4-27-93

MRID No. 423277-02

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: 8/27/92

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora

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_{50} 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 $CaCO_3$, an alkalinity of 22-31 mg/l as $CaCO_3$, a conductivity of 130-150 µmhos/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 ±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_{50} 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_{50} 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.

Page_____ is not included in this copy.

Pages 6 through 7 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.

MOSSLER ENDOTHALL ONCORHYNCHUS MYKISS 8-9-92

****

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

****

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 (%) =		
Species:		Slope = # Animals/Level = Temperature =		
Lab:		48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( )	_____	_____
MRID #		Comments:		
96-Hour LC_{50}	77.9	LC_{50} = 49 mg ai/l* 95% C.L. (45-54) probit Control Mortality (%) = 0 Solvent Control Mortality (%) = n/a		

Species: Oncorhynchus mykiss		Slope = 10.6 # Animals/Level = 20
		 Temperature = 11-13°C		

Lab: Springborn Laboratories		96-Hour Dose Level ~~pp~~ mg ai/l* /(% Mortality) 13 (0), 30 (0), 35 (10), 57 (60), 65 (100) 120 (100)	M. Mossler 8/5/92	Core
MRID # 423277-02		Comments: * based on mean measured concentrations NOEC = 13 mg ai/l*		

9