent of Day 0
0	1	–	–	24	< LOQ	–	–
18.3	2-7	19.5	107	25 26	19.2 19.9	19.6	101
36.6	8, 9	40.2	110	27 28	44.4 53.8	49.1	122
73.2	10, 11	74.5	102	29 30	76.4 77.9	77.2	104
146	12, 13	174	119	31 32	177 174	176	101
293	14, 15	291	99.3	33 34	318 315	317	109
586	16, 17	537	91.6	35 36	560 665	613	114
1171	18-23	1196	102	37 38	1260 1187	1224	102

[1]Day 0 results obtained from Table 4 and Table 5.
[2]The limit of quantitation (LOQ) was 1.15 ppm a.i. based upon the product of the lowest calibration standard analyzed (0.00229 mg a.i./L) and the dilution factor of the matrix blank samples (500).

APPENDIX III

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.

↓

Dry Mix Technique

For the high-level matrix fortification sample, weigh the requisite quantity of Wildlife International Ltd. (WIL) ration into a weigh boat.

↓

Weigh the requisite quantity of test substance (PFOS) into a beaker.

↓

Add ½ of the WIL ration and the test substance to a larger beaker.

↓

Rinse the beaker that contained the PFOS with small portions of the remaining ration and transfer all portions to the larger beaker.

↓

Mix the contents of the larger beaker well and transfer the mixture to a Waring blender.

↓

Blend the mixture for ~5 minutes stopping at 1 minute intervals to scrape down the sides of the blender. During the third interval transfer the fortified feed to a beaker, mix well and return the mixture to the blender to complete mixing in the specified time.

↓

Prepare the next two matrix fortification levels by serial dilutions. Follow the same procedure described for the high-level matrix fortification except weigh the appropriate quantity of fortified matrix (high or mid-level) rather than the test substance.

↓

Weigh 10-g samples of the matrix blank, matrix fortification and test samples into weigh boats and transfer to 16-oz. French-square glass bottles. Record the weights.

For each sample, measure 100 mLs of methanol with a graduated cylinder and transfer volume to the French-square bottle.

↓

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

↓

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

↓

Transfer the filtrate to a 200-mL volumetric flask and bring the flask 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 solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v).

Ampulate and submit samples for LC/MS analysis.

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

APPENDIX III

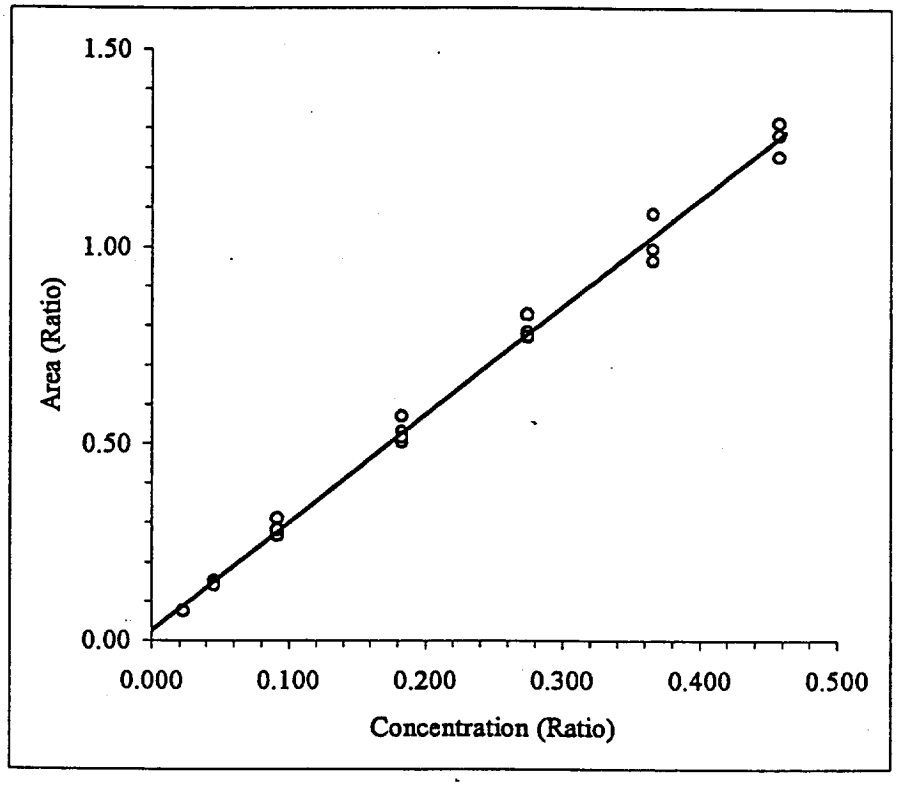


Figure 2. A typical calibration curve for PFOS. Slope = 2.77397; Intercept = 0.01894; r = 0.9981. Curve is weighted (1/x).

APPENDIX III

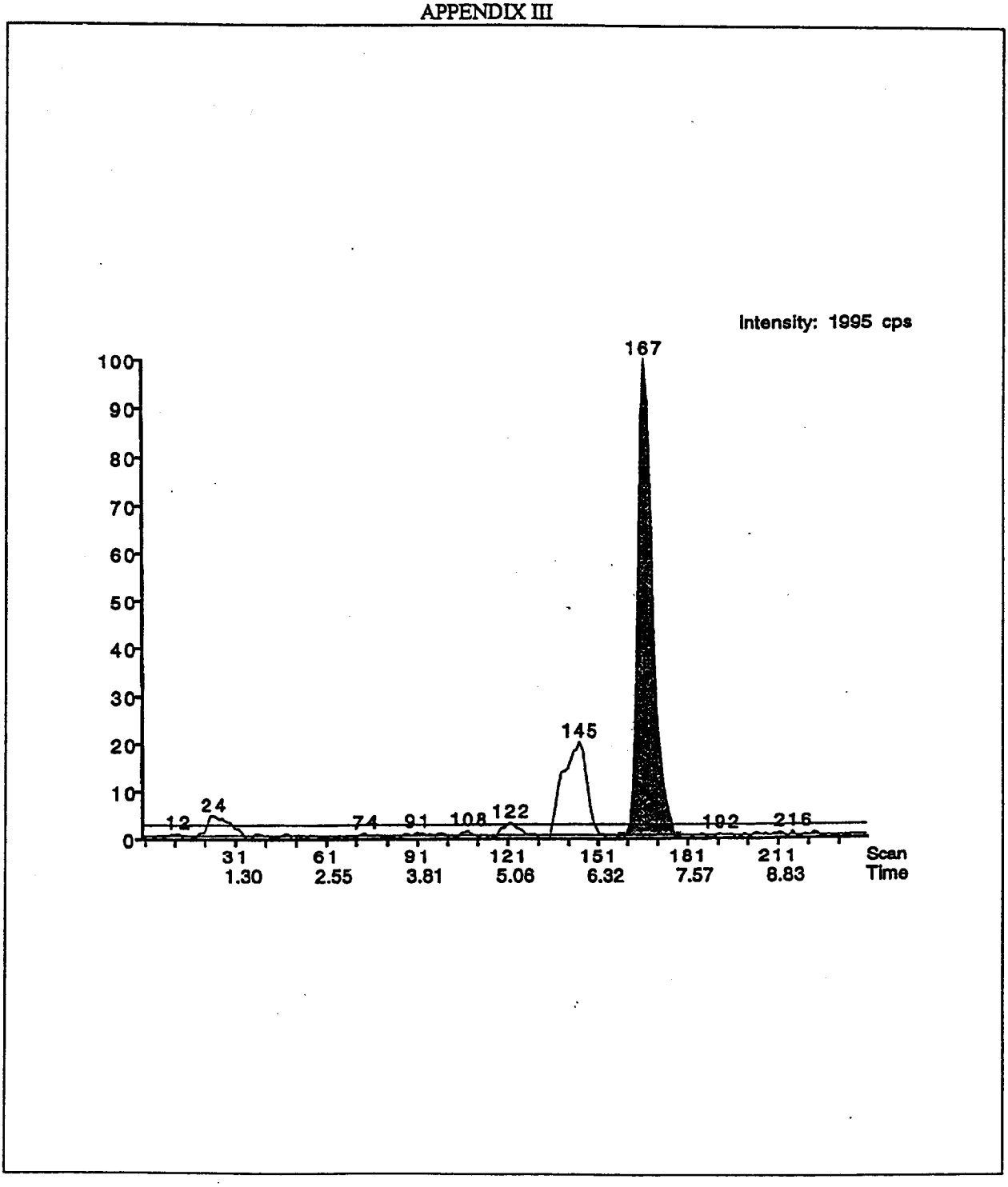


Figure 3. A representative ion chromatogram of a low-level (0.00229 mg a.i./L) PFOS standard.

APPENDIX III

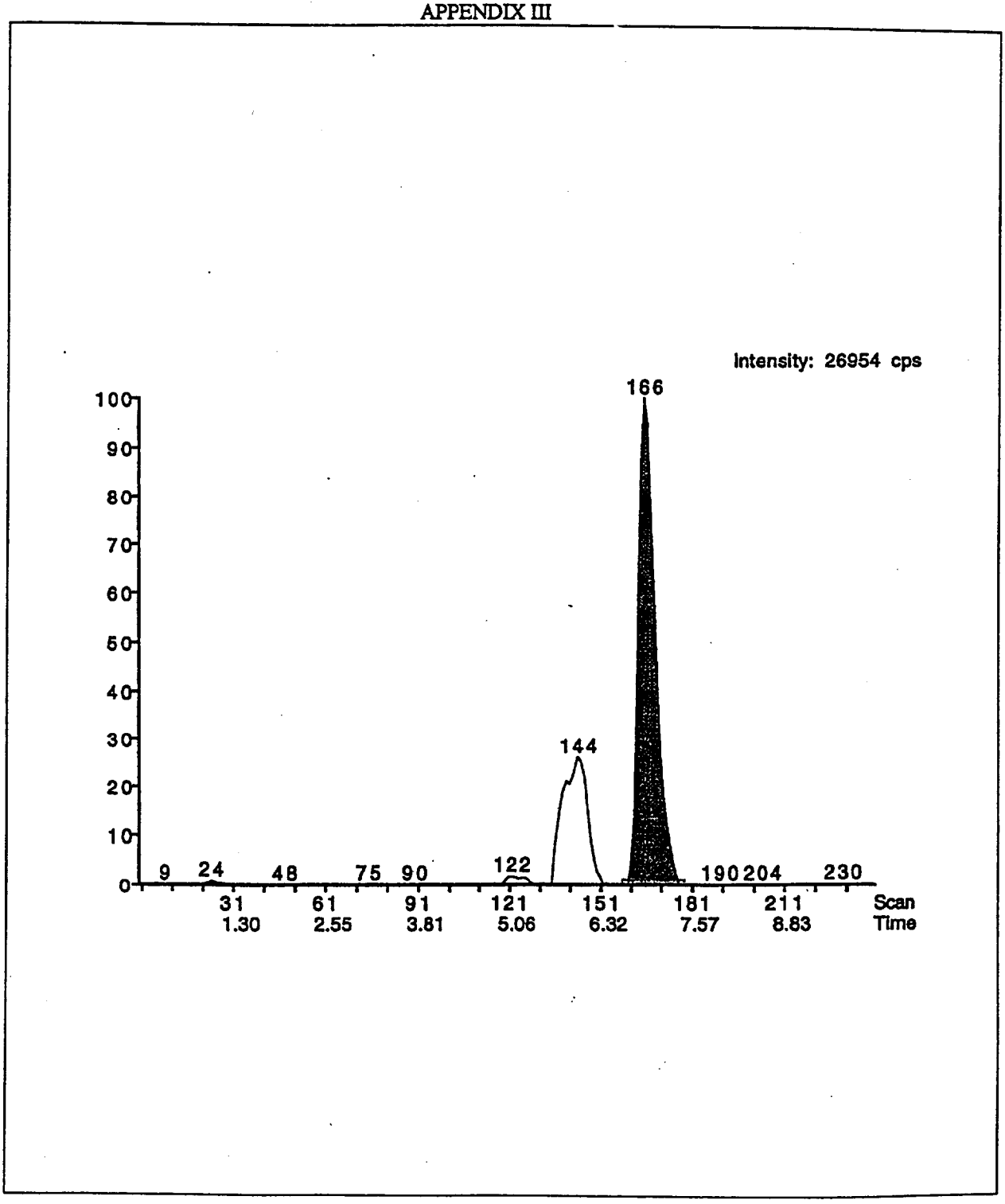


Figure 4. A representative ion chromatogram of a high-level (0.0457 mg a.i./L) PFOS standard.

APPENDIX III

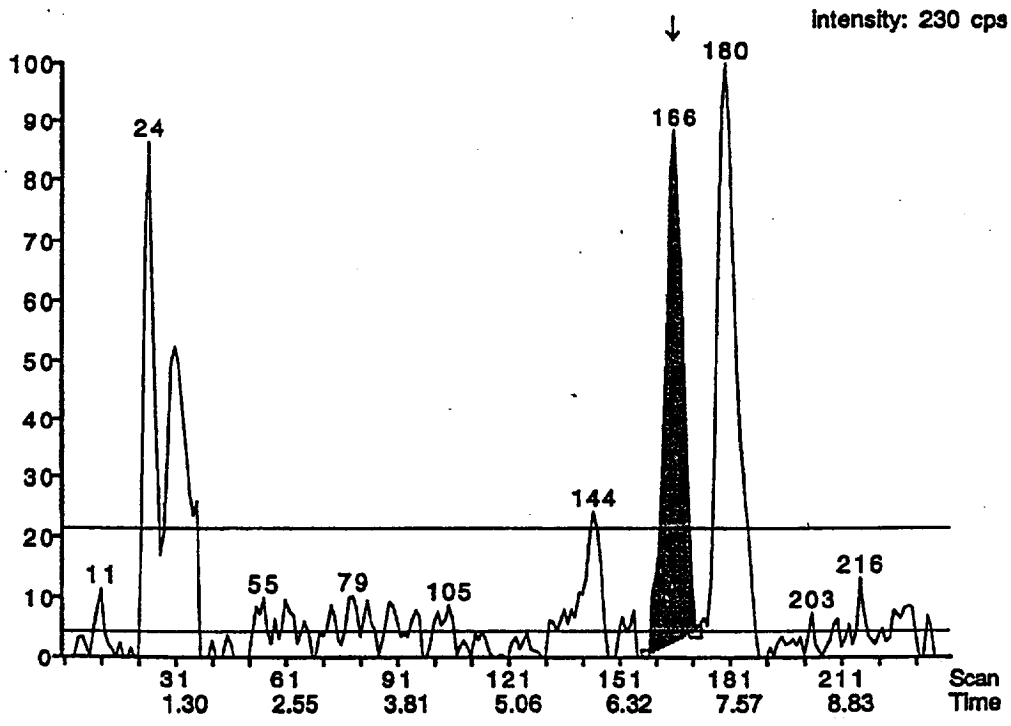


Figure 5. A representative chromatogram of a matrix blank sample (454-103-MAB-1). The arrow indicates the retention time of PFOS.

APPENDIX III

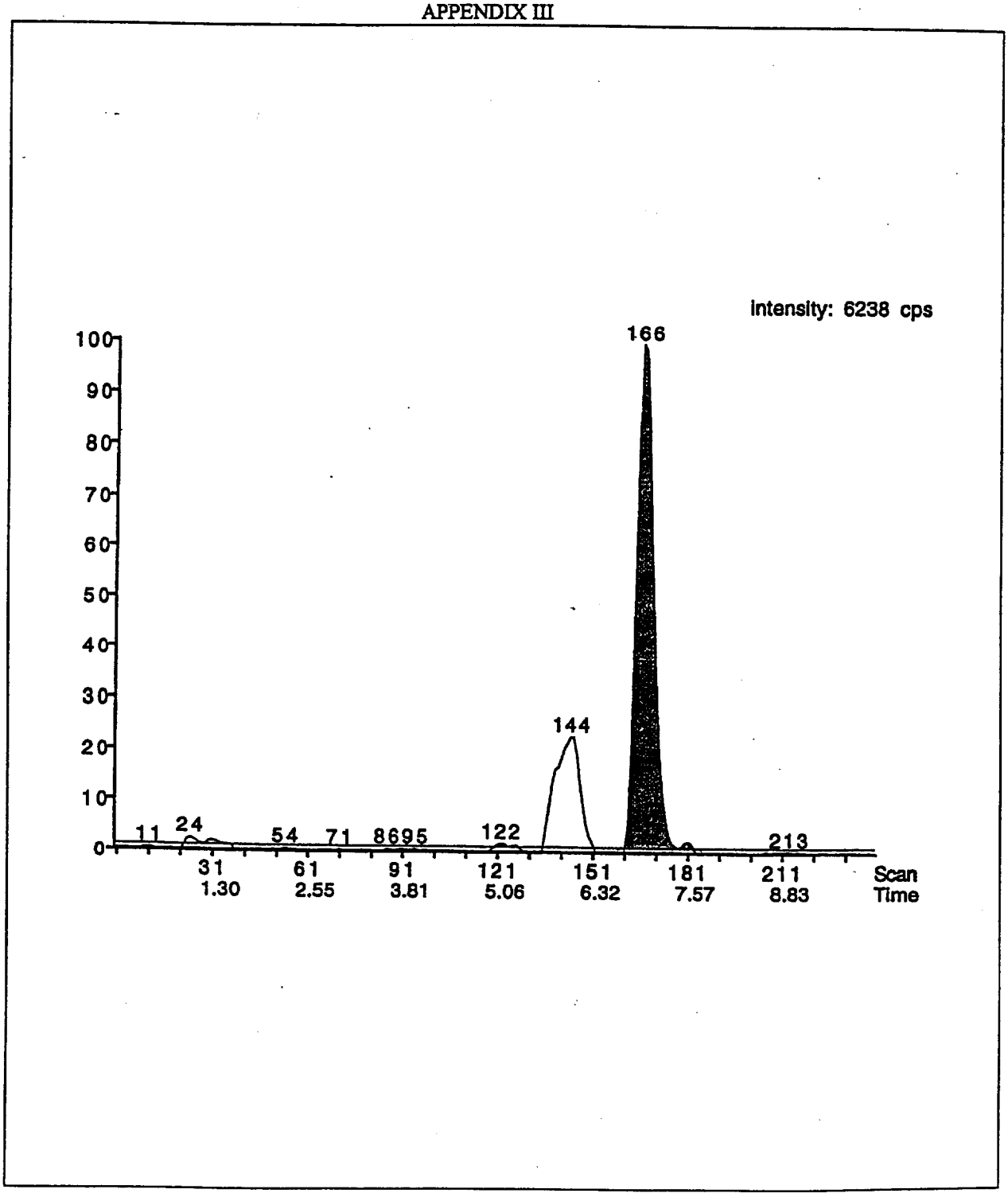


Figure 6. A representative chromatogram of a matrix fortification sample (454-103-MAS-1A).

APPENDIX III

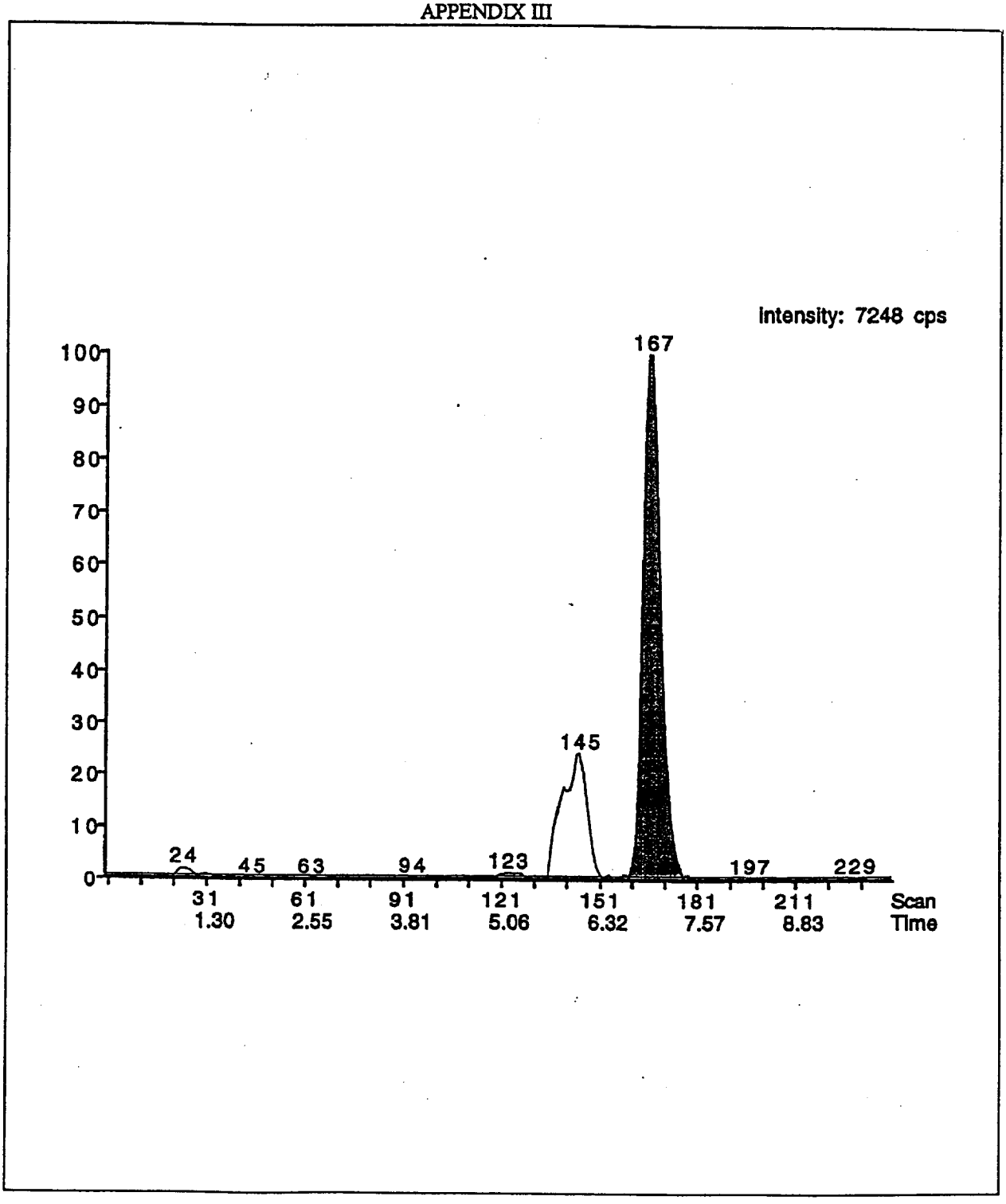


Figure 7. A representative chromatogram of a test sample (454-103-2).

APPENDIX IV
DIET PREPARATION

Weight and volume of constituents used to prepare test diets:

Nominal Concentrations (ppm a.i.)	Test Substance (g)	Basal Ration (g)
0	-	9000.0
18.3	0.1818	8999.8
36.6	0.3638	8999.6
73.2	0.7282	8999.3
146	1.4659	8998.5
293	2.9123.	8997.1
586	5.8239	8994.2
1171	11.6483	8988.4

Diets were prepared as follows:

•5000.0 g of basal ration was weighed into a tared Hobart mixing bowl.

•The test substance was weighed in a tared weigh boat.

•Approximately 100 g of basal ration was taken from the mixing bowl and placed in a Waring blender.

•The test substance was added to the blender and the weigh boat was rinsed with additional ration, with the rinse also being placed in the blender.

•The blender contents were blended for approximately 60 seconds and transferred to the mixing bowl. The blender was rinsed with additional ration, with the rinse also being placed in the mixing bowl.

•The bowl was placed on a Hobart mixer and the contents were mixed for approximately six minutes. The remaining ration as added to the bowl and the contents were mixed for six more minutes.

•The diet was transferred to a labelled paper feed bag.

APPENDIX V
Cumulative Mortality by Pen from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS

Experimental Group (ppm a.i.)	Pen	No. Dead Per No. Exposed Exposure Period						No. Dead Per No. Exposed Post-Exposure Period		
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8[1]
Control										
0	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0	3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0	4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0	5	0/5	0/5	0/5	0/5	0/5	0/5	1/5*	1/5	1/5
0	6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Treatment										
18.3	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
18.3	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
36.6	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
36.6	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
73.2	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
73.2	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
146	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5
146	2	0/5	0/5	0/5	1/5*	1/5	1/5	1/5	1/5	1/5
293	1	0/5	0/5	0/5	0/5	0/5	1/5	1/5	4/5	4/5
293	2	0/5	0/5	0/5	0/5	0/5	1/5	3/5	4/5	4/5
586	1	0/5	0/5	0/5	1/5	2/5	2/5	4/5	5/5	5/5
586	2	0/5	0/5	0/5	0/5	0/5	3/5	4/5	5/5	5/5
1171	1	0/5	0/5	0/5	3/5	5/5	5/5	5/5	5/5	5/5
1171	2	0/5	0/5	0/5	0/5	5/5	5/5	5/5	5/5	5/5

The LC50 value was calculated to be 220 ppm a.i. with a 95% confidence interval of 164 ppm a.i. to 289 ppm a.i.
* - Bird was euthanized due to a broken leg.
1 – No mortalities occurred in any of the control or treatment groups after Day 8.

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 1

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
0	1	19	24	5	27	3	8
	2	20	27	7	34	7	14
	3	21	32	11	42	10	21
	4	22	32	10	40	8	18
	5	23	39	16	50	11	27
	Mean	21	31	10	39	8	18
	SD	2	6	4	9	3	7
0	1	19	26	7	35	9	16
	2	20	25	5	35	10	15
	3	20	29	9	37	8	17
	4	22	33	11	43	10	21
	5	22	31	9	39	8	17
	Mean	21	29	8	38	9	17
	SD	1	3	2	3	1	2
0	1	19	23	4	30	7	11
	2	20	26	6	29	3	9
	3	20	29	9	32	3	12
	4	22	30	8	37	7	15
	5	22	35	13	40	5	18
	Mean	21	29	8	34	5	13
	SD	1	5	3	5	2	4
0	1	19	28	9	37	9	18
	2	19	27	8	35	8	16
	3	19	30	11	36	6	17
	4	21	32	11	42	10	21
	5	22	33	11	43	10	21
	Mean	20	30	10	39	9	19
	SD	1	3	1	4	2	2

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 2

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
0	1	19	28	9	35	7	16
	2	20	27	7	36	9	16
	3	21	32	11	44	12	23
	4	20	30	10	-	-	-
	5	22	34	12	44	10	22
	Mean	20	30	10	40	10	19
	SD	1	3	2	5	2	4
0	1	18	30	12	39	9	21
	2	21	35	14	46	11	25
	3	19	29	10	37	8	18
	4	21	32	11	41	9	20
	5	22	32	10	42	10	20
	Mean	20	32	11	41	9	21
	SD	2	2	2	3	1	3
Group Total	Mean	20	30	10	38	8	18
	SD	1	4	3	5	2	4

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 3

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
18.3	1	19	26	7	34	8	15
	2	19	33	14	39	6	20
	3	21	27	6	35	8	14
	4	22	34	12	46	12	24
	5	22	36	14	46	10	24
	Mean	21	31	11	40	9	19
	SD	2	4	4	6	2	5
18.3	1	19	28	9	38	10	19
	2	19	28	9	37	9	18
	3	20	29	9	38	9	18
	4	22	34	12	44	10	22
	5	22	35	13	44	9	22
	Mean	20	31	10	40	9	20
	SD	2	3	2	3	1	2
Group Total	Mean	21	31	11	40	9	20
	SD	1	4	3	5	2	3

APPENDIX VI
Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 4

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
36.6	1	18	26	8	35	9	17
	2	19	30	11	39	9	20
	3	19	29	10	37	8	18
	4	21	30	9	38	8	17
	5	21	34	13	43	9	22
	Mean	20	30	10	38	9	19
	SD	1	3	2	3	1	2
36.6	1	19	29	10	37	8	18
	2	19	29	10	37	8	18
	3	21	32	11	40	8	19
	4	22	35	13	44	9	22
	5	23	36	13	44	8	21
	Mean	21	32	11	40	8	20
	SD	2	3	2	4	0	2
Group Total	Mean	20	31	11	39	8	19
	SD	2	3	2	3	1	2

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 5

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
73.2	1	18	26	8	32	6	14
	2	19	26	7	33	7	14
	3	20	29	9	36	7	16
	4	21	32	11	39	7	18
	5	21	30	9	36	6	15
	Mean	20	29	9	35	7	15
	SD	1	3	1	3	1	2
73.2	1	18	27	9	34	7	16
	2	20	31	11	38	7	18
	3	21	30	9	37	7	16
	4	21	32	11	39	7	18
	5	22	32	10	41	9	19
	Mean	20	30	10	38	7	17
	SD	2	2	1	3	1	1
Group Total	Mean	20	30	9	37	7	16
	SD	1	2	1	3	1	2

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 6

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
146	1	19	28	9	35	7	16
	2	19	28	9	32	4	13
	3	20	24	4	-	-	-
	4	22	33	11	38	5	16
	5	23	28	5	32	4	9
	Mean	21	28	8	34	5	14
	SD	2	3	3	3	1	3
146	1	18	24	6	31	7	13
	2	19	27	8	35	8	16
	3	19	27	8	33	6	14
	4	21	-	-	-	-	-
	5	23	24	1	28	4	5
	Mean	20	26	6	32	6	12
	SD	2	2	3	3	2	5
Group Total	Mean	20	27*	7*	33*	6**	13**
	SD	2	3	3	3	2	4

(-) = No data available due to mortality.
*Statistically different from the control group at $p < 0.05$ (Dunnett's t-test).
**Statistically different from the control group at $p < 0.01$ (Dunnett's t-test).

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 7

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
293	1	18	20	2	21	1	3
	2	19	17	-2	-	-	-
	3	20	18	-2	-	-	-
	4	22	-	-	-	-	-
	5	22	20	-2	-	-	-
	Mean	20		-1	21	1	3
	SD	2		2	-	-	-
293	1	19	19	0	15	-4	-4
	2	19	-	-	-	-	-
	3	20	20	0	-	-	-
	4	20	17	-3	-	-	-
	5	21	16	-5	-	-	-
	Mean	20	18	-2	15	-4	-4
	SD	1	2	2	-	-	-
Group Total	Mean	20	18**	-2**	18**	-2**	-1**
	SD	1	2	2	4	4	5

(-) = No data available due to mortality.
*Statistically different from the control group at $p < 0.05$ (Dunnett's t-test).
**Statistically different from the control group at $p < 0.01$ (Dunnett's t-test).

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 8

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
586	1	18	-	-	-	-	-
	2	20	13	-7	-	-	-
	3	21	18	-3	-	-	-
	4	21	-	-	-	-	-
	5	22	17	-5	-	-	-
	Mean	20	16	-5	-	-	-
	SD	2	3	2	-	-	-
586	1	19	16	-3	-	-	-
	2	20	-	-	-	-	-
	3	20	16	-4	-	-	-
	4	19	-	-	-	-	-
	5	21	-	-	-	-	-
	Mean	20	16	-4	-	-	-
	SD	1	0	1	-	-	-
Group Total	Mean	20	16**	-4**	-	-	-
	SD	1	2	2	-	-	-

(-) = No data available due to mortality.
**Statistically different from the control group at $p < 0.01$ (Dunnett's t-test).

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 9

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
1171	1	18	-	-	-	-	-
	2	20	-	-	-	-	-
	3	20	-	-	-	-	-
	4	21	-	-	-	-	-
	5	21	-	-	-	-	-
	Mean	20	-	-	-	-	-
	SD	1	-	-	-	-	-
1171	1	18	-	-	-	-	-
	2	20	-	-	-	-	-
	3	20	-	-	-	-	-
	4	22	-	-	-	-	-
	5	21	-	-	-	-	-
	Mean	20	-	-	-	-	-
	SD	1	-	-	-	-	-
Group Total	Mean	20	-	-	-	-	-
	SD	1	-	-	-	-	-

(-) = No data available due to mortality.

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 10

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change Day 15-22	Total Change
0	1	27	42	15	61	19	34
	2	34	56	22	77	21	43
	3	42	69	27	92	23	50
	4	40	63	23	84	21	44
	5	50	78	28	104	26	54
	Mean	39	62	23	84	22	45
	SD	9	14	5	16	3	8
0	1	35	55	20	74	19	39
	2	35	53	18	74	21	39
	3	37	64	27	85	21	48
	4	43	74	31	103	29	60
	5	39	59	20	80	21	41
	Mean	38	61	23	83	22	45
	SD	3	8	6	12	4	9
0	1	30	54	24	79	25	49
	2	29	45	16	63	18	34
	3	32	54	22	77	23	45
	4	37	55	18	75	20	38
	5	40	68	28	95	27	55
	Mean	34	55	22	78	23	44
	SD	5	8	5	11	4	8
Group Total	Mean	37	59	23	82	22	45
	SD	6	10	5	13	3	8

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 11

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change Day 15-22	Total Change
18.3	1	34	53	19	78	25	44
	2	39	65	26	85	20	46
	3	35	60	25	86	26	51
	4	46	73	27	95	22	49
	5	46	71	25	92	21	46
	Mean	40	64	24	87	23	47
	SD	6	8	3	7	3	3

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change Day 15-22	Total Change
36.6	1	35	60	25	81	21	46
	2	39	65	26	91	26	52
	3	37	65	28	91	26	54
	4	38	61	23	83	22	45
	5	43	72	29	97	25	54
	Mean	38	65	26	89	24	50
	SD	3	5	2	7	2	4

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 12

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change Day 15-22	Total Change
73.2	1	32	54	22	76	22	44
	2	33	58	25	77	19	44
	3	36	62	26	79	17	43
	4	39	65	26	87	22	48
	5	36	59	23	78	19	42
	Mean	35	60	24	79	20	44
	SD	3	4	2	4	2	2

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change Day 15-22	Total Change
146	1	35	58	23	81	23	46
	2	32	55	23	77	22	45
	3	-	-	-	-	-	-
	4	38	63	25	81	18	43
	5	32	57	25	78	21	46
	Mean	34*	58	24	79	21	45
	SD	3	3	1	2	2	1

(-) = No data available due to mortality.
*Statistically different from the control group at $p < 0.05$ (Dunnett's t-test).

APPENDIX VI

Individual Body Weights (g) from a Northern Bobwhite
Acute Dietary Toxicity Study with PFOS
Page 13

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
293	1	21	35	14	55	20	34
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
	Mean	21	35	14	55	20	34
	SD	-	-	-	-	-	-

(-) = No data available due to mortality.

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS
Page 1

Experimental Group (ppm a.i.)	Pen	Exposure Period Day 0-5	Post-Exposure Period Day 6-8
Control	1	9	12
	2	7	12
	3	8	7
	4	12	12
	5	9	9
	6	10	9
	Mean	9	10
	SD	2	2

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS
Page 2

Experimental Group (ppm a.i.)	Pen	Exposure Period Day 0-5	Post-Exposure Period Day 6-8
18.3	1	9	10
	2	8	11
	Mean	9	11
36.6	1	8	14
	2	7	9
	Mean	8	12
73.2	1	10	12
	2	10	14
	Mean	10	13
146	1	7	8
	2	10	12
	Mean	9	10
293	1	4	8
	2	6	10
	Mean	5	9
586	1	6	16
	2	5	22
	Mean	6	19
1171	1	4	-
	2	4	-
	Mean	4	-

- No data available due to adult mortality.

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Northern Bobwhite Acute Dietary Toxicity Study with PFOS
Page 3

Experimental Group (ppm a.i.)	Pen	Exposure Period Day 8-15	Post-Exposure Period Day 15-22
Control	1	10	14
	2	10	12
	3	7	12
	Mean	9	13
	SD	2	1

Experimental Group (ppm a.i.)	Pen	Exposure Period Day 8-15	Post-Exposure Period Day 15-22
18.3	1	10	12
36.6	1	14	15
73.2	1	13	15
146	1	11	14
293	1	8	9

APPENDIX VIII

CHANGES TO PROTOCOL

1. The protocol was amended to indicate that bile will be collected from all study birds. The protocol was clarified to indicate the collection of liver from birds that died during the course of the study

2. Blood samples were collected on Day 8 and Day 22 in non-heparinized 5 ml borosilicate glass test tubes. The protocol indicated that heparinized vacutainers would be used.

3. The protocol was amended to change the test concentrations from 0, 20, 40, 80, 160, 640 and 1280 ppm a.i., to 0, 18.3, 36.6, 73.2, 146, 293, 586 and 1171 ppm a.i. Test concentrations were changed to reflect the test substance purity given in the new certificate of analysis.

4. The temperatures from several brooder units on Day 4, 7, 8, and all brooder units on Day 22 were not recorded

5. The afternoon observations were inadvertently not recorded for 2 birds in the 640 ppm a.i. treatment group on April 26, 1999.

APPENDIX IX

PERSONNEL INVOLVED IN THE STUDY

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

(1) Mark Jaber, Wildlife Toxicologist
(2) Joann B. Beavers, Director, Avian Toxicology
(3) Sean P. Gallagher, Senior Biologist
(4) Courtney Casey, M.S., Senior Biologist
(5) Willard B. Nixon, Ph.D., Manager, Analytical Chemistry
(6) Timothy Z. Kendall, Supervisor, Analytical Chemistry
(7) Raymond L. Van Hoven, Ph.D., Scientist
(8) Ellen Mank, Chemist

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A DIETARY LC50 STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/030999/QLC/SUB454 AMENDMENT NO.: 1

SPONSOR: 3M Corporation PROJECT NO.: 454-103

EFFECTIVE DATE: April 27, 1999

AMENDMENT:

On page 8 under Necropsy add:

When possible, bile will be collected from all birds. Livers will also be collected from all birds that die during the course of the test.

REASON:

Bile is being collected at Sponsor request. The protocol is being clarified to indicate the collection of liver from birds that die.

Dean P. DeMyer
Study Director

9/9/99
Date

Laboratory Management

9/9/99
Date

Susan A. Beach
Sponsor Principal Contact

10/28/99
Date

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PFOS: A Dietary LC50 Study with the Northern Bobwhite

PROTOCOL NO.: 454/030999/QLC/SUB454 DEVIATION NO.: 1

SPONSOR: 3M Corporation PROJECT NO.: 454-103

DATE OF DEVIATION: April 30 and May 14, 1999

AMENDMENT:

Blood samples collected on Day 8 and Day 22 of the test were stored in labelled non-heparinized 5 ml borosillicate glass test tubes prior to being refrigerated. The protocol indicated that blood samples would be placed in labelled heparanized vacutainers.

REASON:

Test animals were too small to efficiently draw blood by syringe. Blood was drawn by decapitating birds and draining blood into test tubes. Heparin was not added to blood samples due to a Sponsor request to separate serum from the blood at Wildlife International Ltd. Seperation of serum requires coagulation of blood samples, which heparin prevents.

IMPACT:

Blood samples were drawn in an efficient manner and serum was collected per Sponsor request. Therefore this deviation caused no adverse impact on the study outcome or interpretation.

Sean P. Gallagher 9/9/99

STUDY DIRECTOR DATE

9/9/99

WILDLIFE INTERNATIONAL LTD. MANAGEMENT DATE

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PFOS: A Dietary LC50 Study with the Northern Bobwhite

PROTOCOL NO.: 454/030999/QLC/SUB454 DEVIATION NO.: 2

SPONSOR: 3M Corporation PROJECT NO.: 454-103

DATE OF DEVIATION: April 26- May 14, 1999

DEVIATION:

On several occasions temperatures were inadvertently not recorded for two to eight brooder levels housing study birds.

REASON:

Biologist oversight. With the exception of May 1, 1999, when three brooder temperatures were not recorded, the brooder pens where temperatures were not recorded were empty at the time of afternoon observations. Brooder pen temperatures are typically recorded during afternoon observations. Biologists recording afternoon observations on the occasions where temperatures were missed neglected to record temperatures for empty brooder levels that housed birds earlier the same day.

IMPACT:

Data for these brooder levels on the days prior to and/or following the missed temperatures indicated that temperatures were within the acceptable range. It is reasonable to expect that the brooders were performing properly on the days of the missed observations. Therefore, this deviation caused no adverse impact on the outcome of the study.

Lisa P. Hallmark
STUDY DIRECTOR

9/9/99
DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/9/99
DATE

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PFOS: A Dietary LC50 Study with the Northern Bobwhite

PROTOCOL NO.: 454/030999/QLC/SUB454 DEVIATION NO.: 3

SPONSOR: 3M Corporation PROJECT NO.: 454-103

DATE OF DEVIATION: April 26, 1999

DEVIATION:

On April 26, 1999, Day 4 of the study, the afternoon observations for unit B37-1 housing birds from the 640 ppm a.i. treatment level inadvertently did not include recorded observations of two birds.

REASON:

Biologist oversight.

IMPACT:

None. Observations from the morning of Day 4 were made for all birds in Pen B37-1, detailing which were displaying signs of toxicity. Because observations of two birds were not noted it is reasonable to assume they were normal in appearance and behavior, but the biologist failed to record the observation.

Leon P. Gallagher
STUDY DIRECTOR

9/9/99
DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/9/99
DATE

SOP DEVIATION

STUDY TITLE: PFOS: A DIETARY LC50 STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/030999/QLC/SUB454

SOP NO.: 415 DEVIATION NO.: 1

SPONSOR: 3M Corporation PROJECT NO.: 454-103

DATE OF DEFACTO DEVIATION: May 10-13, 1999

DEVIATION:

Brooder unit B21-1 used to house control group birds was below the specified minimum of 95°F on may 10-13, 1999. This brooder unit ranged between 1° and 3° below the minimum and there are no records of the responsible technician or biologist making a thermostat adjustment to correct the temperatures. Brooder unit B26-1 used to house birds from the 320 ppm a.i. treatment group was 1° below the specified minimum of 95°F on May 10, 1999. On the following day the brooder temperature was within the specified range.

REASON:

Misadjustment of the brooder unit thermostat.

IMPACT:

None. There was no apparent effect on test bird body weight or health.

Sean P. Gallagher
STUDY DIRECTOR

9/9/99
DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/9/99
DATE

SOP DEVIATION

STUDY TITLE: PFOS: A DIETARY LC50 STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/030999/QLC/SUB454

SOP NO.: 423.1A DEVIATION NO.: 2

SPONSOR: 3M Corporation PROJECT NO.: 454-103

DATE OF DEFACTO DEVIATION: April 22, 1998

DEVIATION:

On April 22, 1999, during diet preparation on Day 0 of the test, 1.4659 g of test substance was weighed out for preparation of the 160 ppm a.i. test concentration diet. This amount exceeded the target amount by 0.099g. The SOP indicates that the test substance will be weighed to within 0.0005 g of the target amount.

REASON:

Dietician oversight..

IMPACT:

None. The difference in the amount of test substance weighed resulted in a less than 1% change in final dietary concentration.

Lisa P. Dallmyer
STUDY DIRECTOR

9/9/99
DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/9/99
DATE

PROTOCOL

PFOS: A DIETARY LC50 STUDY WITH THE NORTHERN BOBWHITE

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.2200

FIFRA Subdivision E, Section 71-2 .

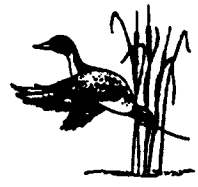
OECD Guideline 205

3M Lab Request No. U2723

Submitted to

3M Corporation
Environmental Laboratory
935 Bush Avenue
St. Paul, Minnesota 55144

WILDLIFE INTERNATIONAL LTD.



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

March 9, 1999

PFOS: A DIETARY LC50 STUDY WITH THE NORTHERN BOBWHITE

SPONSOR: 3M Corporation
Environmental Laboratory
P.O. Box 33331
St. Paul, Minnesota 55133

SPONSOR'S REPRESENTATIVE: Ms. Susan A. Beach

TESTING FACILITY: Wildlife International Ltd.
8598 Commerce Drive
Easton, Maryland 21601

STUDY DIRECTOR: Sean P. Gallagher, Senior Avian Biologist

LABORATORY MANAGEMENT: Joann B. Beavers
Manager of Avian Toxicology

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: 4/22/99 Experimental Termination Date: 5/14/99

Project No.: 454-103 Study Room: 75

Test Concentrations: 0, 20, 40, 80, 160, 320, 640, 1280 ppm a.i.

Test Substance No.: 4675 Reference Substance No. (if applicable): 4526

PROTOCOL APPROVAL

Sean P. Gallagher
STUDY DIRECTOR — DATE 4/22/99

LABORATORY MANAGEMENT — DATE 4/22/99

Susan A. Beach
SPONSOR'S REPRESENTATIVE — DATE 3/12/99

OBJECTIVE

The objective of this study is to evaluate the toxicity of a test substance to the northern bobwhite (*Colinus virginianus*) administered through the diet for five days. An LC50 value will be calculated, if possible.

SUMMARY

Groups of ten bobwhite chicks, 10-14 days old, will be fed diets containing the test substance at selected concentrations for five days. A control group that receives untreated diet will be maintained concurrently. The test substance will be mixed into the diet, usually in a geometric series of concentrations. The exposure period will be followed by a period of at least three days on untreated feed. At the end of the three-day observation period, half of the birds in each treatment group will be euthanized and liver and blood collected for residue analysis. The remaining birds will be maintained on basal diet for an additional 14 days and then will be euthanized, with liver and blood collected. Throughout the test, the birds will be observed for toxicological responses. When possible, an LC50 value will be calculated.

MATERIALS AND METHODS

The methods, species used and route of administration described in this protocol are based upon procedures specified in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.2200 (1); Section 71-2 of the Environmental Protection Agency's Registration Guidelines, *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms* (2), ASTM Standard E857-87, "*Standard Practice For Conducting Subacute Dietary Toxicity Tests With Avian Species*" (3), and OECD Guideline 205, *Avian Dietary Toxicity Test* (4). In order to control bias, birds will be assigned to pens by indiscriminate draw. No other potential sources of bias are expected to affect the results of the study.

The test substance will be administered in the diet. This route of administration was selected because it represents the most likely route of exposure to avian species in the environment.

Test Substance

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLP). The Sponsor is responsible for providing Wildlife International Ltd. written verification that the test substance has been characterized according to GLPs prior to its

use in the study. If written verification of GLP test substance characterization is not provided to Wildlife International Ltd., it will be noted in the compliance statement of the final report. The attached form **IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR** (Appendix I) is to be used to provide information necessary for GLP compliance.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Treatment Groups

Groups of ten bobwhite chicks (five per pen) will be assigned by indiscriminate draw to each of the treatment and control groups. The sex of each individual will not be determined. A test will usually consist of a geometric series of five dietary concentrations and at least three control groups. Test concentrations will be selected after evaluating toxicity data provided by the Sponsor. The highest test concentration need not exceed 5000 ppm. Each group is fed the appropriate test or control diet for five days. This will be followed by a period of at least three days on untreated feed.

Control birds will receive an amount of the solvent or vehicle in their diet equivalent to the highest amount in the treated diets. If more than one study is conducted simultaneously in the same room, with the same solvent or vehicle, a concurrent control may be utilized for all studies.

Duration of Test

The primary phases of the test and their durations are:

1. Acclimation - From receipt or hatch of hatchlings until the start of exposure.
2. Exposure - Five days.
3. Post-exposure observation – 3 days/17 days.

Test Birds

The bobwhite represents an ecologically significant and widely distributed species in the United States. The bobwhite has demonstrated sensitivity to the effects produced by known toxic chemicals. Moreover, this species has proven to be a good laboratory model and a large amount of baseline data are available.

All bobwhite will be from the same hatch and will be 10-14 days old at test initiation. Only those that are apparently healthy will be used in the test. Body weights may vary between 10 and 50 grams depending on the age of the cohort of birds used (e.g. 10 versus 14 days). At least five test and three control groups will be used. Each treatment or control group will contain ten birds (two pens of five birds each) with each group of birds identified by pen number and test concentration. Individual birds will be identified by colored leg bands. Birds will be obtained from Top Flight Quail Farm, Belvidere, NJ 07823, from another reputable supplier or obtained from the Wildlife International Ltd. production flock. All experimental birds will be acclimated to the caging and facilities from the time of hatch or receipt until initiation of the test.

Animal Diet

All test birds will be fed a game bird ration formulated to Wildlife International Ltd. specifications (Table 1). From the time of hatch or receipt of the chicks until the initiation of the test, the birds will receive a water soluble vitamin mix via their water. Feed and water will be provided *ad libitum* during acclimation and during the test. The water source will be the Town of Easton municipal water supply. Feed is analyzed periodically in accordance with Wildlife International Ltd. Standard Operating Procedures. The birds will receive no form of antibiotic medication during the test.

Specifications for acceptable levels of contaminants in game bird ration for avian species have not been established. There are no levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the study.

Diet Preparation

Test diets will be prepared at the start of the test and a sufficient portion estimated for the 5-day exposure period will be presented to the birds. After five days of exposure, all birds will receive untreated basal ration.

The test substance will be mixed directly into the ration or by dissolving or suspending the test substance in acetone and/or table grade corn oil prior to mixing with the feed. If used, acetone will be allowed to volatilize from the diets during the mixing procedure. Table grade corn oil will normally be incorporated into the diet at a maximum of 2% (w/w) of the final diet. Mixing is normally done with a Hobart mixer (Model Number AS200T).

All test substance calculations will be based on the purity of the test substance as received or corrected to 100% active ingredient based on the information provided by the Sponsor in Appendix I of this protocol.

Diet Sampling

Samples of the experimental diets will be collected for chemical analysis to determine the homogeneity and stability of the test substance in the avian diet and to verify/measure test concentrations. All samples will be placed in uniquely identified polypropylene jars.

Samples will be collected on Day 0, at diet preparation, to determine homogeneity, measure test concentrations, and establish Day 0 values for evaluating stability over the course of the exposure period. On Day 5, at the end of the exposure phase, samples will be collected from the feeders containing the experimental diets to assess stability of the test substance under test conditions. If a separate study is conducted with a different species (e.g., mallard), at the same test concentrations, diets that are prepared may be divided between the two studies. Therefore, samples collected for homogeneity and verification will be common to both studies. However, samples collected from the feeders in the study room (e.g. Day 5 samples) will be unique to that respective study. The diet sampling scheme for studies with five test concentrations is summarized below. The sampling scheme may be adjusted based on the actual number of concentrations tested (see Page 2) or the option of the Sponsor.

PROPOSED NUMBERS OF SAMPLES

Experimental Group	Day 0	Day 5
Control	1	1
Level 1 - Low Concentration	6[1]	2
Level 2	2	2
Level 3	2	2
Level 4	2	2
Level 5 - High Concentration	6[1]	2
	19	11
		Total Number of Samples = 30

[1]Samples collected from the left and right sides of the top, middle and bottom layers of feed in the mixing vessel to determine homogeneity.

The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical chemistry phase of the study.

Diet Analyses

Samples of the experimental diets may be analyzed following collection or stored in a freezer until prepared and/or extracted for analysis. Chemical analyses of diets will be performed by Wildlife International Ltd. The analytical method used will be based upon methodology provided by the Sponsor and identified in Appendix II. The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report. Maximum sample holding times, prior to analysis, may be specified by the Sponsor, and if specified, will be added to this protocol by amendment.

Housing and Environmental Conditions

Test birds will be housed in thermostatically controlled brooding pens manufactured by the Beacon Steel Company (Model B735Q) or equivalent. Each pen measures approximately 72 X 90 X 23 cm high. The external walls and ceilings of each pen are constructed of wire mesh and galvanized sheeting. Floors are of wire mesh. Upon initiation of the test, each brooding pen will contain five birds. Two brooding pens will be assigned to each treatment group (10 birds total), while at least six pens will be assigned to the control (at least 30 birds total). Each group will be identified by pen number. During the test, the temperature in the brooding compartment of the pen will be maintained at approximately 38°C. Acceptable ranges for brooding compartment temperatures will be specified in Wildlife International Ltd. Standard Operating Procedures. Brooding compartment temperatures will be recorded once each day during the test. The ambient room temperature will range between approximately 15-30°C. Room temperature and relative humidity will be recorded twice daily during the test. A 16 hours light/8 hours dark photoperiod will be maintained, and the average light intensity provided to the birds during the test will be determined.

Housing and husbandry practices will be conducted so as to adhere to the guidelines established by National Research Council (5).

Observations

All birds will be observed at least once daily for mortality and toxicological responses.

Necropsy

All test birds that die during the course of the test and all birds euthanized will be subjected to a gross necropsy. For those birds euthanized on Days 8 and 22 of the test, livers and ~ 2 mL of blood will be collected. Livers will be weighed, placed in individual labelled containers and stored frozen. Blood will be placed in labelled heparinized vacutainers and placed under refrigeration. All samples will be transferred to the Sponsor for residue analysis.

Disposition of Test Birds

At test termination, test birds will be euthanized by using carbon dioxide gas, cervical dislocation or other appropriate methods. The method used will be documented in the raw data. All carcasses will be stored frozen prior to shipment to the Sponsor.

Statistical Calculations

The concentration response data obtained from the test will be evaluated and, if possible, an LC50 value and 95% confidence limits will be calculated. When the mortality data facilitates statistical analyses, one of three methods will be used to calculate an LC50 value. The data will be analyzed, in order of preference, by probit analysis, the moving average method, or the binomial probability method (6, 7, 8, 9). The choice of method for calculating the LC50 value will be based upon the mortality pattern observed. When possible, a no mortality level will be determined and reported.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated at Wildlife International Ltd. will include, but not be limited to:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Animal history.
5. Husbandry and environmental conditions.
6. Concentration calculations and records of diet preparation.

7. Body weight measurements.
8. Feed consumption measurements.
9. Daily observations.
10. Statistical calculations.
11. Analytical chemistry methods, results and chromatograms, if applicable.
12. A copy of the final report.

FINAL REPORT

A report of the results of the study will be prepared by Wildlife International, Ltd. The report will include, but not be limited to, the following:

1. Name and address of the facility performing the study.
2. Dates on which the study was initiated and completed. It is the responsibility of the Sponsor to provide the final date that data are recorded for chemistry, pathology and/or supporting evaluations that may be generated at other laboratories.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
5. Statistical methods employed for analyzing the data, when applicable. The calculated LC50 value, 95 percent confidence limits, slope of the concentration-response curve, the results of the goodness-of-fit test (e.g. X^2 test), and a description of statistical methods used. The same statistics will be provided for positive controls (when used).
6. The test, control and reference substances identified by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor.
7. Stability and, when relevant to the conduct of the study, the solubility of the test, control and reference substances under the conditions of administration, if provided by the Sponsor.
8. A description of the test system used. Where applicable, the final report shall include the number of animals used, body weight range, source of supply, species (including scientific name), age, and procedure used for identification.
9. A description of the dosage, dosage regimen, route of administration, and duration.
10. A description of the methods used, including but not limited to:

a) Detailed description of the basal diet, including source, diluents (if used), and supplements (if used). A nutrient analysis of the diet will be included in the test report.

b) The number of concentrations used, nominal and (where required) measured dietary concentration of test substance in each level, assay method used to determine actual concentrations, number of birds per concentration and for control, and names of toxicants used for positive controls (if applicable). Results of range-finding test (if conducted).

c) Acclimation procedures and methods of assigning birds to test pens.

d) Frequency, duration and methods of observation. Description of signs of intoxication and other abnormal behavior, including time of onset, duration, severity (including death), and numbers affected in the different dietary concentrations and controls each day of the test period.

e) Description of housing conditions, including type, size and material of pen, pen temperatures, approximate test room humidity, photoperiod and lighting intensity.

f) Average body weights for birds in each pen at the beginning of the test, the end of the exposure period and end of the test.

g) Estimated food consumption for the exposure period and for the postexposure period.

11. A description of all circumstances that may have affected the quality or integrity of the data. Anything unusual about the test, any deviation from these procedures, and any other relevant information.
12. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
13. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.
14. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
15. The location where all specimens, raw data, and the final report are to be stored.
16. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
17. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes shall be in the form of amendment by the Study Director. The amendment should clearly identify the part of the final report that is being added to or corrected and the reasons for the correction or addition. Amendments shall be signed and dated by the Study Director.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (OCDE/GD (92) 32, Environment Monograph No. 45); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). Each study conducted by Wildlife International Ltd. is routinely examined by the Wildlife International Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.2200: *Avian Dietary Toxicity Test.*

2 **U.S. Environmental Protection Agency.** 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms*, subsection 71-2, Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.

3 **American Society for Testing and Materials.** 1987. Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species. ASTM Standard E857-87. Annual Book of ASTM Standards, Vol. 11.04. Philadelphia, PA.

4 **Organization for Economic Cooperation and Development.** 1984. *Avian Dietary Toxicity Test.* OECD Guideline for Testing of Chemicals. Guideline 205. Paris.

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6 **Finney, D. J.** 1971. *Statistical Methods in Biological Assay*, 2nd ed., Griffin Press, London.

7 **Thompson, W. R.** 1947. *Bacteriological Reviews*, Vol 2, No. 2: 115-145.

8 **Stephan, C. E.** 1977. Methods for Calculating an LC50. Pages 65-84 In *Aquatic Toxicology and Hazard Evaluations*, American Society for Testing and Materials. Pub. No. STP 634. Philadelphia, PA.

9 **Stephan, C. E.** 1978. U.S. EPA, Environmental Research Laboratory, Duluth, MN. Personal Communication.

TABLE 1: DIET FORMULATION
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	AMOUNT ADDED 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 I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): PFOS (Perfluorooctane Sulfonic Acid Potassium Salt

Reference Standard (if applicable): Analytical Standard: N/A

Internal Standard: 1,1,2,2H,H,H,H Perfluorooctane Sulfonic Acid

Test Substance Sample Code or Batch Number: Lot 217

Test Substance Purity (% Active Ingredient): 98.9 Expiration Date: 2008

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? ____Yes __x__ No

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International Ltd.

Ambient

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? ____Yes __x__ No

Other pertinent stability information:

IV. Test Concentrations:

__x__ Adjust test concentration to 100% a.i. based upon the purity (%) given above.

____ Do not adjust test concentration to 100% a.i. Test the material AS IS.

V. Toxicity Information:

Mammalian: Rat LD50 251 mg/kg Mouse LD50 N/A

Aquatic:

	Invertebrate Toxicity (EC/LC50)	Fish Toxicity (LC50)
	Daphnia magna: 27 mg/L	Rainbow Trout: 11 mg/L
	Daphnia magna: 50 mg/L	Fathead Minnow: 38 mg/L

Other Toxicity Information (including findings of chronic and subchronic tests):

Please see MSDS

APPENDIX II

Method Outline for the Determination of PFOS in Avian Feed

1. Prepare matrix fortification samples in the desired avian feed stock using the dry mix technique.
2. Weigh 10 g samples of the matrix blank and matrix fortification feed samples into a weigh boat and transfer to 16 oz. French square glass bottles. Record weights.
3. For each weighed sample, measure 100 mLs of methanol with a graduated cylinder and transfer into the French square bottle.
4. Seal bottles with screw caps and place on shaker table. Allow to shake for a minimum of 30 minutes.
5. Vacuum filter with qualitative filter paper and rinse retained feed 3 times with methanol into filtrate.
6. Transfer filtrate into a 200-mL volumetric flask and bring to volume with methanol.
7. Transfer 5 mLs of 200-mL solution into a 10-mL volumetric flask partially-filled with 50% methanol:50% water dilution solvent containing 0.05% v/v formic acid and 100 µg a.i./mL 4HPFOS internal standard. Bring to volume with dilution solvent.
8. Mix and transfer approximately 1 mL of the 10-mL solution into a syringe and filter through a 0.2 µm filter into an HPLC autosampler vial.
9. Ampulate and submit sample for LCMS analysis.