

US EPA ARCHIVE DOCUMENT

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: Rainbow Trout (*Oncorhynchus mykiss*).
- 4. **CITATION:** Bettencourt, M.J. 1992. (Endothall Technical) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. SLI Report No. 91-9-3918. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Atochem North America, Philadelphia, PA. EPA MRID No. 423277-02.

5. **REVIEWED BY:**

Mark Mossler, M.S.  
Associate Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *Mark Mossler*

Date: 8/21/92

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.  
Associate Scientist  
KBN Engineering and  
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Signature: *Rosemary Graham Mora*  
Date: 8-27-93

Date: 8/27/92

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

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<sub>50</sub> value of endothall acid for rainbow trout was 49 mg ai/l. Therefore, endothall acid is classified as slightly toxic to rainbow trout. The NOEC, based on the lack of sublethal effects, was 13 mg ai/l.

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

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Rainbow trout (*Oncorhynchus mykiss*) were obtained from a commercial supplier in California. The fish were maintained in flowing well water (7.9-10 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 28-32 mg/l as CaCO<sub>3</sub>, an alkalinity of 22-31 mg/l as CaCO<sub>3</sub>, a conductivity of 130-150  $\mu$ mhos/cm, a pH of 7.1-7.2, a dissolved oxygen (D.O.) concentration of 86-88% of saturation, and a temperature of 13-15°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 1.3 (0.69-2.1) g and 31 (43-56) mm, respectively. There was 0.4% 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 50 ml/minute (6.5 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 12  $\pm$ 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 30-70 footcandles at the solution surface was used and sudden transitions between light and dark were avoided. The diluter was calibrated before test initiation and at test termination and was checked twice daily during the test. The system was in operation for over 96 hours before test initiation.

A 39 mg active ingredient (ai)/ml diluter stock solution was prepared by diluting 149.27 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 trout 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 0.18 g/l/day. Observations of mortality and test solution characteristics were made every 24 hours. Dead fish were removed at each observation.

The temperature, D.O., and pH were measured once daily in each replicate of the exposure concentrations and the control. The temperature was also monitored continuously in replicate A 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. The mean measured concentrations were 13, 30, 35, 57, 65, and 120 mg ai/l. These values averaged 157% of nominal concentrations (Table 2, attached). Measured concentrations between sampling days were generally consistent.

The responses of rainbow trout are given in Table 3 (attached). The 96-hour  $LC_{50}$  was determined as 49 mg ai/l with a 95% confidence interval of 45-54 mg ai/l. The slope of the probit curve was 8.3. The NOEC was determined to be 13 mg ai/l.

Dissolved oxygen ranged from 9.1 to 10.4 mg/l or 84 to 96% of saturation. The pH ranged from 4.6 to 7.3. The temperature was 11-13°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 rainbow trout.

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 protocols recommended by the guidelines, but deviated as follows:

The age of the test organisms was not reported.

The hardness of the water (28-32 mg/l) was less than recommended (40-200 mg/l).

The fish 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<sub>50</sub> value and obtained the same results as the author (see attached printout). However, a larger slope for the probit curve (10.6) was obtained.

- 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<sub>50</sub> value of endothall acid for rainbow trout was 49 mg ai/l. Therefore, endothall acid is classified as slightly toxic to rainbow trout. The NOEC, based on the lack of sublethal effects, was 13 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, 8-9-92.

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Pages 6 through 7 are not included.

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MOSSLER ENDOTHALL ONCORHYNCHUS MYKISS 8-9-92

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|-------|----------------|-------------|--------------|--------------------------|
| 120   | 20             | 20          | 100          | 9.536742E-05             |
| 65    | 20             | 20          | 100          | 9.536742E-05             |
| 57    | 20             | 12          | 60.00001     | 25.17223                 |
| 35    | 20             | 2           | 10           | 2.012253E-02             |
| 30    | 20             | 0           | 0            | 9.536742E-05             |
| 13    | 20             | 0           | 0            | 9.536742E-05             |

THE BINOMIAL TEST SHOWS THAT 35 AND 65 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 52.18952

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G            | LC50     | 95 PERCENT CONFIDENCE LIMITS |         |
|------|--------------|----------|------------------------------|---------|
| 4    | 3.625671E-02 | 43.16224 | 38.90517                     | 48.6478 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G        | H | GOODNESS OF FIT PROBABILITY |
|------------|----------|---|-----------------------------|
| 7          | .1079448 | 1 | .2361316                    |

SLOPE = 10.64838  
95 PERCENT CONFIDENCE LIMITS = 7.149856 AND 14.14689

LC50 = 49.23465  
95 PERCENT CONFIDENCE LIMITS = 44.62748 AND 53.74734

LC10 = 37.41138  
95 PERCENT CONFIDENCE LIMITS = 31.02309 AND 41.77107

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Study/Species/Lab/  
MRID # \_\_\_\_\_ Chemical  
% S.L. \_\_\_\_\_ Results \_\_\_\_\_ Reviewer/ Validation  
Date \_\_\_\_\_ Status \_\_\_\_\_

48-Hour EC<sub>50</sub> \_\_\_\_\_  
EC<sub>50</sub> - \_\_\_\_\_ PP ( 95% C.L. ) Control Mortality (%) - \_\_\_\_\_  
Slope - \_\_\_\_\_ # Animals/Level - \_\_\_\_\_ Solvent Control Mortality (%) - \_\_\_\_\_  
Temperature - \_\_\_\_\_

Lab: \_\_\_\_\_  
MRID # \_\_\_\_\_  
48-Hour Dose Level pp / (% Effect)  
( ), ( ), ( ), ( ), ( )

Comments: \_\_\_\_\_

96-Hour LC<sub>50</sub> 77.9  
LC<sub>50</sub> - 49 pp ( 95% C.L. ) Control Mortality (%) - 0  
95% C.L. probit  
Slope - 10.6 # Animals/Level - 20 Solvent Control Mortality (%) - n/a  
Temperature - 11-13°C

Species: Oncorhynchus mykiss  
Lab: Springborn Laboratories  
MRID # 423277-02  
96-Hour Dose Level pp / (% Mortality)  
13 (0), 30 (0), 35 (10), 57 (60), 65 (100)  
Comments: \* based on mean measured concentrations  
NOEC = 13 mg ai/l \*  
mg ai/l \*  
Address Conc  
8/9/92

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