24) OECD 205-OPPTS 850.2200,
Acute avian toxicity (Mallard),
454-113

T-7485:
A DIETARY LC50 STUDY WITH THE MALLARD

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454-113
3M LAB PROJECT NO.: E00-1429

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

OECD Guideline 205

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STUDY INITIATION: June 4, 2002

STUDY COMPLETION: January 9, 2003

SUBMITTED TO

3M Corporation
Environmental Laboratory
Building 2-3E-09
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: T-7485: A Dietary LC50 Study with the Mallard

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454-113

3M LAB PROJECT NO.: E00-1429

STUDY COMPLETION: January 9, 2003

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; which is basically in compliance with OECD Principles of Good Laboratory Practice, (ENV/MC/CHEM (98) 17), Paris 1998; and Japan MAFF, 11 NohSan Notification No. 6283, Agricultural Production Bureau, 1 October 1999.

STUDY DIRECTOR:

Sean P. Gallagher DATE 1/9/03

Sean P. Gallagher
Senior Biologist, Avian Toxicology

SPONSOR APPROVAL:

Sponsor Representative DATE 1/30/03

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, (ENV/MC/CHEM (98) 17), Paris 1998; and Japan MAFF, 11 NohSan Notification No. 6283, Agricultural Production Bureau, 1 October 1999. 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 Preparation and Analytical Sample Preparation	June 6, 2002	June 7, 2002	June 18, 2002
Matrix Fortifications	June 7, 2002	June 7, 2002	June 12, 2002
Body Weight	June 11, 2002	June 11, 2002	June 13, 2002
Sample Collection	June 28, 2002	June 28, 2002	August 5, 2002
Analytical Data & Draft Report	August 2, 5, & 6, 2002	August 6, 2002	August 6, 2002
Biological Data & Draft Report	August 6 & 7, 2002	August 8, 2002	August 13, 2002
Final Report	January 9, 2003	January 9, 2003	January 9, 2003

All inspections were study based unless otherwise noted.

Robert N. McGee DATE January 9, 2003

Robert N. McGee, B.S.
Quality Assurance Representative

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: T-7485: A Dietary LC50 Study with the Mallard

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STUDY DIRECTOR:

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Willard B. Nixon, Ph.D. — Date: 1/9/03
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SPONSOR: 3M Corporation

TEST SUBSTANCE: T-7485

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454-113

STUDY: T-7485: A Dietary LC50 Study with the Mallard

RESULTS: The dietary LC50 value for mallard exposed to T-7485 was determined to be greater than 10,000 ppm a.i., the highest concentration tested. No mortalities occured at any of the concentrations tested. The no mortality concentration was 10,000 ppm a.i.

There were no overt signs of toxicity or treatment-related effects on feed consumption or liver weights at any of the concentrations tested. Additionally, there were no apparent treatment-related effects on body weight among birds in the 1000, 1780, 3160 and 5620 ppm a.i. treatment groups. However, there was a treatment-related reduction in body weight gain among birds in the 10,000 ppm a.i. treatment group during the exposure period. Based upon a reduction in body weight gain at the 10,000 ppm a.i. test concentration, the no-observed-effect concentration was 5620 ppm a.i.

TEST DATES: Hatch – May 29, 2002
Acclimation – May 31, 2002 to June 6, 2002
Experimental Start (EPA) – June 6, 2002
Experimental Start (OECD) – June 6, 2002
Experimental Termination – July 19, 2002

NOMINAL TEST CONCENTRATIONS: 0, 1000, 1780, 3160, 5620 and 10,000 ppm a.i.

SUMMARY
(contined)

TEST ANIMALS: Mallard (*Anas platyrhynchos*)

AGE TEST ANIMALS: Approximately 8 days of age at test initiation

SOURCE TEST ANIMALS: Whistling Wings
113 Washington Street
Hanover, Illinois 61041

STUDY COMPLETION: January 9, 2003

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 June 6, 2002 to June 28, 2002. Raw data generated at Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454-113 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to evaluate the toxicity of T-7485 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); OECD Guideline 205, Guideline for Testing of Chemicals, Avian Dietary Toxicity Test (2); and upon ASTM Standard E857-87, "Standard Practice for Conducting SubDietary Toxicity Tests with Avian Species" (3).

Test Substance

The test substance was received from 3M Corporation on March 27, 2000 and was assigned Wildlife International, Ltd. Identification Number 5216 upon receipt. The test substance was white powder identified as: Potassium Perfluorobutane Sulfonate; ; Lot No.: 2; CAS No. 29420-49-3. The reported purity of the test substance was 97.3%, with an expiration date of January 17, 2007 (Appendix I). The test substance was stored under ambient conditions.

Treatment Groups

The test consisted of a geometric series of five test concentrations and a control group. Thirty mallard ducklings were assigned to the control group and twelve mallard ducklings 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 or six ducklings each. Nominal dietary concentrations used

in this study were 0, 1000, 1780, 3160, 5620 and 10,000 parts per million active ingredient (ppm a.i.) of T-7485. 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 subjected to gross necropsy. Livers were weighed and liver and blood samples were also collected for potential analysis. The remaining birds were maintained on basal ration until Day 22. On Day 22, these birds were euthanized, liver weights were recorded, and samples of liver tissue and blood were collected.

Duration of the Test

The primary phases of this test and their approximate durations were:

1. Acclimation - 6 days.
2. Exposure - 5 days.
3. Post-exposure observation - 3 or 17 days

Test Birds

All mallard (*Anas platyrhynchos*) were approximately eight days of age and appeared to be in good health at initiation of the test. The birds were obtained from Whistling Wings, Hanover, IL and were hatched on May 29, 2002. Birds ranged in weight from 79 to 138 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 receipt 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 in basal ration into which corn oil had been added. The concentration of corn oil in the control and treated diets was 2%. Mixing was done with a Hobart mixer (Model Number AS200T). All dietary test concentrations were adjusted to 100% active ingredient 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 1000, 1780, 3160, 5620 and 10,000 ppm a.i. (Appendix III).

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 1000 ppm a.i. a.i. test diet and six samples from the 10,000 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 group diet and two verification samples were collected from each remaining treatment diet at preparation on Day 0. At the end of the exposure period (Day 5), one sample was collected from the control diet and two samples were collected from each treatment diet 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. chemistry laboratory.

Analytical Method

A liquid chromatography mass spectrometry (HPLC/MS/MS) based method was developed at Wildlife International, Ltd. for the analysis of the T-7485 in avian diet. The analytical method consisted of sample weighing, extraction with methanol, filtration, dilution with methanol and micro-filtration. Dilutions into the calibration range of the HPLC/MS/MS methodology were performed with a solution of 50% methanol and 50% NANOpure® water. Samples were then analyzed by direct injection. A method flowchart is provided in Appendix IV, Figure 1.

Concentrations of T-7485 in extracts of the samples were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer API 3000LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Chromatographic separations were achieved using a Keystone PRISM RP column (50 mm × 2.0 mm, 3-µm particle size) fitted with a Keystone Javelin C_{18} guard column (20 mm x 2.0 mm i.d.). The instrument parameters are summarized in Appendix IV, Table 1.

All primary and secondary stock preparations were adjusted for the purity of the test substance (97.3%). A 1.00 mg a.i./mL primary stock solution of T-7485 in methanol was prepared by weighing 0.1027 g of the test substance and bringing to a final volume of 100 milliliters with methanol. Secondary stock solutions (100 and 10.0 mg a.i./L) of T-7485 in methanol were prepared by serial volumetric dilution of the primary stock.

Calibration standards were prepared in a solution of 50% methanol and 50% NANOpure® water by appropriate dilutions of the 100 mg a.i./L stock solution of T-7485 in methanol. Calibration standards of T-7485, ranging in concentration from 0.100 to 0.500 mg a.i./L, were analyzed with each sample set. The calibration standard series was injected at the beginning and end of the run, and one standard was injected, at a minimum, after every five samples. Linear regression equations were generated using peak area responses versus the respective concentrations of the calibration standards. A representative calibration curve is presented in Appendix IV, Figure 2. The concentration of T-7485 in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equation. Representative ion chromatograms of low and high-level calibration standards are presented in Appendix IV, Figures 3 and 4, respectively. Examples of equations used in calculations are presented in Appendix IV, Table 2.

The instrument limit of detection (LOD) was set based upon the injection volume (5.00 µL) and the lowest standard concentration 0.100 mg a.i./L. The LOD was set at 0.500 ng on-column. The method limit of quantitation (LOQ) for these analyses was set at 150 ppm a.i. based upon the lowest matrix fortification level analyzed concurrently with the samples. The 0.100 mg a.i./L T-7485 standard was equivalent to a calculated value of 40 ppm a.i. T-7485 in the matrix blank extract. Measured values greater than or equal to the ppm a.i.equivalent were reported.

Along with the sample analyses, three matrix blanks were analyzed to determine possible interferences. No interferences were observed at or above the ppm a.i. equivalent of the lowest standard during the sample analyses (Appendix IV, Table 3). A representative ion chromatogram of a matrix blank sample is presented in Appendix IV, Figure 5.

Avian diet samples were fortified at 150, 3000, and 12000 ppm a.i. and analyzed concurrently with the samples to determine the mean procedural recovery. The method yielded mean procedural recoveries of 87.7%, 99.0% and 98.2% (Appendix IV, Table 3). Sample measured concentrations were not corrected for the mean procedural recoveries. A representative ion chromatogram of a matrix fortification sample is presented in Appendix IV, Figure 6.

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. (Model No. 5355). Each pen had floor space that measured approximately 62 X 92 cm. Ceiling height was approximately 25.5 cm. External walls, ceilings and floors were constructed of vinyl-coated wire grid. 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 uniquely numbered wing bands.

Through Day 11 of the test, the average temperature in the brooding compartment of the pens was 31°C ±1°C (SD). Brooding was discontinued on Day 11, when test birds were of sufficient size to thermoregulate. Average ambient room temperature for this study was 24.9°C ± 0.7°C (SD) with an average relative humidity of 74% ± 5% (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 196 lux of illumination. Housing and husbandry practices were based on guidelines established by the National Research Council (4).

Observations

During acclimation all birds were observed daily. Birds exhibiting abnormal behavior or physical injury were not used for the study. 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 Days 5 and 8, and Days 15 and 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 16-22 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

On Day 8 of the test, half of the birds in each treatment group and half of the control birds were euthanized and subjected to gross necropsy. On Day 22 of the test, all remaining birds were euthanized and subjected to gross necropsy. Prior to euthanasia, blood was collected from each bird when possible. During necropsy, the liver was removed and weighed. Blood and liver samples were transferred to the sponsor for analysis if deemed appropriate.

Statistical Analyses

There were no treatment related mortalities observed in this study, therefore, it was not possible to perform the calculation (5,6,7) of an LC50 value using the computer program of Stephan (8). The LC50 value was estimated to be greater than 10,000 ppm a.i., the highest concentration tested. Differences in the mean body weights, body weight change, absolute liver weights and relative liver weights between the control group and the treatment groups were determined by an Analysis of Variance (ANOVA). Bonferoni's t-Test, as run with Toxstat Software (9,10) was used to compare the five treatment group means with the control group mean and assessed the statistical significance of the differences observed.

RESULTS

Diet Analysis

Diet samples collected from the 1000 and 10000 ppm a.i. test concentrations were analyzed to evaluate the homogeneity of the test substance in the diet. The mean and standard deviation for the 1000 and 10000 ppm a.i. test concentrations were 938 ± 60.6 ppm a.i. and 9440 ± 627 ppm a.i., respectively. The corresponding coefficients of variation were 6.46% and 6.65%, respectively (Appendix IV, Table 4). A typical ion chromatogram of a test sample is shown in Appendix IV, Figure 7.

Samples collected during Day 0 of the test to verify test substance concentrations for the 1000, 1780, 3160, 5620, and 10000 ppm a.i. diets had mean measured concentrations of 938, 1700, 2940, 5420, and 9440 ppm a.i., respectively. These values represented 93.8, 95.5, 93.0, 96.4, and 94.4% of nominal concentrations, respectively (Appendix IV, Table 5). Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days averaged 106%, 92.9%, 101%, 102%, and 108% of the Day 0 values for the 1000, 1780, 3160, 5620 and 10000 ppm a.i. test concentrations, respectively (Appendix IV, Table 5).

Mortalities and Clinical Observations

There were no mortalities in the control group and all control birds were normal in appearance and behavior throughout the test (Table 1 and Appendix V). In addition, there were no mortalities or overt signs of toxicity at the 1000, 1780, 3160, 5620 and 10,000 ppm a.i. test concentrations. All birds at all test concentrations were normal in appearance and behavior throughout the test.

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 1000, 1780, 3160 or 5620 ppm a.i. treatment groups during the exposure period (Days 0-5). Any differences between the control group and the 1000, 1780, 3160 and 5620 ppm a.i. treatment groups were not statistically significant. However, there was a statistically significant ($p<0.01$) reduction in body weight gain among birds in the 10,000 ppm a.i. treatment group during the exposure period that was considered treatment related (Table 2 and Appendix VI).

When compared to the control group, there was a statistically significant ($p<0.05$) reduction in body weight gain among birds in the 3160 ppm a.i. treatment group from Days 15 to 22 of the post exposure period. However, the reduction was not concentration responsive. Additionally, a similar reduction was not noted during the exposure period or during the subsequent post-exposure intervals (Day 5 to 8 and Day 8 to 15) at this treatment level. Therefore, this reduction was not considered treatment-related.

When compared to the control group, there were no apparent treatment-related effects on feed consumption at any of the concentrations tested (Table 3 and Appendix VII).

Gross Necropsy

Gross necropsy revealed one bird in the control group with a subcapsular hematoma on the right lobe of the liver. Another control bird was noted with a pale and mottled spleen. The necropsy results for all other birds were not remarkable.

Liver Weight

When compared to the control group, there were no apparent treatment-related effects on mean absolute liver weight or mean relative liver weight (liver weight as a percentage of body weight) for any of the concentrations tested. Any differences between the control and each treatment group were not statistically significant. Mean absolute liver weights and mean relative liver weights are presented in Table 4. Individual liver weights are presented in Appendix VIII.

CONCLUSION

The dietary LC50 value for mallard exposed to T-7485 was determined to be greater than 10,000 ppm a.i., the highest concentration tested. No mortalities occured at any of the concentrations tested. The no mortality concentration was 10,000 ppm a.i.

There were no overt signs of toxicity or treatment-related effects on feed consumption or liver weights at any of the concentrations tested. Additionally, there were no apparent treatment-related effects on body weight among birds in the 1000, 1780, 3160 and 5620 ppm a.i. treatment groups. However, there was a treatment-related reduction in body weight gain among birds in the 10,000 ppm a.i. treatment group during the exposure period. Based upon a reduction in body weight gain at the 10,000 ppm a.i. test concentration, the no-observed-effect concentration was 5620 ppm a.i.

REFERENCES

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

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

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

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

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

10. **Gulley, D.D.** 1990. TOXSTAT Release 3.2. The University of Wyoming.

TABLE 1

Cumulative Mortality from a Mallard Dietary Toxicity Study with T-7485

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]	Day 22 [2]
Control										
0	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/15
Treatment										
1000	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/6
1780	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/6
3160	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/6
5620	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/6
10,000	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/6

The LC50 value was estimated to be greater than 10,000 ppm a.i., the highest concentration tested.
[1] – Half of the birds were euthanized on Day 8 for necropsy and tissue collection.
[2] – No mortalities occurred in any of the control or treatment groups from Day 8 to Day 22.

TABLE 2

Mean Body Weight (g) from a Mallard Dietary Toxicity Study with T-7485

Experimental Group (ppm a.i.)		Exposure Period			Post-Exposure Period						Total
		Day 0	Day 5	Change[1]	Day 8	Change[1]	Day 15	Change[1]	Day 22	Change[1]	Change
Control											
0	Mean	105	212	107	299	87	529	226	728	199	622
	SD	14	36	24	45	12	50	13	48	23	39
Treatment											
1000	Mean	110	232	122	327	95	548	223	727	178	615
	SD	16	32	18	44	14	76	27	79	31	65
1780	Mean	109	222	113	309	88	528	213	740	212	630
	SD	14	30	24	44	17	44	17	62	30	58
3160	Mean	108	223	115	320	97	551	228	710	159*	602
	SD	17	34	22	40	13	35	24	35	10	24
5620	Mean	109	203	94	295	92	553	271	737	184	629
	SD	17	32	20	48	22	86	47	72	38	64
10,000	Mean	108	192	84**	287	95	520	230	730	209	621
	SD	17	40	26	51	16	39	26	33	36	31

[1] Mean change is calculated separately from the mean body weights using individual body weights (See Appendix VI).
* - Significantly different from the control at $p<0.05$ (Bonferroni's t-Test).
** - Significantly different from the control at $p<0.01$ (Bonferroni's t-Test).

TABLE 3

Mean Feed Consumption (g/bird/day) from a Mallard Dietary Toxicity Study with T-7485

Experimental Group (ppm a.i)		Exposure Period Days 0-5	Post-Exposure Period Days 6-8	Days 9-15	Days 16-22
Control	Mean[1]	73	112	163	226
	SD[1]	10	14	11	20
Treatment					
1000	Mean	88	128	153	191
1780	Mean	79	132	161	228
3160	Mean	72	142	183	247
5620	Mean	61	109	129	173
10000	Mean	60	102	141	181

[1]Mean values calculated using Excel in full precision mode. Manual calculation may vary.

TABLE 4

Mean Liver Weight and Relative Liver Weight[1] from a Mallard Dietary Toxicity Study with T-7485

Experimental Group (ppm a.i.)		Day 8 Liver Weight (g)	Day 8 Relative Liver Weight	Day 22 Liver Weight (g)	Day 22 Relative Liver Weight
Control					
0	Mean	14.315	4.833	25.807	3.533
	SD	2.588	0.624	4.672	0.498
Treatment					
1000	Mean	17.577	5.332	27.449	3.781
	SD	3.640	0.599	3.870	0.411
1780	Mean	15.727	5.224	25.964	3.516
	SD	2.533	0.646	2.087	0.244
3160	Mean	15.576	4.885	24.694	3.478
	SD	3.992	0.584	1.658	0.202
5620	Mean	15.138	4.918	27.071	3.694
	SD	1.790	0.401	1.847	0.330
10000	Mean	14.828	5.242	28.035	3.837
	SD	3.115	0.544	4.041	0.498

[1]Relative Liver Weight = Liver Weight (g)/Body Weight (g) X 100.
Differences between the control and each experimental group were not significant ($p>0.05$, Bonferroni's t-Test).

APPENDIX I

Certificate of Analysis

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

INTERIM CERTIFICATE OF ANALYSIS

Revision 2 (10/17/01)

Centre Analytical Laboratories COA Reference #: 023-032
3M Product: PFBS, Lot 2
Test Control Reference #: TCR-00017-71
Purity: 97.3%

Test Name	Specifications	Result
Purity[1]		97.3%
Appearance	White, crystalline solid	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium[2] 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		 1. 0.003 wt./wt.% 2. 0.001 wt./wt.% 3. 0.768 wt./wt.% 4. 10.718 wt./wt.% 5. <0.001 wt./wt.% 6. 0.002 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		2.07 wt./wt.%
Total % Impurity (LC/MS)		5.56 wt./wt.%
Related Compounds (POAA)		None Quantified
Total % Impurity (GC/MS)		None Quantified
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate		 1. 0.016 wt./wt.% 2. <0.005 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.030 wt./wt.% 7. <0.040 wt./wt.%
Organic Acids[3] (IC)		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.25 wt./wt.%
Elemental Analysis[4]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 Theoretical Value = 14.2% Theoretical Value = 0.0% Theoretical Value = 0.0% Theoretical Value = 9.5% Theoretical Value = 50.6%	 1. 11.0 wt./wt.% 2. 0.282 wt./wt.% 3. 0.465 wt./wt.% 4. 11.2 wt./wt.% 5. 48.2 wt./wt.%

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

INTERIM CERTIFICATE OF ANALYSIS

Revision 2

Centre Analytical Laboratories COA Reference #: 023-032
3M Product: PFBS, Lot 2
Test Control Reference #: TCR-00017-71

Date of Last Analysis: 01/17/01

Expiration Date: 01/17/07

Storage Conditions: < -10 °C

Re-assessment Date: 01/17/07

[1]Purity = 100% - (Total NMR impurities, 2.07% + Total LC/MS impurities, (5.56% - 4.99%*) + Total Metal impurities, 0.006% + Total inorganic anions, 0.016%)

Total impurity from all tests = 2.662%
Purity = 100% - 2.662% = 97.3%

*1.93% of the NMR total impurity value is due to the presence of This impurity is also observed in the LC/MS analysis at 4.99%. The impurity value is taken from the NMR analysis as it is deemed the more quantitative technique in the absence of standards (See impurity profile on page 3).

[2]Potassium and sodium are present as counterions and thus are excluded from the purity calculation.

3.

[4]Theoretical value calculations based on the empirical formula, $C_4F_9SO_3K^+$ (MW=338.2)

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INTERIM CERTIFICATE OF ANALYSIS

Revision 2

Centre Analytical Laboratories COA Reference #: 023-032
3M Product: PFBS, Lot 2
Test Control Reference #: TCR-00017-71

LC/MS Purity Profile:

Peak #	Retention Time (min)	Mass(s)	Identity	Area	% Impurity
1				186918	0.57
2				1629660	4.99
3		298.9	PFBS	30835100	-
Total	-	-	-	32651678	5.56

NMR Purity Profile:

Component *	Molecular Weight	Relative Weight Percent
CF_3-CF_2-CF_2-CF_2-SO_3^-,	298.9	97.9
		0.05
		1.93
		0.13
		0.01
Total Impurity		2.07

*All components are assumed to be associated with K^+ cation.
Note: NMR detected impurities in boldface type.

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

Prepared By: _____ 10/3/01
Charles Simons Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 10/4/01
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

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
Diet Preparation

Weight and volume of constituents used to prepare test diets:

Nominal Concentrations (ppm a.i.)	Test Substance (g)	Basal Ration (g)	Corn Oil (mL)
0	-	8820.0	195
1000	9.2496	8810.8	195
1780	16.4645	8803.5	195
3160	29.2291	8790.8	195
5620	51.9837	8768.0	195
10000	92.4971	8727.5	195

Diets were prepared as follows:

- 5000.0 g of basal ration was weighed into a tared Hobart mixing bowl.
- 195 mL of corn oil was added to the ration and the bowl contents were mixed for approximately 5 minutes on a Hobart mixer.
- The test substance was weighed in a tared weigh boat.
- Approximately 100 g of basal ration was removed from the mixing bowl and placed in a Waring® blender.
- The test substance was added to the blender, the weigh boat was rinsed with additional ration from the mixing bowl, with the rinse also being placed in the blender.
- The blender contents were blended for approximately 1 minute and transferred to the mixing bowl. The blender was rinsed with uncontaminated ration from the mixing bowl, with the rinse also being returned to the mixing bowl.
- The bowl was placed on a Hobart mixer and the contents were mixed for approximately five minutes. The remaining ration was weighed and added to the bowl and the contents were mixed for approximately 10 more minutes.
- The diet was transferred to a labeled paper feed bag.
- The control diet was prepared by weighing 5000.0 g of basal ration into a tared Hobart mixing bowl. 195 mL of corn oil was added and the bowl was placed on the Hobart mixer and the contents were mixed for approximately five minutes. The remaining ration was weighed and added to the bowl. The contents of the bowl were mixed for approximately ten minutes. Diet was transferred to a labeled paper feed bag.

APPENDIX IV

Analytical Methods and Results

Appendix IV

Table 1

Typical HPLC/MS/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 3000 LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in multiple ion reaction monitoring (MRM) mode.
ANALYTICAL COLUMN:	Keystone Prism RP column (50 mm × 2 mm I.D., 3-µm)
GUARD COLUMN:	Keystone Javelin C_{18} column (20 mm × 2 mm I.D.)
OVEN TEMPERATURE:	40°C
STOP TIME:	5.00 minutes
FLOW RATE:	200 µL/minute
MOBILE PHASE:	10% NANOpure Water® with 0.1% Ammonium Formate: 90% Methanol
INJECTION VOLUME:	5.00 µL
T-7485 RETENTION TIME:	Approximately 2.1 minutes
T-7485 MONITORED MASS:	299.0 amu

Appendix IV

Table 2

Example Calculations

Sample number 454-112,113-7, nominal concentration of 1000 ppm a.i. in avian feed
Peak Area = 1157259
Y-intercept = 287382.5938
Slope = 3571005
Primary Dilution:
Initial Weight (W_i) = 10.0 g
Original Final Volume (V_1)= 200 mL
Secondary Dilution:
Initial Volume (V_i) = 0.0500 mL
Final Volume (V_f) = 10.0 mL
Overall Dilution Factor ($V_1/W_i \times V_f/V_i$) = 4000 mL/g = 4000 L/Kg

$$\text{T-7485 at instrument (mg a.i./L)} = \frac{(\text{peak area} - \text{y-intercept})}{\text{slope}}$$

$$= \frac{(1157259 - 287382.5938)}{3571005}$$

$$= 0.24359 \text{ mg a.i./L}$$

T-7485 in sample (mg a.i./Kg) = T-7485 at instrument (mg a.i./L) × overall dilution factor (L/Kg)

$$\text{T-7485 in sample (mg a.i./Kg)} = \frac{0.24359 \text{ mg a.i.}}{\text{L}} \times \frac{4000 \text{ L}}{\text{Kg}}$$

$$= 974 \text{ mg a.i./Kg}$$

$$= 974 \text{ ppm a.i.}$$

$$\text{Percent of Nominal Concentration} = \frac{\text{T-7485 (ppm a.i.) in sample}}{\text{T-7485 (ppm a.i.) nominal}} \times 100$$

$$= \frac{974}{1000} \times 100 = 97.4\%$$

Instrument Software: MacQuan, version 1.6.

Appendix IV

Table 3

Matrix Blank and Fortifications Analyzed Concurrently with the Samples

Number (454-112,113)	Sample Type	Interval	Concentration of T-7485 (ppm a.i.)		Percent Recovery[2]	Mean Percent of Nominal
			Fortified	Measured[1,2]		
MAB-1	Matrix Blank	Day 0	0	<40	-	
MAS-1	Matrix Fortification	Day 0	150	137	91.1	87.7
MAS-2	Matrix Fortification	Day 0	3000	2600	86.7	
MAS-3	Matrix Fortification	Day 0	12000	10200	85.2	
MAB-2	Matrix Blank	Day 5	0	<40	-	
MAS-4	Matrix Fortification	Day 5	150	157	105	99.0
MAS-5	Matrix Fortification	Day 5	3000	2810	93.5	
MAS-6	Matrix Fortification	Day 5	12000	11800	98.6	
MAB-3[3]	Matrix Blank	Day 0	0	<40	-	
MAS-7[3]	Matrix Fortification	Day 0	150	143	95.6	98.2
MAS-8[3]	Matrix Fortification	Day 0	3000	2990	99.6	
MAS-9[3]	Matrix Fortification	Day 0	12000	11900	99.4	

[1]The lowest level analytical standard was equivalent to a calculated value of 40 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent are reported.

[2]Measured and percent recovery values calculated using MacQuan ver.1.6 and rounded to three significant figures. Manual calculations may vary slightly.

[3]Quality control samples analyzed concurrent with re-extracted sample 454-112,113-8 (Table 5).

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Table 4

Homogeneity of T-7485 in Avian Diet

Nominal Concentration (ppm a.i.)	Sample I.D. Number (454-112,113)	Location Sampled In Mixing Vessel	T-7485 Concentration Measured[1] (ppm a.i.)	Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Mean Percent of Nominal
1000	2	Top Left	829		
	3	Top Right	908		
	4	Middle Left	954	$\bar{x}$ 938	93.8
	5	Middle Right	995	SD =60.6	
	6	Bottom Left	966	CV = 6.46	
	7	Bottom Right	974		
10000	14	Top Left	10000		
	15	Top Right	9230		
	16	Middle Left	10300	$\bar{x}$ 9440	94.4
	17	Middle Right	8720	SD = 627	
	18	Bottom Left	8850	CV = 6.65	
	19	Bottom Right	9510		

[1]Measured values were not corrected for procedural recoveries based on sample sets (Table 3).

Appendix IV

Table 5

Ambient Stability of T-7485 in Avian Diet During the Mallard Dietary LC50 Study

	T-7485 Concentration							
	Week 1, Day 0				Week 1, Day 5			
Nominal Concentration (ppm a.i.)	Sample Number (454-112,113)	Measured[2] (ppm a.i.)	Mean Measured (ppm a.i.)	Mean Percent of Nominal	Sample Number (454-113-)	Measured[2] (ppm a.i.)	Mean Measured (ppm a.i.)	Mean Percent of Day 0
0	1	< 40[3]		-	31	< 40[3]		
1000	2-7[1]	938[1]		93.8	32	1000	997	106
					33	993		
1780	8	1770[4]	1700	95.5	34	1540	1580	92.9
	9	1630			35	1620		
3160	10	3000	2940	93.0	36	2810	2960	101
	11	2880			37	3110		
5620	12	5420	5420	96.4	38	5870	5540	102
	13	5410			39	5220		
10000	14-19[1]	9440[1]		94.4	40	10700	10200	108
					41	9650		

[1]Day 0 values are from homogeneity samples presented in Table 4.
[2]Measured values were not corrected for mean procedural recoveries based on sample sets (Table 3).
[3]The lowest level analytical standard was equivalent to a calculated value of 40 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent are reported.
[4]Result is the mean of duplicate re-extraction of original sample. Original recovery was 78.2% of nominal concentration due to procedural error. Re-extraction duplicate measured values were 1760 and 1780 ppm a.i., representing 99.0% and 99.9% of the nominal concentration, respectively.

Appendix IV

Analytical Method Flowchart for the Analysis of T-7485 in Avian Diet

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

↓

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

↓

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

↓

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

↓

Filter into a 200-mL volumetric flask using a powder funnel and glass wool. Rinse retained feed 3 times with methanol into filtrate. Bring to volume with methanol.

↓

Transfer an aliquot of each sample into a 10-mL B-D syringe fitted with a 0.45 µm Acrodisc® and filter into a scintillation vial.

↓

Prepare appropriate dilution(s) to bring final concentration into the calibration range of the LC/MS/MS methodology. Use 50% methanol:50% NANOpure® water dilution solvent.

↓

Ampulate and submit samples for HPLC/MS/MS analysis.

Figure 1. Analytical method flowchart for the analysis of T-7485 in avian diet.

Appendix IV

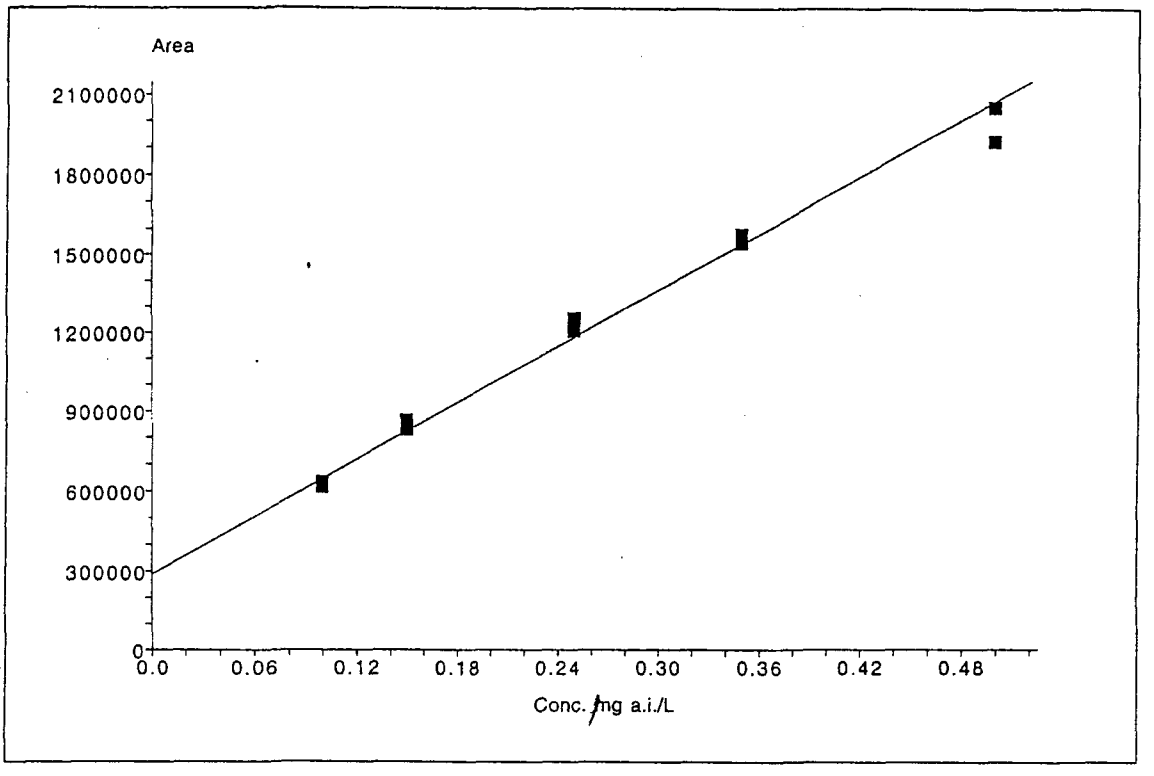

Figure 2. Representative calibration curve for T-7485.
Slope = 3571005, y-Intercept = 287382.5938, r = 0.99518.

Appendix IV

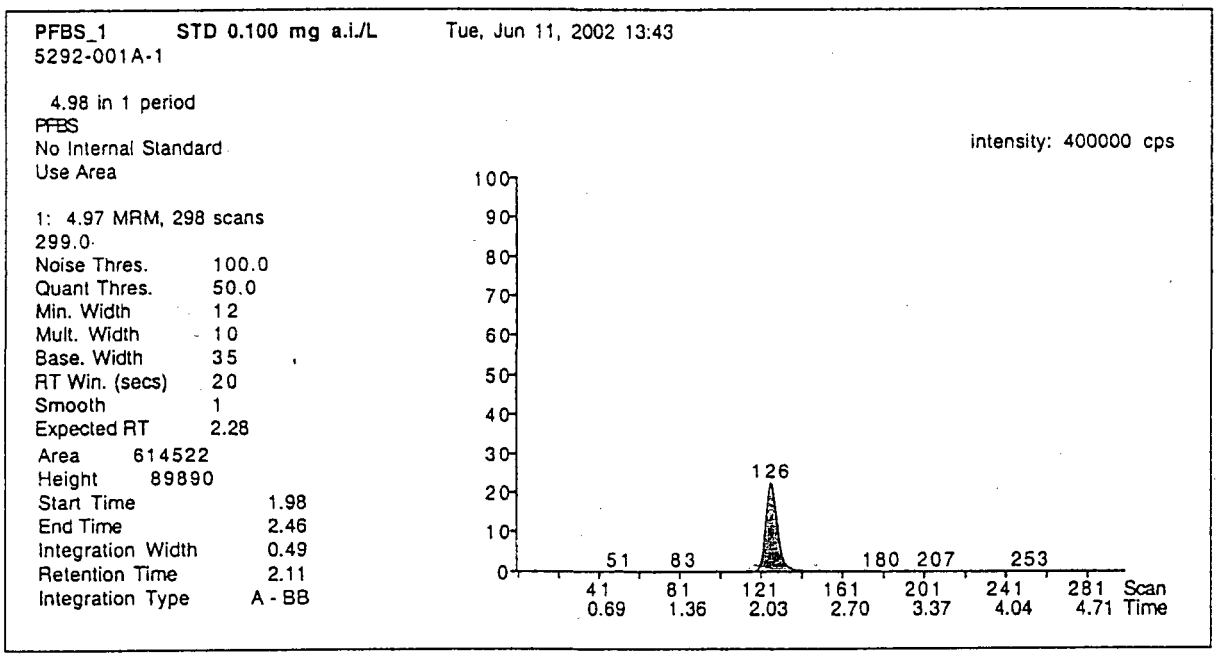

Figure 3. Representative ion chromatogram of a low-level T-7485 standard, 0.100 mg a.i./L.

Appendix IV

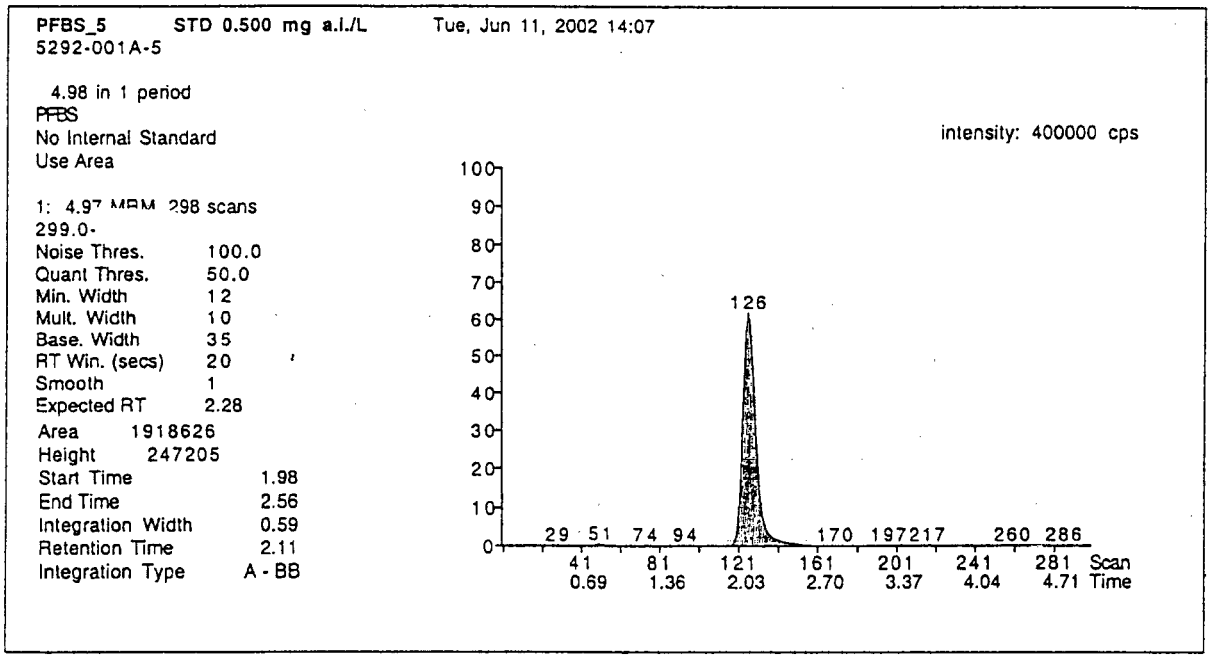

Figure 4. Representative ion chromatogram of a high level T-7485 standard, 0.500 mg a.i./L.

Appendix IV

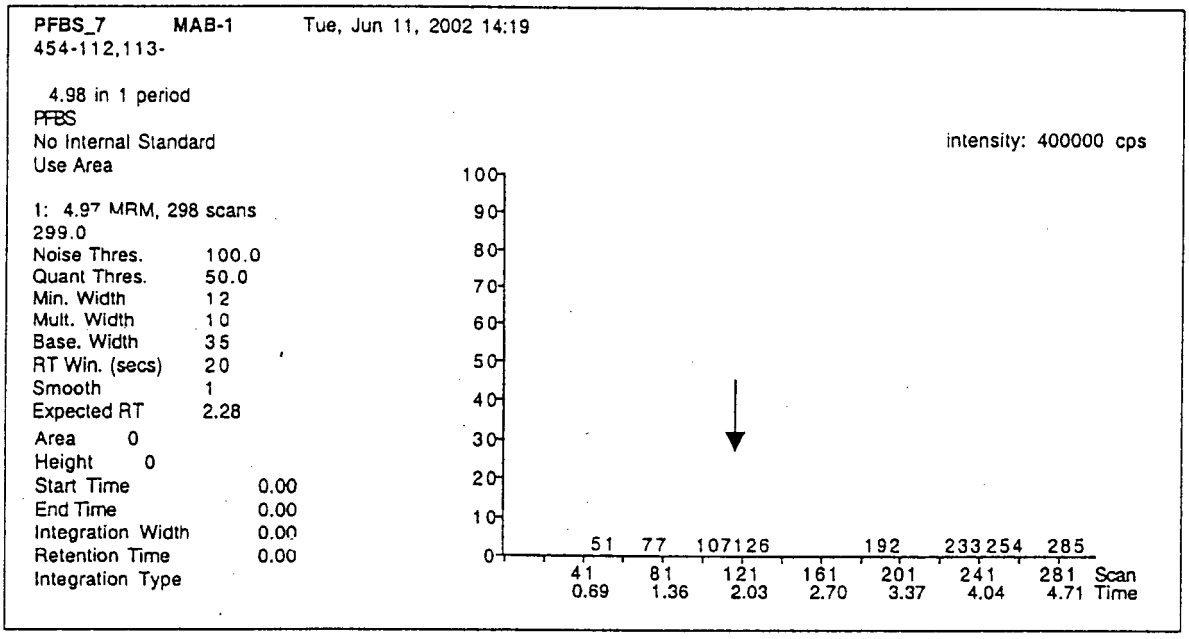

Figure 5. Representative ion chromatogram of a matrix blank sample, (454-112,113-MAB-1). The arrow indicates the approximate retention time of T-7485. 10-g sample, Overall dilution = 400X.

Appendix IV

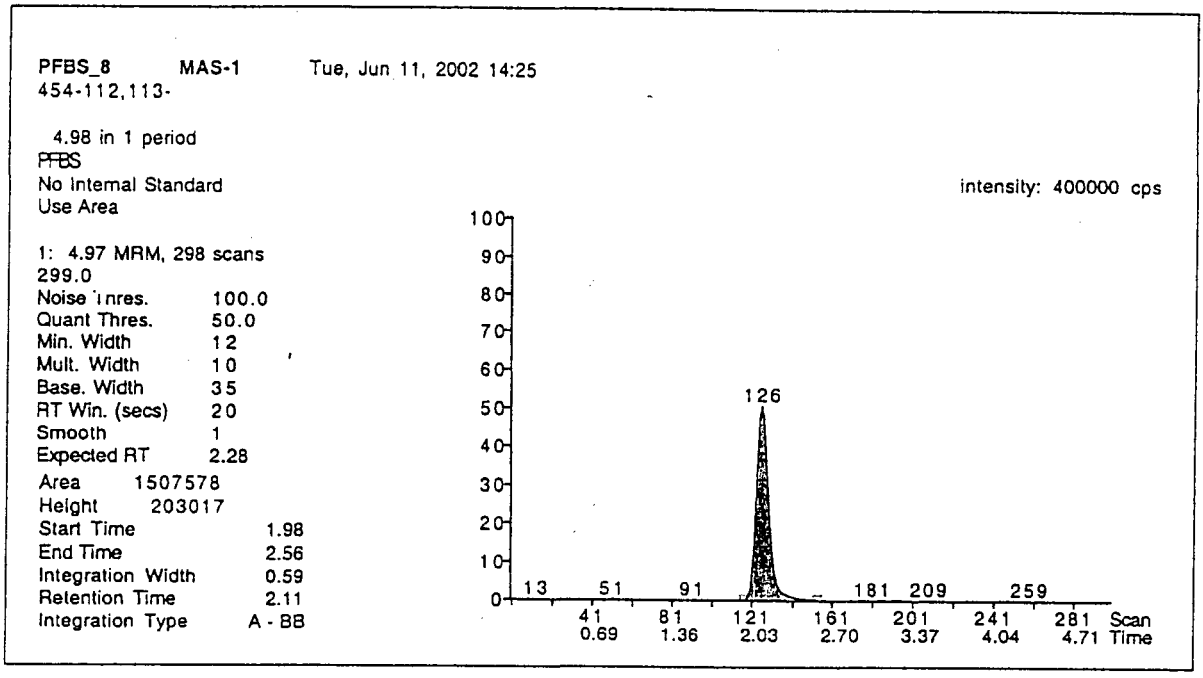

Figure 6. Representative ion chromatogram of a matrix fortification (454-112,113-MAS-1). Nominal concentration 150 ppm a.i., 10-g sample, Overall dilution = 400X.

Appendix IV

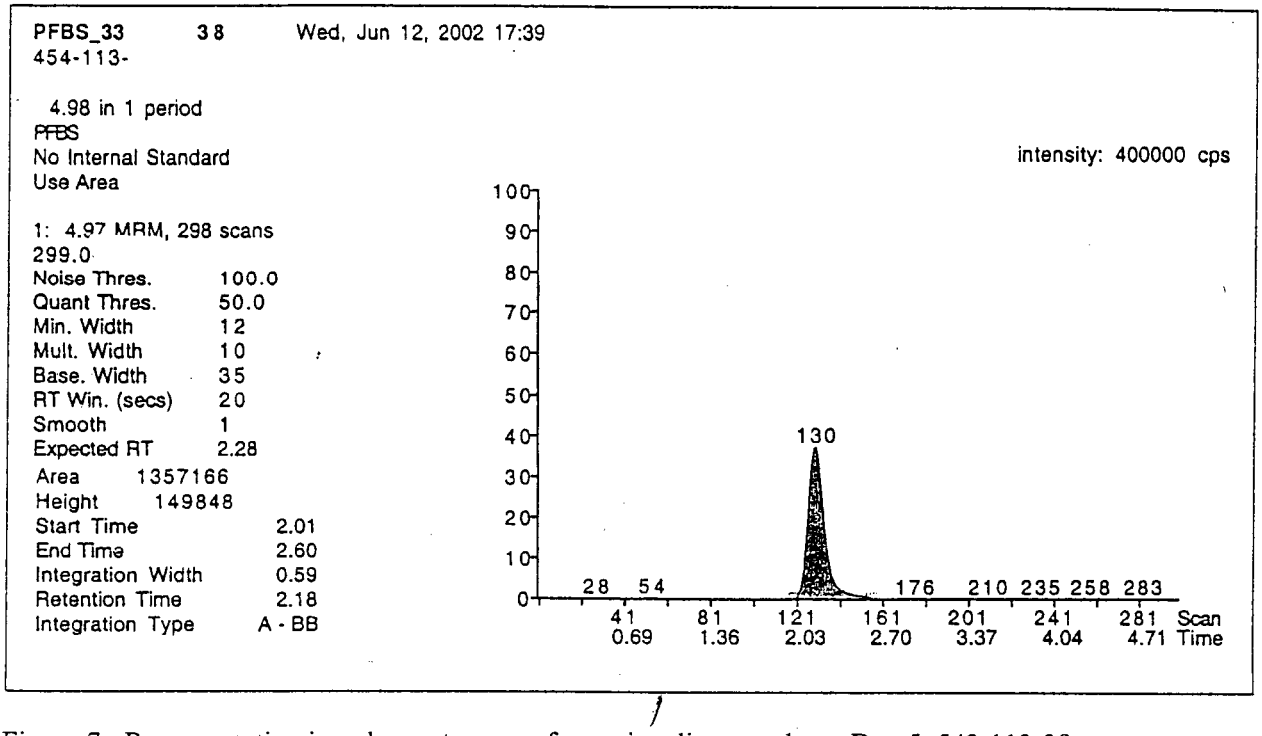

Figure 7. Representative ion chromatogram of an avian diet sample on Day 5, 543-113-38 Nominal concentration 5620 ppm a.i., 10-g sample, Overall dilution = 20000X.

APPENDIX V

Cumulative Mortality by Pen from a Mallard Dietary Toxicity Study with T-7485

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]	Day 22[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	0/5	0/5
0	6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Treatment											
1000	1	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	-
1000	2	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
1780	1	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	-
1780	2	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
3160	1	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	-
3160	2	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
5620	1	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	-
5620	2	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
10,000	1	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	-
10,000	2	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

The LC50 value was estimated to be greater than 10,000 ppm a.i., the highest concentration tested.
[1] – Half of the birds were euthanized on Day 8 for necropsy and tissue collection.
[2] – No mortalities occurred in any of the control or treatment groups after Day 8.

APPENDIX VI

Individual Body Weights (g) from a Mallard
Dietary Toxicity Study with T-7485
Page 1

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change	Day 8	Change	Day 15	Change	Day 22	Change	Total Change
0	1	88	160	72	230	70	-	-	-	-	-
	2	97	188	91	269	81	-	-	-	-	-
	3	102	212	110	291	79	-	-	-	-	-
	4	113	241	128	346	105	-	-	-	-	-
	5	125	265	140	367	102	-	-	-	-	-
	Mean	105	213	108	301	87	-	-	-	-	-
	SD	14	42	27	56	15	-	-	-	-	-
0	1	79	152	73	211	59	-	-	-	-	-
	2	90	191	101	276	85	-	-	-	-	-
	3	99	209	110	291	82	-	-	-	-	-
	4	111	223	112	300	77	-	-	-	-	-
	5	121	255	134	328	73	-	-	-	-	-
	Mean	100	206	106	281	75	-	-	-	-	-
	SD	17	38	22	44	10	-	-	-	-	-
0	1	88	185	97	287	102	-	-	-	-	-
	2	95	186	91	276	90	-	-	-	-	-
	3	105	229	124	315	86	-	-	-	-	-
	4	113	201	88	280	79	-	-	-	-	-
	5	120	255	135	346	91	-	-	-	-	-
	Mean	104	211	107	301	90	-	-	-	-	-
	SD	13	30	21	30	8	-	-	-	-	-

- Indicates bird was euthanized on Day 8 for sampling.

APPENDIX VI

Individual Body Weights (g) from a Mallard
Dietary Toxicity Study with T-7485
Page 2

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change	Day 8	Change	Day 15	Change	Day 22	Change	Total Change
0	1	87	178	91	252	74	468	216	694	226	607
	2	94	192	98	274	82	498	221	684	186	590
	3	103	220	117	310	90	546	236	774	228	671
	4	116	239	123	333	94	546	213	740	194	624
	5	120	258	138	367	109	591	224	820	229	700
	Mean	104	217	113	307	90	530	223	742	213	638
	SD	14	33	19	46	13	48	9	57	21	46
0	1	83	121	38	183	62	433	250	642	209	559
	2	98	195	97	281	86	499	218	681	182	583
	3	104	197	93	294	97	512	218	727	215	623
	4	108	208	100	292	84	509	217	700	191	592
	5	137	290	153	391	101	635	244	789	154	652
	Mean	106	202	96	288	86	518	229	708	190	602
	SD	20	60	41	74	15	73	16	55	24	36
0	1	92	209	117	296	87	515	219	693	178	601
	2	98	216	118	307	91	549	242	756	207	658
	3	107	208	101	310	102	524	214	741	217	634
	4	119	258	139	357	99	577	220	774	197	655
	5	126	217	91	298	81	540	242	707	167	581
	Mean	108	222	113	314	92	541	227	734	193	626
	SD	14	21	18	25	9	24	14	34	21	34
Group Total	Mean	105	212	107	299	87	529	226	728	199	622
	SD	14	636	24	45	12	49	13	48	23	39

APPENDIX VI

Individual Body Weights (g) from a Mallard
Dietary Toxicity Study with T-7485
Page 3

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change	Day 8	Change	Day 15	Change	Day 22	Change	Total Change
	1	85	189	104	268	79	-	-	-	-	-
1000	2	97	232	135	321	89	-	-	-	-	-
	3	107	219	112	308	89	-	-	-	-	-
	4	112	234	122	324	90	-	-	-	-	-
	5	120	266	146	373	107	-	-	-	-	-
	6	130	255	125	373	118	-	-	-	-	-
	Mean	109	233	124	328	95	-	-	-	-	-
	SD	16	27	15	40	14	-	-	-	-	-
	1	89	177	88	252	75	446	194	663	217	574
1000	2	96	212	116	293	81	485	192	616	131	520
	3	109	209	100	308	99	521	213	708	187	599
	4	112	250	138	351	101	605	254	808	203	696
	5	123	251	128	355	104	593	238	752	159	629
	6	138	284	146	395	111	640	245	812	172	674
	Mean	111	231	119	326	95	548	223	727	178	615
	SD	18	38	22	51	14	76	27	79	31	65
Group Total	Mean	110	232	122	327	95	548	223	727	178	615
	SD	16	32	18	44	14	76	27	79	31	65

- Indicates bird was euthanized on Day 8 for sampling.

APPENDIX VI
Individual Body Weights (g) from a Mallard
Dietary Toxicity Study with T-7485
Page 4

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change	Day 8	Change	Day 15	Change	Day 22	Change	Total Change
1780	1	89	183	94	256	73	-	-	-	-	-
	2	93	192	99	270	78	-	-	-	-	-
	3	105	229	124	325	96	-	-	-	-	-
	4	109	249	140	360	111	-	-	-	-	-
	5	122	264	142	365	101	-	-	-	-	-
	6	127	193	66	244	51	-	-	-	-	-
	Mean	108	218	111	303	85	-	-	-	-	-
	SD	15	34	30	54	22	-	-	-	-	-
1780	1	90	206	116	295	89	490	195	740	250	650
	2	97	184	87	269	85	474	205	645	171	548
	3	109	221	112	306	85	544	238	762	218	653
	4	117	251	134	348	97	550	202	741	191	624
	5	119	256	137	367	111	595	228	836	241	717
	6	130	234	104	307	73	517	210	717	200	587
	Mean	110	225	115	315	90	528	213	740	212	630
	SD	15	27	19	36	13	44	17	62	30	58
Group Total	Mean	109	222	113	309	88	528	213	740	212	630
	SD	14	30	24	44	17	44	17	62	30	58

- Indicates bird was euthanized on Day 8 for sampling.

APPENDIX VI

Individual Body Weights (g) from a Mallard
Dietary Toxicity Study with T-7485
Page 5

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change	Day 8	Change	Day 15	Change	Day 22	Change	Total Change
	1	80	167	87	250	83	-	-	-	-	-
3160	2	94	185	91	257	72	-	-	-	-	-
	3	108	228	120	318	90	-	-	-	-	-
	4	118	245	127	344	99	-	-	-	-	-
	5	122	276	154	360	84	-	-	-	-	-
	6	125	253	128	366	113	-	-	-	-	-
	Mean	108	226	118	316	90	-	-	-	-	-
	SD	18	42	25	51	14	-	-	-	-	-
	1	83	189	106	304	115	525	221	681	156	598
3160	2	90	199	109	286	87	522	236	672	150	582
	3	106	230	124	329	99	541	212	700	159	594
	4	119	258	139	359	101	619	260	768	149	649
	5	122	241	119	353	112	546	193	711	165	589
	6	129	207	78	310	103	554	244	730	176	601
	Mean	108	221	113	324	103	551	228	710	159	602
	SD	18	27	21	29	10	35	24	35	10	24
Group	Mean	108	223	115	320	97	551	228	710	159	602
Total	SD	17	34	22	40	13	35	24	35	10	24

- Indicates bird was euthanized on Day 8 for sampling.

APPENDIX VI

Individual Body Weights (g) from a Mallard
Dietary Toxicity Study with T-7485
Page 6

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change	Day 8	Change	Day 15	Change	Day 22	Change	Total Change
	1	84	181	97	256	75	-	-	-	-	-
5620	2	95	203	108	280	77	-	-	-	-	-
	3	107	219	112	306	87	-	-	-	-	-
	4	116	217	101	315	98	-	-	-	-	-
	5	126	229	103	356	127	-	-	-	-	-
	6	133	246	113	339	93	-	-	-	-	-
	Mean	110	216	106	309	93	-	-	-	-	-
	SD	19	22	6	37	19	-	-	-	-	-
	1	85	131	46	194	63	447	253	661	214	576
5620	2	97	170	73	237	67	482	245	653	171	556
	3	102	193	91	272	79	548	276	767	219	665
	4	111	197	86	328	131	691	363	819	128	708
	5	123	238	115	339	101	591	252	808	217	685
	6	128	215	87	319	104	558	239	714	156	586
	Mean	108	191	83	282	91	553	271	737	184	629
	SD	16	37	23	58	26	86	47	72	38	64
Group	Mean	109	203	94	295	92	553	271	737	184	629
Total	SD	17	32	20	48	22	86	47	72	38	64

- Indicates bird was euthanized on Day 8 for sampling.

APPENDIX VI

Individual Body Weights (g) from a Mallard
Dietary Toxicity Study with T-7485
Page 7

Experimental Group (ppm a.i.)	Bird	Day 0	Day 5	Change	Day 8	Change	Day 15	Change	Day 22	Change	Total Change
	1	83	118	35	202	84	-	-	-	-	-
10,000	2	100	167	67	256	89	-	-	-	-	-
	3	92	173	81	249	76	-	-	-	-	-
	4	112	219	107	338	119	-	-	-	-	-
	5	125	237	112	327	90	-	-	-	-	-
	6	132	218	86	327	109	-	-	-	-	-
	Mean	107	189	81	283	95	-	-	-	-	-
	SD	19	44	28	55	16	-	-	-	-	-
	1	84	151	67	217	66	459	242	680	221	596
10,000	2	100	173	73	264	91	521	257	732	211	632
	3	109	163	54	273	110	521	248	777	256	668
	4	111	207	96	307	100	533	226	750	217	639
	5	120	224	104	323	99	508	185	713	205	593
	6	130	250	120	360	110	580	220	726	146	596
	Mean	109	195	86	291	96	520	230	730	209	621
	SD	16	39	25	50	16	39	26	33	36	31
Group	Mean	108	192	84	287	95	520	230	730	209	621
Total	SD	17	40	26	51	16	39	26	33	36	31

- Indicates bird was euthanized on Day 8 for sampling.

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Mallard
Dietary Toxicity Study with T-7485
Page 1

Experimental Group (ppm a.i)	Pen	Exposure Period Days 0-5	Post-Exposure Period Days 6-8	Days 9-15	Days 16-22
Control	1	62	88	-	-
0	2	73	118	-	-
	3	82	113	-	-
	4	70	110	153	218
	5	63	111	159	211
	6	87	131	175	249
	Mean[1]	73	112	163	226
	SD	10	14	11	20

[1] Mean values calculated using Excel in full-precision mode. Manual calculation may vary.

- Indicates bird was euthanized on Day 8 for sampling.

APPENDIX VII

Feed Consumption (g/bird/day) by Pen from a Mallard
Dietary Toxicity Study with T-7485
Page 2

Experimental Group (ppm a.i.)	Pen	Exposure Period Days 0-5	Post-Exposure Period Days 6-8	Post-Exposure Period Days 9-15	Post-Exposure Period Days 16-22
1000	1	85	133	-	-
	2	90	123	153	191
	Mean[1]	88	128	153	191
1780	1	78	128	-	-
	2	79	137	161	228
	Mean[1]	79	132	161	228
3160	1	72	114	-	-
	2	73	169	183	247
	Mean[1]	72	142	183	247
5620	1	68	114	-	-
	2	54	103	129	173
	Mean[1]	61	109	129	173
10,000	1	65	104	-	-
	2	54	101	141	181
	Mean[1]	60	102	141	181

[1] Mean values calculated using Excel in full-precision mode. Manual calculation may vary.
- Indicates bird was euthanized on Day 8 for sampling.

APPENDIX VIII

Individual Liver Weights (g) from a Mallard Dietary Toxicity Study with T-7485

Day 8

	Test Concentration (ppm a.i.)					
Replicate	0	1000	1780	3160	5620	10,000
1	10.423	14.779	11.612	11.422	12.335	9.866
2	13.533	16.787	14.582	11.892	15.357	15.807
3	13.655	13.522	16.453	14.612	14.841	12.771
4	15.072	16.630	19.088	14.821	14.766	18.895
5	16.034	23.205	17.128	19.441	15.620	16.150
6	12.193	20.538	15.496	21.270	17.906	15.479
7	14.766	-	-	-	-	-
8	12.346	-	-	-	-	-
9	14.489	-	-	-	-	-
10	13.183	-	-	-	-	-
11	15.682	-	-	-	-	-
12	12.259	-	-	-	-	-
13	15.602	-	-	-	-	-
14	13.696	-	-	-	-	-
15	21.799	-	-	-	-	-
Mean[1]	14.315	17.577	15.727	15.576	15.138	14.828
SD	2.588	3.640	2.533	3.992	1.790	3.115

Day 22

	Test Concentration (ppm a.i.)					
Replicate	0	1000	1780	3160	5620	10,000
1	29.919	29.016	26.240	22.878	26.811	22.255
2	22.799	20.478	24.570	24.640	24.758	27.412
3	26.250	26.401	24.662	24.093	30.148	28.648
4	31.345	27.167	28.038	25.280	27.828	29.002
5	37.977	30.841	28.755	23.643	25.806	26.268
6	22.717	30.789	23.519	27.632	27.077	34.624
7	22.160	-	-	-	-	-
8	22.965	-	-	-	-	-
9	21.348	-	-	-	-	-
10	26.384	-	-	-	-	-
11	21.619	-	-	-	-	-
12	26.076	-	-	-	-	-
13	28.230	-	-	-	-	-
14	26.553	-	-	-	-	-
15	20.764	-	-	-	-	-
Mean[1]	25.807	27.449	25.964	24.694	27.071	28.035
SD	4.672	3.870	2.087	1.658	1.847	4.041

[1] Mean values calculated using Excel in full-precision mode. Manual calculation may vary.

Appendix IX

Changes to Protocol

The study was conducted in accordance with the approved Protocol with the following change.

1. The protocol was amended to indicate blood and liver samples will be analyzed at the Sponsor's discretion and results of tissue analysis will not be reported. Additionally, change the Sponsor's Representative to Sue Beach.

2. The protocol was amended to indicate statistical comparisons of body weight and liver weight data will be made.

3. The protocol was amended to change the Sponsor's Representative to John Newstead.

APPENDIX X

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 of Avian Toxicology
(3) Linda R. Mitchell, Manager of Ecotox Operations
(4) Sean P. Gallagher, Senior Biologist, Avian Toxicology
(5) Willard B. Nixon, Ph.D., Director of Chemistry
(6) Timothy Z. Kendall, Supervisor, Chemistry
(7) Raymond L. Van Hoven, Ph.D., Scientist, Chemistry