PFOS: A Dietary LC50 Study with the Mallard

DIETARY ACUTE MALLARD DUCK 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: 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: 2000
Species: *Anas platyrhynchos*
Supplier: Whistling Wings, Inc., Hanover, IL
Analytical monitoring: PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.
Test phases: Acclimation – 9 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. Floor space of each pen measured approximately 62 X 90 cm. Ceiling height was approximately 25.5 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 wing bands.
Number of replicates: Six for controls, two for each treatment group
Number of ducks per replicate: five

Number of concentrations: Eight 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 mg
Folic Acid	600 mg
Biotin	64 mg
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 $\pm$ 2°C

Ambient room mean temperature: 25.2 ± 0.7°C
Average relative humidity: 53 ± 18%
Photoperiod: Sixteen hours light per day
Lighting: fluorescent lights which closely approximate noon-day sunlight; average approximately 207 lux.

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 9.1 ppm and six from the lowest and highest 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. Two verification samples from the remaining concentrations and one from the control 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, 9.1, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm
Measured concentrations: <LOQ, 9.8, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm
Element value: Dietary LC_{50} = 628 (448 – 958) ppm
No mortality concentration = 146 ppm
NOEC (body weight gain) = 36.6 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 - 108% 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 94 – 130% 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
9.1	9.52, 9.70, 9.79, 8.09, 10.9, 10.5	9.8	108
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	-	-
9.8	12.3, 11.0	11.7	119
19.5	18.2, 19.7	19.0	97
40.2	47.9, 56.8	52.4	130
74.5	77.6, 77.9	77.8	104
174	167, 160	164	94.3
291	297, 293	295	101
537	552, 530	541	101
1196	1150, 1122	1136	95

Biological observations

Survival and clinical observations: No mortalities occurred in the control group, and all birds were normal in appearance and behavior throughout the test. The first deaths occurred on day 4 in the 1171 ppm treatment. Mortality occurred through Day 8 in all dose groups $\geq$ 293 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 $\leq$ 146 ppm. Birds at all concentrations $\geq$ 293 ppm displayed signs of toxicity including reduced reaction to stimuli (sound and motion), loss of coordination, ruffled appearance, lethargy and lower limb weakness. Birds at the 1171 ppm level also displayed prostrate posture, depression and convulsions through Day 8. Recovery with normal appearance and behavior was noted from Day 9 through test termination

Body weight gain: When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations $\leq$ 36.6 ppm. During the Day 8-15 and Day 15-22 post-exposure periods, body weight gain appeared comparable among all groups. There was a statistically significant ($p < 0.05$) reduction in weight gain at the 9.1 ppm level for the Day 0-5 and Day 5-8 periods. However, differences from the control group at the 9.1 ppm level appear to be due to a lower mean Day 0 body weight for the 9.1 ppm level, and were not dose responsive. Therefore, these differences were not considered treatment related. Marked, treatment-related, concentration responsive effects on body weight was noted in concentrations > 73.2 ppm for Days 0-5; Day 5-8 post-exposure weight gain continued to be reduced at concentrations $\geq$ 293 ppm.

Feed Consumption: When compared to the control group, there was a marked reduction in feed consumption in the treatment groups $\geq$ 293 ppm throughout the study.

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, empty crops, and empty gastrointestinal tracts. These necropsy findings were considered to be treatment related.

Percent 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
9.1	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	0	0	0	0	0	0
293	0	0	0	0	0	0	10	20
586	0	0	0	0	10	20	30	30
1171	0	0	0	20	60	80	90	90

*No mortalities occurred in any of the treatment levels from Day 8 to Day 22

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	135	144	101	230	183	413	823
9.1	119	108	91	230	198	427	773
18.3	146	131	100	241	186	427	811
36.6	147	128	100	243	208	451	828
73.2	143	117	82	216	203	418	782
146	143	100	89	232	124	356	688
293	129	32	57	256	234	490	701
586	144	-6	36	221	219	439	613
1171	147	-37	1531	198	251	449	634

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	92	125	171	180
9.1	73	117	172	198
18.3	91	132	186	204
36.6	94	125	165	179
73.2	77	101	148	173
146	105	159	159	164
293	44	63	109	132
586	36	55	114	143
1171	22	25	106	154

Gross Pathological Observations from Birds that Died in Study

Finding	293 ppm N = 2	586 ppm N = 3	1171 ppm N = 9
Crop empty	0	2	7
Emaciated	1	1	4
G.I. Tract, primarily empty	1	1	1
Gizzard contents bile stained	0	2	4
Gizzard, empty	0	0	1
Intestinal contents, black and tar-like	0	0	1
Keel prominent	0	0	2
Kidneys, pale	0	0	1
Loss of muscle mass	2	2	5
Spleen, grey	0	0	1
Spleen, small and pale	1	1	2
Spleen, pale	0	2	3
Thin	1	1	4

CONCLUSIONS

The dietary LC50 value for Mallard Duck exposed to PFOS was determined to be 628 ppm with a 95% confidence interval of 448 to 958 ppm. The slope of the concentration-response curve was 3.67 and the chi-square value was 2.13. The no mortality concentration was 146 ppm. Based upon reductions in body weight gain at the 73.2 ppm test concentration, the no observed effect concentration was 36.6 ppm.

Submitter: 3M Company, 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: 5/1/00

PFOS:
A DIETARY LC50 STUDY WITH THE MALLARD

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-102
3M 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

AUTHORS: Sean P. Gallagher
Courtney S. Casey
Joann B. Beavers
Raymond L. Van Hoven

STUDY INITIATION: April 21, 1999

STUDY COMPLETION: April 26, 2000

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Dietary LC50 Study with the Mallard

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-102

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, Avian Toxicology

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 & 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
Matrix Fortification, Test Substance Prep., Analytical Sampling	April 22, 1999	April 22, 1999	April 23, 1999
Feed Consumption and Analytical Sampling	April 27, 1999	April 27, 1999	May 4, 1999
Necropsy, Blood Collection, Liver Weights and Bile Collection	May 14, 1999	May 14, 1999	May 21, 1999
Analytical Data and Draft Report	July 7, 8, and 9, 1999	July 9, 1999	July 16, 1999
Biological Data and Draft Report	Aug. 30, 31, Sept. 1, 1999	September 1, 1999	September 3, 1999
Final Report	April 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 Mallard

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-102

3M LAB REQUEST NO.: U2723

STUDY DIRECTOR:

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

CHEMISTRY PRINCIPAL INVESTIGATOR:

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

REPORT APPROVED BY:

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

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

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SUMMARY

SPONSOR: 3M Corporation

TEST SUBSTANCE: PFOS

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-102

STUDY: PFOS: A Dietary LC50 Study with the Mallard

RESULTS: The dietary LC50 value for Mallards exposed to PFOS was determined to be 628 ppm a.i. with a 95% confidence interval of 448 ppm a.i. to 958 ppm a.i. The slope of the concentration-response curve was 3.67 and the chi-square value was 2.13. The no mortality concentration was 146 ppm a.i. Based upon reductions in body weight gain at the 73.2 ppm a.i. test concentration, the no-observed-effect concentration was 36.6 ppm a.i.

TEST DATES: Hatch – April 13, 1999
Acclimation – April 14-22, 1999
Experimental Start –April 22, 1999
Experimental Termination –

NOMINAL TEST CONCENTRATIONS: 0, 9.1, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm a.i.

TEST ANIMALS: Mallard (*Anas platyrhynchos*)

AGE TEST ANIMALS: 10 days of age at test initiation

SOURCE TEST ANIMALS: Whistling Wings, Inc.
PO Box 1-A
113 Washington St.
Hanover, IL 61041-3512

STUDY COMPLETION: April 26, 2000

INTRODUCTION

This study was conducted by Wildlife International Ltd. for 3M Corporation at the Wildlife International Ltd. 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 454-102 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 mallard (*Anas platyrhynchos*) 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 eight test concentrations and a control group. Thirty mallard ducklings were assigned to the control group and ten mallard ducklings were assigned to each of the treatment groups. 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 ducklings each. On Day 0, following experimental start, the Sponsor requested the addition of a 10 ppm test concentration. Birds from the same lot that remained after test initiation were used for the additional test concentration. Nominal dietary concentrations used in this study were 0, 9.1, 18.3, 36.6, 73.2, 146, 293, 586 and 1171 parts per million active ingredient (ppm a.i.). 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 treatment and control birds were euthanized and liver weight and tissue, blood, and bile samples collected for analysis. The remaining birds were fed basal ration until Day 22. On Day 22, these birds were sacrificed and also sampled for liver weight and tissue, blood, and bile.

Duration of the Test

The primary phases of this test and their durations were:

1. Acclimation - 9 days.
2. Exposure - 5 days.
3. Post-exposure observation - 3 or 17 days

Test Birds

All mallard (*Anas platyrhynchos*) were 10 days of age and appeared to be in good health at initiation of the test. The birds were obtained from Whistling Wings Inc., Hanover, IL. and were hatched on April 13, 1999. Birds ranged in weight from 96 to 204 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 after 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). 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 9.1, 18.3, 36.6, 73.2, 146, 293, 586 and 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 9.1 ppm a.i. a.i. and six samples from the 1171 ppm a.i. a.i. test diets 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. 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 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 454-102-2, 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 Safeguard Products, Inc. Each pen had floor space that measured approximately 62 X 90 cm. Ceiling height was approximately 25.5 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 wing 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 25.2°C ± 0.7°C (SD) with an average relative humidity of 53% ± 18% (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 207 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

observed at least once 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, on Day 8 for all birds, 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 half of the 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, liver weight and liver tissue, serum and blood solids, 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 by 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 9.1, 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 9.8 ± 0.969 ppm a.i. (CV = 9.94%), 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 119, 97.4, 130, 104, 94.3, 101, 101 and 95.0% of the Day 0 values for the 9.1, 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

No mortalities occurred in the control group, and all birds were normal in appearance and behavior throughout the test. There were no treatment-related mortalities or overt signs of toxicity in the 9.1, 18.3, 36.6, 73.2, or 146 ppm a.i. treatment groups (Table 1 and Appendix V). However, there was 20% (2 of 10) mortality in the 293 ppm a.i. treatment group, 30% (3 of 10) mortality in the 586 ppm a.i. treatment group and 90% (9 of 10) in the 1171 ppm a.i. treatment group.

In the 293 ppm a.i. treatment group single mortalities were noted on the mornings of Day 7 and Day 8. Signs of toxicity were first observed on the morning of Day 4 and continued until termination for those birds sacrificed on Day 8 and until the afternoon of Day 8 for those birds sacrificed on Day 22. All birds in this treatment level displayed signs of toxicity. These included reduced reaction to stimuli (sound and motion), loss of coordination, ruffled appearance, lethargy, and lower limb weakness. All remaining birds appeared to have recovered and were normal in appearance and behavior from the morning of Day 9 until test termination.

At the 586 ppm a.i. treatment level there were three treatment-related mortalities, one each noted on the morning of Day 5, Day 6, and Day 7. Signs of toxicity were first observed on the morning of Day 3 and continued until termination for those birds sacrificed on Day 8 and until the afternoon of Day 8 for those birds sacrificed on Day 22. All birds in this treatment level displayed signs of toxicity. These included reduced reaction to stimuli (sound and motion), ruffled appearance, lower

limb weakness, and lethargy. All remaining birds appeared to have recovered and were normal in appearance and behavior from the morning of Day 9 until test termination.

In the 1171 ppm a.i. treatment group there were nine treatment-related mortalities. The first two mortalities were found on the morning of Day 4, followed by four mortalities on Day 5, two mortalities on Day 6 and the final mortality on Day 7. Overt signs of toxicity were first observed on the morning of Day 2 and the single surviving bird continued to display signs of toxicity through the afternoon of Day 8. Signs of toxicity observed among birds in the 1171 ppm a.i. treatment group included reduced reaction to stimuli (sound and motion), loss of coordination, prostrate posture, depression, convulsions, ruffled appearance, lower limb weakness, and lethargy. The one surviving bird appeared to have recovered and was normal in appearance and behavior from the morning of Day 9 until test termination.

Body Weight and Feed Consumption

When compared to the control group, there were no apparent treatment related effects on body weight among birds in the 9.1, 18.3, or 36.6 ppm a.i. treatment groups. There was a statistically significant ($p<0.05$) reduction in weight gain at the 9.1 ppm a.i. level for the Day 0-5 period that was reflected in significantly lower ($p< 0.05$) mean Day 5 body weight. When compared to the control group, reductions in weight gain were also statistically significant ($p<0.05$) for the Day 5-8 period, resulting in a significantly ($p<0.05$) lower Day 8 mean body weight. However, differences from the control group at the 9.1 ppm a.i. level appear to be due to a lower mean Day 0 body weight for the 9.1 ppm a.i. level and were not dose responsive. Therefore, these differences were not considered treatment related.

There was a marked, treatment-related, concentration-responsive effect on body weight in the 73.2, 146, 293, 586, and 1171 ppm a.i. treatment groups during the exposure period (Days 0-5) (Table 2 and Appendix VI). These reductions were statistically significant at $p<0.05$ for the 73.2 ppm a.i. level and at $p<0.01$ at the 146, 293, 586 and 1171 ppm a.i. levels. During the Day 5-8 post-exposure period, weight gain continued to be reduced for surviving birds in the 293, 586 and 1171 ppm a.i. treatment groups. These reductions were statistically significant at $p<0.01$ for the 293 and 586 ppm a.i. levels. Differences at the 1171 ppm a.i. level were marked but could not be statistically compared. Body weight losses and reductions in weight gain at the 293, 586 and 1171 ppm a.i. levels were reflected in significantly ($p<0.05$ or $p<0.01$) lower mean body weights on Days 5, 8, 15, and 22

of the test. At the 146 ppm a.i. level mean body weights on Days 8 and 22 were also significantly ($p<0.05$) lower than the control group. During the Day 8-15 and Day 15-22 post-exposure periods, body weight gain appeared comparable among all groups.

When compared to the control group, there was a marked reduction in feed consumption in the 293, 586, and 1171 ppm a.i. treatment groups during the exposure period (Days 0-5). A reduction in feed consumption continued to be observed through Day 15 in the 293, 586 and 1171 ppm a.i. treatment groups.

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, empty crops, and empty gastrointestinal tracts. Findings were considered to be treatment related. 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 on Day 22, following test termination. The necropsy results for birds euthanized on Day 8 and Day 22 from the 18.3, 146 and 1171 ppm a.i. treatment groups were unremarkable. One bird in the control group and one bird in the 36.6 ppm a.i. treatment group were noted with retained yolk sacs during necropsy of birds on Day 8. A single bird in the 9.1 ppm a.i. treatment group necropsied on Day 8 was noted as small, with a lack of muscle mass. In the 73.2 ppm a.i. treatment group one bird necropsied on Day 8 was small, with a lack of muscle mass, and had a 2 x 0.5 cm abscess containing caceous necrotic material on the right side of the abdominal wall. Due to the nature of the lesions observed in birds, and the isolated incidence of occurrence, the findings listed above in euthanized birds from the 9.1, 36.6, and 73.2 ppm a.i. treatment groups were not considered to be related to treatment. In addition, all remaining birds in the control, 9.1, 36.6, and 73.2 ppm a.i. treatment groups were non remarkable.

In the 293 ppm a.i. treatment group, one bird euthanized on Day 8 was noted with an incidental finding of a retained yolk sac. A second bird in the 293 ppm a.i. group, necropsied on Day 8, was noted as emaciated with a lack of muscle mass. This loss of muscle mass was consistent with reduced weight gain for the level and was considered treatment related. Another bird in the 293 ppm a.i. treatment group, euthanized on Day 22, was noted with 2 cm cysts containing yellow fluid on the

anterior portion of the left lobe of the liver. Due to the nature of the cysts and the isolated incidence of occurrence, this observation was not considered treatment related.

Three birds in the 586 ppm a.i. treatment level were euthanized on Day 8. At necropsy, they were noted to be thin and lacking muscle mass. One bird was noted with a slightly pale liver. These findings are considered to be treatment related. Four birds in the 586 ppm a.i. treatment level survived until Day 22. Necropsy results for these four birds were not remarkable.

CONCLUSION

The dietary LC50 value for Mallards exposed to PFOS was determined to be 628 ppm a.i. with a 95% confidence interval of 448 ppm a.i. to 958 ppm a.i.. The slope of the concentration-response curve was 3.67 and the chi-square value was 2.13. The no mortality concentration was 146 ppm a.i.. Based upon reductions in body weight gain at the 73.2 ppm a.i. test concentration, the no-observed-effect concentration was 36.6 ppm a.i..

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TABLE 1

Cumulative Mortality from a Mallard 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	0/30	0/30	0/30
Treatment									
9.1	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
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	0/10	0/10	0/10	0/10	0/10	0/10
293	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	2/10
586	0/10	0/10	0/10	0/10	0/10	1/10	2/10	3/10	3/10
1171	0/10	0/10	0/10	0/10	2/10	6/10	8/10	9/10	9/10

The LC50 value was calculated to be 628 ppm a.i. with a 95% confidence interval of 448 ppm a.i. to 958 ppm a.i.

[1] – No mortalities occurred in any of the treatment levels from Day 8 to Day 22.

TABLE 2
Page 1

Mean Body Weight (g) from a Mallard 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 (Day 0-8)[1]
Control							
0	Mean	135	279	144	380	101	245
	SD	24	42	22	53	16	34
Treatment							
9.1	Mean	119	226**	108**	317*	91	198*
	SD	14	36	23	66	33	55
18.3	Mean	146	277	131	377	100	231
	SD	14	26	18	32	11	25
36.6	Mean	147	275	128	375	100	228
	SD	16	32	22	37	12	30
73.2	Mean	143	260	117*	343	82	200*
	SD	30	55	33	90	42	73
146	Mean	143	242	100**	331	89	188**
	SD	19	37	21	48	16	32
293	Mean	129	161**	32**	220**	57	94**
	SD	17	28	33	55	44	54
586	Mean	144	137**	-6**	175**	36	39**
	SD	23	27	26	34	41	36
1171	Mean	147	112**	-37**	185[2]	31[2]	15[2]
	SD	25	28	13	-	-	-

[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 using Dunnett's t-test.
(-) = 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 Mallard 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	410	640	230	823	183	413
	SD	30	56	47	49	24	42
Treatment							
9.1	Mean	345*	575	230	773	198	427
	SD	33	32	20	38	15	34
18.3	Mean	384	625	241	811	186	427
	SD	27	42	22	81	41	62
36.6	Mean	377	620	243	828	208	451
	SD	33	44	40	78	34	71
73.2	Mean	364	579	216	782	203	418
	SD	82	97	21	135	39	56
146	Mean	332*	564	232	688*	124*	356
	SD	58	56	22	86	90	106
293	Mean	211**	467**	256	701*	234	490
	SD	49	51	35	41	35	54
586	Mean	174**	394**	221	613**	219	439
	SD	46	82	37	91	13	47
1171	Mean	185[2]	373[2]	198[2]	634[2]	251[2]	449[2]
	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 using Dunnett's t-test.
(-) = 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 3
Page 1

Mean Feed Consumption (g/bird/day) from a Mallard 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	92	125
	SD	10	17
Treatment			
9.1		73	117
18.3		91	132
36.6		94	125
73.2		77	101
146		105	159
293		44	63
586		36	55
1171		22	25

(-) = No data available due to mortality.

TABLE 3
Page 2

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

Experimental Group (ppm a.i.)		Post-Exposure Period Day 8-15	Post-Exposure Period Day 15-22
Control			
0	Mean	171	180
	SD	13	20
Treatment			
9.1		172	198
18.3		186	204
36.6		165	179
73.2		148	173
146		159	164
293		109	132
586		114	143
1171		106	154

(-) = No data available due to mortality.

TABLE 4

Gross Pathological Observations
from a Mallard Acute Dietary Toxicity Study with PFOS

Birds that died during the course of the study

Finding	Male, Female, and Undetermined PPM A.I.		
	293 N=2	586 N=3	1171 N=9
Crop empty	0	2	7
Emaciated	1	1	4
G.I. Tract, primarily empty	1	1	1
Gizzard contents bile stained	0	2	4
Gizzard, empty	0	0	1
Intestinal contents, black and tar-like	0	0	1
Keel prominent	0	0	2
Kidneys, pale	0	0	1
Loss of muscle mass	2	2	5
Spleen, grey	0	0	1
Spleen, small and pale	1	1	2
Spleen, pale	0	2	3
Thin	1	1	4

Table 5
Cumulative Mortality (Estimated Cumulative Dose, mg/kg[1]) from a Mallard
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	0/5	0/5	0/5
0	6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Treatment										
9.1	1	0/5	0/5 (4)	0/5 (8)	0/5 (12)	0/5 (16)	0/5 (20)	0/5	0/5	0/5
9.1	2	0/5	0/5 (4)	0/5 (8)	0/5 (12)	0/5 (16)	0/5 (20)	0/5	0/5	0/5
18.3	1	0/5	0/5 (8)	0/5 (16)	0/5 (24)	0/5 (32)	0/5 (40)	0/5	0/5	0/5
18.3	2	0/5	0/5 (9)	0/5 (18)	0/5 (27)	0/5 (36)	0/5 (45)	0/5	0/5	0/5
36.6	1	0/5	0/5 (19)	0/5 (38)	0/5 (57)	0/5 (76)	0/5 (95)	0/5	0/5	0/5
36.6	2	0/5	0/5 (16)	0/5 (32)	0/5 (48)	0/5 (64)	0/5 (80)	0/5	0/5	0/5
73.2	1	0/5	0/5 (31)	0/5 (62)	0/5 (93)	0/5 (124)	0/5 (155)	0/5	0/5	0/5
73.2	2	0/5	0/5 (30)	0/5 (60)	0/5 (90)	0/5 (120)	0/5 (150)	0/5	0/5	0/5
146	1	0/5	0/5 (74)	0/5 (148)	0/5 (222)	0/5 (296)	0/5 (370)	0/5	0/5	0/5
146	2	0/5	0/5 (101)	0/5 (202)	0/5 (303)	0/5 (404)	0/5 (505)	0/5	0/5	0/5
293	1	0/5	0/5 (64)	0/5 (128)	0/5 (192)	0/5 (256)	0/5 (320)	0/5	0/5	0/5
293	2	0/5	0/5 (126)	0/5 (252)	0/5 (378)	0/5 (504)	0/5 (630)	0/5	1/5	2/5
586	1	0/5	0/5 (175)	0/5 (350)	0/5 (525)	0/5 (700)	0/5 (875)	0/5	1/5	1/5
586	2	0/5	0/5 (153)	0/5 (306)	0/5 (459)	0/5 (612)	1/5 (765)	2/5	2/5	2/5
1171	1	0/5	0/5 (240)	0/5 (480)	0/5 (720)	1/5 (960)	4/5 (1200)	5/5	5/5	5/5
1171	2	0/5	0/5 (207)	0/5 (414)	0/5 (621)	1/5 (828)	2/5 (1035)	3/5	4/5	4/5

The LC50 value was calculated to be approximately 628 ppm a.i. with a 95% confidence interval of 448 to 958 ppm a.i..

[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 approximation 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_xF_{2x+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 (454-102-)	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 (454-102-)	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-454-102-)	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
9.1	54	Top Left	9.52		
	55	Top Right	9.70	$\bar{x}$ = 9.8 ppm a.i.	
	56	Middle Left	9.79	SD = 0.969 ppm a.i.	108
	57	Middle Right	8.09	CV = 9.94%	
	58	Bottom Left	10.9		
	59	Bottom Right	10.5		
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-454-102-)	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	--	--	--
9.1	--	--	--	--	9.8[2]	108[2]
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 Mallard LC50 Study

Nominal Concentration (ppm a.i.)	Day 0[1]			Day 5			
	Sample Number (S-454-102-)	Mean Measured Concentration (ppm a.i.)	Mean Percent of Nominal	Sample Number (S-454-102-)	Measured Concentration[2] (ppm a.i.)	Mean Measured Concentration (ppm a.i.)	Mean Percent of Day 0
0	1	–	–	39	< LOQ	–	–
9.1	54-59	9.8	108	60 61	12.3 11.0	11.7	119
18.3	2-7	19.5	107	40 41	18.2 19.7	19.0	97.4
36.6	8, 9	40.2	110	42 43	47.9 56.8	52.4	130[3]
73.2	10, 11	74.5	102	44 45	77.6 77.9	77.8	104
146	12, 13	174	119	46 47	167 160	164	94.3
293	14, 15	291	99.3	48 49	297 293	295	101
586	16, 17	537	91.6	50 51	552 530	541	101
1171	18-23	1196	102	52 53	1150 1122	1136	95.0

[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).
[3]The Day 5 value is consistent with the Day 0 mean measured concentration value for this level. No analytical measuring bias is indicated.

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

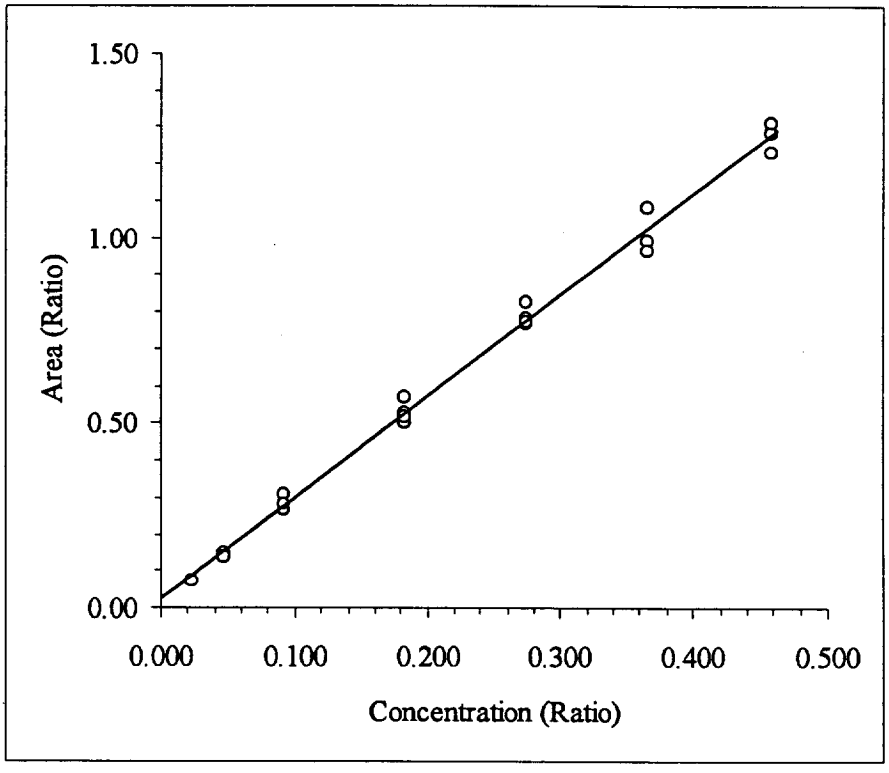


Figure 2. A typical calibration curve for PFOS. Slope = 2.77397; Intercept = 0.01894; r = 0.9981. Curve is weighted (1/x).

Appendix III

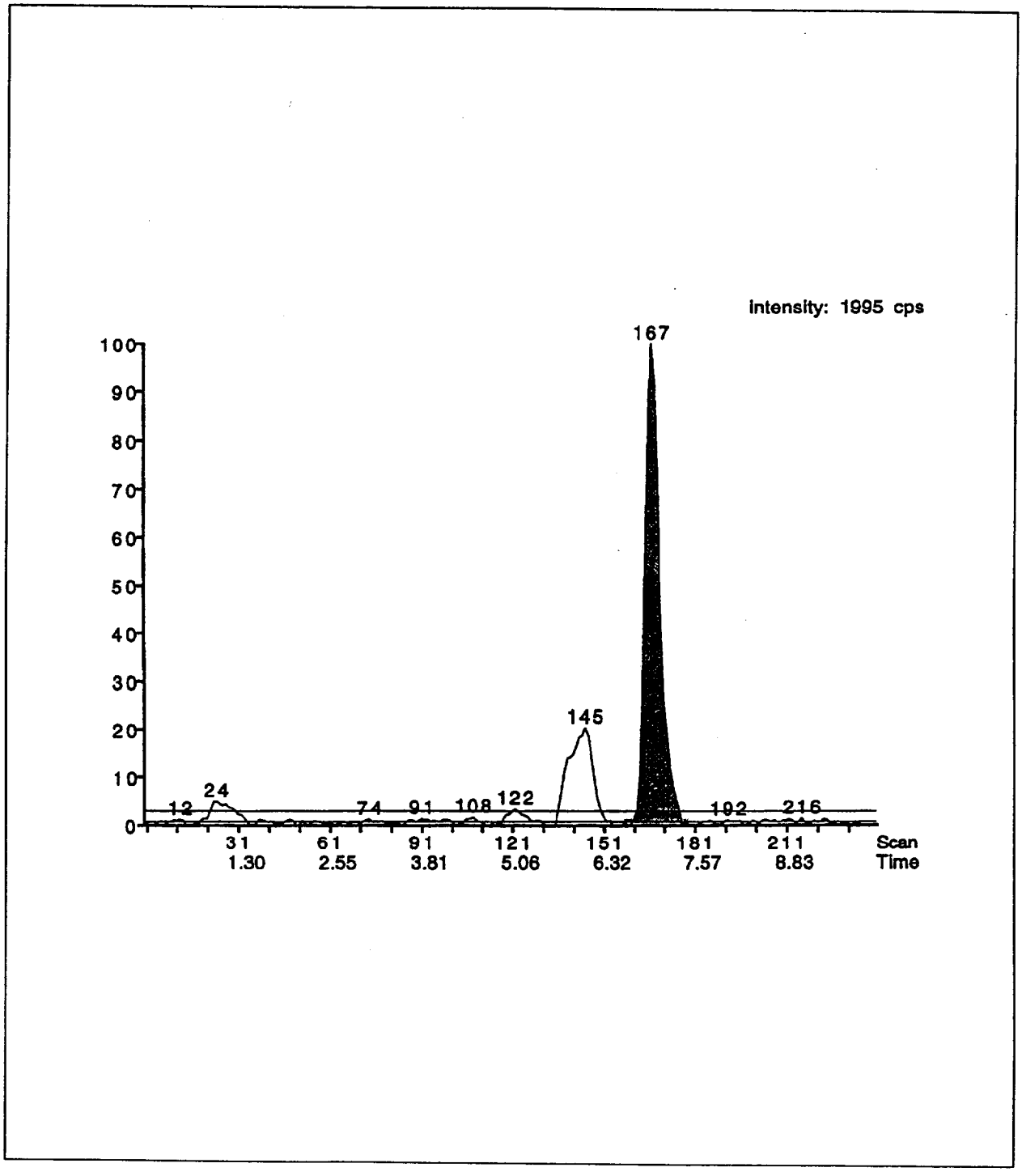


Figure 3. A representative ion chromatogram of a low-level (0.00229 mg a.i./L) PFOS standard.

Appendix III

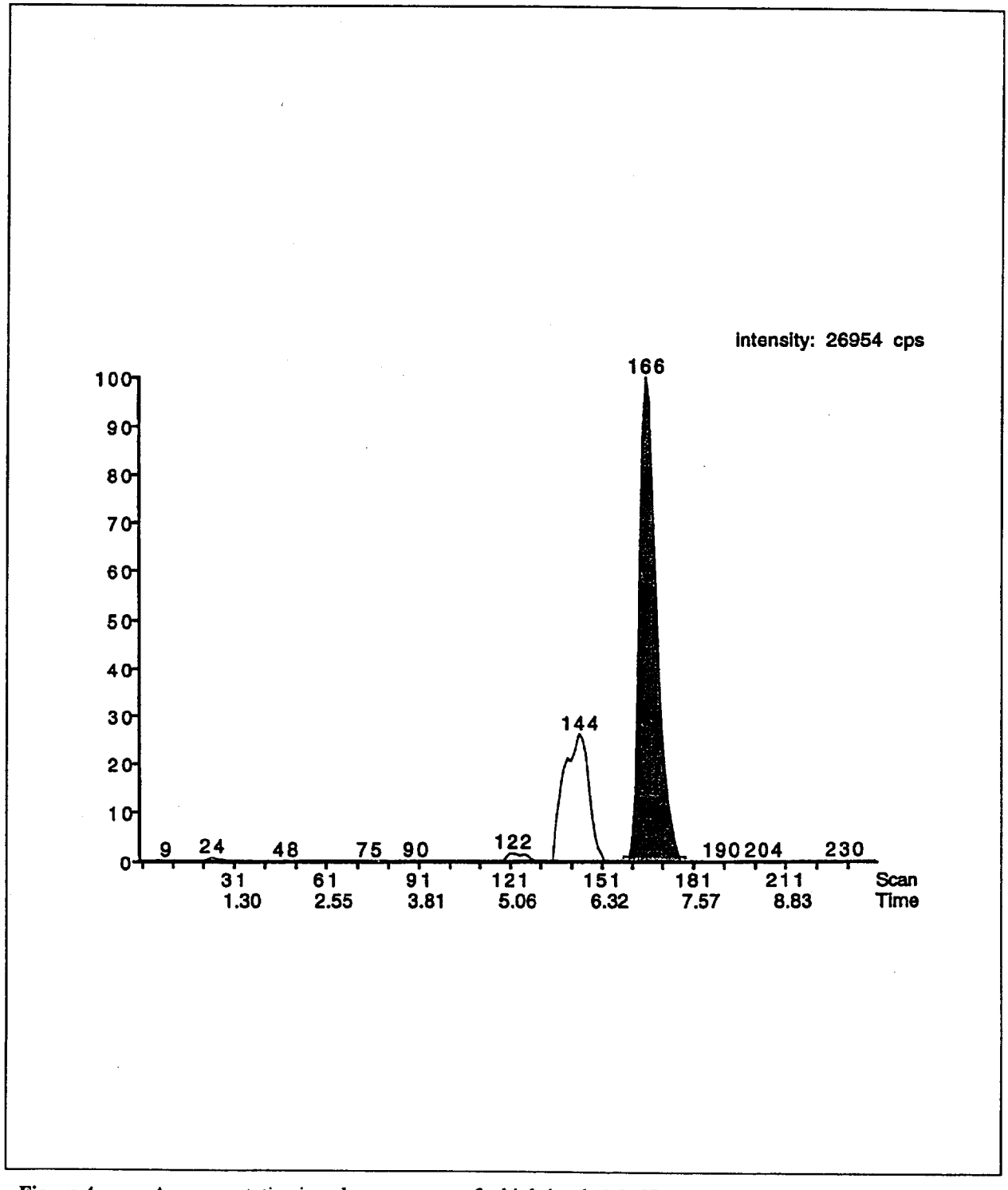


Figure 4. A representative ion chromatogram of a high-level (0.0457 mg a.i./L) PFOS standard.

Appendix III

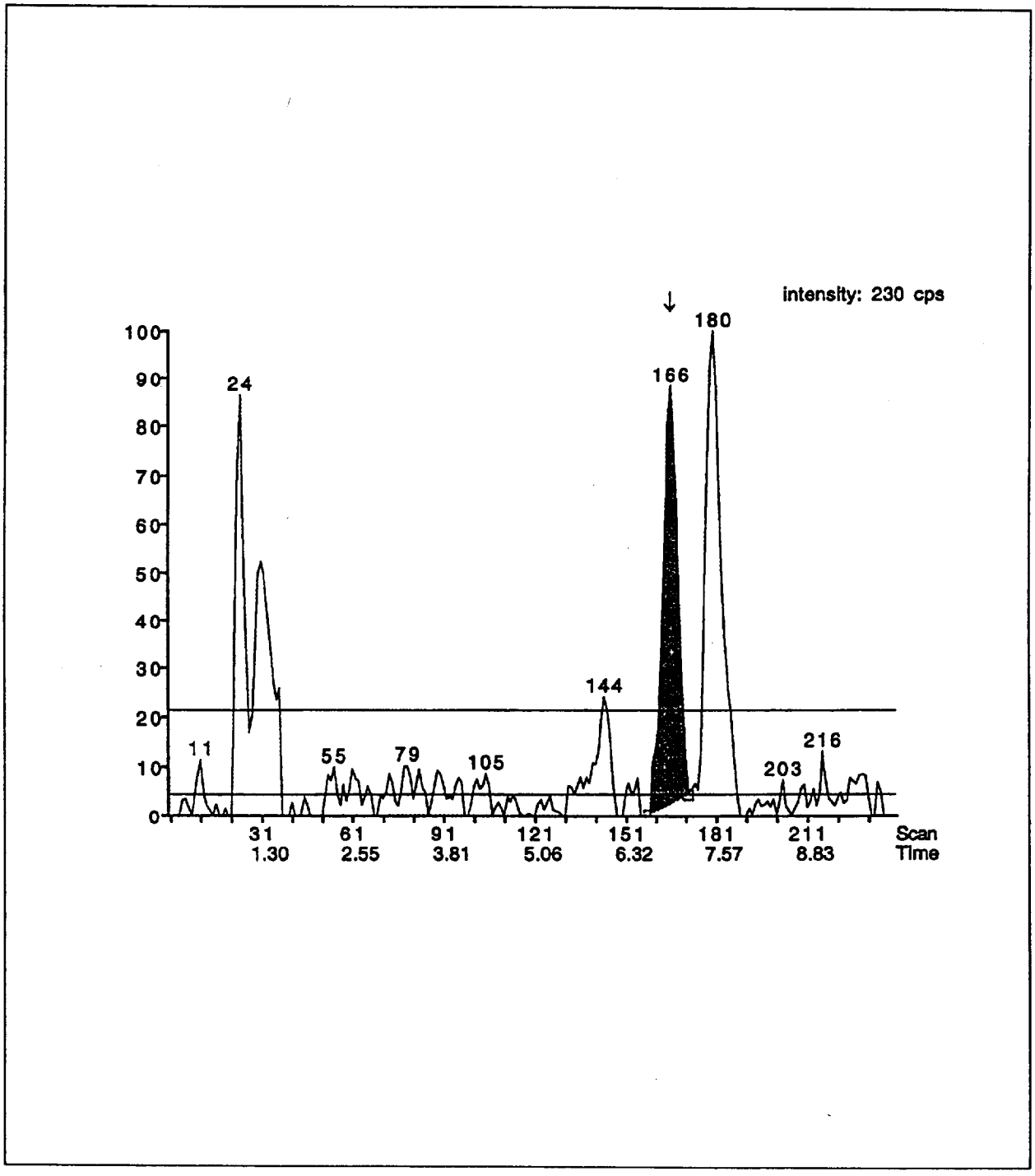


Figure 5. A representative chromatogram of a matrix blank sample (454-102-MAB-1). The arrow indicates the retention time of PFOS.

Appendix III

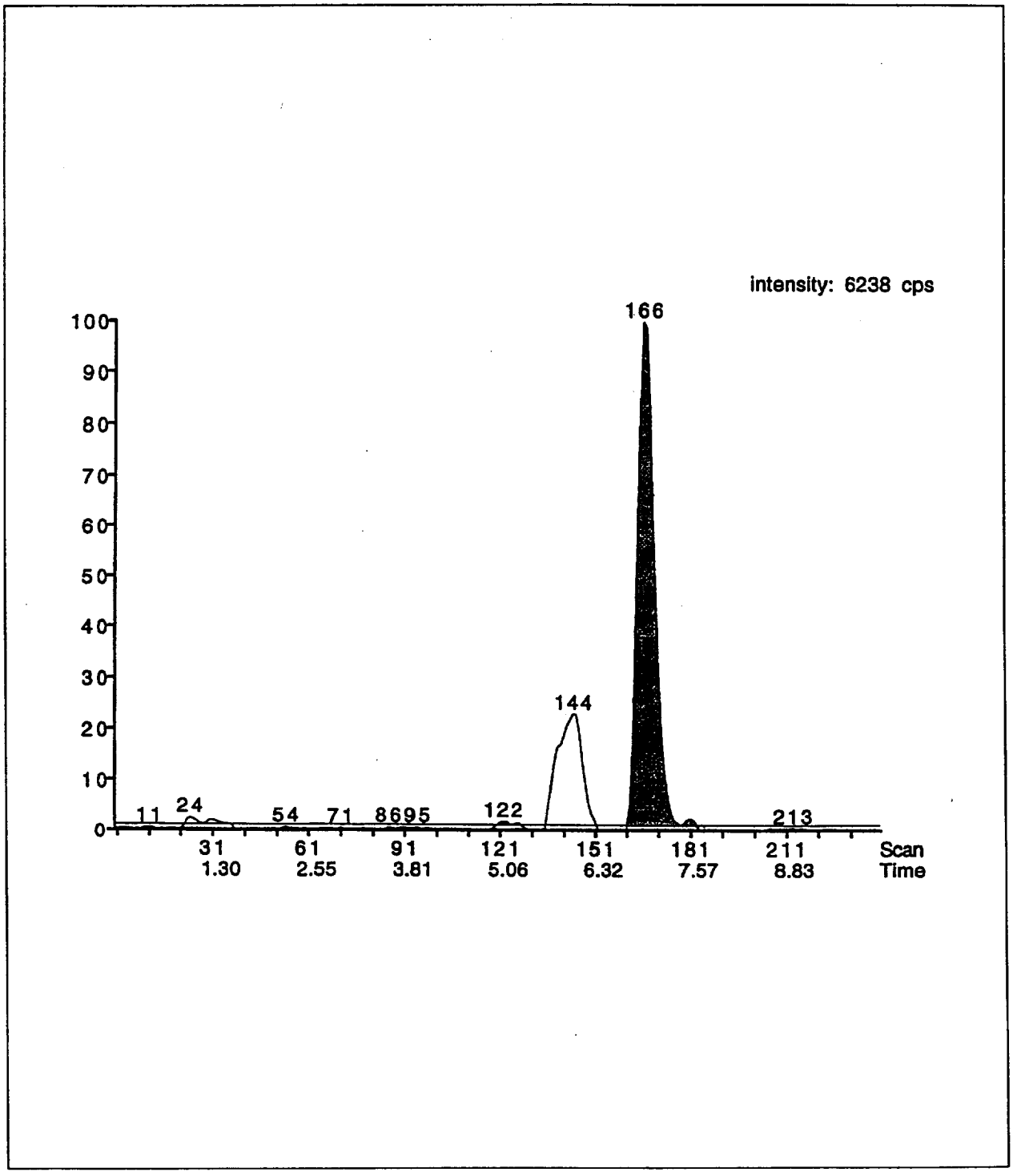


Figure 6. A representative chromatogram of a matrix fortification sample (454-102-MAS-1A).

Appendix III

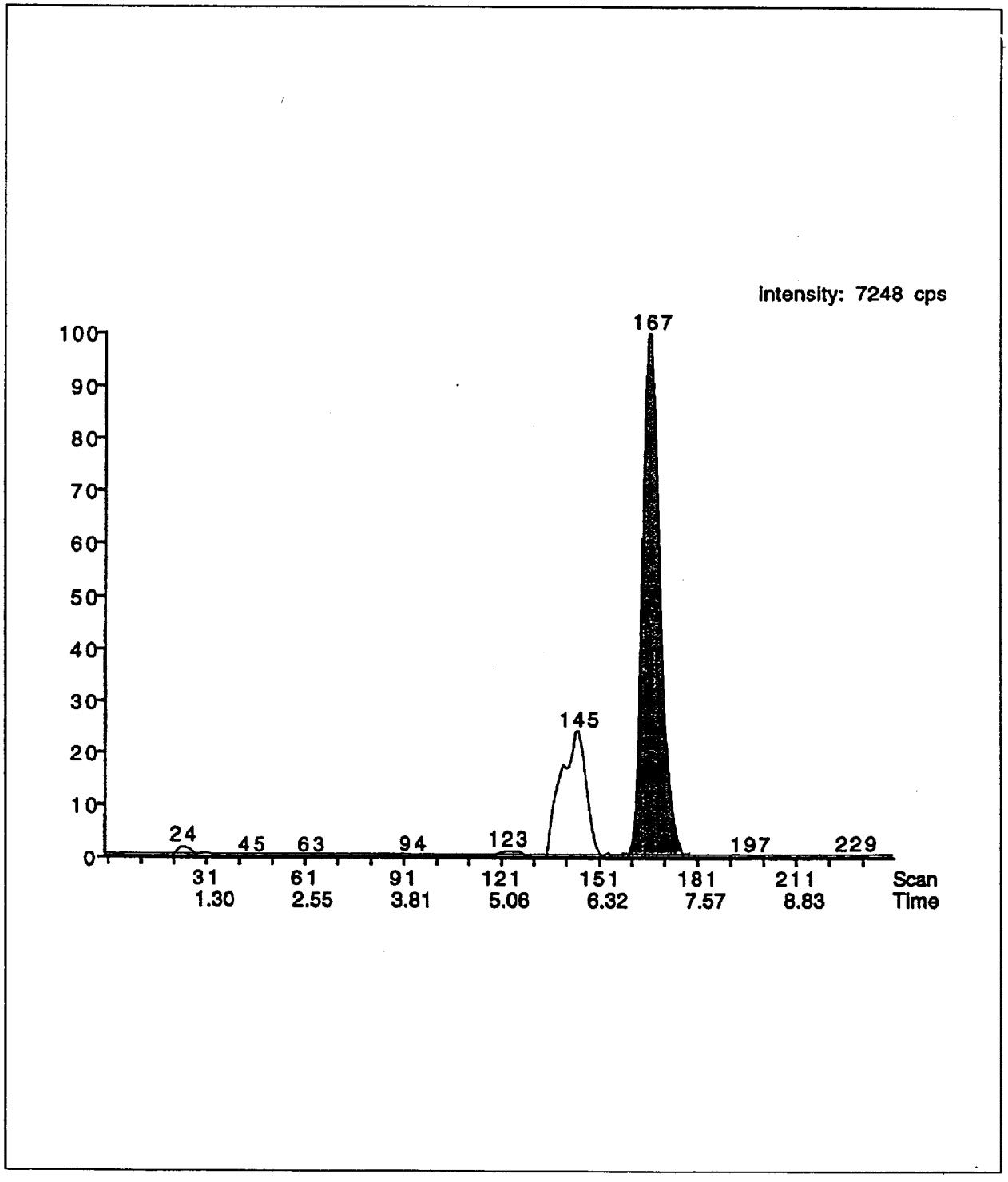


Figure 7. A representative chromatogram of a test sample (454-102-2).

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
9.1	0.0910	8999.9
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 Mallard 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*
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	0/5	0/5	0/5
0	6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Treatment										
9.1	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
9.1	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
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	0/5	0/5
146	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
293	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
293	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	2/5
586	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5
586	2	0/5	0/5	0/5	0/5	0/5	1/5	2/5	2/5	2/5
1171	1	0/5	0/5	0/5	0/5	1/5	4/5	5/5	5/5	5/5
1171	2	0/5	0/5	0/5	0/5	1/5	2/5	3/5	4/5	4/5

The LC50 value was calculated to be 628 ppm a.i. with a 95% confidence interval of 448 ppm a.i. to 958 ppm a.i.
* - No mortalities occurred in any of the treatment or control groups after Day 8.

APPENDIX VI

Individual Body Weights (g) from a Mallard
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 (0-8)
0	1	132	302	170	404	102	272
	2	136	292	156	381	89	245
	3	154	319	165	415	96	261
	4	158	319	161	429	110	271
	5	169	309	140	418	109	249
	Mean	150	308	158	409	101	260
	SD	15	12	12	18	9	12
0	1	136	282	146	399	117	263
	2	142	284	142	394	110	252
	3	152	299	147	409	110	257
	4	152	312	160	430	118	278
	5	177	345	168	484	139	307
	Mean	152	304	153	423	119	271
	SD	16	26	11	37	12	22
0	1	125	265	140	372	107	247
	2	134	282	148	371	89	237
	3	149	304	155	440	136	291
	4	154	292	138	378	86	224
	5	166	328	162	430	102	264
	Mean	146	294	149	398	104	253
	SD	16	24	10	34	20	26
0	1	124	271	147	376	105	252
	2	135	263	128	360	97	225
	3	151	300	149	408	108	257
	4	163	333	170	432	99	269
	5	170	351	181	453	102	283
	Mean	149	304	155	406	102	257
	SD	19	38	21	38	4	22

APPENDIX VI

Individual Body Weights (g) from a Mallard
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	107	222	115	298	76	191
	2	126	267	141	360	93	234
	3	116	203	87	266	63	150
	4	107	203	96	284	81	177
	5	111	248	137	348	100	237
	Mean	113	229	115	311	83	198
	SD	8	28	24	41	15	37
0	1	100	235	135	335	100	235
	2	100	232	132	328	96	228
	3	102	233	131	317	84	215
	4	108	267	159	383	116	275
	5	102	210	108	299	89	197
	Mean	102	235	133	332	97	230
	SD	3	20	18	32	12	29
Group Total	Mean	135	279	144	380	101	245
	SD	24	42	22	53	16	34

APPENDIX VI

Individual Body Weights (g) from a Mallard
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 (0-8)
9.1	1	123	244	121	349	105	226
	2	118	231	113	334	103	216
	3	151	286	135	399	113	248
	4	108	209	101	313	104	205
	5	119	231	112	332	101	213
	Mean	124	240	116	345	105	222
	SD	16	29	13	33	5	17
9.1	1	96	145	49	146	1	50
	2	123	250	127	345	95	222
	3	127	237	110	343	106	216
	4	112	216	104	316	100	204
	5	111	215	104	294	79	183
	Mean	114	213	99	289	76	175
	SD	12	41	29	83	43	71
Group Total	Mean	119	226**	108**	317*	91	198*
	SD	14	36	23	66	33	55

*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 Mallard
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
18.3	1	123	261	138	355	94	232
	2	141	297	156	421	124	280
	3	150	270	120	361	91	211
	4	152	286	134	390	104	238
	5	160	294	134	391	97	231
	Mean	145	282	136	384	102	238
	SD	14	16	13	27	13	25
18.3	1	130	240	110	327	87	197
	2	137	266	129	359	93	222
	3	145	268	123	366	98	221
	4	151	258	107	366	108	215
	5	171	333	162	433	100	262
	Mean	147	273	126	370	97	223
	SD	16	35	22	39	8	24
Group Total	Mean	146	277	131	377	100	231
	SD	14	26	18	32	11	25

APPENDIX VI

Individual Body Weights (g) from a Mallard
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
36.6	1	131	261	130	372	111	241
	2	132	256	124	356	100	224
	3	142	269	127	369	100	227
	4	160	270	110	353	83	193
	5	179	335	156	433	98	254
	Mean	149	278	129	377	98	228
	SD	21	32	17	33	10	23
36.6	1	131	230	99	316	86	185
	2	136	287	151	402	115	266
	3	147	263	116	379	116	232
	4	151	255	104	344	89	193
	5	160	324	164	427	103	267
	Mean	145	272	127	374	102	229
	SD	12	36	29	44	14	39
Group Total	Mean	147	275	128	375	100	228
	SD	16	32	22	37	12	30

APPENDIX VI

Individual Body Weights (g) from a Mallard
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
73.2	1	113	203	90	281	78	168
	2	113	219	106	288	69	175
	3	142	286	144	390	104	248
	4	154	290	136	379	89	225
	5	179	338	159	480	142	301
	Mean	140	267	127	364	96	223
	SD	28	56	28	82	29	55
73.2	1	109	222	113	302	80	193
	2	129	254	125	332	78	203
	3	139	243	104	340	97	201
	4	147	196	49	175	-21	28
	5	204	352	148	458	106	254
	Mean	146	253	108	321	68	176
	SD	36	59	37	101	51	86
Group Total	Mean	143	260	117*	343	82	200*
	SD	30	55	33	90	42	73

*Statistically different from the control group at $p < 0.05$ (Dunnett's t-test).

APPENDIX VI

Individual Body Weights (g) from a Mallard
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
146	1	108	169	61	236	67	128
	2	138	248	110	330	82	192
	3	151	271	120	375	104	224
	4	151	254	103	341	87	190
	5	164	282	118	380	98	216
	Mean	142	245	102	332	88	190
	SD	21	44	24	58	14	38
146	1	115	204	89	280	76	165
	2	139	219	80	287	68	148
	3	144	229	85	348	119	204
	4	159	261	102	358	97	199
	5	158	285	127	374	89	216
	Mean	143	240	97	329	90	186
	SD	18	33	19	43	20	29
Group Total	Mean	143	242	100**	331	89	188**
	SD	19	37	21	48	16	32

**Statistically different from the control group at $p < 0.01$ (Dunnett's t-test).

APPENDIX VI

Individual Body Weights (g) from a Mallard
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
293	1	115	142	27	179	27	64
	2	139	119	-20	165	-20	26
	3	140	156	16	217	16	77
	4	155	187	32	291	32	136
	5	112	134	22	203	22	91
	Mean	132	148	15	211	15	79
	SD	18	26	21	49	21	40
293	1	123	205	82	289	82	166
	2	120	183	63	273	63	153
	3	151	139	-12	-	-12	-
	4	106	158	52	223	52	117
	5	124	185	61	138	61	14
	Mean	125	174	49	231	49	113
	SD	16	26	36	68	36	69
Group	Mean	129	161**	32**	220**	57**	94**
Total	SD	17	28	33	55	44	54

(-) = 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 Mallard
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
586	1	122	105	-17	126	21	4
	2	135	143	8	209	66	74
	3	152	160	8	217	57	65
	4	154	129	-25	-	-	-
	5	165	195	30	143	-52	-22
	Mean	146	146	1	174	23	30
	SD	17	34	22	46	54	47
586	1	105	114	9	159	45	54
	2	120	130	10	185	55	65
	3	149	-	-	-	-	-
	4	153	128	-25	185	57	32
	5	182	128	-54	-	-	-
	Mean	142	125	-15	176	52	50
	SD	30	7	31	15	6	17
Group Total	Mean	144	137**	-6**	175**	36**	39**
	SD	23	27	26	34	41	36

(-) = 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 Mallard
Acute Dietary Toxicity Study with PFOS
Page 10

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change Day 0-5	Day 8	Change Day 5-8	Total Change
1171	1	124	88	-39	-	-	-
	2	140	-	-	-	-	-
	3	145	96	-49	-	-	-
	4	150	118	-32	-	-	-
	5	166	-	-	-	-	-
	Mean	145	100	-40	-	-	-
	SD	15	17	9	-	-	-
1171	1	128	87	-41	-	-	-
	2	110	-	-	-	-	-
	3	145	132	-13	-	-	-
	4	157	-	-	-	-	-
	5	200	154	-46	185	31	-15
	Mean	148	124	-33	185	31	-15
	SD	34	34	18	-	-	
Group Total	Mean	147	112**	-37**	185[2]	31[2]	15[2]
	SD	25	28	13	-	-	-

(-) = No data available due to mortality.
[2]No mortalities occurred in any of the treatment or control groups after Day 8.
**Statistically different from the control group at $p < 0.01$ (Dunnett's t-test).

APPENDIX VI

Individual Body Weights (g) from a Mallard
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 (8-22)
0	1	404	721	317	895	174	491
	2	381	633	252	780	147	399
	3	415	685	270	861	176	446
	4	429	692	263	822	130	393
	5	418	679	261	847	168	429
	Mean	409	682	273	841	159	432
	SD	18	32	26	43	20	40
0	1	399	606	207	810	204	411
	2	394	604	210	791	187	397
	3	409	638	229	819	181	410
	4	430	543	113	730	187	300
	5	484	752	268	924	172	440
	Mean	423	629	205	815	186	392
	SD	37	77	57	70	12	54
0	1	372	583	211	779	196	407
	2	371	613	242	823	210	452
	3	440	636	196	859	223	419
	4	378	594	216	794	200	416
	5	430	627	197	816	189	386
	Mean	398	611	212	814	204	416
	SD	34	22	19	31	13	24
Group Total	Mean	410	640	230	823	183	413
	SD	30	56	47	49	24	42

APPENDIX VI

Individual Body Weights (g) from a Mallard
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	Total Change
9.1	1	349	602	253	821	219	472
	2	334	549	215	730	181	396
	3	399	616	217	802	186	403
	4	313	562	249	767	205	454
	5	332	546	214	744	198	412
	Mean	345*	575	230	773	198	427
	SD	33	32	20	38	15	34

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	355	611	256	811	200	465
	2	421	693	272	938	245	517
	3	361	578	217	713	135	352
	4	390	626	238	794	168	404
	5	391	618	231	799	181	408
	Mean	384	625	242	811	186	427
	SD	27	42	22	81	41	62

*Statistically different from the control group at $p < 0.05$ (Dunnett's t-test).

APPENDIX VI

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

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	372	677	305	942	265	570
	2	356	607	251	817	210	461
	3	369	613	244	793	180	424
	4	353	559	206	746	187	393
	5	433	642	209	840	198	407
	Mean	377	620	243	828	208	451
	SD	33	44	40	73	34	71

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	281	482	201	648	166	367
	2	288	480	192	646	166	358
	3	390	636	246	864	228	474
	4	379	599	220	796	197	417
	5	480	699	219	955	256	475
	Mean	364	579	216	782	203	418
	SD	82	97	21	135	39	56

APPENDIX VI

Individual Body Weights (g) from a Mallard
Acute Dietary Toxicity Study with PFOS
Page 14

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change	Total Change
146	1	236	475	239	659	184	423
	2	330	569	239	739	170	409
	3	375	631	256	738	107	363
	4	341	570	229	754	184	413
	5	380	576	196	550	-26	170
	Mean	332*	564	232	688*	124*	356
	SD	58	56	22	86	90	106

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change Day 15-22	Total Change
293	1	179	447	268	731	284	552
	2	165	415	250	646	231	481
	3	217	526	309	748	222	531
	4	291	516	225	704	188	413
	5	203	430	227	676	246	473
	Mean	211**	467**	256	701*	234	490
	SD	49	51	35	41	35	54

*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 Mallard
Acute Dietary Toxicity Study with PFOS
Page 14

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change Day 15-22	Total Change
586	1	126	308	182	525	217	399
	2	209	469	260	706	237	497
	3	217	460	243	675	215	458
	4	-	-	-	-	-	-
	5	143	340	197	546	206	403
	Mean	174	394	221	613	219	439
	SD	46	82	37	91	13	47

Experimental Group (ppm a.i.)	Bird	Day 8	Day 15	Change Day 8-15	Day 22	Change Day 15-22	Total Change
1171	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	185	383	198	634	251	449
	Mean	185[2]	383[2]	198[2]	634[2]	251[2]	449[2]
	SD	-	-	-	-	-	-

[2]n=1, could not be evaluated statistically using Dunnett's t-test.

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Mallard 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	109	145
	2	92	140
	3	85	117
	4	93	132
	5	82	100
	6	94	116
	Mean	92	125
	SD	10	17

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Mallard 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
9.1	1	73	130
	2	72	104
	Mean	73	117
18.3	1	90	135
	2	91	128
	Mean	91	132
36.6	1	103	129
	2	85	120
	Mean	94	125
73.2	1	79	109
	2	74	94
	Mean	77	101
146	1	89	102
	2	121	217
	Mean	105	159
293	1	28	61
	2	59	65
	Mean	44	63
586	1	40	43
	2	32	67
	Mean	36	55
1171	1	23	25
	2	22	26
	Mean	22	25

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Mallard 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	185	159
	2	161	182
	3	166	199
	Mean	171	180
	SD	13	20

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Mallard Acute Dietary Toxicity Study with PFOS
Page 4

Experimental Group (ppm a.i.)	Pen	Exposure Period Day 8-15	Post-Exposure Period Day 15-22
9.1	1	172	198
18.3	1	186	204
36.6	1	165	179
73.2	1	148	173
146	1	159	164
293	1	109	132
586	1	114	143
1171	1	106	154

APPENDIX VIII

CHANGES TO PROTOCOL

This study was conducted in accordance with the approved Protocol with the following changes:

1. The protocol was amended to add an additional test concentration of 10 ppm a.i..

2. 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.

3. The protocol was amended to change the test concentrations from 0, 10, 20, 40, 80, 160, 320, 640 and 1280 ppm a.i., to 0, 9.1, 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. Individually numbered wing bands were used to uniquely identify each duckling. The protocol required leg bands to be used to identify the birds.

5. 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.

6. The final temperature from one brooder unit on Day 8 and all brooder units on Day 22 were not recorded.

7. The afternoon observations were inadvertently not recorded for birds in a single brooder unit on May 8, 1999.

8. Observations for control birds from two brooder levels were inadvertently not recorded on Day 6 of the test. Temperatures for these brooder levels were also not recorded on this day. Observations for control birds from one brooder level were inadvertently not recorded on Day 22 of the test.

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) Tim 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 MALLARD

PROTOCOL NO.:454/111098/MLCSDT.WC/OPPTS AMENDMENT NO.: 1

SPONSOR:3M Corporation PROJECT NO.: 454-102

EFFECTIVE DATE: April 22, 1999

AMENDMENT:

On page 2 of the protocol add an additional test concentration of 10 ppm a.i.

REASON:

Sponsor request.

Study Director — Date: 9/3/99

Laboratory Management — Date: 9/3/99

Sponsor Principal Contact — Date: 10/28/99

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A DIETARY LC50 STUDY WITH THE MALLARD

PROTOCOL NO.: 454/111098/MLCSDT.WC/OPPTS AMENDMENT NO.: 2

SPONSOR: 3M Corporation PROJECT NO.: 454-102

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.

Study Director — Date: 9/3/99

Laboratory Management — Date: 9/3/99

Sponsor Principal Contact — Date: 10/28/99

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PFOS: A DIETARY LC50 STUDY WITH THE MALLARD

PROTOCOL NO.: 454/111098/MLVSDT.WC/OPPTS DEVIATION NO.: 1

SPONSOR: 3M Corporation PROJECT NO.: 454-102

DATE OF DEFACTO DEVIATION: April 22-May 14, 1999

DEVIATION:

Individually numbered wing bands were used to uniquely identify each duckling. The protocol stated that birds would be identified by colored leg bands.

REASON:

Protocol error. Due to the extended duration of this study, ducklings would have outgrown leg bands. The protocol should have been clarified to indicate that all ducklings would be identified by wing bands.

IMPACT:

None. Wing bands are routinely used to identify ducklings and all birds were uniquely identified.

Sean P. Gallagher
STUDY DIRECTOR

9/3/99
DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/3/99
DATE

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PFOS: A Dietary LC50 Study with the Mallard

PROTOCOL NO.: 454/111098/MLCSDT.WC/OPPTS DEVIATION NO.: 2

SPONSOR: 3M Corporation PROJECT NO.: 454-102

DATE OF DEVIATION: April 30 and May 14, 1999

DEVIATION:

Blood samples collected on Day 8 and Day 22 of the test were stored in 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 effeciently draw blood by syringe. Blood was draw by decapitating birds and draining blood into test tubes. Heparin was not added to blood samples due to a Sponsor request to seperate serum from the blood at Wildlife International Ltd. Seperation of serum requires coagulation of blood samples.

IMPACT:

None.

Dean P. Dallmer
STUDY DIRECTOR

9/3/99
DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/3/99
DATE

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PFOS: A Dietary LC50 Study with the Mallard

PROTOCOL NO.: 454/111098/MLCSDT.WC/OPPTS DEVIATION NO.: 3

SPONSOR: 3M Corporation PROJECT NO.: 454-102

DATE OF DEVIATION: April 30 and May 14, 1999

DEVIATION:

On April 30, 1999, the final temperature from a brooder unit housing birds in the 640 ppm a.i. treatment level was not recorded prior to termination of those birds. Additionally, on May 14 the final temperatures on all brooder units were not recorded prior to termination of those birds.

REASON:

Biologist oversight. 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 occassions where temperatures were missed neglected to record temperatures for empty pens that housed birds earlier the same day.

IMPACT:

None. Temperatures recorded for brooders the day prior to the missed data indicated that the brooders were functioning properly.

Leon P. Walbaugh

STUDY DIRECTOR

9/3/99

DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/3/99

DATE

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PFOS: A Dietary LC50 Study with the Mallard

PROTOCOL NO.: 454/111098/MLCSDT.WC/OPPTS

DEVIATION NO.: 4

SPONSOR: 3M Corporation

PROJECT NO.: 454-102

DATE OF DEVIATION: May 8, 1999

DEVIATION:

On May 8, 1999, the afternoon observations for unit S17-1 housing birds from the 320 ppm a.i. treatment level were inadvertently not recorded.

REASON:

Biologist oversight.

IMPACT:

None. Observations from the morning of May 8 and May 9, 1999 indicate that all birds in this level were normal in appearance and behavior. Since the temperature for that unit was recorded at the same time observations were performed, it is apparent that the birds were observed, but the observation not recorded.

STUDY DIRECTOR — DATE 9/3/99

WILDLIFE INTERNATIONAL LTD. MANAGEMENT — DATE 9/3/99

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PFOS: A DIETARY LC50 STUDY WITH THE MALLARD

PROTOCOL NO.: 454/111098/MLVSDT.WC/OPPTS DEVIATION NO.: 5

SPONSOR: 3M Corporation PROJECT NO.: 454-102

DATE OF DEFACTO DEVIATION: April 28 and May 14, 1999

DEVIATION:

On April 28, 1999, Day 6 of the test, observations for control group birds in brooder levels S62-2 and S62-3 were inadvertently not recorded. Temperatures for these brooder levels were also not recorded on this date. On May 14, 1999, Day 22 of the test, observations for control group birds in level S70-3 were inadvertently not recorded. The protocol required that birds would be observed at least once daily and that the brooder compartment temperature would be recorded once daily.

REASON:

Biologist oversight.

IMPACT:

With the exception of the missed observations, observations indicate all control birds were normal in behavior and appearance and brooder temperatures were within the acceptable range for the duration of the test. It is reasonable to expect that birds were normal in behavior and appearance and that brooder temperatures were within the acceptable range at the time the observations were not recorded. Therefore, this deviation caused no adverse impact on the outcome and interpretation of the study.

Dean P. Gallagher — 3/21/00

STUDY DIRECTOR — DATE

Linda R. Westell — 3/21/00

WILDLIFE INTERNATIONAL LTD. MANAGEMENT — DATE

SOP DEVIATION

STUDY TITLE: PFOS: A DIETARY LC50 STUDY WITH THE MALLARD

PROTOCOL NO.: 454/111098/MLCSDT.WC/OPPTS

SOP NO.: 415 DEVIATION NO.: 1

SPONSOR: 3M Corporation PROJECT NO.: 454-102

DATE OF DEFACTO DEVIATION: April 23, 1998

DEVIATION:

On April 23, 1999 the temperature of one brooder level used to house the 1280 ppm a.i. treatment group was 1°F below the specified minimum of 95°F. There are no records of the responsible technician or biologist making a thermostat adjustment to correct the temperature. However, on the following day the temperature was within normal 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

9/3/99
DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/3/99
DATE

SOP DEVIATION

STUDY TITLE: PFOS: A DIETARY LC50 STUDY WITH THE MALLARD

PROTOCOL NO.: 454/111098/MLCSDT.WC/OPPTS

SOP NO.: 423.1A DEVIATION NO.: 2

SPONSOR: 3M Corporation PROJECT NO.: 454-102

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

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.

Sean P. Mallaghan
STUDY DIRECTOR

9/3/99
DATE

WILDLIFE INTERNATIONAL LTD. MANAGEMENT

9/3/99
DATE

PROTOCOL

PFOS: A DIETARY LC50 STUDY WITH THE MALLARD

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

FIFRA Subdivision E, Section 71-2

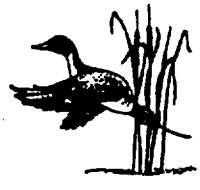
OECD Guideline 205

3M Lab Request No. U2723

Submitted to

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

WILDLIFE INTERNATIONAL LTD.



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

November 10, 1998

PFOS: A DIETARY LC50 STUDY WITH THE MALLARD

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.:	454-102	Study Room:	76
Test Concentrations:	① 0, 10, 20, 40, 80, 160, 320, 640 and 1280 ppm a.i.	Int/Date:	SD 4/21/99
Test Substance No.:	4675, 4526 ~~analytical~~ internal standard	Receipt Date:	① SD 4/22/99 10/29/98, 7/2/98

PROTOCOL APPROVAL

Sean P. Gallagher
STUDY DIRECTOR — DATE 4/21/99

[signature]
LABORATORY MANAGEMENT — DATE 4/22/99

Susan A. Beach
SPONSOR'S REPRESENTATIVE — DATE 11/30/98

① SD 4/22/99 The client requested the addition of a 10 ppm a.i. level prior to test initiation.

OBJECTIVE

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

SUMMARY

Groups of ten mallard ducklings, 5-10 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 mallard ducklings (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 mallard represents an ecologically significant and widely distributed species in the United States. The mallard 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 mallards will be from the same hatch and will be 5-10 days old at test initiation. Only those that are apparently healthy will be used in the test. Body weights may vary between 75 and 300 grams depending on the age of the cohort of birds used (e.g. 5 versus 10 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 Whistling Wings, 113 Washington Street, Hanover, IL 61041 or from another reputable supplier. 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). 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. However, 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. If necessary, appropriate diet will be added as needed. 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. 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 Safeguard Products, Inc. or equivalent. Each pen measures approximately 62 X 92 X 25.5 cm high. The external walls, floors and ceilings of each pen are constructed of vinyl coated wire grid. 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. On Day 8 of the test, birds in one pen from each treatment group (5 birds) and three pens from the control group (15 birds) will be euthanized. Remaining birds will be maintained for an additional 14 days. During that period, remaining birds may be separated into two pens.

During the first eight days of the test, the temperature in the brooding compartment of the pen will be maintained at approximately 29°C. Brooders will be turned off during the last 14 days of the test. 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 first eight days of the test. The ambient room temperature will range between approximately 15-30°C. 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.

Animal Body Weights/Feed Consumption

Individual body weights will be measured at the initiation of the test, at the end of the exposure period on Day 5, and on Day 8. Average estimated feed consumption will be determined by pen for each treatment and control group for the exposure (Days 0-5) and post-exposure observation (Days 6-8) periods. For those birds maintained for an additional 14 days, body weight and feed consumption measurements will be performed weekly and at test termination. The accuracy of feed consumption values may be affected by unavoidable wastage of feed by birds. No attempts will be made to quantify the amount of wasted feed, as it is normally scattered and mixed with water and excreta.

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. Necropsy findings.
11. Statistical calculations.
12. Analytical chemistry methods, results and chromatograms, if applicable.
13. 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.

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

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

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 ____ No X

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International Ltd.

Ambient room temperature

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes ____ No X

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.