4-27-93

MRID No. 423277-01

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Endothall Technical; Batch No. 366; Drum No. 002 J19A; 77.9% active ingredient; a tan solid.

3. **STUDY TYPE:** Freshwater Fish Acute Flow-Through Toxicity Test. Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).

4. **CITATION:** Bettencourt, M.J. 1992. (Endothall Technical) - Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions. SLI Report No. 91-9-3917. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Atochem North America, Philadelphia, PA. EPA MRID No. 423277-01.

5. **REVIEWED BY:**

Mark Mossler, M.S.
Associate Scientist
KBN Engineering and
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Signature: [signature]

Date: 8/27/92 4-27-93

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
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Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Signature:

Date:

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater fish. Based on mean measured concentrations, the 96-hour LC_{50} value of endothall acid for bluegill sunfish was 77 mg ai/l. Therefore, endothall acid is classified as slightly toxic to bluegill sunfish. The NOEC, based on the lack of sublethal effects, was 18 mg ai/l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Bluegill sunfish (*Lepomis macrochirus*) were obtained from a commercial supplier in Connecticut. The fish were maintained in flowing well water (5.5-6.0 tank volume replacements/day) and fed a commercially available pelleted fish food, *ad libitum*, daily. Water quality characteristics of the well water were: a total hardness of 28-36 mg/l as $CaCO_3$, an alkalinity of 22-27 mg/l as $CaCO_3$, a conductivity of 140-160 µmhos/cm, a pH of 7.0-7.2, a dissolved oxygen (D.O.) concentration of 81-88% of saturation, and a temperature of 20-22°C. The laboratory was maintained on a 16-hour daylight photoperiod. A record of daily observations was kept.

The fish were acclimated to the laboratory for a minimum of two weeks. Feeding was discontinued 48 hours before the test. The fish were from the same year class and mean weight and length of a representative group were 1.2 (0.79-1.9) g and 45 (40-50) mm, respectively. There was no mortality in the population in the 48 hours before test initiation.

B. **Test System:** The system consisted of 14 glass aquaria (39 x 20 x 25 cm), each containing approximately 11 l of test solution for a solution depth of 14.5 cm. A diluter delivered 500 ml/cycle (or 8.9 volume replacements per day) of test solution or control water to the individual aquaria. The test aquaria were impartially placed in a circulating water bath set to maintain 22 ±1°C. The dilution water was the same as that used in holding. A 16-hour light/8-hour dark photoperiod with a light intensity of 24-100 footcandles at the solution surface was used and sudden transitions between light and dark were avoided. The diluter was calibrated before test initiation and again at test termination and was checked twice daily during the test. The system was in operation for over 96 hours before test initiation.

A 50 mg active ingredient (ai)/ml diluter stock solution was prepared by diluting 192.55 g of endothall acid to 3 l with distilled water. The stock was delivered to the mixing chamber via a peristaltic pump.

C. **Dosage:** Ninety-six-hour flow-through test. Based on preliminary testing, six nominal concentrations (7.8, 13, 22, 36, 60, and 100 mg ai/l) and a dilution water control were used.

D. **Design:** Twenty bluegill were impartially selected and distributed to two aquaria (10 per aquaria) for each treatment and control. The aquaria were not aerated during the test. The biomass loading was 0.12 g/l/day. Observations of mortality and test solution characteristics were made every 24 hours. Dead fish were removed from the containers at each observation.

The temperature, dissolved oxygen (DO), and pH were measured once daily in each replicate of the exposure concentrations and the control. The temperature was also monitored continuously in replicate B of the dilution water control.

Endothall concentrations from each replicate aquarium were measured by gas chromatography and mass spectrophotometry from samples taken at test initiation and termination.

E. **Statistics:** The median lethal concentration (LC_{50}) and associated 95% confidence interval for each 24-hour interval were calculated using a computer program that employed probit analysis, moving average angle analysis, and binomial probability. Mean measured concentrations and mortality data were used to determine the EC value. The no-observed-effect concentration (NOEC) was defined as the highest concentration tested at and below which there were no toxicant-related mortalities or physical and behavioral abnormalities.

12. **REPORTED RESULTS:** The diluter functioned properly throughout the test period and no precipitate was observed in the delivery system or aquaria. Mean measured concentrations were 8.4, 15, 18, 23, 74, and 130 mg ai/l. These values averaged 103% of nominal concentrations (Table 2, attached). Measured concentrations between sampling days were generally consistent.

The responses of bluegill sunfish are given in Table 3 (attached). The 96-hour LC_{50} was determined as 77 mg ai/l with a 95% confidence interval of 23-130 mg ai/l. The NOEC was determined to be 18 mg ai/l.

Dissolved oxygen ranged from 8.0 to 8.8 mg/l or 93 to 102% of saturation. The pH ranged from 4.6 to 7.3. The temperature was 22-23°C throughout the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author concluded that the test material would be considered slightly toxic to bluegill sunfish.

Quality Assurance and Good Laboratory Practice (GLP) Regulation Statements were included in the report, indicating that the study was conducted in accordance with EPA GLP Regulations (40 CFR Part 160). The GLP statement also indicated that maintenance of records on the stability, characterization, and verification was the responsibility of the sponsor and that routine water and food analyses were conducted at a laboratory that did not collect the data under GLPs.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, but deviated as follows:

Age of the test organisms was not reported.

The hardness of the dilution water (28-36 mg/l) was less than recommended (40-200 mg/l).

The test organisms were impartially distributed to the aquaria. Random distribution is required.

Although stated in the protocol, the report did not indicate that the fish were not fed during the test.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained the same result as the author (see attached printout).

C. **Discussion/Results:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater fish. Based on mean measured concentrations, the 96-hour LC_{50} value of endothall acid for bluegill sunfish was 77 mg ai/l. Therefore, endothall acid is classified as slightly toxic to bluegill sunfish. The NOEC, based on the lack of sublethal effects, was 18 mg ai/l.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 8-8-92.

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Pages 6 through 7 are not included.

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MOSSLER ENDOTHALL LEPOMIS MACROCHIRUS 8-8-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
130	20	20	100	9.536742E-05
74	20	9	45	41.19014
23	20	0	0	9.536742E-05
18	20	0	0	9.536742E-05
15	20	0	0	9.536742E-05
8.4	20	0	0	"

THE BINOMIAL TEST SHOWS THAT 23 AND 130 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 76.80487

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Shaughnessey # 038901 Chemical Name Endothall Acid Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} = pp 95% C.L. ( ) Control Mortality (%) = Solvent Control Mortality (%) = Slope = # Animals/Level = Temperature = 48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) Comments:	_____	_____

96-Hour LC_{50}
 Species: *Lepomis macrochirus*
 Lab: Springborn Laboratories
 MRID # 423277-01
	77.9%	LC_{50} = 77 ~~pp~~ mg ai/l* 95% C.L. (23-130) binomial probability Control Mortality (%) = 0
		 Solvent Control Mortality (%) = n/a
		 Slope = n/a # Animals/Level = 20
		 Temperature = 23°C
		 96-Hour Dose Level ~~pp~~ mg ai/l* /(% Mortality)
		 8.4 (0), 15 (0), 18 (0), 23 (0), 74 (45) 130 (100)
		 Comments:
		 * - based on mean measured concentrations
		 NOEC = 18 mg ai/l*
			M. Mossler 8/5/92	Core

NOEC = 18 mg ai/l*