

26) OECD 206-OPPTS
850.2300, Pilot chronic avian
toxicity (Northern Bobwhite),



DEC 09 2003

July 11, 2003

Dr. John Newsted
Entrix
East Lansing Office
4295 Okemos Road, Suite 101
Okemos, MI 48864

Dear John:

The following is a summary of the findings for the study: T-7485: A Pilot Reproduction Study with the Northern Bobwhite (WLI Project No.454-115; 3M Environmental Lab Project No. E03-0214). Adult northern bobwhite were exposed to T-7485 at nominal dietary concentrations of 75, 200, 550 and 1500 ppm a.i. for a period of 6 weeks. A control group, fed non-treated diet, was maintained concurrently with the treatment groups. Each treatment and the control group consisted of five pairs of birds, housed with one male and one female per pen. At the end of Week 6, all surviving birds were euthanized and subjected to gross necropsy. Effects on adult health, body weight gain, feed consumption and egg production were evaluated. Egg, blood and liver samples were collected for possible analyses.

The adult birds were observed daily for mortality, abnormal behavior and signs of toxicity. Body weights were measured at test initiation, on Weeks 2 and 4, and at adult termination. Feed consumption for each pen was measured over a seven-day period each week throughout the test. Necropsies were performed on all birds. Liver weights were recorded for all surviving birds. Liver and blood samples (when available) were collected at the time of necropsy for analysis.

During the test, the number of eggs laid in each pen was recorded to evaluate egg production. Following enumeration, eggs laid during the first five weeks of the test were disposed of by incineration. All eggs laid during the final week of exposure were stored refrigerated. Two eggs (when available) were indiscriminately chosen from each pen for possible analysis. All blood, liver and egg samples were shipped to 3M Corporation for possible analysis.

Mortalities

There were no treatment-related mortalities in any of the treatment groups. While no mortalities occurred in the control group or in the 75, 200, 550 ppm a.i. treatment groups during the course of the test, one incidental mortality occurred in the 1500 ppm a.i. treatment group. The single mortality in the 1500 ppm a.i. treatment group was a hen (Pen 322) that was noted with an apparent neck injury on Day 2 of the test and was subsequently euthanized, three days following experimental start. Prior to euthanasia, the hen exhibited ventral head curl, wing droop, ataxia (loss of coordination), a ruffled appearance and lethargy. At necropsy the bird was thin, with a body weight of 162 grams. Externally, feather loss was noted on the head and neck. Internally, there was a loss of muscle mass and a small bruise around the left ear. The liver contained scattered

white foci. The abdominal cavity showed evidence of egg yolk peritonitis and the ovary was regressing. Since the bird was euthanized within three days of experimental start and the mortality was clearly not treatment-related, the hen was replaced. The replacement bird was from the same lot that had acclimated with the test birds for use as a potential replacement. The data reported for Pen 322 are that of the replacement bird.

Clinical Observations

Several birds were noted with foot lesions and feather loss as a result of pen wear and/or pen mate aggression during the course of the test. An incidental clinical sign, lameness, was noted for one bird (Pen 320) in the 550 ppm a.i. treatment group during Week 2 of the test. All other birds were normal in appearance and behavior for the duration of the test.

Adult Body Weight and Feed Consumption

When compared to the control group, there were no apparent treatment-related effects on adult male or female body weight at the 75, 200 or 550 ppm a.i. test concentrations, or on female body weight at the 1500 ppm a.i. test concentration. There was a slight, but consistent, reduction in adult male body weight at the 1500 ppm a.i. test concentration during each weight interval that may have been treatment-related. Mean body weight measurements are presented in Table 1, and individual body weight measurements are presented in Appendix I.

Due to excessive wastage by some birds, feed consumption was variable between pens. However, when compared to the control group, there appeared to be no treatment-related effects on feed consumption at the 75, 200 or 550 ppm a.i. test concentrations. There were slight, but consistent, reductions in feed consumption at the 1500 ppm a.i. treatment group during each feeding interval that may have been related to treatment. Mean feed consumption measurements are shown in Table 2, and feed consumption measurements by pen are presented in Appendix II.

Egg Production

When compared the control group, there were no apparent treatment-related effects upon egg production at the 75 and 200 ppm a.i. test concentrations. While there was a slight reduction in egg production at the 75 ppm a.i. concentration, the reduction was not dose-responsive and was primarily due to two hens (Pens 309 and 310) which laid poorly during the final week of the test. Subsequent gross necropsy of these two hens revealed egg remnants in the abdominal cavity. However, there were reductions in mean egg production at the 550 and 1500 ppm a.i. test concentrations for which a treatment-related effect could not be precluded. Mean egg production by concentration is presented in Table 3. Individual egg production data are presented in Appendix III.

Gross Necropsy

At the end of Week 6 (Day 42), all adult birds were euthanized and subjected to gross necropsy. All findings observed were considered to be incidental to treatment. Necropsy findings are reported in Table 4 and Appendix IV.

Liver Weight

When compared to the control group, there were no apparent treatment-related effects on liver weight at the 75, 200 or 550 ppm a.i. test concentrations, or among males at the 1500 ppm a.i. test concentration. There was a slight reduction in mean female liver weight at the 1500 ppm a.i. test concentration. Liver weights are presented in Table 5 and Appendix V.

Results of Diet Analyses

The procedures followed to analyze the dietary samples are presented in Appendix VI. None of the control samples showed any indication of the presence of the test substance or of the presence of co-eluting substances at the characteristic retention time of the test substance. Diet samples were collected on Week 1, Day 0 of the test from the 75, 200, 550, and 1500 ppm a.i. test levels to evaluate the homogeneity of the test substance in the diet. The means and standard deviations for the 75, 200, 550, and 1500 ppm a.i. test levels were 75.6 ± 4.85 ppm a.i., 218 ± 8.87 ppm a.i., 561 ± 17.2 ppm a.i., and 1530 ± 41.2 ppm a.i., respectively. The corresponding coefficients of variation were 1.32%, 4.06%, 3.06%, and 2.69% (Appendix VI, Table 4). The mean measured T-7485 concentrations from the 75, 200, 550, and 1500 ppm a.i. test levels were 101%, 109%, 102%, and 102%, respectively, of the nominal concentrations. A typical ion chromatogram of a test sample is shown in Appendix VI, Figure 7.

Diet samples collected on Week 6, Day 0 of the test from the 75, 200, 550, and 1500 ppm a.i. test levels were analyzed to verify the concentration of the test substance in the diet. The mean and standard deviation for the 75, 200, 550, and 1500 ppm a.i. test concentrations were 71.7 ± 1.72 ppm a.i., 192 ± 3.21 ppm a.i., 519 ± 15.6 ppm a.i., and 1410 ± 25.2 ppm a.i., respectively. The corresponding coefficients of variation were 2.40%, 1.68%, 3.01%, and 1.78% (Appendix VI, Table 5). The mean measured T-7485 concentrations from the 75, 200, 550, and 1500 ppm a.i. test levels were 96%, 96%, 94%, and 94%, respectively, of the nominal concentrations. Analysis of diet samples collected from feeders after being held at ambient temperature for seven days averaged 128%, 116%, 115%, and 109% of the Day 0 values for the 75, 200, 550, and 1500 ppm a.i. test concentrations, respectively (Appendix VI, Table 6).

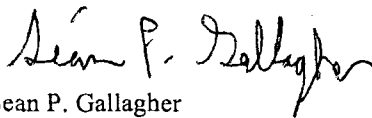
Results of Blood, Liver and Egg Analysis

Blood, liver and egg samples were transferred to 3M Corporation for possible analysis.

Conclusion

Northern bobwhite were exposed to T-7485 at dietary concentrations of 0, 75, 200, 550 and 1500 ppm a.i. for 6 weeks. No treatment-related mortalities or overt signs of toxicity were observed at any of the concentrations tested. Additionally, there were no apparent treatment-related effects upon body weight or upon feed consumption at the 75, 200 or 550 ppm a.i. test concentrations. There were no apparent treatment-related reductions in egg production at the 75 or 200 ppm a.i. test concentrations. However, there were slight, but consistent, reductions in male body weight and feed consumption in the 1500 ppm a.i. treatment group that may have been related to treatment. There were also reductions in mean egg production at the 550 and 1500 ppm a.i. test concentrations and in female liver weight at the 1500 ppm a.i. test concentration that may have been related to treatment. Based upon the results of this study, the conservative no-observed-effect concentration for northern bobwhite exposed to T-7485 in the diet was 200 ppm a.i.

Sincerely,



Sean P. Gallagher
Senior Avian Biologist

Table 1
Mean Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

Experimental Group	Sex	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
Control	Male	212	3	215	0	214	2	217	4
	Female	229	6	236	3	239	1	240	11
75 ppm a.i.	Male	213	8	221	2	223	1	224	11
	Female	234	6	240	1	241	-25	216	-18
200 ppm a.i.	Male	215	1	216	1	217	2	219	4
	Female	231	7	238	-1	236	-3	234	3
550 ppm a.i.	Male	218	10	229	5	233	-8	226	7
	Female	227	2	229	-8	221	1	222	-5
1500 ppm a.i.	Male	215	1	217	-9	208	0	208	-8
	Female	230	4	234	-5	229	3	232	2

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

Table 2
Mean Feed Consumption (g/bird/day) from a Northern Bobwhite Pilot
Reproduction Study with T-7485

Experimental Group ppm a.i.	Weeks					
	1	2	3	4	5	6
Control	22	26	23	29	27	27
75	23	25	23	28	26	28
200	22	25	23	27	28	27
550	22	25	22	26	25	23
1500	19	22	20	22	23	24

Table 3
Egg Production Data by Week from a Northern Bobwhite
Pilot Reproduction Study with T-7485

Experimental Group ppm a.i.	Weeks						Totals	Eggs/Female/Day
	1	2	3	4	5	6		
Control	25	24	33	34	32	34	182	0.87
75	25	27	31	33	30	20	166	0.79
200	29	31	31	33	32	33	189	0.90
550	20	23	24	24	26	21	138	0.66
1500	16	24	27	26	22	27	142	0.69

Table 4
Summary of Gross Pathological Observations
from a Northern Bobwhite Pilot Reproduction Study with T-7485
Surviving Birds Euthanized at Test Termination

Finding	Males					Females				
	ppm a.i.					ppm a.i.				
	Control	75	200	550	1500	Control	75	200	550	1500
Number of birds	5	5	5	5	5	5	5	5	5	5
Feather loss	0	2	1	0	0	4	1	2	2	1
Spleen - slightly enlarged	1	0	0	0	0	0	0	0	0	0
Liver - friable	0	0	0	0	0	0	0	0	1	0
Abdominal cavity - slight egg yolk peritonitis	-	-	-	-	-	0	1	0	0	0
Abdominal cavity - yolk remnants	-	-	-	-	-	0	3	0	0	0
Ovary - regressing	-	-	-	-	-	0	1	0	0	0
Right oviduct - partially developed	-	-	-	-	-	0	0	1	0	0
Testes - small (one or both <1.25 cm)	0	0	1	2	0	-	-	-	-	-
Not remarkable	4	3	3	3	5	1	1	3	2	4

Table 5
Mean Liver Weights
from a Northern Bobwhite Pilot Reproduction Study with T-7485

Experimental Group	Liver Weights (g)			
	Males		Females	
	Mean	SD	Mean	SD
Control	3.624 ±	0.756	6.514 ±	1.406
75 ppm a.i.	3.757 ±	0.511	6.197 ±	0.948
200 ppm a.i.	3.743 ±	0.428	6.646 ±	1.181
550 ppm a.i.	3.916 ±	0.600	6.222 ±	1.328
1500 ppm a.i.	3.655 ±	0.525	5.886 ±	1.110

Appendix I - Table 1
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

Control Males

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
301	194	-2	192	-1	191	2	193	-1
302	210	1	211	1	212	1	213	3
303	198	2	200	1	201	-3	198	0
304	227	3	230	-2	228	0	228	1
305	232	9	241	-1	240	11	251	19
Mean	212	3	215	0	214	2	217	4
SD	17	4	20	1	20	5	24	8

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

Appendix I - Table 2
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

Control Females

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
301	236	5	241	3	244	-2	242	6
302	206	10	216	10	226	9	235	29
303	242	10	252	6	258	-1	257	15
304	219	1	220	-5	215	1	216	-3
305	243	6	249	1	250	0	250	7
Mean	229	6	236	3	239	1	240	11
SD	16	4	17	6	18	4	16	12

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

Appendix I - Table 3
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

75 ppm a.i. Males

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
306	205	7	212	0	212	-4	208	3
307	231	15	246	9	255	2	257	26
308	211	5	216	0	216	2	218	7
309	183	7	190	-7	183	0	183	0
310	235	5	240	8	248	4	252	17
Mean	213	8	221	2	223	1	224	11
SD	21	4	23	7	29	3	31	11

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

Appendix I - Table 4
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

75 ppm a.i. Females

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
306	236	-6	230	3	233	-4	229	-7
307	208	17	225	-1	224	-1	223	15
308	277	0	277	4	281	-68	213	-64
309	217	11	228	2	230	-28	202	-15
310	232	7	239	-3	236	-24	212	-20
Mean	234	6	240	1	241	-25	216	-18
SD	27	9	21	3	23	27	10	29

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

Appendix I - Table 5
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

200 ppm a.i. Males

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
311	224	2	226	0	226	1	227	3
312	229	3	232	5	237	2	239	10
313	222	1	223	-4	219	4	223	1
314	189	-5	184	4	188	1	189	0
315	211	5	216	-2	214	1	215	4
Mean	215	1	216	1	217	2	219	4
SD	16	4	19	4	18	1	19	4

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

Appendix I - Table 6
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

200 ppm a.i. Females

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
311	229	3	232	4	236	-29	207	-22
312	234	14	248	-2	246	5	251	17
313	209	7	216	-7	209	5	214	5
314	237	0	237	-7	230	9	239	2
315	244	11	255	6	261	-3	258	14
Mean	231	7	238	-1	236	-3	234	3
SD	13	6	15	6	19	15	22	15

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

Appendix I - Table 7
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

550 ppm a.i. Males

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
316	216	16	232	8	240	10	250	34
317	216	8	224	-6	218	-3	215	-1
318	220	8	228	14	242	-40	202	-18
319	208	13	221	1	222	-8	214	6
320	232	6	238	7	245	2	247	15
Mean	218	10	229	5	233	-8	226	7
SD	9	4	7	8	12	19	22	19

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

Appendix I - Table 8
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

550 ppm a.i. Females

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
316	248	-5	243	-4	239	-31	208	-40
317	214	3	217	-8	209	10	219	5
318	232	20	252	-50	202	36	238	6
319	213	-8	205	2	207	-3	204	-9
320	227	-1	226	20	246	-7	239	12
Mean	227	2	229	-8	221	1	222	-5
SD	14	11	19	26	20	25	16	21

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

Appendix I - Table 9
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

1500 ppm a.i. Males

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
321	231	3	234	-3	231	0	231	0
322	193	5	198	2	200	0	200	7
323	207	4	211	0	211	-4	207	0
324	231	-7	224	-42	182	4	186	-45
325	215	1	216	-1	215	-1	214	-1
Mean	215	1	217	-9	208	0	208	-8
SD	16	5	14	19	18	3	17	21

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

Appendix I - Table 10
Adult Body Weight (g) from a Northern Bobwhite
Pilot Reproduction Study with T-7485

1500 ppm a.i. Females

Pen	Week 0	Change Week 0-2	Week 2	Change Week 2-4	Week 4	Change Week 4-6	Week 6	Total Change
321	230	4	234	-5	229	5	234	4
322	204	-6	198	10	208	-2	206	2
323	230	14	244	-32	212	18	230	0
324	221	11	232	-1	231	-11	220	-1
325	265	-2	263	1	264	7	271	6
Mean	230	4	234	-5	229	3	232	2
SD	22	8	24	16	22	11	24	3

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

Appendix II - Table 1
Feed Consumption (g/bird/day) from a Northern Bobwhite Pilot
Reproduction Study with T-7485

Control						
Pen	Weeks					
	1	2	3	4	5	6
301	24	26	25	30	30	28
302	17	20	21	24	22	23
303	21	24	24	27	25	26
304	21	23	22	27	26	27
305	26	36	25	39	33	33
Mean	22	26	23	29	27	27
SD	3	6	2	6	4	3

Appendix II - Table 2
Feed Consumption (g/bird/day) from a Northern Bobwhite Pilot
Reproduction Study with T-7485

75 ppm a.i.

Pen	Weeks					
	1	2	3	4	5	6
306	20	21	21	28	27	29
307	24	27	27	34	29	31
308	22	26	22	26	26	33
309	25	27	22	26	20	25
310	20	24	22	25	26	22
Mean	23	25	23	28	26	28
SD	2	3	2	4	3	5

Appendix II - Table 3
Feed Consumption (g/bird/day) from a Northern Bobwhite Pilot
Reproduction Study with T-7485

200 ppm a.i.

Pen	Weeks					
	1	2	3	4	5	6
311	20	25	24	24	27	22
312	24	27	23	30	27	31
313	21	24	23	26	29	27
314	18	23	22	26	25	24
315	25	25	24	29	29	30
Mean	22	25	23	27	28	27
SD	3	1	1	2	2	4

Appendix II - Table 4
Feed Consumption (g/bird/day) from a Northern Bobwhite Pilot
Reproduction Study with T-7485

550 ppm a.i.						
Pen	Weeks					
	1	2	3	4	5	6
316	21	23	24	23	18	18
317	21	24	14	27	27	25
318	28	28	27	25	26	25
319	17	22	22	27	29	23
320	22	25	23	27	26	26
Mean	22	25	22	26	25	23
SD	4	2	5	2	4	3

Appendix II - Table 5
Feed Consumption (g/bird/day) from a Northern Bobwhite Pilot
Reproduction Study with T-7485

1500 ppm a.i.						
Pen	Weeks					
	1	2	3	4	5	6
321	22	23	24	27	28	30
322	-	20	20	21	23	23
323	18	23	19	19	22	23
324	18	20	15	18	19	22
325	17	23	22	24	22	23
Mean	19	22	20	22	23	24
SD	2	2	4	4	3	3
- Data not reported. On day 3 of the test, the female in Pen 322 was replaced due to possible neck injury.						

Appendix III - Table I
Eggs Laid per Pen per Week from a Northern Bobwhite
Pilot Reproduction Study with T-7485

Control

Pen	Weeks						Totals	Eggs/Female/Day
	1	2	3	4	5	6		
301	6	5	7	7	7	7	39	0.93
302	4	4	7	6	6	7	34	0.81
303	5	4	7	7	6	6	35	0.83
304	5	5	6	7	6	7	36	0.86
305	5	6	6	7	7	7	38	0.90
Totals	25	24	33	34	32	34	182	0.87

Appendix III - Table 2
Eggs Laid per Pen per Week from a Northern Bobwhite
Pilot Reproduction Study with T-7485

75 ppm a.i.

Pen	Weeks						Totals	Eggs/Female/Day
	1	2	3	4	5	6		
306	6	5	6	7	7	6	37	0.88
307	3	3	5	5	5	6	27	0.64
308	5	5	6	7	6	7	36	0.86
309	6	7	7	7	4	0	31	0.74
310	5	7	7	7	8	1	35	0.83
Totals	25	27	31	33	30	20	166	0.79

Appendix III - Table 3
Eggs Laid per Pen per Week from a Northern Bobwhite
Pilot Reproduction Study with T-7485

200 ppm a.i.

Pen	Weeks						Totals	Eggs/Female/Day
	1	2	3	4	5	6		
311	6	7	6	6	7	7	39	0.93
312	5	6	6	6	7	7	37	0.88
313	6	6	7	7	6	6	38	0.90
314	5	5	5	7	5	6	33	0.79
315	7	7	7	7	7	7	42	1.00
Totals	29	31	31	33	32	33	189	0.90

Appendix III - Table 4
Eggs Laid per Pen per Week from a Northern Bobwhite
Pilot Reproduction Study with T-7485

550 ppm a.i.

Pen	Weeks						Totals	Eggs/Female/Day
	1	2	3	4	5	6		
316	5	5	6	7	5	1	29	0.69
317	5	5	3	2	6	6	27	0.64
318	5	7	7	6	3	3	31	0.74
319	3	3	5	5	6	5	27	0.64
320	2	3	3	4	6	6	24	0.57
Totals	20	23	24	24	26	21	138	0.66

Appendix III - Table 5
Eggs Laid per Pen per Week from a Northern Bobwhite
Pilot Reproduction Study with T-7485

1500 ppm a.i.

Pen	Weeks						Totals	Eggs/Female/Day
	1	2	3	4	5	6		
321	5	5	7	6	7	7	37	0.88
322	-	2	3	3	4	4	16	0.42
323	4	6	6	6	0	6	28	0.67
324	3	5	5	4	5	5	27	0.64
325	4	6	6	7	6	5	34	0.81
Totals	16	24	27	26	22	27	142	0.69

- Data not reported. On day 3 of the test, the female in Pen 322 was replaced due to possible neck injury.

Appendix IV - Table 1
 Individual Gross Pathological Observations
 from a Northern Bobwhite Pilot Reproduction Study with T-7485
 Birds Euthanized at Test Termination

Control

Males

Finding	Pen Number				
	301	302	303	304	305
Spleen - slightly enlarged	X	-	-	-	-
Not remarkable	-	X	X	X	X

Females

Finding	Pen Number				
	301	302	303	304	305
Feather loss	X	X	-	X	X
Not remarkable	-	-	X	-	-

Appendix IV - Table 2
 Individual Gross Pathological Observations
 from a Northern Bobwhite Pilot Reproduction Study with T-7485
 Birds Euthanized at Test Termination

75 ppm a.i.

Males

Finding	Pen Number				
	306	307	308	309	310
Feather loss	X	-	-	X	-
Not remarkable	-	X	X	-	X

Females

Finding	Pen Number				
	306	307	308	309	310
Feather loss	-	X	-	-	-
Abdominal cavity - slight egg yolk peritonitis	-	-	X	-	-
Abdominal cavity - yolk remnants	-	-	X	X	X
Ovary - regressing	-	-	X	-	-
Not remarkable	X	-	-	-	-

Appendix IV - Table 3
 Individual Gross Pathological Observations
 from a Northern Bobwhite Pilot Reproduction Study with T-7485
 Birds Euthanized at Test Termination

200 ppm a.i.

Males

Finding	Pen Number				
	311	312	313	314	315
Feather loss	-	-	-	X	-
Testes - small (~1.25 cm)	-	X	-	-	-
Not remarkable	X	-	X	-	X

Females

Finding	Pen Number				
	311	312	313	314	315
Feather loss	-	X	-	X	-
Right oviduct - partially developed	-	X	-	-	-
Not remarkable	X	-	X	-	X

Appendix IV - Table 4
 Individual Gross Pathological Observations
 from a Northern Bobwhite Pilot Reproduction Study with T-7485
 Birds Euthanized at Test Termination

550 ppm a.i.

Males

Finding	Pen Number				
	316	317	318	319	320
Testes - small (~1.0 cm)	-	-	X	-	-
Right testis - small (~1.25 cm)	-	-	-	-	X
Not remarkable	X	X	-	X	-

Females

Finding	Pen Number				
	316	317	318	319	320
Feather loss	X	-	-	X	-
Liver - friable	-	X	-	-	-
Not remarkable	-	-	X	-	X

Appendix IV - Table 5
 Individual Gross Pathological Observations
 from a Northern Bobwhite Pilot Reproduction Study with T-7485
 Birds Euthanized at Test Termination

1500 ppm a.i.

Males

Finding	Pen Number				
	321	322	323	324	325
Not remarkable	X	X	X	X	X

Females

Finding	Pen Number				
	321	322	323	324	325
Feather loss	-	X	-	-	-
Not remarkable	X	-	X	X	X

Appendix V - Table 1
Liver Weights by Pen
from a Northern Bobwhite Pilot Reproduction Study with T-7485

Male				
Experimental Group	Pen	Liver Weights (g)	Group Mean	SD
Control	301	3.832	3.624 ±	0.756
	302	2.995		
	303	3.110		
	304	3.336		
	305	4.848		
75 ppm a.i.	306	3.706	3.757 ±	0.511
	307	12.315 ¹		
	308	4.099		
	309	3.053		
	310	4.168		
200 ppm a.i.	311	4.426	3.743 ±	0.428
	312	3.239		
	313	3.647		
	314	3.719		
	315	3.685		
550 ppm a.i.	316	4.612	3.916 ±	0.600
	317	4.474		
	318	3.387		
	319	3.326		
	320	3.781		
1500 ppm a.i.	321	4.036	3.655 ±	0.525
	322	3.427		
	323	3.272		
	324	4.375		
	325	3.164		

¹ - Abnormally large liver. Data was not included in mean calculation.

Appendix V - Table 2
 Liver Weights by Pen
 from a Northern Bobwhite Pilot Reproduction Study with T-7485

Female				
Experimental Group	Pen	Liver Weights (g)	Group Mean	SD
Control	301	7.880	6.514 ±	1.406
	302	4.994		
	303	6.461		
	304	5.255		
	305	7.978		
75 ppm a.i.	306	6.786	6.197 ±	0.948
	307	6.370		
	308	4.707		
	309	7.166		
	310	5.957		
200 ppm a.i.	311	5.875	6.646 ±	1.181
	312	5.994		
	313	6.236		
	314	6.399		
	315	8.726		
550 ppm a.i.	316	5.301	6.222 ±	1.328
	317	7.044		
	318	8.175		
	319	5.253		
	320	5.338		
1500 ppm a.i.	321	5.825	5.886 ±	1.110
	322	4.324		
	323	6.269		
	324	5.618		
	325	7.392		

Appendix VI

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- μ m)
GUARD COLUMN:	Keystone Javelin C ₁₈ column (20 mm × 2 mm I.D.)
OVEN TEMPERATURE:	40°C
STOP TIME:	3.00 minutes
FLOW RATE:	200 μ L/minute
MOBILE PHASE:	10% NANOpure Water [®] with 0.1% Ammonium Formate: 90% Methanol
INJECTION VOLUME:	5.0 μ L
T-7485 RETENTION TIME:	Approximately 1.7 minutes
T-7485 MONITORED MASS:	299.0 amu → 98.9 amu

Appendix VI

Table 2

Example Calculations

Sample number 454-115-9, nominal concentration of 200 ppm a.i. in avian feed

Peak Area = 581411

Y-intercept = 29621.3262

Slope = 2682496.7500

Primary Dilution:

Initial Weight (W_i) = 10.0 g

Original Final Volume (V_i) = 100 mL

Secondary Dilution:

Initial Volume (V_i) = 0.100 mL

Final Volume (V_f) = 10.0 mL

Overall Dilution Factor ($V_i/W_i \times V_f/V_i$) = 1000 mL/g = 1000 L/Kg

$$\begin{aligned} \text{T-7485 at instrument (mg a.i./L)} &= \frac{(\text{peak area} - \text{y-intercept})}{\text{slope}} \\ &= \frac{(581411 - 29621.3262)}{2682496.7500} \\ &= 0.2057 \text{ mg a.i./L} \end{aligned}$$

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

$$\begin{aligned} \text{T-7485 in sample (mg a.i./Kg)} &= \frac{0.2057 \text{ mg a.i.}}{\text{L}} \times \frac{1000 \text{ L}}{\text{Kg}} \\ &= 205.7 \text{ mg a.i./Kg} \\ &= 205.7 \text{ ppm a.i.} \end{aligned}$$

$$\begin{aligned} \text{Percent of Nominal Concentration} &= \frac{\text{T-7485 (ppm a.i.) in sample}}{\text{T-7485 (ppm a.i.) nominal}} \times 100 \\ &= \frac{205.7}{200} \times 100 = 103\% \end{aligned}$$

Instrument Software: MacQuan, version 1.6.

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Table 3

Matrix Blank and Fortifications Analyzed Concurrently with the Samples

Number (454-115-)	Sample		Concentration of T-7485 (ppm a.i.)		Percent Recovery ²	Mean Percent of Nominal
	Type	Interval	Fortified	Measured ^{1, 2}		
MAB-1	Matrix Blank	Week 1, Day 0	0	< 25	--	--
MAS-1	Matrix Fortification	Week 1, Day 0	50.0	47.0	94.0	96.3
MAS-2	Matrix Fortification	Week 1, Day 0	500	496	99.1	
MAS-3	Matrix Fortification	Week 1, Day 0	2000	1920	95.9	
MAB-2	Matrix Blank	Week 1, Day 7	0	< 25	--	--
MAS-4	Matrix Fortification	Week 1, Day 7	50.0	51.9	104	101
MAS-5	Matrix Fortification	Week 1, Day 7	500	522	104	
MAS-6	Matrix Fortification	Week 1, Day 7	2000	1900	94.9	
MAB-3	Matrix Blank	Week 6, Day 0	0	< 25	--	--
MAS-7	Matrix Fortification	Week 6, Day 0	50.0	47.2	94.4	97.4
MAS-8	Matrix Fortification	Week 6, Day 0	500	499	99.8	
MAS-9	Matrix Fortification	Week 6, Day 0	2000	1960	97.9	

¹The lowest level analytical standard was equivalent to a calculated value of 25 ppm a.i. in the matrix blank extract.

Measured values greater than or equal to the ppm a.i. equivalent are reported.

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

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Table 4

Homogeneity of T-7485 in Avian Diet

Nominal Concentration (ppm a.i.)	Sample I.D. Number (454-115-)	Location Sampled In Mixing Vessel	T-7485 Concentration	Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Mean Percent of Nominal
			Measured ¹ (ppm a.i.)		
75	3	Top Left	71.0	$\bar{x} = 75.6$ SD = 4.85 CV = 1.32	101
	4	Top Right	72.3		
	5	Middle Left	73.9		
	6	Middle Right	72.8		
	7	Bottom Left	82.1		
	8	Bottom Right	81.3		
200	9	Top Left	206	$\bar{x} = 218$ SD = 8.87 CV = 4.06	109
	10	Top Right	214		
	11	Middle Left	223		
	12	Middle Right	215		
	13	Bottom Left	220		
	14	Bottom Right	232		
550	15	Top Left	542	$\bar{x} = 561$ SD = 17.2 CV = 3.06	102
	16	Top Right	547		
	17	Middle Left	572		
	18	Middle Right	572		
	19	Bottom Left	584		
	20	Bottom Right	549		
1500	21	Top Left	1500	$\bar{x} = 1530$ SD = 41.2 CV = 2.69	102
	22	Top Right	1500		
	23	Middle Left	1500		
	24	Middle Right	1560		
	25	Bottom Left	1600		
	26	Bottom Right	1530		

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

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Table 5

Verification of T-7485 Concentrations in Avian Diet

Nominal Concentration (ppm a.i.)	Sample I.D. Number (454-115-)	Interval (Day 0 of Week -)	Measured T-7485 Concentrations ¹ (ppm a.i.)	Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Percent of Nominal	Mean
0	41	6	< 25	< 25	-	-
	42	6	< 25	-	-	-
75	43	6	70.5	$\bar{x} = 71.7$	94	96
	44	6	71.0	SD = 1.72	95	
	45	6	73.7	CV = 2.40%	98	
200	46	6	193	$\bar{x} = 192$	97	96
	47	6	188	SD = 3.21	94	
	48	6	194	CV = 1.68%	97	
550	49	6	521	$\bar{x} = 519$	95	94
	50	6	533	SD = 15.6	97	
	51	6	502	CV = 3.01%	91	
1500	52	6	1390	$\bar{x} = 1410$	93	94
	53	6	1440	SD = 25.2	96	
	54	6	1410	CV = 1.78%	94	

¹The lowest level analytical standard was equivalent to a calculated value of 25 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent are reported.

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Table 6

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

Nominal Concentration (ppm a.i.)	T-7485 Concentration							
	Week 1, Day 0				Week 1, Day 7			
	Sample Number (454-115-)	Measured ² (ppm a.i.)	Mean Measured (ppm a.i.)	Mean Percent of Nominal	Sample Number (454-115-)	Measured ² (ppm a.i.)	Mean Measured (ppm a.i.)	Mean Percent of Day 0
0	1	<25 ³	< 25	--	27	< 25	< 25	--
	2	< 25			28	< 25		
75	3-8 ¹	-- ¹	75.6 ¹	101	29	93.3	96.4	128
					30	97.4		
					31	98.6		
200	9-14 ¹	-- ¹	218 ¹	109	32	249	253	116
					33	255		
					34	255		
550	15-20 ¹	-- ¹	561 ¹	102	36	630	645	115
					37	667		
					38	638		
1500	21-26 ¹	-- ¹	1530 ¹	102	39	1600	1670	109
					40	1720		
					41	1690		

¹Day 0 values are from homogeneity samples presented in Table 4.

²Measured values were not corrected for mean procedural recoveries based on sample sets (Table 3).

³The lowest level analytical standard was equivalent to a calculated value of 25 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent are reported.

Appendix VI

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

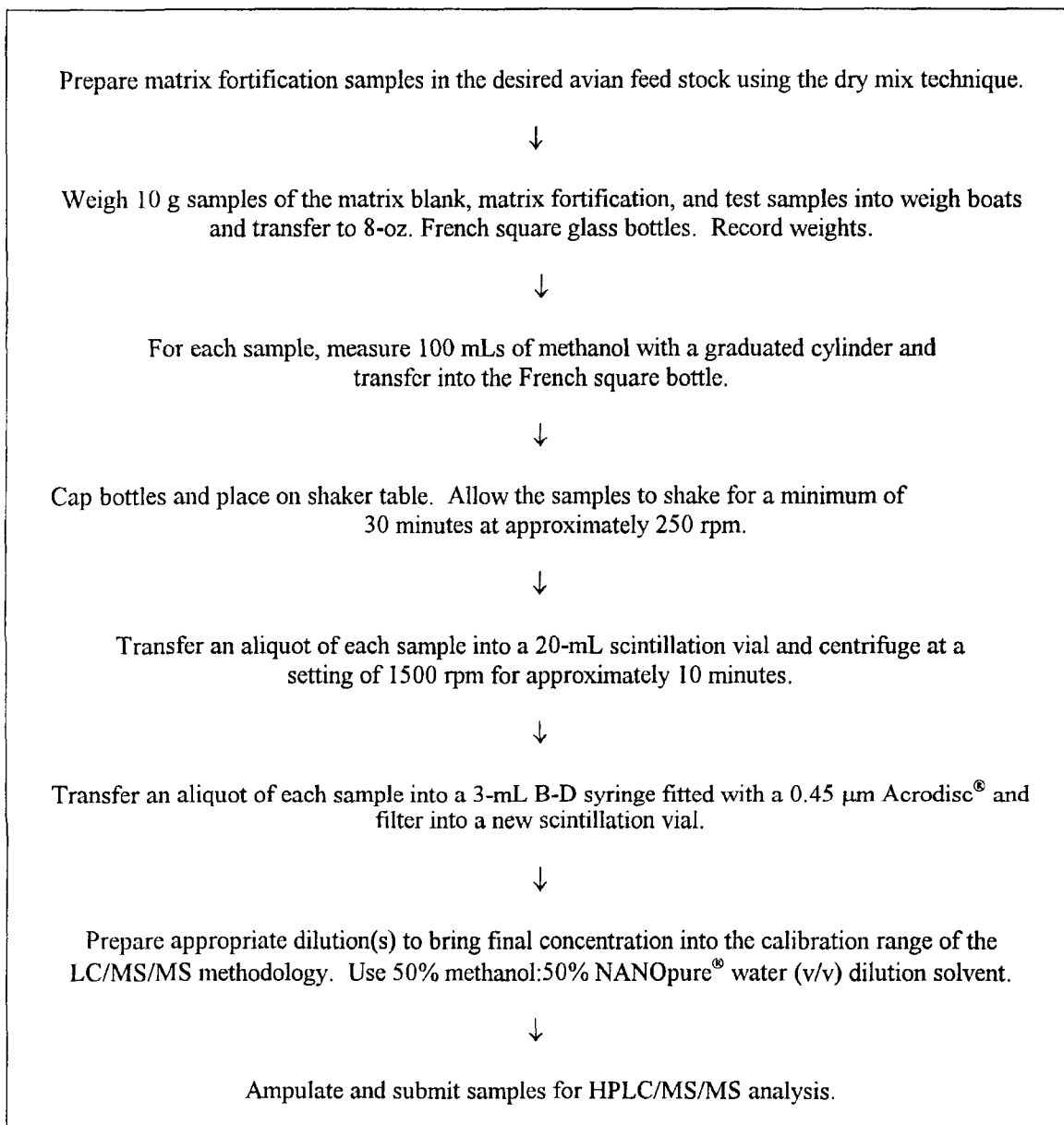


Figure 1. Analytical method flow-chart for the analysis of T-7485 in avian diet.

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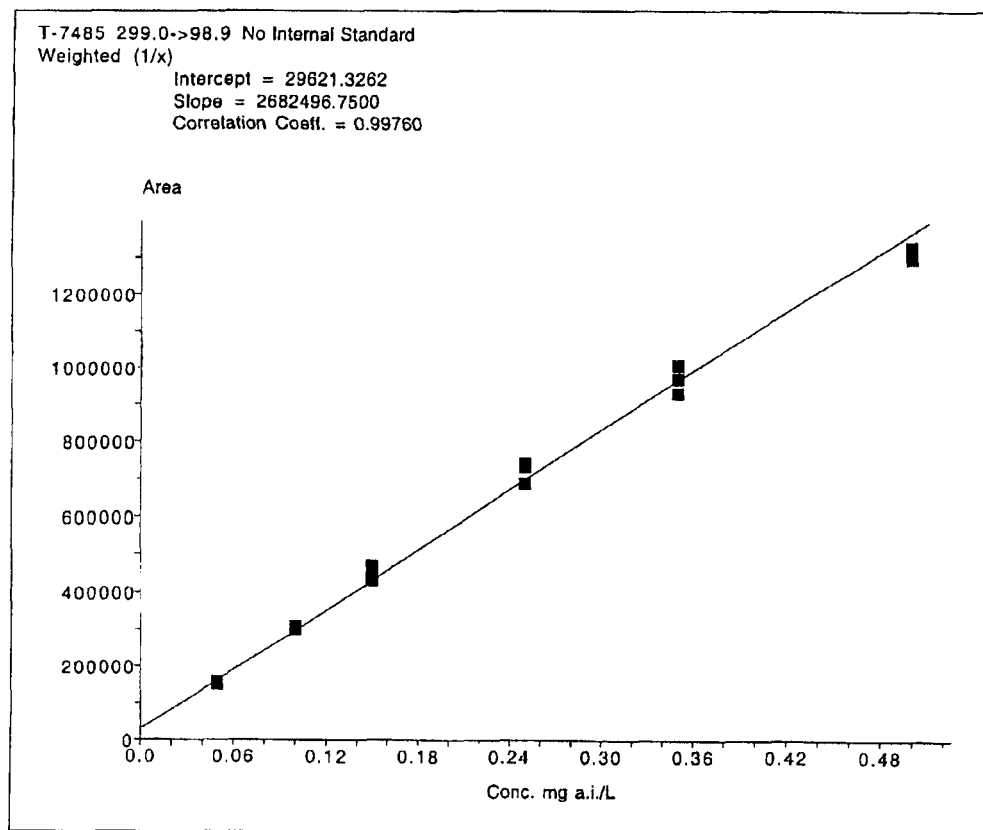


Figure 2. Representative calibration curve for T-7485.

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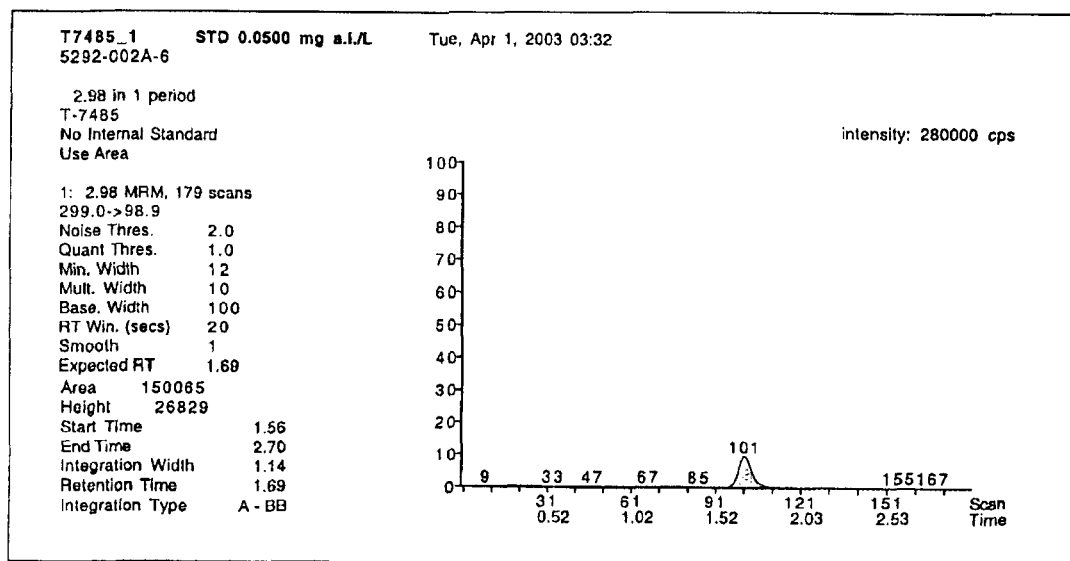


Figure 3. Representative ion chromatogram of a low-level T-7485 standard, 0.0500 mg a.i./L.

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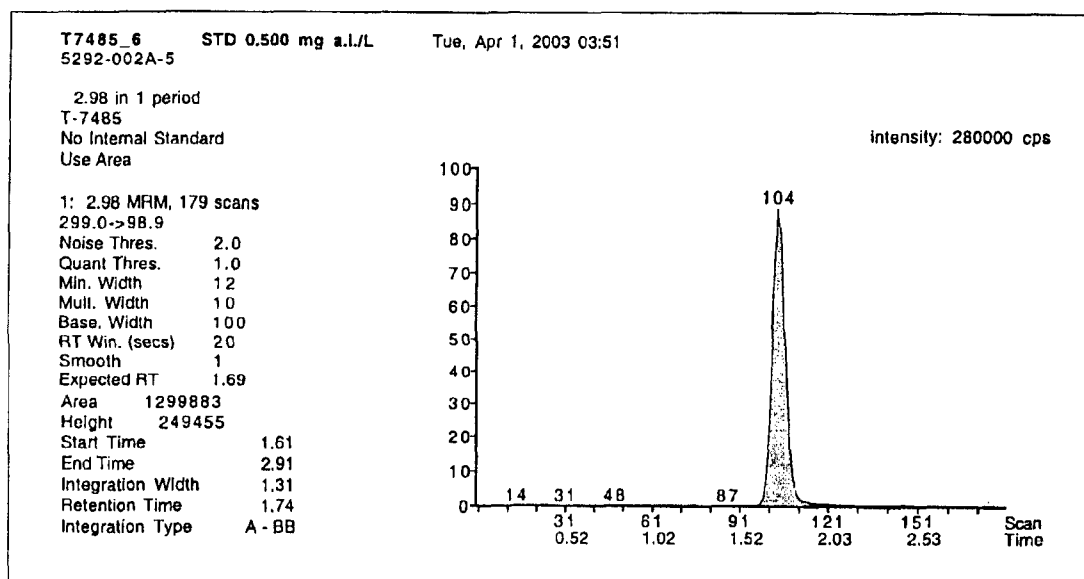


Figure 4. Representative ion chromatogram of a high-level T-7485 standard, 0.500 mg a.i./L.

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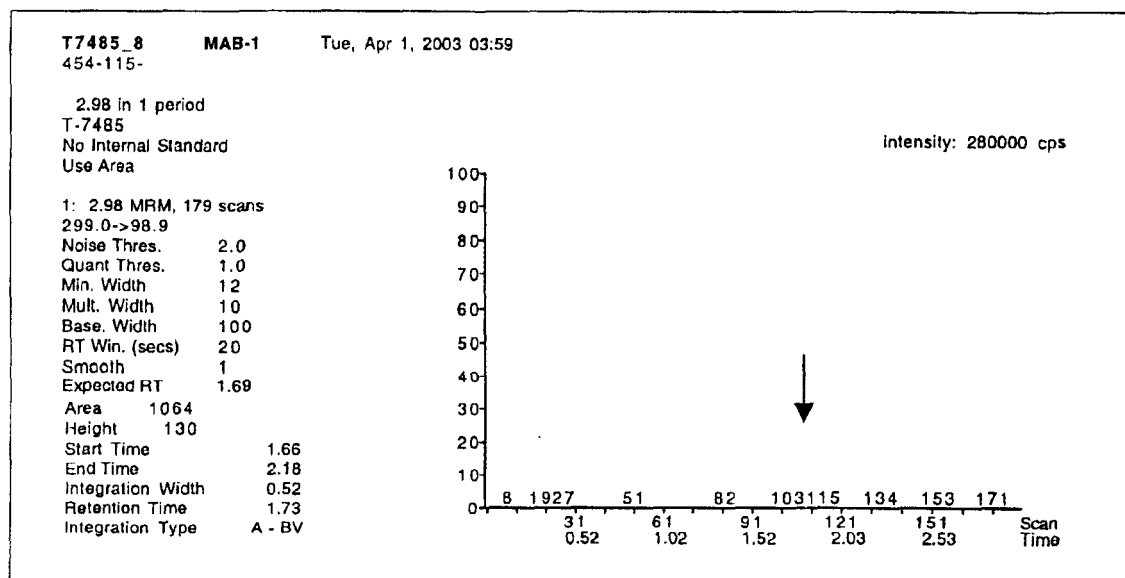


Figure 5. Representative ion chromatogram of a matrix blank sample, (454-115-MAB-1).
The arrow indicates the approximate retention time of T-7485.
10-g sample, Overall dilution = 500X.

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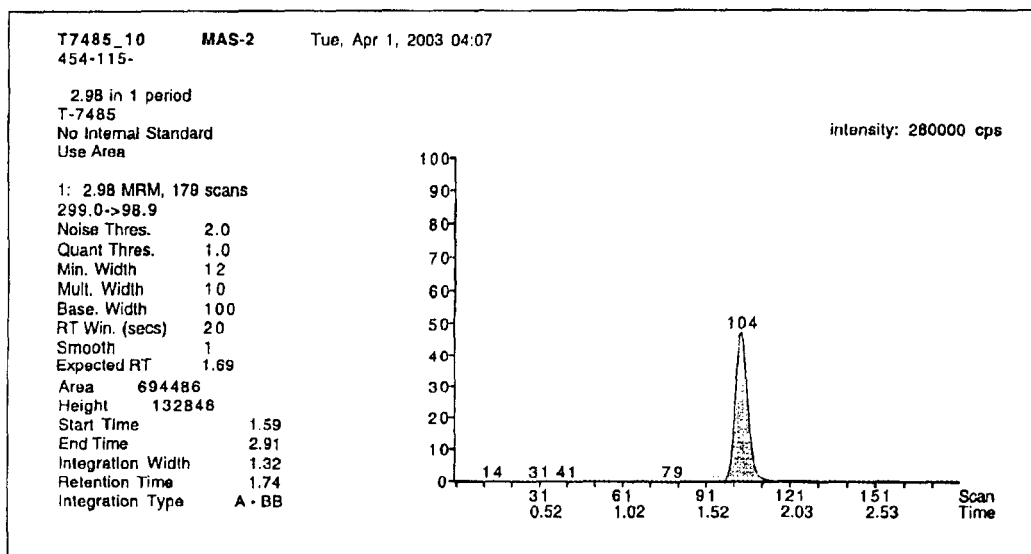


Figure 6. Representative ion chromatogram of a matrix fortification (454-115-MAS-2).
Nominal concentration 500 ppm a.i., 10-g sample, Overall dilution = 2000X.

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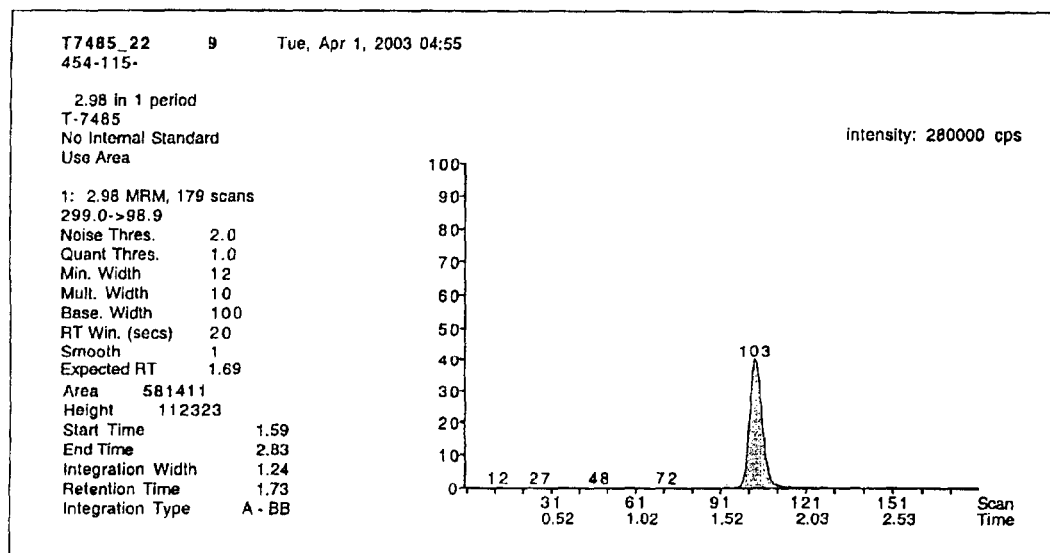


Figure 7. Representative ion chromatogram of an avian diet sample on Day 0, 454-115-9
Nominal concentration 200 ppm a.i., 10-g sample, Overall dilution = 1000X.