5-25-93

MRID No. 425073-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Endothall Technical.
Shaughnessey No. 038901.

2. **TEST MATERIAL:** Endothall Technical; 7-oxabicyclo[2.2.1] heptane-2,3-dicarboxylic acid; CAS No. 145-73-3; Lot No. G 05A; Batch No. 259; 83.02% active ingredient; an off-white, crystalline solid with a distinctive odor.

3. **STUDY TYPE:** 71-4. Avian Reproduction Study.
Species Tested: Mallard duck (*Anas platyrhynchos*).

4. **CITATION:** Pedersen, C.A., D.W. Fletcher, and C.L. Lesar. 1992. Endothall Technical: Toxicity and Reproduction Study in Mallard Ducks. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Laboratory Project ID No. BLAL No. 89 DR 37. Submitted by Elf Atochem North America, Philadelphia, PA. EPA MRID No. 425073-01.

5. **REVIEWED BY:**

Dennis J. McLane, Wildlife Biologist **Signature:** Dennis J. McLane
Section 1
Ecological Effects Branch
Environmental Fate and Effects Division **Date:** 5-24-93

6. **APPROVED BY:**

Les Touart, Chief, Section 1 **Signature:** L T
Ecological Effects Branch
Environmental Fate and Effects Division **Date:** 5/25/93

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study. The analytical results were not included in the report. There was a significant increase in early embryo mortality at 250 ppm a.i., when compared to the control (200%). The no-observed-effect concentration (NOEC) was 50 ppm a.i., nominal concentration.

8. **RECOMMENDATIONS:** Provide a study which addresses the items discussed in 14C, because control population used in this study may have obscured effects at lower test levels.

9. **BACKGROUND:** As per the List B - Phase IV, Dicarboxylic Endothall (038905) (March 14, 1991), §71-4(a) avian reproduction/duck study is,..." Required for products registered for multiple treatments per season. May also required depending on environmental fate information". The

List B indicates that the N with the above quotation as a footnote.

The List B - Phase IV, Dipotassium Endothall (038904) indicates Reserved with this footnote, "Reserved pending additional use and environmental fate information. Required for products registered for multiple treatments per season."

The List B - Phase IV, Disodium Endothall (038903) indicates "Reserved" with this footnote, "Reserved pending additional use information Required for products registered for multiple treatments per season."

The List B - Phase IV, Endothall Acid (038901) indicates NA.

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in this study were mallard ducks (*Anas platyrhynchos*) purchased from Whistling Wings, Inc. The birds were 26 weeks of age at study initiation and were acclimated to the laboratory environment for 46 days. All birds were phenotypically indistinguishable from wild birds. At test initiation, all birds were examined for physical injuries and general health.

B. **Dose/Diet Preparation/Food Consumption:** Diets were prepared by dissolving appropriate amounts of the test substance in tap water. This mixture was then dispensed into the basal feed and mixed for 10-15 minutes. Diets were prepared fresh weekly, approximately 24 hours prior to administration. The control diet consisted of tap water and basal feed. Each of the three treatment groups and the control group were fed the appropriate diet for 20 weeks.

Basal diet for adult birds during the first 8 weeks of the study was Purina® Duck Grower W/O. The birds received Purina® Game Bird Breeder Layena from week 9 until study termination. The composition of these diets were 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.

Samples of control and treated diets were collected and frozen during test weeks 1, 6, 12, 15, 16, 17, 18, 19, and 20. Samples were shipped under dry ice to Columbia

Laboratories, Inc. for concentration verification. Beginning during test week 17, duplicate samples were collected and frozen. The duplicate set of samples from test week 8 were sent to Hazleton Washington, Inc. for chemical analysis.

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

Endothall Technical Nominal Concentration	Number of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm a.i.)	16	1	1
10 ppm a.i.	16	1	1
50 ppm a.i.	16	1	1
250 ppm a.i.	16	1	1

Treatment levels were based upon the results of a 28-day dietary pilot study. The diets were adjusted for percent purity and are reported as parts per million (ppm) of active ingredient (a.i.). Adult birds were identified by individual leg bands. Study phases and their approximate durations were as follows:

1. Quarantine Period - 46 days
2. F_0 Generation Test Period - 20 weeks
3. F_1 Generation Growth Period - 14 weeks.

D. **Pen Facilities:** Adult birds were housed in pens constructed of steel mesh. Each pen measured 61.0 x 121.9 x 61.0 cm and was equipped with a feeder and an automatic waterer. The pens were placed in a thermostatically-controlled room with an average temperature of 59°F (15°C) and an average relative humidity of 82%.

The photoperiod during the first 8 weeks of the study was 7 hours of light per day. The photoperiod was increased to 17 hours of light per day for the duration of the study. Throughout the study, the birds received a minimum of 6 foot-candles of illumination.

E. **Adult Observations/Gross Pathology:** Adult birds were observed daily throughout the study for signs of toxicity, injuries, or illness. Mortalities occurring prior to terminal adult sacrifice were recorded and necropsied. Necropsies were also conducted on half of all surviving adult birds from each group at termination of the study. Adult body weights were measured at study initiation, biweekly through week 6,

and at study termination (week 20). Feed consumption was measured by cage biweekly throughout the adult phase of the study.

F. **Eggs/Eggshell Thickness:** Eggs were collected and candled daily during the production period and were labeled according to pen of origin. Normal eggs were stored at 59°F (15°C) with a relative humidity of 75%. The eggs were turned once daily during each seven-day collection period. Eggs were removed from the egg cooler weekly and eggs not cracked or used for eggshell thickness measurements were placed in an incubator maintained at 99.5°F (37.5°C) with a relative humidity of approximately 51%. All eggs were turned automatically every four hours while in the incubator. Eggs were candled on day 14 of incubation to determine fertility and embryo viability and again on day 21 to determine embryo survival. On incubation day 23, the eggs were placed in hatching trays. On day 27, all hatchlings, unhatched eggs (embryos not showing life at day 21) and full term eggs (embryos that did not liberate from the shell) were removed from the incubator.

Eggs were collected from all pens on the first day of alternate weeks during the test period for eggshell thickness measurements. Eggs used for eggshell thickness were opened and the contents removed. The shell washed and allowed to air dry for a minimum of 48 hours at room temperature. The average thickness of the dried shell was determined by measuring (to the nearest 0.01 mm) three points around the equator of the egg.

G. **Hatchlings:** Hatchlings were removed from the hatcher and housed according to week of hatch and parental treatment group in pens measuring 61.0 x 61.0 x 45.7 cm. All ducklings were observed daily and received untreated diet during the 14-day observation period. The temperature that the ducklings were maintained at ranged from 71-83°F (22-28°C) and the relative humidity from 75-94%. Hatchling body weights were measured and recorded at hatch and on day 14. Feed consumption was not monitored.

Hatchlings were observed daily throughout the 14-day period for signs of toxicity, injuries, or illness. Gross pathological examinations were conducted on birds found dead during the 14-day observation period and on selected survivors from each group and hatch.

H. **Statistics:** Analysis of variance (ANOVA) was used to statistically analyze the following parameters:

Adult Body Weight	Hatchling Body Weight
Adult Feed Consumption	Eggshell Thickness

Contingency Table Analysis was used to statistically analyze the following parameters:

Eggs Set of Net Eggs Laid	Eggs Laid Per Hen
One Week Eggs of Viable Embryos	Midterm Eggs of Viable Embryos
Full-Term Eggs of Viable Embryos	Viable Embryos of Eggs Set
Infertile Eggs of Eggs Set	Live 3-Week Embryos of Viable Embryos
Hatchlings of Viable Embryos	Defective Eggs of Eggs Laid
Cracked Eggs of Eggs Laid	14-Day Old Survivors of Hatchlings
Normal Eggs of Eggs Laid	

12. **REPORTED RESULTS:**

A. **Diet Analysis:** The results of the diet analysis were not presented in the report.

B. **Mortality and Behavioral Reactions:** One male at 10 ppm a.i. died during the study. A *post-mortem* examination of this bird showed abnormal findings, however, they were attributed to factors other than the test material. Examinations of one-half of the surviving adult birds in each group revealed no treatment-related effects. There were no clinical signs of toxicity or abnormal behavior noted in any of the birds during the study.

C. **Adult Body Weight and Food Consumption:** There were no significant differences in adult body weight and weight gain throughout the study (Tables 1A and 1B, attached). Feed consumption at 250 ppm a.i. was significantly higher than that of the control during week 4 (Table 1A). This increase was not considered to be treatment-related.

D. **Reproduction:** Early embryo mortality (number of one-week eggs) at 250 ppm a.i. was significantly higher when compared to that of the control (Table 3B,

attached). The authors considered this difference to be due to the test substance.

An atypical number of midterm eggs (late embryo mortality) were observed in all test groups including the control. These differences were attributed to atypical pens and not to the test substance.

A significantly higher number of cracked eggs was observed at 10 ppm a.i., however, this was attributed to an atypical pen and not to the test substance.

No significant differences were reported in any other reproduction parameters (Tables 3B and 4, attached).

E. **Egg Shell Thickness:** There were no significant differences in the overall mean eggshell thickness when compared to control eggs (Table 6B, attached).

F. **Offspring:** Significantly lower mean body weights for certain hatches (when compared to controls) were reported at 250 ppm a.i. on day 1 and at 10 and 250 ppm a.i. on day 14 (Tables 8 and 9, attached). Significantly higher mean body weights for certain hatches (when compared to controls) were reported at all levels on days 1 and 14. When all hatches were combined, mean body weights were significantly higher at all levels when compared to the controls. The authors concluded that these differences were due to normal biological variation.

There were no treatment-related clinical signs observed during the 14-day period. Gross pathological examinations of ducklings that died and of selected ducklings on day 14 revealed no treatment-related findings.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "The ingestion of Endothall Technical at 250 ppm a.i. by the parental generation appeared to adversely affect early embryonic development. The no-observed-effect level was determined to be 50 ppm a.i."

The report stated that the 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 Bio-Life Associates, Ltd.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

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:

The adult birds were maintained at 15°C and 82% relative humidity; 21°C and 55% are recommended.

The test material should be administered for 10 weeks before egg collection; eggs were collected as early as week 7.

The eggs were incubated at 37.5°C and 51% relative humidity; 39°C and 70% are recommended.

Observations on food palatability were not reported.

B. **Statistical Analysis:** Statistical analyses of reproductive parameters were performed by the KBN reviewer using analysis of variance (ANOVA) following square-root transformation of the count data and arcsine square-root transformation of the ratio data. The comparison between control data and data from each treatment level was made using multiple comparison tests. The computer program used is based on the EEB Birdall program, with an exception that the count data were square-root transformed before the ANOVA. The significance level was $p \leq 0.05$. EEB used the BIRDREPR.SAS which is ANOVA with Duncan's, Dunnett's, and Bonferroni. The following table shows the results for both EEB and KBN with no transformation of the count data and arcsine square-root transformation of the ratio data. The significance level was $p \leq 0.05$.

The following table compares the results.

EEB			KBN			
Parameter	P value	Dose Level	Parameter	P value	Dose Level (ppm)	Mean >or< con-trol
Female Body Weight	0.0202	N/A	Female Body Weight	0.030	10	<
Male Body Weight	0.0262	N/A	-	-	-	
-	-	-	Food Consump-tion	0.033	250	>
-	-	-	EC	0.011	10	>
-	-	-	EC/EL	0.045	10	>
HS/NH	0.0326	N/A	HS/NH	0.026	50	<

The KBN's statistical results were in general agreement with those reported by the authors, with the following exceptions: female body weight was significantly lower at 10 ppm a.i. and a slight, but not significant, decrease at 250 ppm a.i. was also observed. The number of eggs cracked/number of eggs laid was significantly higher at 10 ppm a.i. than the control, the number of two week survivors/number of hatchlings was significantly lower at 50 ppm a.i. than the control, and overall food consumption at 250 ppm a.i. was significantly higher than the control. None of these differences were considered to be related to the test material.

EEB's preformed a Williams Test (Toxstat) which shows that the 250 ppm dosage for one week eggs ("one week eggs are those embryos which are not showing life at day 14 candling"). This agrees with the author's analysis.

C. **Discussion/Results:** The control bird population or the other the birds in other levels were not typical of most bobwhite quail populations. The study author states, "The number in parentheses (Pen #10) represents

the an atypically high number of midterm eggs (Table 5. footnote)." "Review of historical control data from 19 reproductive studies shows that up to two pens per study may show high numbers (greater than ten eggs per pen) of midterm eggs (eggs not showing life at the 21 day candling)." The following Table 1. shows the number of midterms by treatment and pen number (hen) within treatment:

Table 1. Midterm Eggs

Treatment	Pen Number	Midterms
Vehicle Control	10	13
10 ppm	1	10
	4	10
	14	16
	16	14
	Total	50
50 ppm	5	11
	6	12
	8	22
	Total	45
250 ppm	4	18
	9	22
	Total	38

The author points out that this is an inverse relationship and not likely related to the chemical. Unless the relationship is asymptotic and the three levels tested are on the portion of the curve which has leveled off. Not only does this embryo mortality appear to be not from a normal population, the high number of eggs cracked inthe 10 ppm level appears to be reflected in the other parameters. The following table show that the 10 ppm level is always the lowest value:

	Control	10 ppm	50 ppm	250 ppm
Eggs Laid	56.3	49.8	49.8	49.6
Eggs Cracked	1.125	2.5	.875	.812
Eggs Set	42.8	36.5	40	38
Viable Embryos	46.25	39.2	42.4	41.7
Live Embryos	38.25	29.7	33.1	30.6
Normal Hatchlings	34.4	26.9	30.4	27.4

Therefore, the one hen in the 10 ppm level which cracked 20 eggs may have masked other effects.

Diet samples had been collected at intervals during the test but the analytical results were not included in the report.

The test material should be administered for 10 weeks before egg collection; eggs were collected as early as week 7.

There was a treatment-related effect in reproduction (i.e., 200% increase in early embryo mortality vs control) at 250 ppm a.i. The NOEC was 50 ppm a.i. (nominal concentration). Based on the above items, this study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Three items indicate that an atypical population may have been selected for this study. The high incident of midterm eggs, high number of eggs cracked and the exclusion of an entire hatch due to the incubator malfunction. These items may have masked the effects occurring in the study.

(3) **Repairability:** No

15. **COMPLETION OF ONE-LINER:** Yes; 01/19/93.

Mallard Endothal
Mid term Eggs

APPENDIX 5

Historical Control Data

Page_____ is not included in this copy.

Pages 13 through 14 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Midterm eggs
File: b:toxst.end Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	1.000	1.000	470.500
2	10 ppm	0.750	0.750	415.000
3	50 ppm	1.625	1.625	529.500
4	250 ppm	2.063	2.063	665.000

Calculated H Value = 0.018 Critical H Value Table = 7.810
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Midterm eggs
File: b:toxst.end Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 2	0 1	0 3	0 4
2	10 ppm	0.750	0.750	\			
1	Control	1.000	1.000	.	\		
3	50 ppm	1.625	1.625	.	.	\	
4	250 ppm	2.063	2.063	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,4) = 2.639 SE = 6.306

Midterm eggs
File: b:toxst.end Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	16	1.000	1.000	0.875
2	10 ppm	16	0.750	0.750	0.875
3	50 ppm	16	1.625	1.625	1.625
4	250 ppm	16	2.063	2.063	2.063

32
16
16

1.000
0.75

Midterm eggs
File: b:toxst.end Transform: NO TRANSFORMATION

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.875				
10 ppm	0.875	0.218		1.67	k= 1, v=60
50 ppm	1.625	1.090		1.75	k= 2, v=60
250 ppm	2.063	1.854	*	1.77	k= 3, v=60

s = 1.621

Note: df used for table values are approximate when v > 20.

DATA EVALUATION RECORD

1. **CHEMICAL:** Endothall Technical.
Shaughnessey No. 038901.

2. **TEST MATERIAL:** Endothall Technical; 7-oxabicyclo[2.2.1] heptane-2,3-dicarboxylic acid; CAS No. 145-73-3; Lot No. G 05A; Batch No. 259; 83.02% active ingredient; an off-white, crystalline solid with a distinctive odor.

3. **STUDY TYPE:** 71-4. Avian Reproduction Study.
Species Tested: Mallard duck (*Anas platyrhynchos*).

4. **CITATION:** Pedersen, C.A., D.W. Fletcher, and C.L. Lesar. 1992. Endothall Technical: Toxicity and Reproduction Study in Mallard Ducks. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Laboratory Project ID No. BLAL No. 89 DR 37. Submitted by Elf Atochem North America, Philadelphia, PA. EPA MRID No. 425073-01.

5. **REVIEWED BY:**

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Date: 2/8/93

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Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study. The analytical results were not included in the report. There was a significant increase in early embryo mortality at 250 ppm a.i., when compared to the control. No treatment-related effects on any other reproductive parameters, survival, body weight, or food consumption were observed. The no-observed-effect concentration (NOEC) was 50 ppm a.i., nominal concentration.

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

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