PFOS:
A DIETARY LC50 STUDY WITH THE NORTHERN BOBWHITE

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

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

FIFRA Subdivision E, Section 71-2

OECD Guideline 205

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STUDY INITIATION: April 21, 1999

STUDY COMPLETION: April 26, 2000

SUBMITTED TO

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

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

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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

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QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, (OCDE/GD (92) 32, Environment Monograph No. 45, Paris, 1992); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all audits and inspections and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO: STUDY DIRECTOR	MANAGEMENT
Test Substance Prep. & Analytical Sampling	April 22, 1999	April 22, 1999	April 23, 1999
Matrix Fortification	April 22, 1999	April 22, 1999	April 23, 1999
Feed Consumption & Analytical Sampling	April 27, 1999	April 27, 1999	May 4, 1999
Analytical Data and Draft Report	July 7, 8, 9, 1999	July 9, 1999	July 16, 1999
Biology Data and Draft Report	August 26, 27, 30, 31, 1999	August 31, 1999	September 13, 1999
Final Report	April 17-18, 2000	April 18, 2000	April 19, 2000

Susan L. Coleman DATE 4-19-00

Susan L. Coleman
Senior Quality Assurance Representative

REPORT APPROVAL

SPONSOR: 3M Corporation

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

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SPONSOR: 3M Corporation

TEST SUBSTANCE: PFOS

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454-103

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

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

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

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

TEST ANIMALS: Northern Bobwhite (*Colinus virginianus*)

AGE TEST ANIMALS: 10 days of age at test initiation

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

STUDY COMPLETION: April 26, 2000

INTRODUCTION

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

OBJECTIVE

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

MATERIALS AND METHODS

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

Test Substance

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

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

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

Treatment Groups

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

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

Duration of the Test

The primary phases of this test and their durations were:

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

Test Birds

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

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

Animal Diet

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

Diet Preparation

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

Diet Sampling

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

Analytical Method

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

Avian diet samples were extracted with methanol. Methanol was added to a requisite quantity of feed contained in a French-square glass bottle. Bottles were capped and shaken on a shaker table. Samples were vacuum filtered using qualitative filter paper. The retained feed was rinsed three times with methanol into the filtrate. The filtrate was transferred to a volumetric flask and brought to volume with methanol. As appropriate, samples were further diluted with methanol. Each sample then was diluted with a 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v) so that they fell within the calibration range of the PFOS methodology. A method flowchart is provided in Appendix III, Figure 1.

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

Calibration standards of PFOS prepared in a 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v), ranging in concentration from 0.00229 to 0.0457 mg a.i./L were analyzed with the samples. The same and most prominent peak response for PFOS was utilized to monitor PFOS in all calibration, quality control, and study samples. No attempt was made to quantify PFOS on the basis of individual isomeric components. Linear regression equations were generated using peak area response ratios (PFOS : internal standard) versus the respective concentration ratios (PFOS : internal standard) of the calibration standards. A typical calibration curve is presented in Appendix III, Figure 2. The concentration of PFOS in the samples was determined by substituting the peak area response ratios into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards are presented in Appendix III, Figures 3 and 4, respectively.

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

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

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

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

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

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

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

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

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

Note: I.S. = internal standard.

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

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

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

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

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

Housing and Environmental Conditions

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

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

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

Observations

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

Animal Body Weights/Feed Consumption

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

Gross Necropsy

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

Statistical Analyses

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

RESULTS

Diet Analysis

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

Mortalities and Clinical Observations

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

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

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

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

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

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

Body Weight and Feed Consumption

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

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

Gross Necropsy

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

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

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

CONCLUSION

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

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TABLE 1

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

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

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

TABLE 2
Page 1

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

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

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

TABLE 2
Page 2

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

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

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

TABLE 3
Page 1

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

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

(-) = No data available due to mortality.

TABLE 3
Page 2

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

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

(-) = No data available due to mortality.

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

Birds that died during the course of the study

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

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

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

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

*- Bird was euthanized due to a broken leg.

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

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

APPENDIX I

Certificate Of Analysis

FC-95, Lot 217

March 9, 2000

Richard M. Payfer

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

$C_2F_5SO_3^-K^+$	0.04 %
$C_3F_7SO_3^-K^+$	0.83 %
$C_4F_9SO_3^-K^+$	1.38 %
$C_5F_{11}SO_3^-K^+$	1.30 %
$C_6F_{13}SO_3^-K^+$	3.71 %
$C_7F_{15}SO_3^-K^+$	1.19 %
$C_8F_{17}SO_3^-K^+$	90.49 %
$C_9F_{19}SO_3^-K^+$	0.49 %
$C_{10}F_{21}SO_3^-K^+$	0.13 %
$C_{11}F_{23}SO_3^-K^+$	0.04 %
CF_3-$CO_2^-K^+$	0.05 %
$SF_5C_4F_{16}SO_3^-K^+$	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]
Number (454-103-)	Type	(ppm a.i.)
MAB-1	Matrix Blank	< LOQ
MAB-2	Matrix Blank	< LOQ

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

APPENDIX III

Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

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

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

APPENDIX III

Table 4

Homogeneity of PFOS in Avian Diet

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

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

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

APPENDIX III

Table 5

Verification of PFOS Concentrations in Avian Diet

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

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

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

[2]Result obtained from Table 4.

APPENDIX III

Table 6

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

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

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

APPENDIX III

METHOD OUTLINE FOR THE ANALYSIS OF PFOS IN AVIAN DIET

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

↓

Dry Mix Technique

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

↓

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

↓

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

↓

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

↓

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

↓

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

↓

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

↓

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

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

↓

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

↓

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

↓

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

↓

Prepare appropriate dilution(s) to bring final concentration into the calibration range of the LCMS methodology.
Use methanol for intermediate dilutions, if required.
For all final dilutions use 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v).

Ampulate and submit samples for LC/MS analysis.

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

APPENDIX III

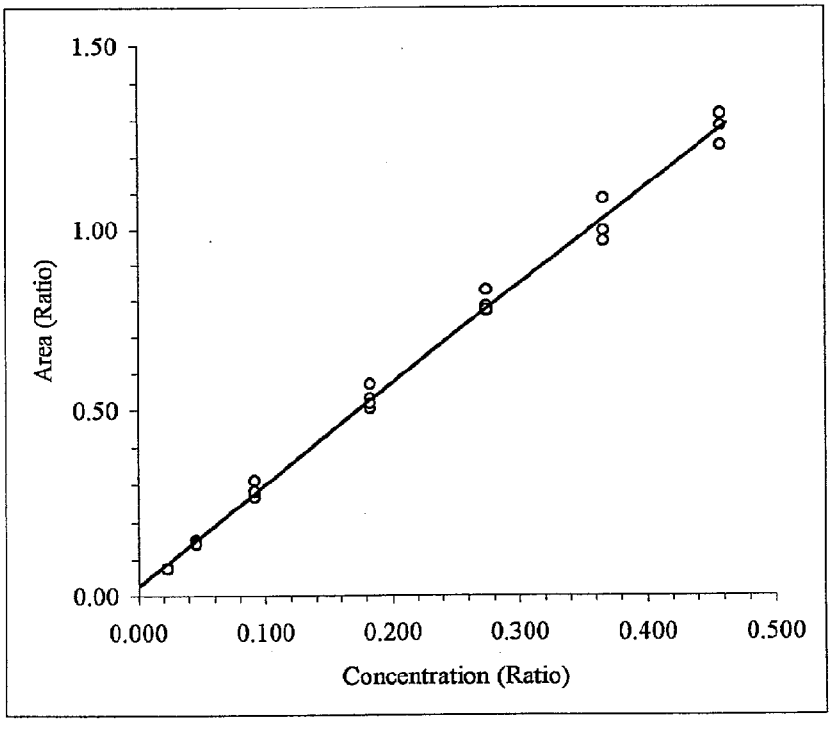

Figure 2. A typical calibration curve for PFOS. Slope = 2.77397; Intercept = 0.01894; r = 0.9981. Curve is weighted (1/x).

APPENDIX III

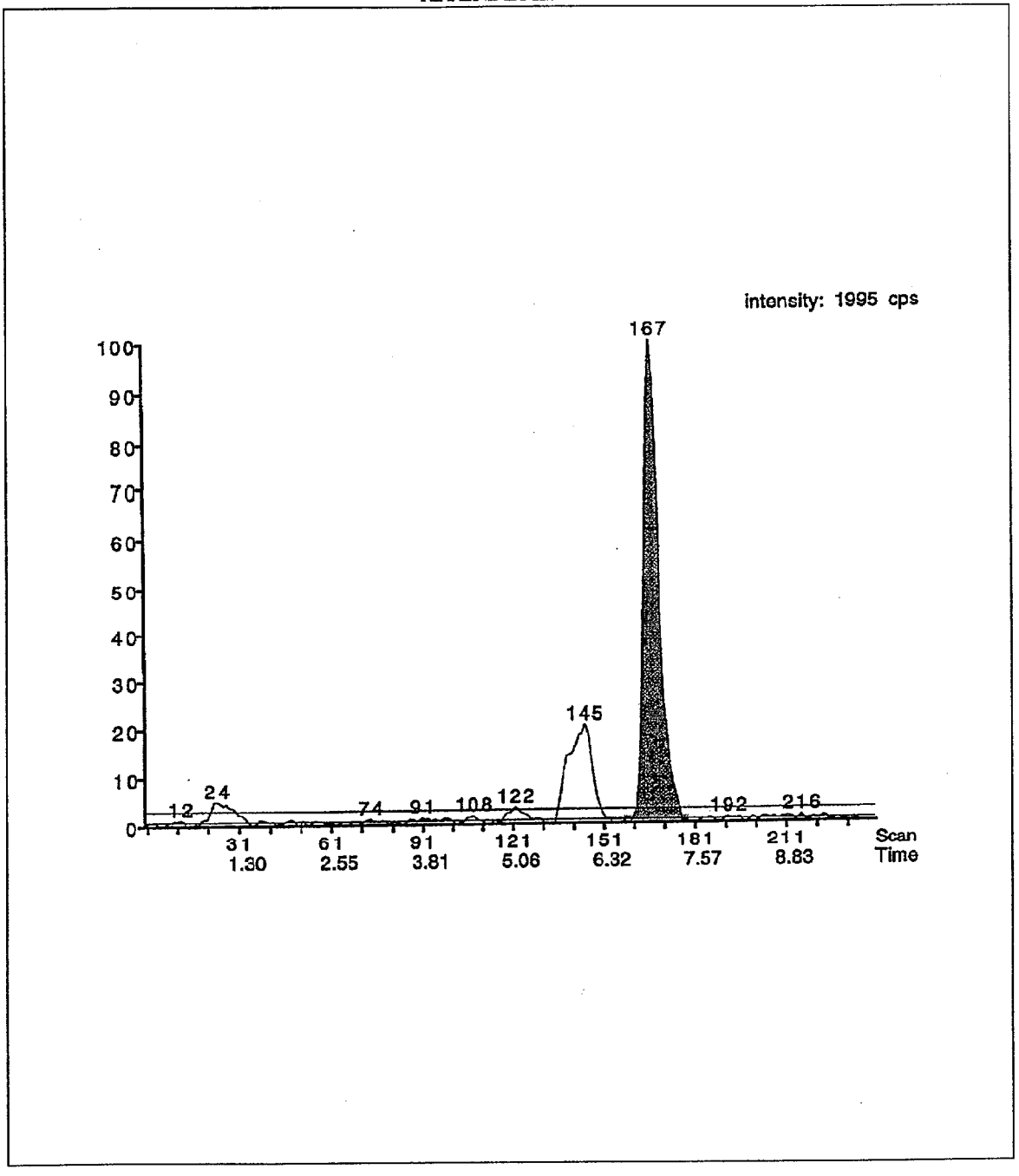

Figure 3. A representative ion chromatogram of a low-level (0.00229 mg a.i./L) PFOS standard.

APPENDIX III

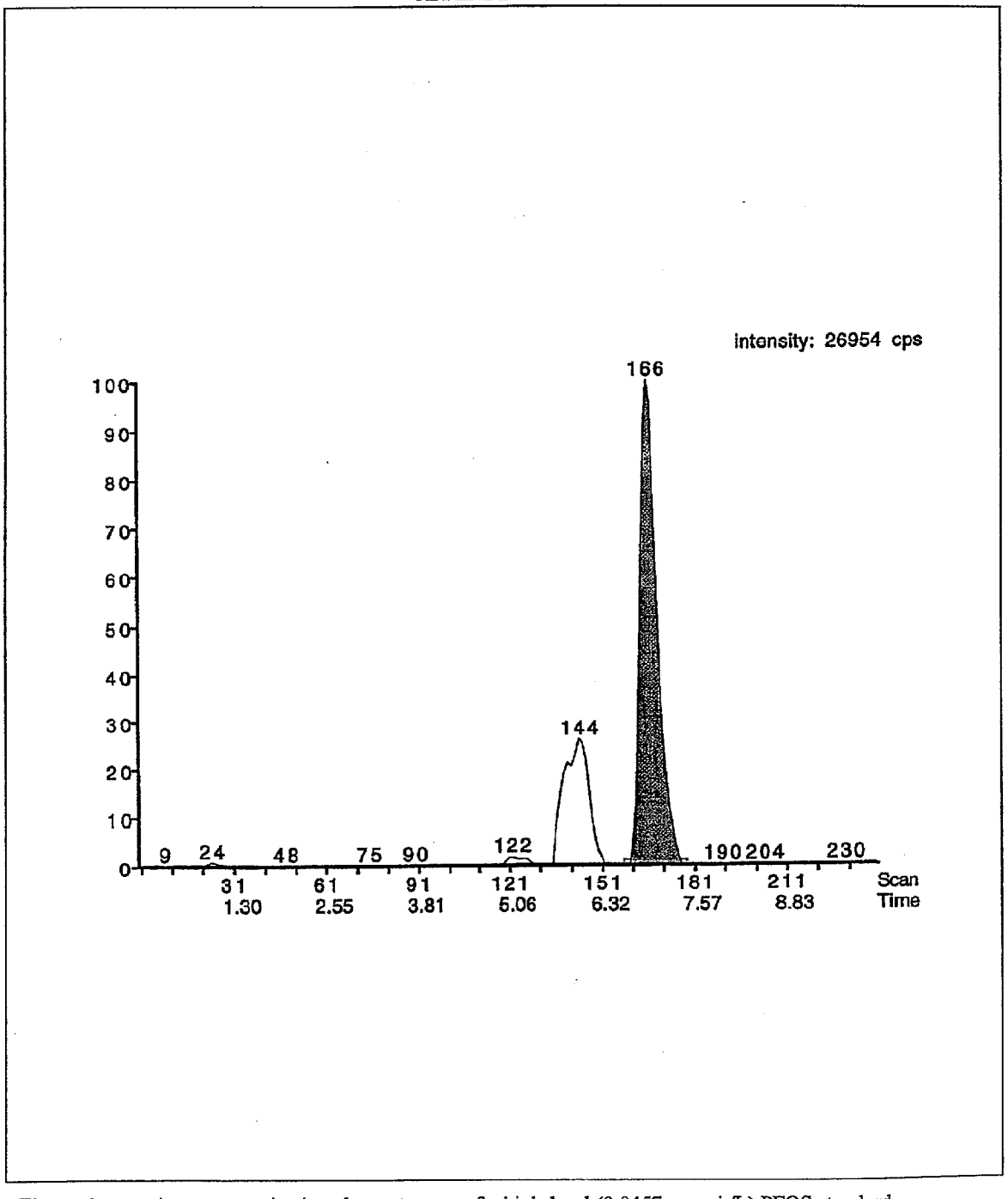

Figure 4. A representative ion chromatogram of a high-level (0.0457 mg a.i./L) PFOS standard.

APPENDIX III

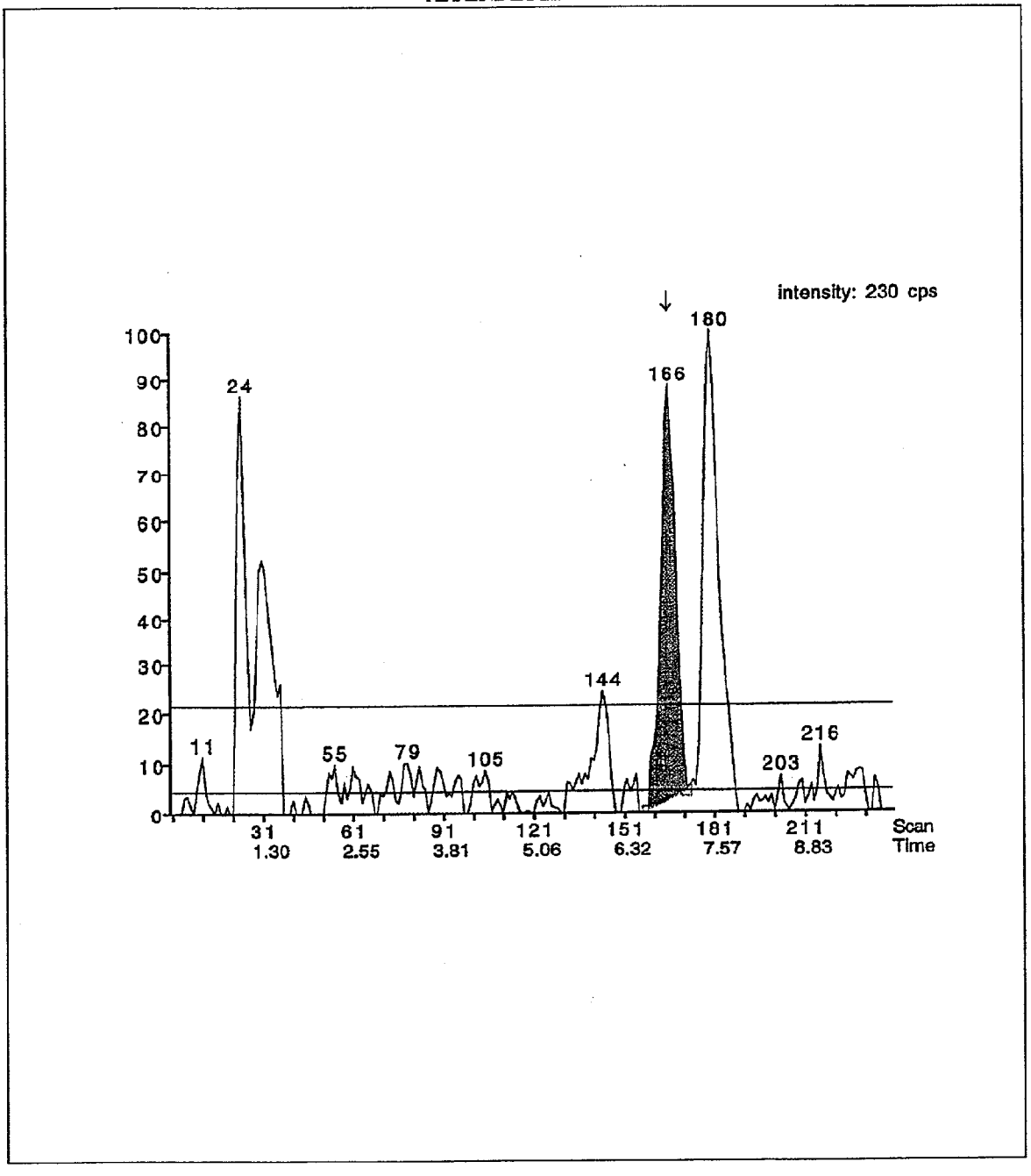

Figure 5. A representative chromatogram of a matrix blank sample (454-103-MAB-1). The arrow indicates the retention time of PFOS.

APPENDIX III

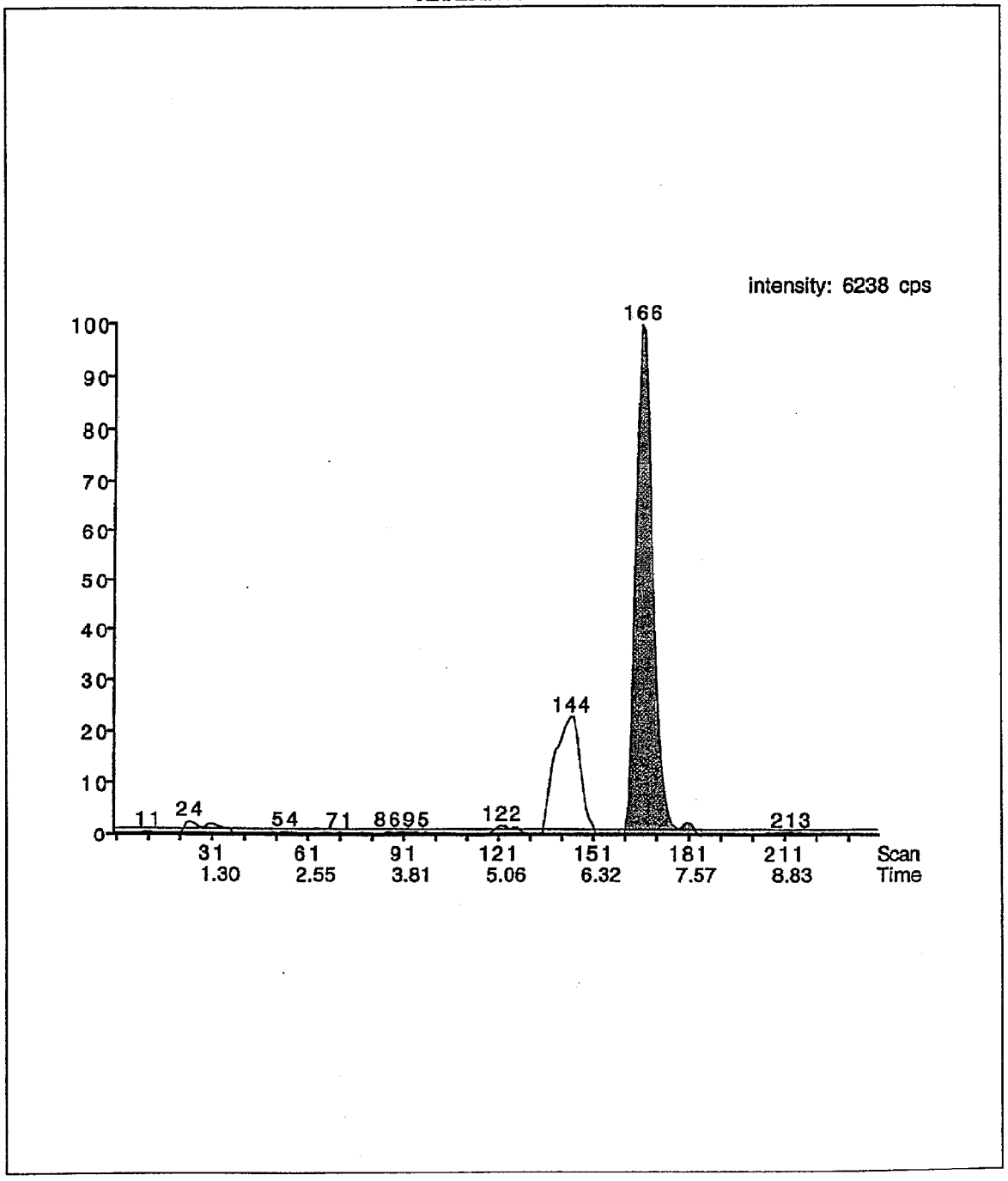

Figure 6. A representative chromatogram of a matrix fortification sample (454-103-MAS-1A).

APPENDIX III

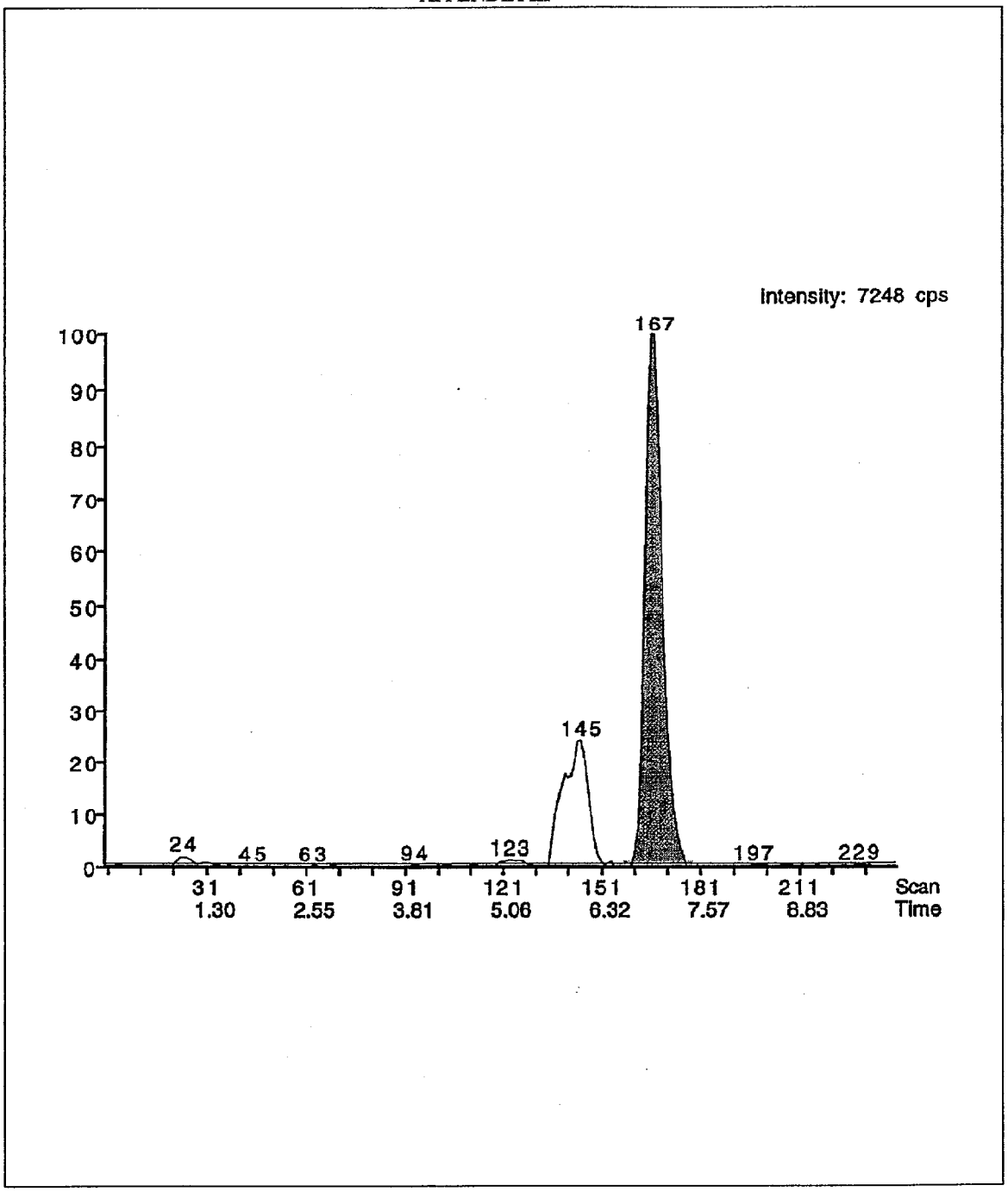

Figure 7. A representative chromatogram of a test sample (454-103-2).

APPENDIX IV
DIET PREPARATION

Weight and volume of constituents used to prepare test diets:

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

Diets were prepared as follows:

- 5000.0 g of basal ration was weighed into a tared Hobart mixing bowl.
- The test substance was weighed in a tared weigh boat.
- Approximately 100 g of basal ration was taken from the mixing bowl and placed in a Waring blender.
- The test substance was added to the blender and the weigh boat was rinsed with additional ration, with the rinse also being placed in the blender.
- The blender contents were blended for approximately 60 seconds and transferred to the mixing bowl. The blender was rinsed with additional ration, with the rinse also being placed in the mixing bowl.
- The bowl was placed on a Hobart mixer and the contents were mixed for approximately six minutes. The remaining ration as added to the bowl and the contents were mixed for six more minutes.
- The diet was transferred to a labelled paper feed bag.

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

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

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

APPENDIX VI

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

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

APPENDIX VI

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

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

APPENDIX VI

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

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

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

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

APPENDIX VI

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

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

APPENDIX VI

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

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

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

APPENDIX VI

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

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

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

APPENDIX VI

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

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

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

APPENDIX VI

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

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

(-) = No data available due to mortality.

APPENDIX VI

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

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

APPENDIX VI

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

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

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

APPENDIX VI

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

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

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

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

APPENDIX VI

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

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

(-) = No data available due to mortality.

APPENDIX VII

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

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

APPENDIX VII

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

Page 2

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

- No data available due to adult mortality.

APPENDIX VII

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

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

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

APPENDIX VIII

CHANGES TO PROTOCOL

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

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

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

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

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

APPENDIX IX

PERSONNEL INVOLVED IN THE STUDY

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

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