

## DATA EVALUATION RECORD

1. **CHEMICAL:** Endothall (Amine Salt).  
Shaughnessey No. 038901.
2. **TEST MATERIAL:** Endothall Technical; 77.9% active  
ingredient; Batch No. 366, Lot No. J19A; a tan solid.
3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Flow-Through  
Acute Toxicity Test. Species Tested: *Daphnia magna*.
4. **CITATION:** McNamara, P.C. 1992. Endothall Technical -  
Acute Toxicity to Daphnids (*Daphnia magna*) Under Flow-  
Through Conditions. Report No. 91-10-3946. Prepared by  
Springborn Laboratories, Inc., Wareham, MA. Submitted by  
Atochem North America, Philadelphia, PA. EPA MRID No.  
423597-02.
5. **REVIEWED BY:**  
  
Carolyn F. Poppell, Sc.M.                      Signature: P. Kosalwat  
Senior Scientist    for CFP  
KBN Engineering and                      Date: 9/30/92  
Applied Sciences, Inc.                      Denis M. G. 4/28/93
6. **APPROVED BY:**  
  
Pim Kosalwat, Ph.D.                      Signature: P. Kosalwat  
Senior Scientist    Date: 9/30/92  
KBN Engineering and  
Applied Sciences, Inc.  
  
Henry T. Craven, M.S.                      Signature:  
Supervisor, EEB/EFED                      Date:  
USEPA
7. **CONCLUSIONS:** This study is scientifically sound and meets  
the guideline requirements for a freshwater invertebrate  
acute toxicity test using *Daphnia magna*. The 48-hour EC<sub>50</sub>  
was 92 mg a.i./l mean measured concentration. Endothall is  
classified as slightly toxic to *Daphnia magna*. The NOEC was  
24 mg a.i./l mean measured concentration.
8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:10. DISCUSSION OF INDIVIDUAL TESTS: N/A.11. MATERIALS AND METHODS:

A. Test Animals: First instar *Daphnia magna* (<24 hours old) were obtained from in-house cultures. The cultures were maintained at  $20 \pm 2^\circ\text{C}$  under a 16-hour daylight photoperiod. Culture water was a fortified and filtered well water. Daphnids were fed a combination of a trout food suspension and a unicellular green algae (*Ankistrodesmus falcatus*) once daily.

B. Test System: The test was conducted using an intermittent flow proportional diluter and a set of 12 exposure vessels (two replicate vessels for each treatment level and the control). Each glass test vessel maintained a constant solution volume of 1.4 l and a solution depth of approximately 15 cm. A peristaltic pump delivered a stock solution (20 mg a.i./ml of Endothall) into a mixing chamber with dilution water. The solution in the mixing chamber constituted the highest treatment level (170 mg a.i./l) and was subsequently diluted to provide four lower concentrations. Each test chamber received approximately 6.2 solution volume replacements per day. The diluter system was allowed to run for 9 days prior to test initiation.

The vessels were maintained at  $20 \pm 1^\circ\text{C}$  in a temperature controlled water bath. The photoperiod was the same as that used for culturing with a light intensity of 30-100 footcandles. Sudden transitions from light to dark and vice versa were avoided.

The dilution water was from the same source as the culture water with the following characteristics: a hardness of 170 mg/l as  $\text{CaCO}_3$ , an alkalinity of 110 mg/l as  $\text{CaCO}_3$ , a conductivity of 500  $\mu\text{mhos/cm}$ , and a pH range of 8.2 to 8.3.

C. Dosage: Forty-eight-hour flow-through test. Based on a preliminary test, five nominal concentrations (22, 37, 61, 100, and 170 mg a.i./l) and a dilution water control.

- D. **Design:** Ten daphnids were impartially placed in each replicate vessel (2 replicates per treatment). The number of immobilized daphnids was recorded at 24 and 48 hours. Other biological observations (i.e., abnormal behavior or appearance) and observations of physical characteristics of the test solutions were made at test initiation and at 24 and 48 hours. Daphnids were not fed during the test.

Temperature, dissolved oxygen, and pH were measured once daily in each vessel throughout the exposure period. The temperature was also continuously monitored in one replicate of the 22 mg a.i./l solution. Total hardness, alkalinity, and specific conductance were measured at test initiation in one replicate vessel of each treatment level and control solution.

One water sample was removed from both replicate solutions of each treatment level and control on test days 0 and 2 for analyses of Endothall concentration.

- E. **Statistics:** The median effect concentration (EC<sub>50</sub>) and 95% confidence intervals were determined using a computer program developed by Stephan.

12. **REPORTED RESULTS:** Mean measured concentrations were 24, 35, 62, 92, and 180 mg a.i./l, representing 92 to 109% of nominal concentrations (Table 3, attached). The responses of *Daphnia magna* are given in Table 4 (attached). At test termination, 100% immobilization was observed among daphnids exposed to the highest test concentration (180 mg a.i./l), while immobilization of 50% was observed among daphnids exposed to the 92 mg a.i./l test concentration. No immobilization was observed among daphnids at lower test concentrations or among controls.

Sublethal effects (e.g., pale color, small) were observed among all of the surviving daphnids exposed to concentrations of  $\geq 35$  mg a.i./l. The slope of the 48-hour concentration response curve was calculated to be 7.8587. The 48-hour EC<sub>50</sub> value was estimated by non-linear interpolation to be 92 mg a.i./l with a 95% confidence interval calculated by binomial probability of 62-180 mg a.i./l. The NOEC established for this study was 24 mg a.i./l, based on mean measured concentrations.

Water quality measurements during the test are presented in Tables 1 and 2 (attached). Throughout the exposure period,

the pH and alkalinity of the test solutions were observed to decrease with increasing test concentrations. The remaining water quality parameters were unaffected, and remained within acceptable ranges. Continuous temperature monitoring in one test solution confirmed that temperature remained between 20 and 21°C for the duration of the study.

**13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

Based on the results of the test, the author concluded that Endothall Technical is classified as slightly toxic to daphnids.

Quality Assurance and Study Compliance Statements were included in the report. The Good Laboratory Practices (GLP) Compliance Statement indicated that the study was conducted in accordance with USEPA Good Laboratory Practice Standards set forth in 40 CFR Part 160, with the exception of stability, characterization and verification of the test substance identity. Routine water and food contaminant screening analyses for pesticides, PCBs, and metals were conducted using standard EPA procedures. However, these data were not collected in accordance with GLP procedures. The report was inspected by a quality assurance officer and study inspections were performed.

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

- A. **Test Procedure:** The test procedures follows the protocols recommended by the SEP.
- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the EC<sub>50</sub> value. The 48-hour EC<sub>50</sub> was 92 mg a.i./l, based on mean measured concentrations of Endothall. The 95% confidence interval, using the binomial test, was estimated to be 62 and 180 mg a.i./l (see attached printout).
- C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a flow-through acute toxicity test using *Daphnia magna*. With a 48-hour EC<sub>50</sub> of 92 mg a.i./l mean measured concentration, Endothall is classified as slightly toxic to *Daphnia magna*. The NOEC was 24 mg a.i./l, based on daphnid abnormalities and immobility observed at higher test concentrations.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, September 10, 1992.

## WETLAND RANKING

A subjective ranking of wetland quality was completed as a preliminary evaluation. These rankings reflect professional judgement based on brief field observations recorded during the field work for wetland delineation.

Seven characteristics of these wetlands were selected and rated from lowest quality to highest quality. The highest quality reflects largely undisturbed wetlands. The characteristics with the lowest quality were given a 1 and those with the highest quality were given a 5. The categories chosen are as follows:

Regulatory and/or Jurisdictional Status: How many agencies (county, regional planning council, US Army Corps of Engineers, water management district, Florida Department of Environmental Regulation) have jurisdiction over the wetland.

Hydrological Integrity: How does the wetland function for water collection and distribution. Natural water retention with little disturbance was given the highest value and ditches or other disturbance changing all or nearly all of original water flow was given the lowest value.

Disturbance: Ditching, vehicle traffic, grazing activity, mowing, pasture improvements, and logging are among the factors evaluated. Natural wetlands with little disturbance ranged to those which were completely disturbed.

Exotics: Melaleuca, Brazilian Pepper Tree, Torpedo Grass, and Bahia Grass are species present in this tract which are native to other areas of the world. These species have escaped from cultivation, invaded natural areas and become weeds. No exotics present was the highest rating and heavily invaded was the lowest.

Rare/Endangered: Rare or endangered species known and/or thought to be in similar Martin County habitats were evaluated. Those wetlands with rare and endangered species known to be present were given the highest rating. If the species is likely to be present the wetland was given an in between value. Wetlands with no known rare or endangered species and no likely or suitable habitat were given the lowest value.

Aesthetic/Recreation: Aesthetic values and recreation potential (scenic view, potential nature trails, site for scientific research, good example of ecological habitat for teaching and demonstration) were examined and evaluated. An unusual habitat example which would attract international

and national visitors was the highest evaluation and aesthetically unappealing with little scientific or educational value was the lowest.

Hydrologically Connected: Flow ways of whatever nature that allow water to run between wetlands were rated. Natural connections were high, man made or disturbed connections were medium quality, and wetlands with no connections (isolated) were the lowest quality.

#### Summary

These seven categories when added total 35 for the highest quality wetland and 7 for the lowest quality wetland. The lowest quality wetlands on the T P & J property have a score of 9 and the highest a score of 30. The lowest quality wetlands are functioning in a reduced capacity as they have been artificially maintained as fish ponds. The highest quality wetlands such as the wooded areas along the South Fork of the St. Lucie River have had little disturbance and contain some endangered or threatened species.

#### T P & J Preliminary Wetland Evaluation Values:

1-10 = Barrow pits/fish ponds, artificially created and maintained.

11-14 = Ditches/drainage trenches, artificially created.

15-18 = Disturbed natural wetlands with extensive ditching, vehicular traffic ruts, heavily grazed vegetation, exotic species of plants, planted with turf and forage grasses or crops, heavily invaded by plant species more indicative of drier habitats, extensive populations of weeds.

19-24 = Good quality natural wetlands with little invasion by plant species indicative of drier habitats, little or no ditching, little grazing, no or little vehicle traffic, populations of weedy species absent or small.

30 = High quality natural wetlands with no or very little disturbance, no vehicle traffic, presence of rare/endangered species, no or very minor ditching, natural community with intact canopy.

Carolyn Poppell Endothall Technical Daphnia magna 09-10-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
180	20	20	100	9.536742E-05
92	20	10	50	58.80985
62	20	0	0	9.536742E-05
35	20	0	0	9.536742E-05
24	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 62 AND 180 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 92

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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Pages 9 through 12 are not included.

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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.
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Shaughnessey # 038901

Chemical Name

Endothall (Amine salt)

Chemical Class

Page

of

Study/Species/Lab/  
MRID # Chemical  
% a.i.

48-Hour EC<sub>50</sub>

77.9

EC<sub>50</sub> = 92

95% C.L.  
ppm (62-180)

Control Mortality (%) = 0

Solvent Control Mortality (%) = N/A

Species: *Daphnia magna*

Slope = 7.8

# Animals/Level = 20

Temperature = 20°C

Lab: Springborn Laboratories

MRID # 423597-02

48-Hour Dose Level ppm / (% Effect)

24 (0), 35 (0), 62 (0), 92 (50), 180 (100)

Comments: \* Based on mean measured concn. of active ingredient

N/A = not applicable

NOEC = 24 mg ai./l

CTHoyall Core  
9-18-92

96-Hour LC<sub>50</sub>

LC<sub>50</sub> =

pp

95% C.L.

( )

Control Mortality (%) =

Solvent Control Mortality (%) =

Species:

Slope =

# Animals/Level =

Temperature =

Lab:

96-Hour Dose Level pp / (% Mortality)

( ), ( ), ( ), ( ), ( ), ( )

MRID #

Comments:

CP