10-17-94 EEB files
S#38901

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 17 1994

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Endothall Technical - Invertebrate Life Cycle Test Study - D199241

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) 10/5/94

To: Kathryn Davis, PM Team 52 Reviewer
Special Review and Reregistration Division (7508C)

The following Data Evaluation Record (DER) Conclusions (see attached complete DER) have been provided for the subject study.

Action I.D.: D199241 818815 S#38901
Guideline: 72-4(b) MRID No.: 43007801

CONCLUSIONS: This study is scientifically sound but does not fulfill the guideline requirements for a life-cycle study with freshwater aquatic invertebrates. The NOEC less than 2.2 mg a.i./L for both length and offspring/female.

In the Bean Sheet (D199241) for this action you ask if the subject study with endothall acid (S#038901) would satisfy the requirement for the dipotassium salt of endothall (S# 038904) and/or the N, N- dimethyl alkyl amine salt of endothall (S# 038905). In EEB's March 14, 1991 list B responses placed the requirement for subject study in reserve for both of these chemicals. The reserve status was pending the receipt of lower tier studies, additional use information, and environmental fate data. This information has not come available. Testing on the endothall acid (S# 038901) was not required in the EEB's March 12, 1991 list B response because it did not have any outdoor uses and of course the subject study does not fulfill the guidelines because it did not reach the NOEC level for length or reproduction.

Please contact Dennis J. McLane (305-5096) if you have any further questions.

DP BARCODE: D199241 REREG CASE # 2245

CASE: 818815 DATA PACKAGE RECORD DATE: 02/10/94
SUBMISSION: S458046 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 627 CORE DATA
CHEMICALS: 038901 Endothall

ID#: 038901-
COMPANY:
PRODUCT MANAGER: 52 KATHRYN DAVIS 703-308-8156 ROOM: CS1 3F3
PM TEAM REVIEWER: KATHRYN DAVIS 703-308-8156 ROOM: CS1 3F3
RECEIVED DATE: 11/15/93 DUE OUT DATE: 02/13/94

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 199241 EXPEDITE: N DATE SENT: 02/08/94 DATE RET.: / /
CHEMICAL: 038901 Endothall
DP TYPE: 999 Miscellaneous Data Package
CSF: LABEL:

| ASSIGNED TO | DATE IN | DATE OUT |
|---|---|---|
| DIV : EFED | 02/14/94 | / / |
| BRAN: EEB | 02/15/94 | / / |
| SECT: | / / | / / |
| REVR : | / / | / / |
| CONTR: | / / | / / |

ADMIN DUE DATE: 05/09/94
NEGOT DATE: / /
PROJ DATE: / /

* * * DATA REVIEW INSTRUCTIONS * * *

Please review attached study. Does it satisfy for 72-4(b) Life cycle - invertebrate MRID # 430078-01 for the dipotassium salt and amine salt as well?

If you have any questions please contact Kathy Davis at 308-8156

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

| DP BC | BRANCH/SECTION | DATE OUT | DUE BACK | INS | CSF | LABEL |
|---|---|---|---|---|---|---|

2

DP Barcode : D199241
PC Code No : 038901
EEB Out : / /

To: Kathryn Davis
Product Manager 52
Special Review and Reregistration Division (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 038901
Chemical Name : Endothall
Type Product : herbicide
Product Name :
Company Name :
Purpose : Review invertebrate life cycle study.

Action Code: 627 Date Due: 5/9/94
Reviewer: Dennis McLane

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

| GDLN NO | MRID NO | CAT | GDLN NO | MRID NO | CAT | GDLN NO | MRID NO | CAT |
|---|---|---|---|---|---|---|---|---|
| 71-1(A) | | | 72-2(A) | | | 72-7(A) | | |
| 71-1(B) | | | 72-2(B) | | | 72-7(B) | | |
| 71-2(A) | | | 72-3(A) | | | 122-1(A) | | |
| 71-2(B) | | | 72-3(B) | | | 122-1(B) | | |
| 71-3 | | | 72-3(C) | | | 122-2 | | |
| 71-4(A) | | | 72-3(D) | | | 123-1(A) | | |
| 71-4(B) | | | 72-3(E) | | | 123-1(B) | | |
| 71-5(A) | | | 72-3(F) | | | 123-2 | | |
| 71-5(B) | | | 72-4(A) | | | 124-1 | | |
| 72-1(A) | | | 72-4(B) | 43007801 | S | 124-2 | | |
| 72-1(B) | | | 72-5 | | | 141-1 | | |
| 72-1(C) | | | 72-6 | | | 141-2 | | |
| 72-1(D) | | | | | | 141-5 | | |

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

**DATA EVALUATION RECORD**
**INVERTEBRATE LIFE CYCLE TEST**
**GUIDELINE 72-4(B)**

1. **CHEMICAL:** Endothall Technical (acid) Shaughnessey #: 038901

2. **TEST MATERIAL:** Technical Purity: 91.2 %

3. **CITATION:**

| | |
|---|---|
| Author: | Arthur E. Putt |
| Title: | Endothall Technical (Acid)- The Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions |
| Date: | August 13, 1993 |
| Laboratory Report #: | 93-11-4525 |
| Any Other Study #: | 12442.0692.6148.103 |
| Sponsor: | Atochem North America, Inc |
| Sponsor #: | N/A |
| Laboratory: | Springborn Laboratories, Inc. |
| MRID No.: | 43007801 |

4. **REVIEWED BY:**

Dennis J/ McLane, Wildlife Biologist
Ecological Effects Branch (7507C)
U.S.E.P.A.

Signature: Dennis J McLane
Date: 9-15-94

5. **APPROVED BY:**

Les W. Touart
Section Head
Ecological Effects Branch (7507C)
U.S.E.P.A.

Signature: L W Touart
Date: 9/28/94

6. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a life-cycle study with freshwater aquatic invertebrates. The $NOEC_1^{15}$ less than 2.2 mg a.i./L for both length and offspring/female.

7. **MAJOR GUIDELINE DEVIATIONS:**

The study did not establish a NOEC or LOEC for daphnids.

8. **MATERIALS AND METHODS:**

A. **Biological System:**

| Guideline Criteria | Reported Information |
|---|---|
| **Species:** *Daphnia* | *Daphnia magna* |

| Guideline Criteria | Reported Information |
|---|---|
| **Source** | Springborn's own culture |
| **Food** variety - 1) synthetic (trout chow); 2) Alfalfa yeast and algae; 3) Algae *Ankistrodesmus falcatus*; *Chalamydamonas reinhardtii*; and *Selenastrum capricornutum* | *Ankistrodesmus falcatus* |
| **Age at beginning of test:**<br>1) ≤ 24 hours old;<br>2) Prior to the test, at least 10-12 days old (those that have had at least on brood) should be separated from the culture, put in a separate culture container and maintained for at least 21 days to insure that good health and conditions are present. | 1) ≤24 hours old<br>2) Not reported |
| **Renewal**: 7 beakers containing 1 parent daphnid each, the offspring, both live and dead, are counted and discarded. | This test was a flow-through not renewal. |
| **Duration:** Last day 21 count and measure to nearest 0.01 mm from apex of helmet to the base of the spine. # young, both alive and dead. | Yes |
| **Test Rejection:**<br>1) 30 % die;<br>2) must ≥ 40 young after 21 days;<br>3) Production of "resting eggs" or ephippia;<br>4) Temp. changes over 5°C from 20°C in 48 hrs;<br>5) DO >50% for 48 hrs;<br>6) pH changes > one unit in 48 hrs. | 1) No<br>2) Yes<br>3) Not reported<br>4) No<br>5) No<br>6) No |

| Guideline Criteria | Reported Information |
|---|---|
| **Data Endpoints:**<br>1) survival of 1st generation<br>2) production of young at various times for each treatment<br>3) Length of 1st generation at end of test | <br>1) yes<br>2) Yes<br><br>3) Yes |

Comments: None

**B. Physical System:**

| Guideline Criteria | Reported Information |
|---|---|
| **Test Water:**<br>1) Well or spring provided that the source is not polluted<br>2) Tested for contaminants<br>3) Measured pH 7.6 and 8.0.<br>4) Hardness 160 to 180 mg/L<br>5) reconstituted water detailed descriptions (ASTM) | <br>1) well water adjusted to meet ASTM hard water criteria plus filtering through Amberlite® resin column and carbon filter.<br>2) yes<br>3) 7.9-8.3<br>4) see above |
| **Test Temperature:** 20°C±2°C | 20±2°C |
| **Photoperiod:** Recommend 16L/8D; Intensity should be 400 to 800 Lux (37 to 74 footcandles) at surface with wide-spectrum fluorescent lamps. | 16L/8D;<br>Intensity was not reported. Source was a combination of Marvel® Cool White and Sylvania® Lifeline Natural White fluorescent |
| **Test Vessels:** All glass, No. 316 stainless steel, or perfluorocarbon plastics; volume 200 mls. 250 ml (borosilicate glass beakers are typically used). vessels should be covered with glass plates to prevent evaporation. | glass, Nitex® 40-mesh screen, silicone: tubing, stoppers and sealant. |
| **Aeration:** Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated. | Sufficient DO concentrations were maintained. Test tanks were not aerated. |



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Comments: The following are criteria for fish chronic flow-through testing.

| ASTM Criteria for a Flow-Through Study | Reported Information |
|---|---|
| **Dosing Apparatus:**<br>1) Intermittent flow proportional diluters or continuous flow serial diluters should be used.<br>2) Checked twice daily operation of metering system | 1) Mount and Brungs (1967) intermittent flow proportional diluter;<br><br>2) Yes |
| **Toxicant Mixing:**<br><br>1) Aeration should not be used for mixing;<br>2) It must be demonstrated that the test solution is completely mixed before intro. into the test system;<br>3) Flow splitting accuracy must be within 10%. | <br><br>1) Not reported<br><br>2) Pretest measurements (see Table 2)<br><br>3) The 1.9 mg/L level failed to show less than a 10% difference between A & B replicates during Pretest #1 all other measurements met the guidelines. |
| **Retention and Pairing Chambers:** Glass with mesh nylon or stainless steel screens | Glass with nylon screens. |
| **Concentrations**- Toxicant conc. must be measured in one tank at each toxicant level every week. | - see Table 2 |
| **Flow Rate:** 1) Flow rates should provide 5 to 10 volume additions per 24 hr;<br>2) flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. | 1) Flow rate provided approx. 6 volume additions per day.<br>2) DO maintained at > 60% saturation. |

C. Chemical System:

| Guideline Criteria | Reported Information |
|---|---|



7

| | |
|---|---|
| **Concentrations:** 1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.<br><br>2) - food conc. dry weight 5 mg/L for synthetic; algal cells $10^8$ | 1) 5 concentrations and a control<br><br>2)-Trout food 5 mg/ml+algal suspension 4 x $10^7$+Selco® food supplement .6 mg/ml 3 x daily on weekdays 2 x daily on weekends |
| **Other Variables:**<br>1) DO must be measured at each conc. at least once a week;<br>2) Freshwater parameters in a control & 1 conc. must be analyzed once a week; (pH, alkalinity, hardness, and conductance | 1) Once a week<br><br>2) Once a week |

Comments: No comments.

**9. REPORTED RESULTS:**

| Guideline Criteria | Reported Information |
|---|---|
| **Data Endpoints** must include:<br>1) Survival of first-generation daphnids<br>2) Production of young<br><br>3) Length of first generation daphnids at the end of the test. | 1) Yes (see Table 3)<br><br>2) Yes (see Table 5)<br><br>3) Yes (see Table 7) |
| **Raw data included?** (Y/N) | No |

A. Effects Data:

| Toxicant Conc. (mg/L) | | Young per fem.per repro. day | | | | Survival (21 days) | | | | Total Length (mm) | | | | Dry weight (mg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nom. | Meas. | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |

| Toxicant Conc. (mg/L) | | Young per fem.per repro. day | | | | Survival (21 days) | | | | Total Length (mm) | | | | Dry weight (mg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ctrl | | 11.1 | 10.2 | 10.6 | 10.4 | 90 | 100 | 80 | 100 | 5.16 | 5.11 | 5.2 | 5.14 | 1.19 | 1.36 | 1.31 | 1.45 |
| 1.9 | 2.2 | 10.1 | 8.6 | 9.3 | 8.7 | 100 | 100 | 100 | 100 | 5.13 | 5.06 | 5.11 | 4.92 | 1.4 | 1.37 | 1.37 | 1.28 |
| 3.8 | 5.0 | 10.6 | 9.9 | 10.1 | 11.0 | 90 | 100 | 100 | 100 | 5.24 | 5.18 | 5.11 | 5.3 | 1.58 | 1.49 | 1.44 | 1.57 |
| 7.5 | 8.9 | 9.9 | 8.6 | 9.5 | 8.1 | 100 | 90 | 100 | 100 | 5.27 | 5.22 | 5.24 | 5.01 | 1.76 | 1.61 | 1.6 | 1.51 |
| 15 | 17 | 4.2 | 6.0 | 5.8 | 5.9 | 90 | 100 | 100 | 100 | 5.01 | 4.91 | 4.99 | 4.88 | 1.43 | 1.4 | 1.61 | 1.37 |
| 30 | 35 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

B. Statistical Results:

Statistical Methods: Shapiro-Wilks test for normality, Bartlett's test for homogeneity, and William's test for statistical comparison

NOEC: 5.0 mg a.i./L LOEC: 8.9 mg a.i./L

Most sensitive endpoint: Mean Total Weight and Offspring/daphnid

Comments: Table 7. and Figure 5. indicate that the mean dry weight is NOEC is 35 mg a.i./L and the LOEC is 17 mg a.i./L.

**10. Reviewer's Statistical Results:**

Statistical Method: Toxstat Dunnett's for offspring/female
Toxstat Bonferroni's for total body length

NOEC: <2.2 mg a.i./L LOEC: UNKNOWN

Most sensitive endpoint: Total body length and offspring/female

Of additional interest is that the mean cumulative percent survival decreased from 100% on day 2 to 48% on day 4 at the 35 mg a.i./L. The other treatment levels ranged from 95% to 100% through day 21. This is illustrate in Table 3.

Comments:

The attached Toxstat printout entitled Endothall 21 Day Daphnia - Offspring/Female shows the number offspring per female was most sensitive parameter (Dunnett's) followed by body length. EEB's statistical analysis shows that all treatments except that 5 mg a.i./L offspring/female were different from the control. The total body length (printout entitled 21 Day Daphnia magna Total Length) data shows two levels which were not different from the control at 5.0 mg a.i./L level and 8.9 mg a.i./L level. The Dunn's multiple comparison - Kruskal-Wallis shows that the dry body weight at the 8.9 mg a.i./L level is different from the control, 2.2, and 35 mg a.i./L, whereas, all the other doses were only different from the 35 mg a.i./L level. In addition the effects appear to contradict each other, the weight appears to increase up to 8.9 then decreases while the length decreases from the control to the 2.2 dose and increases to the 5.0 dose and decreases from the 5.0 dose to the 35 mg a.i./L dose. On the other hand, the offspring per female decreases from control to 2.2 dose and increases from 2.2 to 5.0 dose and then decreases through to the last dose (see attached Springborn's Figures 3 - 5). Also, the mean total cumulative offspring per female was 192.75 with a minimum and maximum of 181 and 211, respectively, for the 2.2 mg a.i./L, whereas, the 8.9 mg a.i./L (Springborn's LOEC) was 190 mg a.i./L with a minimum of 171 and maximum of 208. In either case the minimum and maximum values for the control group are not overlapped by either of these two concentrations, further emphasizing the differences from the control. Notice however the offspring/female confidence levels for the 5.0 level are similar to the controls 208 and 231. Based on all these items, it appears the study responses at the 5.0 and/or 8.9 mg a.i./L levels to may be an anomalies, and because the lowest level tested was significantly different from the controls for both offspring/female and length an NOEC can not be determined using this test.

**11. ADEQUACY OF THE STUDY:**

A. Classification: Supplemental

B. Rationale: The study failed to show a no-observed-effect concentration.

C. Reparability: No

**12. COMPLETION OF ONE-LINER FOR STUDY:**

One-liner completed

Page_____ is not included in this copy.

Pages 11 through 21 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.

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat  Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA  TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | Control | 4 | 215.000 | 232.000 | 221.750 |
| 2 | 1.9 | 4 | 181.000 | 211.000 | 192.750 |
| 3 | 3.8 | 4 | 208.000 | 231.000 | 218.000 |
| 4 | 7.5 | 4 | 171.000 | 208.000 | 190.000 |
| 5 | 15 | 4 | 88.000 | 126.000 | 115.000 |
| 6 | 30 | 4 | 0.000 | 0.000 | 0.000 |

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat  Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA  TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM |
|---|---|---|---|---|
| 1 | Control | 54.917 | 7.411 | 3.705 |
| 2 | 1.9 | 192.250 | 13.865 | 6.933 |
| 3 | 3.8 | 111.333 | 10.551 | 5.276 |
| 4 | 7.5 | 288.667 | 16.990 | 8.495 |
| 5 | 15 | 326.667 | 18.074 | 9.037 |
| 6 | 30 | 0.000 | 0.000 | 0.000 |

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat  Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 146761.000 | 29352.200 | 180.845 |
| Within (Error) | 18 | 2921.500 | 162.306 | |
| Total | 23 | 149682.500 | | |

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat  Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | Control | 221.750 | 221.750 | | |
| 2 | 1.9 | 192.750 | 192.750 | 3.219 | * |
| 3 | 3.8 | 218.000 | 218.000 | 0.416 | |
| 4 | 7.5 | 190.000 | 190.000 | 3.524 | * |
| 5 | 15 | 115.000 | 115.000 | 11.850 | * |
| 6 | 30 | 0.000 | 0.000 | 24.616 | * |

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | Control | 4 | | | |
| 2 | 1.9 | 4 | 21.710 | 9.8 | 29.000 |
| 3 | 3.8 | 4 | 21.710 | 9.8 | 3.750 |
| 4 | 7.5 | 4 | 21.710 | 9.8 | 31.750 |
| 5 | 15 | 4 | 21.710 | 9.8 | 106.750 |
| 6 | 30 | 4 | 21.710 | 9.8 | 221.750 |

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 146761.000 | 29352.200 | 180.845 |
| Within (Error) | 18 | 2921.500 | 162.306 | |
| Total | 23 | 149682.500 | | |

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | ORIGINAL MEAN | 0 0 0 0 0 0<br>6 5 4 2 3 1 |
|---|---|---|---|---|
| 6 | 30 | 0.000 | 0.000 | \ |
| 5 | 15 | 115.000 | 115.000 | * \ |
| 4 | 7.5 | 190.000 | 190.000 | * * \ |
| 2 | 1.9 | 192.750 | 192.750 | * * . \ |
| 3 | 3.8 | 218.000 | 218.000 | * * . . \ |
| 1 | Control | 221.750 | 221.750 | * * * * . \ |

* = significant difference (p=0.05) . = no significant difference
Tukey value (6,18) = 4.49 s = 162.306

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | RANK SUM |
|---|---|---|---|---|
| 1 | Control | 221.750 | 221.750 | 84.500 |
| 2 | 1.9 | 192.750 | 192.750 | 53.000 |
| 3 | 3.8 | 218.000 | 218.000 | 77.500 |
| 4 | 7.5 | 190.000 | 190.000 | 49.000 |
| 5 | 15 | 115.000 | 115.000 | 26.000 |
| 6 | 30 | 0.000 | 0.000 | 10.000 |

Calculated H Value = 20.789 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Endothall 21 Day Daphnia - Offspring/Female
File: a:offsprin.dat Transform: NO TRANSFORM

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

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | ORIGINAL MEAN | GROUP<br>0 0 0 0 0 0<br>6 5 4 2 3 1 |
|---|---|---|---|---|
| 6 | 30 | 0.000 | 0.000 | \ |
| 5 | 15 | 115.000 | 115.000 | . \ |
| 4 | 7.5 | 190.000 | 190.000 | . . \ |
| 2 | 1.9 | 192.750 | 192.750 | . . . \ |
| 3 | 3.8 | 218.000 | 218.000 | * . . . \ |
| 1 | Control | 221.750 | 221.750 | * . . . . \ |

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 4.985

21 Day Daphnia magna Total Length
File: c:\chem\endothal\daph21.dat Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 863.655 | 172.731 | 6397.444 |
| Within (Error) | 225 | 5.980 | 0.027 | |
| Total | 230 | 869.635 | | |

Critical F value = 2.29 (0.05,5,120)
Since F > Critical F REJECT Ho:All groups equal

21 Day Daphnia magna Total Length
File: c:\chem\endothal\daph21.dat Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | Control | 5.149 | 5.149 | | |
| 2 | 1.9 | 5.054 | 5.054 | 2.513 | * |
| 3 | 3.8 | 5.207 | 5.207 | -1.512 | |
| 4 | 7.5 | 5.182 | 5.182 | -0.881 | |
| 5 | 15 | 4.942 | 4.942 | 5.432 | * |
| 6 | 30 | 0.000 | 0.000 | 136.390 | * |

Bonferroni T table value = 2.36 (1 Tailed Value, P=0.05, df=120,5)

21 Day Daphnia magna Total Length
File: c:\chem\endothal\daph21.dat Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | Control | 36 | | | |
| 2 | 1.9 | 40 | 0.089 | 1.7 | 0.095 |
| 3 | 3.8 | 37 | 0.091 | 1.8 | -0.058 |
| 4 | 7.5 | 39 | 0.090 | 1.7 | -0.033 |
| 5 | 15 | 39 | 0.090 | 1.7 | 0.206 |
| 6 | 30 | 40 | 0.089 | 1.7 | 5.149 |

21 Day Daphnia magna Total Length
File: c:\chem\endothal\daph21.dat Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|---|---|---|---|---|---|
| 1 | Control | 36 | 5.149 | 5.149 | 5.149 |
| 2 | 1.9 | 40 | 5.054 | 5.054 | 5.146 |
| 3 | 3.8 | 37 | 5.207 | 5.207 | 5.146 |
| 4 | 7.5 | 39 | 5.182 | 5.182 | 5.146 |
| 5 | 15 | 39 | 4.942 | 4.942 | 4.942 |
| 6 | 30 | 40 | 0.000 | 0.000 | 0.000 |

21 Day Daphnia magna Total Length
File: c:\chem\endothal\daph21.dat Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|---|---|---|---|---|---|
| Control | 5.149 | | | | |
| 1.9 | 5.146 | 0.078 | | 1.66 | k= 1, v=225 |
| 3.8 | 5.146 | 0.077 | | 1.73 | k= 2, v=225 |
| 7.5 | 5.146 | 0.078 | | 1.75 | k= 3, v=225 |
| 15 | 4.942 | 5.475 | * | 1.77 | k= 4, v=225 |
| 30 | 0.000 | 137.463 | * | 1.77 | k= 5, v=225 |

s = 0.163
Note: df used for table values are approximate when v > 20.

```
*************************************************************************
* User name:   HWINNIK (13)                Queue:  DCOPP1/PRINTQ_1012_BAY *
* File name:                               Server  PRINT_SERVER_1         *
* Directory:                                                              *
* Description: Quattro Pro                                                *
*              September 14, 94                        9:29am             *
*************************************************************************
*                                                                         *
*                  H   H W   W  III  N   N N   N  III  K   K              *
*                  H   H W   W   I   NN  N NN  N   I   K  K               *
*                  H   H W   W   I   N N N N N N   I   K K                *
*                  HHHHH W W W   I   N N N N N N   I   KK                 *
*                  H   H W W W   I   N N N N N N   I   K K                *
*                  H   H WW WW   I   N  NN N  NN   I   K  K               *
*                  H   H W   W  III  N   N N   N  III  K   K              *
*                                                                         *
*************************************************************************
*                                                                         *
*                                                                         *
*                                                                         *
*                                                                         *
*                                                                         *
*                                                                         *
```

26

Endothall 21 Day Daphnia dry body weight
File: c:\chem\endothal\dapwt.dat Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | control | 36 | 0.900 | 1.650 | 1.336 |
| 2 | 1.9 | 40 | 0.860 | 1.740 | 1.357 |
| 3 | 3.8 | 37 | 1.110 | 1.800 | 1.519 |
| 4 | 7.5 | 39 | 1.160 | 1.970 | 1.618 |
| 5 | 15 | 39 | 1.020 | 1.830 | 1.455 |
| 6 | 30 | 40 | 0.000 | 0.000 | 0.000 |

Endothall 21 Day Daphnia dry body weight
File: c:\chem\endothal\dapwt.dat Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM |
|---|---|---|---|---|
| 1 | control | 0.046 | 0.216 | 0.036 |
| 2 | 1.9 | 0.048 | 0.220 | 0.035 |
| 3 | 3.8 | 0.032 | 0.180 | 0.030 |
| 4 | 7.5 | 0.040 | 0.200 | 0.032 |
| 5 | 15 | 0.038 | 0.196 | 0.031 |
| 6 | 30 | 0.000 | 0.000 | 0.000 |

Endothall 21 Day Daphnia dry body weight
File: c:\chem\endothal\dapwt.dat Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 72.377 | 14.475 | 425.735 |
| Within (Error) | 225 | 7.649 | 0.034 | |
| Total | 230 | 80.026 | | |

Critical F value = 2.29 (0.05,5,120)
Since F > Critical F REJECT Ho:All groups equal

Endothall 21 Day Daphnia dry body weight
File: c:\chem\endothal\dapwt.dat Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | control | 1.336 | 1.336 | | |
| 2 | 1.9 | 1.357 | 1.357 | -0.512 | |
| 3 | 3.8 | 1.519 | 1.519 | -4.260 | |
| 4 | 7.5 | 1.618 | 1.618 | -6.632 | |
| 5 | 15 | 1.455 | 1.455 | -2.803 | |
| 6 | 30 | 0.000 | 0.000 | 31.528 | * |

Bonferroni T table value = 2.36 (1 Tailed Value, P=0.05, df=120,5)

Endothall 21 Day Daphnia dry body weight
File: c:\chem\endothal\dapwt.dat Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | control | 36 | | | |
| 2 | 1.9 | 40 | 0.100 | 7.5 | -0.022 |
| 3 | 3.8 | 37 | 0.102 | 7.6 | -0.184 |
| 4 | 7.5 | 39 | 0.100 | 7.5 | -0.283 |
| 5 | 15 | 39 | 0.100 | 7.5 | -0.119 |
| 6 | 30 | 40 | 0.100 | 7.5 | 1.336 |

Endothall 21 Day Daphnia dry body weight
File: c:\chem\endothal\dapwt.dat Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | RANK SUM |
|---|---|---|---|---|
| 1 | control | 1.336 | 1.336 | 3883.000 |
| 2 | 1.9 | 1.357 | 1.357 | 4472.500 |
| 3 | 3.8 | 1.519 | 1.519 | 5608.000 |
| 4 | 7.5 | 1.618 | 1.618 | 6794.000 |
| 5 | 15 | 1.455 | 1.455 | 5218.500 |
| 6 | 30 | 0.000 | 0.000 | 820.000 |

Calculated H Value = -5736.008 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

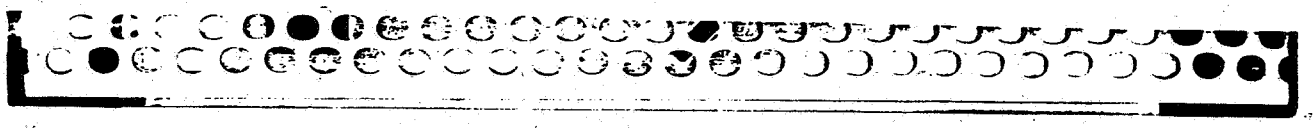
Endothall 21 Day Daphnia dry body weight
File: c:\chem\endothal\dapwt.dat Transform: NO TRANSFORM

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

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | ORIGINAL MEAN | GROUP 0 0 0 0 0 0 6 1 2 5 3 4 |
|---|---|---|---|---|
| 6 | 30 | 0.000 | 0.000 | \ |
| 1 | control | 1.336 | 1.336 | * \ |
| 2 | 1.9 | 1.357 | 1.357 | * . \ |
| 5 | 15 | 1.455 | 1.455 | * . . \ |
| 3 | 3.8 | 1.519 | 1.519 | * . . . \ |
| 4 | 7.5 | 1.618 | 1.618 | * * * . . \ |

* = significant difference (p=0.05)
Table q value (0.05,6) = 2.936

. = no significant difference
Unequal reps - multiple SE values



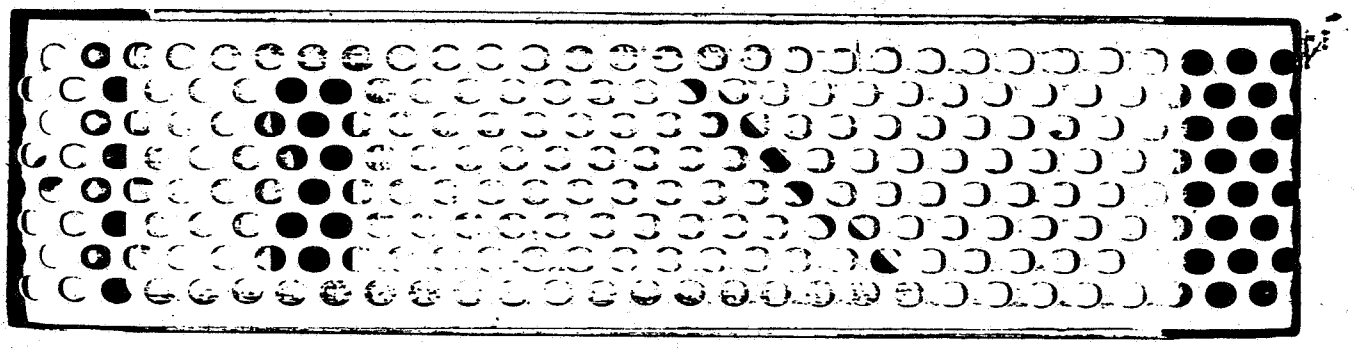


Figure 1
21 Day Daphnia Study
Percent Difference Between Measured Concentrations of Endothall

| Nominal Conc. | Pretest #1 | Pretest #2 | Day 0 | Day 7 | Day 14 | Day 21 | Means |
|---|---|---|---|---|---|---|---|
| 30 | 31 | 33 | 37 | 32 | 37 | 35 | 34.17 |
| | 31 | 33 | 34 | 33 | 39 | 32 | 33.67 |
| | 0.00% | 0.00% | 8.11% | -3.13% | -5.41% | 8.57% | 1.46% |
| 15 | NA | NA | 17 | 16 | 18 | 17 | 11.33 |
| | NA | NA | 17 | 16 | 18 | 16 | 11.17 |
| | | | 0.00% | 0.00% | 0.00% | 5.88% | 1.47% |
| 7.5 | 8.9 | 9.2 | 9.4 | 8.2 | 9.7 | 8.3 | 8.95 |
| | 8.9 | 9.7 | 9.7 | 8.3 | 9.7 | 8.2 | 9.08 |
| | 0.00% | -5.43% | -3.19% | -1.22% | 0.00% | 1.20% | -1.49% |
| 3.8 | NA | NA | 5.2 | 4.7 | 5.7 | 4.1 | 3.28 |
| | NA | NA | 5.3 | 4.8 | 5.7 | 4.5 | 3.38 |
| | | | -1.92% | -2.13% | 0.00% | -9.76% | -3.05% |
| | ** | | | | | | |
| 1.9 | 4.1 | 2.8 | 2.5 | 2.1 | 2.3 | 1.9 | 2.62 |
| | 2.7 | 2.9 | 2.5 | 1.9 | 2.3 | 1.9 | 2.37 |
| | 34.15% | -3.57% | 0.00% | 9.52% | 0.00% | 0.00% | 9.55% |

* The number of times the 10% difference was exceeded for a given conce
** The difference between the two replicates exceeds the 10% allowed by t

Figure 2

Daphnia magna Reproduction Test Summary

| Conc. mg/L | REP. | Offspring / Female | Offspring / Female /Day @ Day 21 |
|---|---|---|---|
| Control | A | 232 | 11.05 |
| | B | 215 | 10.24 |
| | C | 222 | 10.57 |
| | D | 218 | 10.38 |
| Average | | 221.75 | 10.56 |
| 1.9 | A | 211 | 10.05 |
| | B | 181 | 8.62 |
| | C | 196 | 9.33 |
| | D | 183 | 8.71 |
| Average | | 192.75 | 9.18 |
| 3.8 | A | 222 | 10.57 |
| | B | 208 | 9.90 |
| | C | 211 | 10.05 |
| | D | 231 | 11.00 |
| Average | | 216.67 | 10.32 |
| 7.5 | A | 208 | 9.90 |
| | B | 181 | 8.62 |
| | C | 200 | 9.52 |
| | D | 171 | 8.14 |
| Average | | 190 | 9.05 |
| 15 | A | 88 | 4.19 |
| | B | 126 | 6.00 |
| | C | 122 | 5.81 |
| | D | 124 | 5.90 |
| Average | | 115 | 5.48 |
| 30 | A | 0 | 0.00 |
| | B | 0 | 0.00 |
| | C | 0 | 0.00 |
| | D | 0 | 0.00 |
| Average | | 0 | 0.00 |