DP Barcode : D179116
PC Code No : 106201
EEB Out : 9/17/92

To: Dennis Edwards
Product Manager 19
Registration Division (H7505C)

From: Douglas J. Urban, Acting Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 045639-RUA
Chemical Name : Amitraz
Type Product : Insecticide
Product Name : Ovasyn
Company Name : Nor-Am Chemical Company
Purpose : Review of avian reproduction studies and supplemental data to upgrade avian dietary studies conducted on the two Amitraz metabolites.
Action Code : 181 Date Due : 09/15/92
Reviewer : Tracy Perry

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)	42124604 42124605	Y Y	72-3(A)			122-1(A)		
71-2(B)	42124606 42124607	Y Y	72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)	42336001	Y	72-3(D)			123-1(A)		
71-4(B)	42336002	Y	72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)

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DP BARCODE: D179116

CASE: 193369 DATA PACKAGE RECORD DATE: 06/09/92
SUBMISSION: S419140 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REGISTRATION ACTION: 181 RESB NW PRD-OC-NW F/F U
CHEMICALS: 106201 Dimethylphenyl)-N-(((2,4-dimethylphenyl)imino)meth 19.8000%

ID#: 045639-RUA Ovasyn
COMPANY: 045639 NOR-AM CHEMICAL COMPANY
PRODUCT MANAGER: 19 DENNIS JR EDWARDS 703-305-6386 ROOM: CM2 207
PM TEAM REVIEWER: MEREDITH JOHNSON 703-305-7080 ROOM: CM2 201
RECEIVED DATE: 06/01/92 DUE OUT DATE: 12/08/92

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 179116 EXPEDITE: N DATE SENT: 06/09/92 DATE RET.: / /
CHEMICAL: 106201 Dimethylphenyl)-N-(((2,4-dimethylphenyl)imino)methyl)-N-met
DP TYPE: 001 Submission Related Data Package
ADMIN DUE DATE: 10/07/92 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	06/11/92	09/10/92
BRAN: EEB	06/12/92	9/14/92
SECT:	/ /	/ /
REVR :	/ /	/ /
CONTR:	/ /	/ /

* * * DATA REVIEW INSTRUCTIONS * * *

* Please review these avian reproduction studies (71-4a,b) conducted on technical amitraz (necessary to support the registration of amitraz for use on cotton). Please also review the avian dietary studies conducted on the two metabolites. Attention: Harry Craven

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

* MRID Nos. 42336001
42336002
42336003
42336004

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 17 1992

MEMORANDUM

SUBJECT: Amitraz: review of avian reproduction studies (71-4 a,b); review of supplemental data submitted to upgrade the avian dietary studies conducted on the two metabolites.

FROM: Douglas Urban, Acting Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Douglas J. Urban 9/16/92

TO: Dennis Edwards, PM 19
Insecticide-Rodenticide Branch
Registration Division (H7505C)

Avian Reproduction Studies

In order to support the registration of Amitraz for use on cotton, Nor-Am Chemical Company has submitted two avian reproduction studies for review. The study citations and EEB's conclusions are as follows:

I. Beavers, J.B. et al. 1992. Technical Amitraz: Mallard Duck Dietary One Generation Reproduction Study. Performed by Wildlife International Ltd., Easton, Maryland. MRID No. 423360-02.

Conclusions: This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study as amitraz was not tested at high enough concentrations to produce reproductive effects. The NOEL was 24.6 ppm ai, based on reduced hatchling weight and increased male body weight at 50.5 ppm ai.

II. Beavers, J.B. et al. 1992. Technical Amitraz: Bobwhite Quail Dietary One Generation Reproduction Study. Performed by Wildlife International Ltd., Easton, Maryland. MRID No. 423360-01.

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Conclusions: This study is scientifically sound and fulfills the guideline requirements for an avian reproduction study. The NOEL was 24.6 ppm ai, based upon reductions in viable embryos/eggs set at 50.5 ppm ai.

Even though not all of the avian reproduction studies are core, the combination of studies performed by Wildlife International Ltd. (MRID No.s 423360-01, 423360-02, 00072411, 00072412) can be used to fulfill the guideline requirements 71-4(a) and 71-4(b).

Avian Dietary Studies Conducted with Amitraz Metabolites

In EEB's Amitraz review of March 27, 1992, the four avian dietary studies conducted on the two amitraz metabolites were classified as supplemental as the results of the homogeneity, stability and concentration verification tests were not included in the report. The missing information was provided by the registrant in two supplemental volumes (MRID #'s 423360-03, 423360-04).

In the avian dietary studies conducted with the metabolite BTS 27271 (421246-04, 421246-06), the test material was found to be homogeneously distributed in the test diets. The mean measured concentrations of the test diets were all within the range of 80.3% to 107.8% of nominal at the time of preparation (see Table 2). Stability tests showed that BTS 27271 is not stable in chick diet at room temperature. Therefore, samples were frozen (-20°C) immediately after mixing until needed for feeding. Mean analyses of stability samples from the quail study showed that the concentration of BTS 27271 in the diet was never below 61.9% of nominal (see Table 3).

In the avian dietary studies with the metabolite BTS 27919 (421246-05, 421246-07), the test material was found to be homogeneously distributed in the test diets. The mean measured concentrations of the test diets were all within the range of 70.9% to 101.2% of nominal at the time of preparation (see Table 2A). Stability tests showed that BTS 27919 is not stable in chick diet at room temperature. Therefore, samples were frozen (-20°C) immediately after mixing until needed for feeding. Mean analyses of stability samples from the quail study showed that the concentration of BTS 27919 in the diet was never below 39.2% of nominal (see Table 3A).

After review of the supplemental information, the four dietary studies conducted with the Amitraz metabolites are now upgraded from supplemental to core.

If you have any questions, please contact Tracy Perry at 305-6451 or Henry Craven at 305-5320.

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Table 2

Summary of mean analyses of test diet samples

Study type	Sample type	BTS 27 271 found (% nominal) 81 ppm	163 ppm	325 ppm	650 ppm	1300 ppm	5200 ppm
Trial mix	H - Top	93.8	n	n	n	n	96.9
	- Middle	91.7	n	n	n	n	96.3
	- Bottom	86.3	n	n	n	n	93.2
Trial mix	S - Day 0[m]	(90.6)					(95.5)
	- Day 3*	46.3	n	n	n	n	56.5
	- Day 6*	33.6	n	n	n	n	45.5
Quail	- Day 0	91.7	80.3	-	83.9	94.0	94.6
	- Day 5**	63.7	n	n	51.9	n	103.0
Duck	- Day 0	96.1	-	82.1	-	89.9	107.8

H Homogeneity
S Stability
n No sample supplied
(-) Not analysed
(*) Number of days stored at room temperature
(**) Stability samples stored at -20°C for days 1-4 then in animal room conditions for day 5.
m Mean of 12, T, M and B analyses

The mean results for the dietary mixes analysed from the main studies were within the range 80.3% to 107.8% of nominal at the time of preparation.

Homogeneity between top, middle and bottom portions of the 81 5200 ppm diet mixes was shown to be satisfactory in the trial mix.

Analysis of the stability samples from the trial mixes, stored 3 and 6 days, shows that BTS 27 272 is not stable in chick diet at room temperature. To minimise these losses, in the main studies test diets were frozen (-20°C) immediately after mixing until needed for feeding. As a result the diet remained at room temperature for a maximum time of one day i.e. the day of use

Samples of the mixes prepared on 5 February for the quail study were frozen on that day, placed in the animal (bird) room on 9 February and frozen again on 10 February. Analysis of the 81, 650 and 5200 ppm levels were shown in Table 2 but are repeated in Table 3.

Table 3

Mean analyses of stability samples from quail study

	BTS 27 271 found (% of nominal)		
	81 ppm	650 ppm	5200 ppm
Prepared on 05.02.91	91.7 (100.0)	83.9 (100.0)	94.6 (100.0)
After 1 day in animal room	63.7 (69.5)	51.9 (61.9)	103.0 (108.9)

Based on these analyses, the concentration in the diet was never below 61.9% of its concentration at the time of mixing.

5.4 Archives

All raw data relating to these studies and a copy of this report will be filed under the study numbers 082/10/020 and 082/10/021 in the GLP Archive of Schering Agrochemicals Limited at Chesterford Park Research Station.

6 CONCLUSIONS

6.1 The mean analyses of the test diets analysed from the main studies were all within the range 80.3% to 107.8% of nominal at the time of preparation.

6.2 The mixing process produced homogeneous mixes.

6.3 Based on the analyses of diets from the quail study, the test material concentration in the diet was never below 61.9% of its concentration at the time of mixing, as a result of exposure to animal room temperature.

7 REFERENCE

1 BRIGHT, J.H.M., Schering Report RESID/88/4 "ANALYTICAL METHOD FOR AMITRAZ, BTS 27 271 AND BTS 27 919 IN AVIAN DIET"
Registration reference Amitraz/W88

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Table 2 A

Summary of mean analyses of test diet samples

Study type	Sample type		BTS 27 919 found (% nominal) 163 ppm	650 ppm	1300 ppm	5200 ppm
Trial mix	Homogeneity	- Top	93.6	n	n	97.3
		- Middle	94.9	n	n	97.5
		- Bottom	92.8	n	n	96.0
Trial mix	Stability	- Day 0	99.5	n	n	108.0
		- Day 3*	24.1	n	n	84.9
		- Day 6*	16.8	n	n	84.7
Quail		- Day 0	70.9	84.4	101.2	100.8
		- Day 5**	70.2	63.9	n	88.5
Duck		- Day 0	77.9	80.1	92.4	97.0
		- Day 5**	30.5	42.6	n	82.0

n No sample supplied
(*) Number of days stored at room temperature
(**) Stability samples stored at -20°C for days 1-4 then in animal room conditions for day 5.

The mean results for the dietary mixes analysed from the main studies were within the range 70.9% to 101.2% of nominal at the time of preparation.

Homogeneity between top, middle and bottom portions of the 163 and 5200 ppm diet mixes was shown to be satisfactory in the trial mix.

Analysis of the stability samples from the trial mixes, stored 3 and 6 days, show that BTS 27 919 is not stable in chick diet at room temperature. To minimise these losses in the main studies test diets were frozen (-20°C) immediately after mixing until needed for feeding. As a result the diet remaining at room temperature for a maximum time of one day i.e. the day of use.

Samples of the mixes prepared on 18 January (for the quail study) were frozen on that day, placed in the animal (bird) room (after 4 days frozen) and frozen again after 1 day of exposure to animal room temperature. A similar exercise was done with the mixes prepared for the duck study. Analyses of these samples are shown in Table 2 but are repeated in Table 3.

Table 3 B

Mean analyses of stability samples from quail and duck studies

	BTS 27 919 found (% of nominal)		
	163 ppm	650 ppm	5200 ppm
Quail study Prepared on 18.01.91	70.9 (100.0)	84.4 (100.0)	100.8 (100.0)
After 1 day in animal room	70.2 (99.0)	63.9 (75.7)	88.5 (87.8)
Duck study Prepared on 28.01.91	77.9 (100.0)	80.1 (100.0)	97.0 (100.0)
After 1 day in animal room	30.5 (39.2)	42.6 (53.2)	82.0 (84.5)

Based on these analyses, the concentration in the diet was never below 39.2% of its concentration at the time of mixing.

5.4 Archives

All raw data relating to these studies and a copy of this report will be filed under the study numbers 082/10/022 and 082/10/023 in the GLP Archive of Schering Agrochemicals Limited at Chesterford Park Research Station.

6 CONCLUSIONS

6.1 The mean analyses of the test diets analysed from the main studies were all within the range 70.9% to 101.2% of nominal at the time of preparation.

6.2 The mixing process produced homogeneous mixes.

6.3 Based on analyses of diets from the quail and duck studies, the test material concentration in the diet was never below 39.2% of its concentration at the time of mixing, as a result of exposure to animal room temperature.

7 REFERENCE

1 BRIGHT, J.H.M., Schering Report RESID/88/4
"ANALYTICAL METHOD FOR AMITRAZ, BTS 27 271 AND BTS 27 919 IN AVIAN DIET"
Registration reference Amitraz/W88

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DATA EVALUATION RECORD

1. **CHEMICAL:** Amitraz. Shaughnessey No. 106201.

2. **TEST MATERIAL:** Amitraz, technical grade; Batch # CR 19619/1; 98.9% purity; a white powder.

3. **STUDY TYPE:** Avian Reproduction Study.
Species Tested: Bobwhite quail (Colinus virginianus).

4. **CITATION:** Beavers, J.B., T. Ross, G.J. Smith, S.P. Lynn, and M. Jaber. 1992. Technical amitraz: Bobwhite quail dietary one generation reproduction study. Prepared by Wildlife International Ltd., Easton, Maryland. Laboratory Project No. 312-101. Submitted by NOR-AM Chemical Co., Wilmington, Delaware. EPA MRID No. 423360-01.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 9/15/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven 9/16/92
Date:

7. **CONCLUSIONS:** Estimated dietary concentrations of amitraz at 6.3, 11.4, and 24.6 ppm ai had no effects upon mortality, behavior, or reproductive performance of bobwhite quail during the exposure period. The NOEC was 24.6 ppm ai, based upon reductions in viable embryos/eggs set at 50.5 ppm ai. This study is scientifically sound and fulfills the guideline requirements for an avian reproduction study.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Pen-reared, unmated bobwhite quail (*Colinus virginianus*) were purchased from Fritts Quail Farm, Phillipsburg, New Jersey. The birds were acclimated to the facilities for 10 weeks prior to initiation of the test. All birds were from the same hatch and were phenotypically indistinguishable from wild birds. At test initiation, all birds were examined for physical injuries and general health. Birds that did not appear healthy were discarded. The birds were 29 weeks of age at test initiation.

B. **Dose/Diet Preparation/Food Consumption:** Diets were prepared without solvent or carrier by mixing test substance with a small amount of diet to form a premix. Test diets were prepared twice weekly from premixes by adding basal diet to the premix. Control birds were given basal (untreated) diet. Due to the instability of the test substance in the diet, both the control and each test diet (10, 20, 40, and 80 ppm) were deep frozen at approximately -20°C immediately after preparation. Birds were fed fresh or freshly defrosted diet daily. Each of the five groups of adult birds was fed the appropriate diet from test initiation until terminal sacrifice. Dietary concentrations were not adjusted for purity of the test substance.

Basal diet for adult birds and their offspring was formulated by Agway, Inc. The composition of the diet was presented in the report. The test substance was not mixed into the diet of the offspring. Food and water were supplied *ad libitum* during acclimation and during the test.

For each test concentration, samples were collected from the trial mix and the first mix of the treatment period to determine the homogeneity of the test material in the diet. One sample was collected from each concentration of the trial mix on Days 0, 1, and 2 to evaluate the stability of the test material in the diet. In addition, one frozen sample was taken from each concentration of the trial mix on Days 0, 8, and 15. Additional freezer stability samples were collected during week 4. To verify the concentration of the test substance in the diet, two samples were collected from each concentration of diets prepared at the beginning of week 1, and from all even numbered mixes. Selected

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verification samples were sent to the laboratory for analysis. Samples were frozen immediately after collection, and analyzed by Bushy Run Research Center, Export, Pennsylvania.

C. **Design:** The birds were randomly distributed into four groups as follows:

Amitraz Nominal Concentration	Number of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	16	1	1
10 ppm	16	1	1
20 ppm	16	1	1
40 ppm	16	1	1
80 ppm	16	1	1

Treatment levels were based upon known toxicity data provided by the sponsor. Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

1. Acclimation - 10 weeks.
2. Pre-photostimulation - 7 weeks.
3. Pre-egg laying (with photostimulation) - 3 weeks
4. Egg laying - 10 weeks
5. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 6 weeks.

D. **Pen Facilities:** Adult birds were housed indoors in pens constructed of galvanized wire grid and sheeting. Pen floors measured approximately 30 x 51 cm. The floors were sloped, resulting in a ceiling height ranging from 21 to 26 cm. The average temperature in the adult study room was 22.3 $\pm$ 1.8°C (SD) with an average relative humidity of 60 $\pm$ 17% (SD).

The photoperiod during acclimation and during the first 7 weeks of the study was 8 hours of light per day. The photoperiod was increased to 17 hours of light per day during week 8 and was maintained at that level until sacrifice of adult birds. The birds were exposed to approximately 380 lux of illumination throughout the study.

E. **Adult Observations/Gross Pathology:** All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. All birds that died during the study were necropsied. As soon as practical after the death of the bird, the pen mate was sacrificed and necropsied. At study termination, all

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surviving birds were sacrificed and necropsied.

Adult birds were weighed at test initiation, at the end of weeks 2, 4, 6, 8, and at study termination. Food consumption for each pen was measured every weekday, and presented on a weekly basis.

F. **Eggs/Eggshell Thickness:** Eggs were collected daily from all pens, marked according to pen of origin, and fumigated to reduce pathogen contamination. The eggs were then stored at 13.7 ± 1.2°C (SD) and 64 ± 8% relative humidity. Once each week, eggs were taken for eggshell thickness measurements. The remaining eggs were candled to detect abnormal or cracked eggs. Cracked or abnormal eggs were discarded. All eggs that were not cracked, abnormal, or used for egg shell thickness measurements were placed in an incubator at 37.5 ± 0.1°C (SD) and 56% relative humidity. Eggs were candled on day 11 of incubation to determine fertility and embryo viability and on day 21 to determine embryo survival. All eggs were turned automatically while in the incubator. The eggs were placed in a hatcher on incubation day 21. The average temperature in the hatcher was 37.2°C (SD) with an average relative humidity of 76%.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the odd numbered weeks, and from each of the even numbered pens during the even numbered weeks. These eggs were opened, the contents removed, the shell washed thoroughly and allowed to air dry for at least one week. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.005 mm) five points around the equator of the egg using a micrometer.

G. **Hatchlings:** All hatchlings and unhatched eggs were removed from the hatcher on day 25 or 26 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were leg banded for identification by pen of origin and placed in brooding pens until 14 days of age. Each brooding pen measured 72 cm x 90 cm x 23 cm high, and was constructed of wire mesh and sheeting. Temperatures in the brooding compartments were approximately 38°C. Average ambient room temperature was 28.7 ± 1.2°C. The photoperiod was maintained at 16 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age, the average body weight by parental pen of all survivors was

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determined.

H. **Statistics:** Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data.

Each of the following parameters was analyzed statistically:

Adult Body Weight
Adult Feed Consumption
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of Viable Embryos
Hatchlings of 3-Week Embryos
Hatchlings of Eggs Set
Offspring Body Weight
Hatchlings of Maximum Set
14-Day Old Survivors of Maximum Set
14-Day Old Survivors of Eggs Set
14-Day Old Survivors of of Hatchlings
Egg Shell Thickness

12. **REPORTED RESULTS**

A. **Diet Analysis:** The results of the diet stability, homogeneity, and verification testing are presented in Appendix XIII, Tables 3-6 (attached). Analysis of test diets showed that amitraz was mixed homogeneously into the diet (Appendix XIII, Table 5, attached). Testing also indicated, however, that the test material was unstable in the avian diet at room temperature (Appendix XIII, Tables 3 and 4, attached). Therefore, test diets were frozen immediately after preparation during the treatment period, and birds were fed either freshly prepared or freshly defrosted diet.

Nominal and mean measured concentrations of freshly prepared diets from weeks 1 through 20 were as follows (Appendix XIII, Table 6, attached):

Amitraz Technical (ppm)		
Nominal Concentration (ppm Technical)	Mean Measured Concentration (ppm ai)	Percent of Target
0	0	0
10	8.55	85.5%
20	15.37	76.8%
40	33.18	82.9%
80	68.34	85.5%

B. Mortality and Behavioral Reactions: There were no treatment related mortalities at any of the concentrations tested. One incidental mortality each occurred at 20, 40, and 80 ppm (based on nominal concentrations).

The single mortality in the 20 ppm treatment group was a female found dead during week 11. Necropsy revealed a very pale and friable liver with frank hemorrhage from the liver apparent in the abdominal cavity. Both the spleen and kidneys were noted to be pale.

The single mortality in the 40 ppm group was a female euthanized during week 13 because of a broken leg. The hen also displayed an ovary which showed no evidence of reproductive activity.

At the 80 ppm test concentration, the single mortality was a hen, found dead during week 11. Necropsy revealed bruising on the top of the head and a fracture of the cervical vertebra.

No other mortalities occurred during the course of the study. Due to the nature of the lesions observed at necropsy, these mortalities were considered not to be treatment related.

No overt signs of toxicity were observed at any of the concentrations tested. Incidental clinical signs such as ventral head curl, lethargy, a ruffled appearance, foot and head lesions, and feather loss were noted in various birds during the study. Except for the mortality and these incidental clinical signs, all birds appeared normal throughout the study.

C. **Adult Body Weight and Food Consumption:** There were no apparent treatment related effects upon adult body weight at test concentrations of 10, 20, or 40 ppm, or among hens at the 80 ppm test concentrations (Table 1, attached). There were no statistically significant differences between the body weights of birds in these groups and the control birds at any weight interval. There was a slight, but statistically significant increase in the body weight of cocks from the 80 ppm treatment group at the terminal body weight interval. The minimal nature of this response was not considered by the authors to be of toxicological significance.

There were no apparent treatment related effects upon feed consumption among birds at any of the concentrations tested. At the 10 ppm and 20 ppm test concentrations there was a slight, but statistically significant increase in feed consumption during the first week of the study (Table 2, attached). The observed differences were not considered to be treatment related.

D. **Reproduction:** When compared to the control group, there were no statistically significant differences in reproductive parameters at the 10, 20, or 40 ppm test concentrations. However, at the 80 ppm test concentration, there was a treatment-related statistically significant reduction in viable embryos as a percent of eggs set. There was also a slight reduction in the number of live three-week embryos as a percent of viable embryos, although this difference was not statistically significant. Both findings are reflected in a reduction in the number of hatchlings and 14-day old survivors as percentages of eggs set and on a per hen basis (Tables 3 and 3A, attached).

E. **Egg Shell Thickness:** When compared to the control group, there were no apparent or significant differences in egg shell thickness at any treatment concentration (Table 4, attached).

F. **Offspring Body Weight:** There were no apparent or significant differences between the control and any treatment group in body weights of offspring at hatching or at 14 days of age (Tables 5 & 6, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Dietary concentrations of amitraz at 10, 20, or 40 ppm had no effect on the adult bobwhite quail or their reproductive performance. At 80 ppm there was a slight treatment related

reduction in reproductive performance as evidenced by slight reductions in viable and live three-week embryos and a slight consequential reduction in the number of offspring per hen.

The report stated that study was conducted in conformance with Good Laboratory Practice regulations (40 CFR Part 160). Quality assurance audits were conducted during the study and the final report was signed by a Quality Assurance Officer for Wildlife International Ltd.

14. **Reviewer's Discussion and Interpretation of the Study:**

A. **Test Procedure:** The test procedures were in accordance with Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, ASTM, and SEP guidelines except for the following deviations:

Eggs were stored at a temperature of approximately 13.7°C and 64% relative humidity; 16°C and 65% are recommended.

Eggs were incubated at 37.5°C and 56% relative humidity; 39°C and 70% are recommended.

Eggs were candled on day 21 to determine embryo survival; day 18 is recommended.

Eight hours of light, not seven as recommended, was provided during the first seven weeks of the study.

Behavioral observations of offspring were not reported.

Observations on food palatability were not reported.

A solvent (test vehicle) was not used in the preparation of the test diets.

B. **Statistical Analysis:** Statistical analyses of reproductive parameters were performed by the reviewer using EEB's "SAS" program "Bigbird" followed by Duncan's Multiple Range Test (Table A, attached). Significant differences ($p < 0.05$) were found in male body weight change at 20 ppm and 80 ppm; 14-day survivor weight at 80 ppm; and viable embryos/eggs set at 80 ppm.

C. **Discussion/Results:** Analytical results of homogeneity tests at each concentration found that the test substance was homogeneously mixed. However, the test material was not stable in the diet over time. A

stability study indicated that the concentration of amitraz in each test diet stored at room temperature decreased to approximately 79% and 58% of the original concentration after 1 and 2 days, respectively (Appendix XIII, Table 3, attached). The concentration of amitraz in each test diet also decreased significantly after frozen storage at -20°C. Concentrations decreased to approximately 74% and 63% of the original concentrations after 6 and 15 days, respectively. Additional testing showed that the test substance remained stable in avian diet for at least 7 days when stored frozen in a closed container at -80°C (Table 4, Appendix XIII, attached).

The authors report that test diets were stored at -20°C, due to instability of the test material. However, as described above, stability testing found significant losses of amitraz, even when frozen at -20°C. The authors did not address potential implications of such losses on reported findings. Furthermore, verification samples were typically analyzed within one day of preparation (or after being frozen at -80°C), and thus do not reflect potential losses resulting from storage at -20°C. Therefore, the reported mean measured concentrations are likely to be overestimates of actual levels of amitraz in the administered diets.

In the absence of more specific data, the reviewer has estimated dietary concentrations of amitraz, as administered, based on the results of stability testing which showed 74% recovery of amitraz after 6 days of storage at -20°C. The reported mean measured concentrations were reduced by a factor of 0.74 (74%), to compensate for potential losses during storage. Although this approach is conservative, and may underestimate actual administered dosages, the resulting values are likely to be more representative of actual administered dosages. The following table summarizes the resulting estimates.

Estimated Dietary Concentrations of Amitraz (ppm)

Nominal Concentration	Mean Measured Concentration[1]	Estimated Concentration[2]
0	0	0
10	8.55	6.3
20	15.4	11.4
40	33.2	24.6
80	68.3	50.5

[1] Mean values of freshly prepared diets.
[2] Based on 74% of initial mean values

One mortality occurred in each of the three highest treatment groups (20, 40, and 80 ppm). The mortality in the 20 ppm treatment group occurred during week 11, without prior clinical signs of toxicity. The hen was noted to have a pale liver, kidney, and spleen, and the liver was friable with frank hemorrhage in the abdominal cavity. The authors concluded that this mortality was not treatment-related. Although the abnormal liver findings are typical of many toxic chemicals, its death late in the study (with no previous symptoms) suggests that perhaps the mortality was not treatment-related. The mortalities which occurred at higher dosage levels also occurred late in the study (weeks 11 and 13), and appeared to be the result of physical trauma rather than toxicological effects of the test compound. One bird in the 40 ppm group was euthanized because of a broken leg. At the 80 ppm test concentration, the bird apparently died as a result of head and neck injuries. Based on these findings, the single mortality in the 20 ppm group appears to be anomalous. The reviewer concurs with the authors' conclusion that these mortalities were not treatment-related.

There were no treatment-related mortalities or overt signs of toxicity in any treatment group.

The reduced values for viable embryos/eggs set at 80 ppm is believed to be treatment-related. There were also reductions (though not significantly different from controls) at 80 ppm for live 3-week embryos/viable embryos, hatchlings/eggs set, and 14-day old survivors/eggs set.

The reviewer's analyses showed that at 80 ppm, the body weights of 14-day old survivors were significantly less than controls.

The reviewer's analyses showed that, from initiation to termination, male body weight gain at 20 ppm and 80 ppm was significantly greater than in controls. These differences are attributed to low values in the control group, rather than to exposure to the test material.

Therefore, (using nominal concentrations) based on the above treatment-related effects at 80 ppm, the NOEC was 40 ppm. Using the values adjusted for stability of the active ingredient (ai), the NOEC was 24.6 ppm ai.

This study is scientifically sound and fulfills the guideline requirements for an avian reproduction study.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** Deviations from protocols were minor and did not affect the validity of the study.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes; July 22, 1992.

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Table A Summary of Statistical Analyses of Various Reproductive Parameters tested with the Bobwhite Quail.

PARAMETER	0	6.3*	11.4*	24.6*	50.5*
Egg Shell Thickness	0.207	0.211	0.204	0.206	0.209
Total Food Consumption	371	385	392	388	371
Female Body Weight Change	+41.5	+40.5	+42.9	+37.5	+40.9
Male Body Weight Change	+6.2	+10.8	+17.9**	+12.0	+26.2**
Hatchling Weight	5.9	6.0	6.0	5.9	5.9
14-Day Survivor Weight	24.9	23.4	24.1	22.9	22.1**
Eggs Laid/Hen (EL)	50.7	54.6	48.1	45.9	48.4
Eggs Cracked/Hen (EC)	1.5	1.1	0.7	0.9	1.5
Eggs Set/Hen (ES)	43.8	47.7	42.3	40.3	41.0
Viable Embryos/Hen (VE)	41.3	42.6	37.3	37.9	35.2
Live 3-Week Embryos/Hen (LE)	40.8	42.1	36.7	37.5	34.1
Number of Hatchlings/Hen (NH)	38.0	39.5	34.5	34.9	31.2
14-Day Survivors/Hen (HS)	34.9	37.3	32.5	31.7	27.6
ES/EL#	68.6	69.2	69.8	69.5	67.2
VE/ES#	78.5	73.5	72.4	78.7	69.2**
LE/VE#	86.3	87.1	84.9	87.2	83.2
NH/LE#	76.3	76.9	78.7	77.7	76.3
NH/EL#	60.3	58.7	58.5	60.9	53.7
HS/NH#	75.5	78.8	77.0	74.7	72.0
EC/EL#	6.8	6.1	4.4	5.2	8.1
NH/ES#	69.6	67.0	66.0	70.4	61.8
HS/ES#	64.0	63.1	62.1	63.8	55.6

* Estimated concentration (ppm) based on 74% of initial mean measured concentration.

** Significantly different from the control value.

\# Reported as arcsine transformed data.

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DATA EVALUATION RECORD

1. **CHEMICAL:** Amitraz. Shaughnessey No. 106201.

2. **TEST MATERIAL:** Amitraz technical; Batch # CR 19619/1; 98.9% purity; a white powder.

3. **STUDY TYPE:** Avian Reproduction Study.
Species Tested: Mallard duck (Anas platyrhynchos).

4. **CITATION:** Beavers, J.B., T. Ross, G.J. Smith, S.P. Lynn, and M. Jaber. 1992. Technical amitraz: Mallard duck dietary one-generation reproduction study. Prepared by Wildlife International Ltd., Easton, Maryland. Laboratory Project No. 312-102. Submitted by NOR-AM Chemical Co., Wilmington, Delaware. EPA MRID No. 423360-02.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 9/15/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven
Date: 9/16/92

7. **CONCLUSIONS:** Nominal dietary concentrations of amitraz technical at 6.3 ppm ai, 11.4 ppm ai and 24.6 ppm ai had no effects upon mortality, behavior, food consumption, or reproduction in mallards during the 20-week exposure period. The NOEC was 24.6 ppm ai, based upon reduced hatchling weight and increased male body weight at 50.5 ppm ai. Egg production was reduced at 24.6 ppm ai and 50.5 ppm ai when compared to the controls, although the differences are not statistically significant. This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study, as amitraz was not tested at high enough concentrations to produce reproductive effects.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in the test were pen-reared, unmated mallard ducks (*Anas platyrhynchos*) and were purchased from Whistling Wings, Hanover, Illinois. The birds were acclimated to the facilities for 3 weeks prior to initiation of the test. All birds were from the same hatch and were phenotypically indistinguishable from wild birds. At test initiation, all birds were examined for physical injuries and general health. Birds that did not appear healthy were discarded. The birds were 21 weeks of age at test initiation.

B. **Dose/Diet Preparation/Food Consumption:** Test diets were prepared twice weekly by mixing the test material into a pre-mix which was used for preparation of the final diets. The diets were prepared without solvent or carrier. The control diet and four test concentrations (10, 20, 40, and 80 ppm) were frozen at -20°C immediately after preparation, and fresh or freshly defrosted food was presented to the birds daily. Each of the five groups of adult birds was fed the appropriate diet from test initiation until terminal sacrifice. Dietary concentrations were not adjusted for purity of the test substance.

Basal diet for adult birds and their offspring was formulated by Agway, Inc. The composition of the diet was presented in the report. The test substance was not mixed into the diet of the offspring. Food and water were supplied *ad libitum* during acclimation and during the test for adults and offspring.

Three samples from each treatment concentration were taken from a trial mix and from the first mix of week 1 to determine the homogeneity of the test material in the diet. Stability samples were collected from the trial mix (stored at ambient temperature) on days 0, 1, and 2, and from the trial mix (stored frozen) on days 0, 8, and 15. Additionally, 4-day frozen samples were taken from the second mix of week 4. Samples were analyzed from weeks 1, 3, 4, 6, 8, 11, 16, 17, and 20 to verify the concentration of the test substance. Samples were frozen immediately after collection, and analyzed by Bushy Run Research Center, Export, Pennsylvania.

C. **Design**: The birds were randomly distributed into five groups as follows:

Amitraz Nominal Concentration	Number of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	16	1	1
10 ppm	16	1	1
20 ppm	16	1	1
40 ppm	16	1	1
80 ppm	16	1	1

Treatment levels were based upon known toxicity data. Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

1. Acclimation - 3 weeks.
2. Pre-egg laying - 10 weeks.
3. Egg laying - 10 weeks.
4. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 5 weeks.

D. **Pen Facilities**: Adult birds were housed indoors in pens constructed of galvanized wire grid. Pens measured approximately 75 x 90 x 45 cm high. The average temperature in the adult study room was 22.2 ±2.2°C (SD) with an average relative humidity of 63 ±13% (SD).

The photoperiod during acclimation and during the first 9 weeks of the study was 8 hours of light per day. The photoperiod was increased to 17 hours of light per day during week 10 and was maintained at that level until sacrifice of adult birds. The birds were exposed to approximately 349 lux of illumination throughout the study.

E. **Adult Observations/Gross Pathology**: All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. All birds that died during the study were necropsied. As soon as practical after the death of the bird, the pen mate was sacrificed and necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at test initiation, at the end of weeks 2, 4, 6, 8, and at study termination. Food consumption in each pen was measured daily from Monday through Friday of each week throughout the study.

F. **Eggs/Eggshell Thickness:** Eggs were collected daily from all pens, marked according to pen of origin, and washed to prevent pathogen contamination. The eggs were then stored at 13.5 ±1.3°C (SD) and 63% relative humidity until incubated. Eggs were removed from the storage room weekly, and eggs were then taken for eggshell thickness measurements. Remaining eggs were candled. Cracked or abnormal eggs were discarded. All eggs that were not cracked, abnormal or used for egg shell thickness measurements were placed in an incubator at 37.5 ±0.1°C (SD) and 56% relative humidity. Eggs were candled on day 14 of incubation to determine embryo viability and on day 21 to determine embryo survival. All eggs were turned automatically while in the incubator. The eggs were placed in a hatcher on incubation day 24. The average temperature in the hatcher was 37.2 ±0.0°C (SD) with an average relative humidity of 76%.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the odd numbered weeks, and from each of the even numbered pens during the even numbered weeks. These eggs were opened at the widest point, the contents removed, the shell washed thoroughly and allowed to dry for at least one week. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.005 mm) five points around the waist of the egg using a micrometer.

G. **Hatchlings:** All hatchlings and unhatched eggs were removed from the hatcher on day 27 or 28 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were wing-banded for identification by pen of origin and placed in brooding pens until 14 days of age. Each brooding pen measured 62 cm x 92 cm x 25.5 cm high, and was constructed of vinyl coated wire mesh. Temperatures in the brooding compartments were approximately 38°C until the birds were 5 to 7 days of age, and 30°C thereafter. The average ambient room temperature was 26.1 ±1.8°C (SD). The photoperiod was maintained at 16 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age, the average body weight by parental pen of all survivors was determined.

H. **Statistics:** Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens

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within each experimental group. Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data.

Each of the following parameters was analyzed statistically:

Adult Body Weight
Adult Feed Consumption
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of Viable Embryos
Hatchlings of 3-Week Embryos
Hatchlings of Eggs Set
Offspring Body Weight
Hatchlings of Maximum Set
14-Day Old Survivors of Maximum Set
14-Day Old Survivors of Eggs Set
14-Day Old Survivors of of Hatchlings
Egg Shell Thickness

12. **REPORTED RESULTS**

A. **Diet Analysis:** Analyses of treatment diets showed that the test chemical was mixed homogeneously, but was relatively unstable in the diet at room temperature. Therefore, the diets were frozen immediately after preparation and the birds fed either freshly prepared or freshly defrosted diet. Nominal and mean measured concentrations of freshly prepared diets were as follows:

Amitraz Technical (ppm)		
Nominal Concentration	Mean Measured Concentration	Percent of Nominal
10	8.55	85.5%
20	15.4	76.8%
40	33.2	82.9%
80	68.3	85.5%

B. **Mortality and Behavioral Reactions:** There were no treatment related mortalities at any of the concentrations tested. No mortalities occurred in the control group or in the 10-, 40-, or 80-ppm groups. One incidental mortality occurred in the 20-ppm group.

The single mortality in the 20-ppm group was a female found dead during week 17. No signs of toxicity were noted prior to death. Gross necropsy results were presented in the report. Due to the nature of the

lesions observed at necropsy, the mortality was considered to be incidental to treatment.

No overt signs of toxicity were observed at any concentration. Necropsy results of sacrificed birds were included in the report. All lesions observed in sacrificed birds were considered to be unrelated to treatment.

C. **Adult Body Weight and Food Consumption:** There were no apparent treatment-related effects on adult body weight at 10, 20, or 40 ppm. There was a slight, but significant ($P<0.05$) increase in female bodyweight at 40 ppm at the end of week 4.

At 80 ppm, male body weights were generally higher than in controls from week 6 and thereafter (Table 1, attached). There was a significant increase ($P<0.05$) of 8% in the mean body weight of males at termination. When compared to the controls, females at 80 ppm had significantly higher body weights at week 4 ($P<0.05$), week 6 ($P<0.01$), and week 8 ($P<0.05$). The terminal body weight of females was similar to control values. The increased body weights at 80 ppm appeared to be treatment related.

There was a slight increase (approximately 16%) in mean food consumption at 40 and 80 ppm during the first four weeks of the study. The increase was significant ($P<0.05$) at 40 ppm during weeks 1 and 2. At 80 ppm, the increase was significant ($P<0.05$) during week 2. While the differences were slight, they appeared to be treatment related (Figure 3 and Table 2, attached).

D. **Reproduction:** When compared to the control group, there were no statistically significant differences in reproductive parameters at any concentration tested (Tables 3 & 3A, attached). However, while not significant, there appeared to be a slight reduction in egg production at 40 and 80 ppm. "The reduction was most apparent during the first four weeks of egg production, and appeared more closely related to the number of hens laying eggs, rather than egg production per laying hen."

E. **Egg Shell Thickness:** When compared to the control group, there were no apparent treatment effects or significant differences in egg shell thickness at any concentration tested (Table 4, attached).

F. **Offspring Body Weight:** There were no significant differences between the control and any treatment group in body weights of offspring at hatching or at 14 days of age (Tables 5 & 6, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Dietary concentrations of technical amitraz at 10 ppm and 20 ppm had no effects on adult mallards or their reproductive performance. There was a slight reduction in egg production at 40 ppm and 80 ppm. Body weight gain and food consumption were slightly increased at 80 ppm.

The report stated that study was conducted in conformance with Good Laboratory Practice regulations (40 CFR Part 160). Quality assurance audits were conducted during the study and the final report was signed by the Quality Assurance Manager of Wildlife International Ltd.

14. **Reviewer's Discussion and Interpretation of the Study:**

A. **Test Procedure:** The test procedures were in accordance with Subdivision E, ASTM, and SEP guidelines except for the following deviations:

Eggs were stored at a temperature of approximately 13.5°C; 16°C is recommended.

Behavioral observations of offspring were not reported.

Observations on food palatability were not reported.

Eight hours of light, not seven as recommended, was provided during the first eight weeks of the study.

All eggs were transferred to the hatcher on day 24. The guidelines recommend the transfer on day 23.

B. **Statistical Analysis:** Statistical procedures differed from recommended methods. Specifically, there is no basis for transforming the number of eggs laid and the number of hatchlings to percentile values of the maximum number of eggs laid or set in any test group.

Statistical analyses of reproductive parameters were performed by the reviewer using EEB's "SAS" program "Bigbird" followed by Duncan's Multiple Range Test (Table A, attached). Significant differences ($p < 0.05$) from the control were found in the hatchling weight and in male body weight change at the 80 ppm (nominal) treatment level.

Egg production was reduced at 40 and 80 ppm, when compared to controls, although these differences are not statistically different (Table 3 & Appendix VI, attached).

C. **Discussion/Results:** The results of EEB's statistical analysis generally agreed with that of the author's, although the reviewer did not find statistical differences in food consumption or female body weight change. When compared to the controls, male body weights were increased at 80 ppm. The authors considered these increases to be treatment effects. While **increased** food consumption and body weight are not normally associated with exposure to treated food, they could indeed be treatment effects. The authors did not speculate regarding the reason for these unusual results. A potential problem with treatment-related increases in food consumption is that, in the wild, this could effectively increase the exposure of the birds to the chemical (i.e., the greater the ingestion rate, the greater the dose), with a concomitant increase in toxicity.

The authors reported no significant differences among groups for hatchling weight, while the reviewer's analysis showed that values at 80 ppm were significantly lower than control values. These decreased values are considered to be treatment effects.

Analytical results of treatment diets showed that initial mean measured concentrations were 8.55, 15.4, 33.2, and 68.3 ppm for the nominal concentrations of 10, 20, 40, and 80 ppm, respectively. Appendix XIII states that the concentration of amitraz in the treatment diets stored frozen for 6 days at -20°C decreased to approximately 74% of the original concentrations. Therefore, since the test material was not stable even when frozen at -20°C, an estimation of the actual concentrations to which the birds were exposed is in order.

The diets were mixed twice each week, frozen at -20°C immediately after preparation, and fresh or freshly defrosted food was presented to the birds daily. Consequently, the maximum time the diet was held frozen prior to presentation to the birds was 4 days. However, stability of diets frozen at -20°C for 4 days is not available. Since stability of diets frozen at -20°C for 6 days is available, this value (74% of initial values) is used herein to calculate exposure concentrations.

Seventy-four percent of the initial mean measured concentrations of 8.55, 15.4, 33.2, and 68.3 ppm results in concentrations of 6.3, 11.4, 24.6, and 50.5 ppm. These values are accepted as the estimated actual concentrations of active ingredient (ai).

With the information acquired in this study, one might determine the LOEL to be 80 ppm based on increased male body weights and reduced hatchling weights at this level. However, one of the most important objectives of an avian reproduction study is to determine the dosage level at which reproductive effects are seen. It is the reviewer's opinion that the dosage levels in this study were spaced too closely together and did not test at high enough concentrations to produce definitive reproductive effects. This study tested four dosage levels separated by a factor of two, while the SEP guidelines suggest that dosage levels be separated by a factor of five.

This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study as amitraz was not tested at high enough concentrations to produce reproductive effects. The LOEL was 50.5 ppm based on increased male body weight and decreased hatchling weight at this test concentration. The NOEL was 24.6 ppm.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Test material was not tested at high enough concentrations to produce reproductive effects.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes; July 22, 1992.

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Table A Summary of Statistical Analyses of Various Reproductive Parameters tested with the Mallard.

PARAMETER	0	6.3*	11.4*	24.6*	50.5*
Egg Shell Thickness	0.38	0.38	0.39	0.38	0.37
Total Food Consumption	3130	3292	3317	3354	3253
Female Body Weight Change	+184	+182	+156	+145	+173
Male Body Weight Change	-12.1	+9.6	+39.6	+30.8	+78.7**
Hatchling Weight	36.5	35.3	34.1	34.0	32.8**
14-Day Survivor Weight	270	264	264	284	263
Eggs Laid/Hen (EL)	42.6	39.3	40.7	31.7	32.4
Eggs Cracked/Hen (EC)	0.38	0.25	0.60	0.56	0.38
Eggs Set/Hen (ES)	38.6	35.1	36.4	27.7	28.6
Viable Embryos/Hen (VE)	33.1	30.1	30.7	22.5	26.1
Live 3-Week Embryos/Hen (LE)	32.4	29.8	30.1	22.1	25.7
Number of Hatchlings/Hen (NH)	21.0	20.9	18.2	13.9	17.8
14-Day Survivors/Hen (HS)	20.8	20.8	17.9	13.6	17.6
ES/EL#	72.3	71.1	71.1	69.6	70.0
VE/ES#	72.2	70.3	69.7	66.0	74.4
LE/VE#	84.3	87.7	85.3	85.2	86.6
NH/LE#	54.3	57.7	52.2	53.1	57.4
NH/EL#	43.2	46.8	41.4	40.1	47.9
HS/NH#	86.6	88.7	86.1	87.3	88.4
EC/EL#	3.1	2.8	4.6	4.2	3.5
NH/ES#	46.5	50.7	44.8	44.1	52.6
HS/ES#	46.1	50.4	44.2	43.5	52.2

* Estimated concentration (ppm) based on 74% of initial mean measured concentration.

** Significantly different from the control value.

Reported as arcsine transformed data.