

MRID No. 438774-01

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

1. **CHEMICAL:** Ethoprop **PC Code No.:** 041101

2. **TEST MATERIAL:** Ethoprop Technical **Purity:** 96.8%

3. **CITATION:**

Author: Arthur E. Putt
Title: Ethoprop Technical - The Chronic Toxicity
to *Daphnia magna* Under Flow-Through
Conditions

Study Completion Date: October 26, 1995

Laboratory: Springborn Laboratories, Inc., Wareham,
MA

Sponsor: Rhone-Poulenc Ag Company, Research
Triangle Park, NC

Laboratory Report ID: 95-5-5857

MRID No.: 438774-01

DP Barcode: D222853 and D226794

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,
KBN Engineering and Applied Sciences, Inc.

Signature:

Date:

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
KBN Engineering and Applied Sciences, Inc.

Signature:

Date:

5. **APPROVED BY:**

Signature:

Date:

6. **STUDY PARAMETERS:**

Age of Test Organism: ≤24 hours
Definitive Test Duration: 21 days
Study Method: Flow-Through
Type of Concentrations: Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater invertebrate life-cycle test using *Daphnia magna*. Based on the most sensitive parameter (length), the MATC was between 0.80 and 2.4 ppb ai. The geometric mean MATC was 1.4 ppb ai.

Results Synopsis:

NOEC: 0.80 ppb ai **LOEC:** 2.4 ppb ai **MATC:** 1.4 ppb ai



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4. **REVIEWED BY:** William Evans, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Signature:

William Evans

Date: 2/12/97

Added

5. **APPROVED BY:** Ann Stavola, Section Chief, Review Section 5
Ecological Effects Branch
Environmental Fate and Effects Division

Signature:

Ann Stavola

Date: 2/25/97

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Results Synopsis:

NOEC: 0.80 ppb ai **LOEC:** 2.4 ppb ai **MATC:** 1.4 ppb ai

LOEC's for specific effects:

Neonates Produced: 23 ppb ai
 Daphnid Survival: 23 ppb ai
 Growth (weight): 11 ppb ai
 Growth (length): 2.4 ppb ai

8. ADEQUACY OF THE STUDY:**A. Classification:** Core**B. Rationale:** N/A**C. Repairability:** N/A**9. GUIDELINE DEVIATIONS:** Since there is no EPA's SEP for a flow-through daphnid life-cycle test, the SEP for static renewal tests was used as a general guidance in this data validation.

1. The concentration of solvent (acetone) was not the same in all treatment concentrations. The solvent control contained the maximum amount of acetone (0.036 mL/L) present in any test vessel (p.15).
2. The age of parental stock was not reported.
3. The pH (8.1 - 8.3) of the test solutions was higher than recommended (7.6 - 8.0).

10 SUBMISSION PURPOSE:**11. MATERIALS AND METHODS:****A. Test Organisms/Acclimation:**

Guideline Criteria	Reported Information
<u>Species</u> <i>Daphnia magna</i>	<i>Daphnia magna</i>
<u>Source</u>	In-house culture
<u>Parental Acclimation Conditions</u> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Daphnid cultures were maintained under similar conditions as those used during the test.
<u>Parental Acclimation Period</u> At least 21 days.	Not reported

Guideline Criteria	Reported Information
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	Not reported
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	<i>Ankistrodesmus falcatus</i> (4 x 10 ⁶ cells/mL) and <i>Seleastrum capricornutum</i> (3.5 x 10 ⁶ cells/mL)
<u>Food Concentration</u> 5 mg/l (dry wt.) of synthetic food or 10 ⁶ cells/l of algae is recommended.	2.0 ml of algal suspension (4 X 10 ⁶ cells/ml) two to three times daily
<u>Were daphnids in good health during acclimation period?</u>	Yes

B. Test System:

Guideline Criteria	Reported Information
<u>Test Water</u> Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).	Fortified well water which was filtered to remove potential organic contaminants.
<u>Water Temperature</u> 20°C ±2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.	Range: 20 ±0.5°C (daily measurements); 19-21°C (continuous measurement)
<u>pH</u> 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.	8.1-8.3
<u>Total Hardness</u> 160 to 180 mg/l as CaCO ₃ is recommended.	Mean total hardness of 170-180 mg/l as CaCO ₃

Guideline Criteria	Reported Information
<u>Dissolved Oxygen</u> <u>Renewal</u>: must not drop below 50% for more than 48 hours. <u>Flow-through</u>: $\geq$ 60% throughout test.	Mean of $\geq$95% of saturation throughout the test
<u>Test Vessels or Compartments</u> 1. <u>Material</u>: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <u>Size</u>: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.	1. Glass. 2. 1.6-liter battery jars with a fill volume of approximately 1.4 l.
<u>Covers</u> <u>Renewal</u>: Test vessels should be covered with a glass plate. <u>Flow-through</u>: openings in test compartments should be covered with mesh nylon or stainless steel screen.	Test vessels had screen-covered holes on each side.
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.	Intermittent-flow proportional diluter
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	6 volume replacements/24 hours
<u>Aeration</u> Dilution water should be vigorously aerated, but the test tanks should not be aerated.	Not reported. D.O. levels were $\geq$95% of saturation throughout the test.
<u>Photoperiod</u> 16 hours light, 8 hours dark.	16 hours light, 8 hours dark

Guideline Criteria	Reported Information
<u>Solvents</u> Not to exceed 0.5 ml/l for static tests or 0.1 ml/l for flow-through tests. Acceptable solvents are dimethyl formamide, triethylene glycol, methanol, acetone and ethanol.	Solvent: acetone Maximum conc.: 0.036 ml/l

C. Test Design:

Guideline Criteria	Reported Information
<u>Duration</u> 21 days	21 days
<u>Nominal Concentrations</u> Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.	Dilution water control, solvent control (0.036 ml acetone/l) and 5 nominal concentrations: 1.6, 3.1, 6.3, 13, and 25 µg ai/l
<u>Number of Test Organisms</u> 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.	10 daphnids/replicate; 4 replicates per treatment and control
Test organisms randomly or impartially assigned to test vessels?	Yes
<u>Renewal</u> Parent daphnids in all beakers must be transferred to containers with fresh test solution (< 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).	N/A

Guideline Criteria	Reported Information
<u>Water Parameter Measurements</u> 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	1. D.O. and pH were measured weekly in each replicate and daily in one replicate of each treatment and control group. 2. Alkalinity, hardness, and conductivity were measured on Days 0, 7, 14, and 21 in one replicate of each treatment and control group. 3. Temperature was measured continuously in one replicate vessel of the highest level, and daily in one replicate of each treatment and control group.
<u>Chemical Analysis</u> Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.	Measured in two alternate replicates of each treatment and control group on Days 0, 7, 14, and 21.

Other Applicable Information: Percent recoveries of ethoprop in treatment solutions ranged from 50 to 93%.

12. REPORTED RESULTS:

A. General Results:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Control Mortality</u> ≤ 30%	0% in dilution water control; 5% (2 out of 40) in solvent control
Did daphnids in each control produce at least 40 young after 21 days?	Yes

Guideline Criteria	Reported Information
Were any ehippia produced in any of the controls?	No
Data Endpoints - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs.	- Survival of first-generation daphnids, - Number of young produced per female, - Total number of live offspring after 21 days, - Dry weight and length of surviving first-generation daphnids, - Clinical observations.
Raw data included?	Yes

Effects Data

Toxicant Concentration (µg ai/L)		# dead daphnids/ total (%)	Mean Number Young per Female	Mean Total Length (mm)	Mean Dry Weight (mg)
Nominal	Mean Measured				
Control	--	0/40 (0%)	70	4.3	0.71
Solvent Control	--	2/40 (5%)	75	4.4	0.82
1.6	0.80	2/40 (5%)	82	4.4	0.85
3.1	2.4	0/40 (0%)	76	4.2	0.71
6.3	5.7	1/40 (3%)	85	4.3	0.75
13	11	1/40 (3%)	66	4.1 ^a	0.52 ^a
25	23	40/40 (100%)	22 ^a	--	--

^a Significantly different from the pooled controls ($p \leq 0.05$).

Toxicity Observations: Throughout the study, no offspring were observed to be immobilized in any treatment or control group.

B. Statistical Results:

Most sensitive endpoint:

Endpoint	Method	NOEC ($\mu\text{g ai/L}$)	LOEC ($\mu\text{g ai/L}$)
Length	Williams' Test	5.7	11
Weight	Williams' Test	5.7	11

13. VERIFICATION OF STATISTICAL RESULTS:Most sensitive endpoint^a:

Endpoint	Method	NOEC (ppb ai)	LOEC (ppb ai)
Length	Bonferroni T test	0.80	2.4

^aCompared to the solvent control.

- 14. REVIEWER'S COMMENTS:** The author analyzed the growth data using only the means from each replicate in the treatment and control groups. In contrast, the reviewer used data from individual daphnids in the verification of statistical results as specified in the guideline requirements for chronic aquatic toxicity studies. Using this method of analysis, there were significant differences in length of daphnids at all but the lowest concentration when compared to the solvent control. However, when the data are analyzed using the interaction between treatment concentrations and replicates (trt*rep) as the error term, the results were similar to the author's.

The data for number of offspring produced per female reproductive day were not included in the report and, therefore, could not be statistically analyzed by the reviewer. Only the raw data for the total number of offspring were included in the report. Results from the analysis were similar to the author's and are included in the attachment.

This study is scientifically sound and fulfills the guideline requirements for a daphnid life-cycle test. Based on the most sensitive endpoint (length), the NOEC and LOEC were 0.80 and 2.4 ppb ai, respectively. The geometric mean MATC was 1.4 ppb ai. This study is classified as **Core**.

----- TRT=I -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	38	4.4078947	0.2613702	3.6000000	4.8000000
WEIGHT	38	0.8360526	0.2193792	0.4200000	1.5700000

----- TRT=II -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	40	4.2225000	0.1404891	3.8000000	4.4000000
WEIGHT	40	0.7070000	0.1381415	0.4300000	1.2500000

----- TRT=III -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	39	4.2743590	0.1956339	4.0000000	4.7000000
WEIGHT	39	0.7528205	0.2235709	0.4000000	1.8600000

----- TRT=IV -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	38	4.0736842	0.1329178	3.7000000	4.4000000
WEIGHT	38	0.5315789	0.2173492	0.1200000	1.3700000

----- TRT=cont -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	40	4.2550000	0.1999359	3.8000000	4.9000000
WEIGHT	40	0.7187500	0.2027653	0.4400000	1.3200000

----- TRT=solv -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	38	4.4131579	0.1596805	4.1000000	4.7000000
WEIGHT	38	0.8076316	0.1322249	0.6200000	1.0700000

ETHOPROP TECHNICAL - DAPHNIA CHRONIC TEST
 14:40 Wednesday, July 24, 1996

General Linear Models Procedure
 Class Level Information

Class	Levels	Values
TRT	6	I II III IV cont solv
REP	4	a b c d

ETHOPROP TECHNICAL - DAPHNIA CHRONIC TEST
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General Linear Models Procedure

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	4.3586363	0.5448295	18.46	0.0001
Error	224	6.6116641	0.0295164		
Corrected Total	232	10.9703004			

R-Square	C.V.	Root MSE	LENGTH Mean
0.397312	4.019899	0.1718	4.2738

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	3.0624546	0.6124909	20.75	0.0001
REP	3	1.2961817	0.4320606	14.64	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	3.0082288	0.6016458	20.38	0.0001
REP	3	1.2961817	0.4320606	14.64	0.0001

ETHOPROP TECHNICAL - DAPHNIA CHRONIC TEST
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General Linear Models Procedure

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2.8014894	0.3501862	10.04	0.0001
Error	224	7.8154686	0.0348905		
Corrected Total	232	10.6169579			

R-Square	C.V.	Root MSE	WEIGHT Mean
0.263869	25.74508	0.1868	0.7255

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	2.1943907	0.4388781	12.58	0.0001
REP	3	0.6070987	0.2023662	5.80	0.0008

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	2.1589167	0.4317833	12.38	0.0001
REP	3	0.6070987	0.2023662	5.80	0.0008

ETHOPROP TECHNICAL - DAPHNIA CHRONIC TEST
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General Linear Models Procedure
 Least Squares Means

TRT	LENGTH LSMEAN	Pr > T i/j	HO: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5	6
I	4.41164198	1	.	0.0001	0.0006	0.0001	0.0001	0.9628	
II	4.22250000	2	0.0001	.	0.1725	0.0002	0.3985	0.0001	
III	4.27541457	3	0.0006	0.1725	.	0.0001	0.5980	0.0007	
IV	4.07743146	4	0.0001	0.0002	0.0001	.	0.0001	0.0001	
cont	4.25500000	5	0.0001	0.3985	0.5980	0.0001	.	0.0001	
solv	4.40980191	6	0.9628	0.0001	0.0007	0.0001	0.0001	.	

TRT	WEIGHT LSMEAN	Pr > T i/j	HO: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5	6
I	0.83860480	1	.	0.0021	0.0473	0.0001	0.0050	0.4232	
II	0.70700000	2	0.0021	.	0.2682	0.0001	0.7787	0.0226	
III	0.75365856	3	0.0473	0.2682	.	0.0001	0.4072	0.2367	
IV	0.53413112	4	0.0001	0.0001	0.0001	.	0.0001	0.0001	
cont	0.71875000	5	0.0050	0.7787	0.4072	0.0001	.	0.0447	
solv	0.80419690	6	0.4232	0.0226	0.2367	0.0001	0.0447	.	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

ETHOPROP TECHNICAL - DAPHNIA CHRONIC TEST
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General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: LENGTH

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 224 MSE= 0.029516
Critical Value of T= 2.96701

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
solv - I	-0.11168	0.00526	0.12221	
solv - III	0.02261	0.13880	0.25499	***
solv - cont	0.04269	0.15816	0.27363	***
solv - II	0.07519	0.19066	0.30613	***
solv - IV	0.22253	0.33947	0.45642	***
I - solv	-0.12221	-0.00526	0.11168	
I - III	0.01734	0.13354	0.24973	***
I - cont	0.03742	0.15289	0.26837	***
I - II	0.06992	0.18539	0.30087	***
I - IV	0.21727	0.33421	0.45115	***
III - solv	-0.25499	-0.13880	-0.02261	***
III - I	-0.24973	-0.13354	-0.01734	***
III - cont	-0.09535	0.01936	0.13407	
III - II	-0.06285	0.05186	0.16657	***
III - IV	0.08448	0.20067	0.31687	
cont - solv	-0.27363	-0.15816	-0.04269	***
cont - I	-0.26837	-0.15289	-0.03742	***
cont - III	-0.13407	-0.01936	0.09535	
cont - II	-0.08148	0.03250	0.14648	

cont - IV	0.06584	0.18132	0.29679	***
II - solv	-0.30613	-0.19066	-0.07519	***
II - I	-0.30087	-0.18539	-0.06992	***
II - III	-0.16657	-0.05186	0.06285	
II - cont	-0.14648	-0.03250	0.08148	
II - IV	0.03334	0.14882	0.26429	***
IV - solv	-0.45642	-0.33947	-0.22253	***
IV - I	-0.45115	-0.33421	-0.21727	***
IV - III	-0.31687	-0.20067	-0.08448	***
IV - cont	-0.29679	-0.18132	-0.06584	***
IV - II	-0.26429	-0.14882	-0.03334	***

ETHOPROP TECHNICAL - DAPHNIA CHRONIC TEST
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General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: WEIGHT

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 224 MSE= 0.03489
Critical Value of T= 2.96701

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
I - solv	-0.09872	0.02842	0.15557	
I - III	-0.04309	0.08323	0.20956	
I - cont	-0.00824	0.11730	0.24285	
I - II	0.00351	0.12905	0.25460	***
I - IV	0.17733	0.30447	0.43162	***
solv - I	-0.15557	-0.02842	0.09872	
solv - III	-0.07152	0.05481	0.18114	
solv - cont	-0.03666	0.08888	0.21443	
solv - II	-0.02491	0.10063	0.22618	
solv - IV	0.14891	0.27605	0.40320	***
III - I	-0.20956	-0.08323	0.04309	
III - solv	-0.18114	-0.05481	0.07152	
III - cont	-0.09065	0.03407	0.15879	
III - II	-0.07890	0.04582	0.17054	
III - IV	0.09492	0.22124	0.34757	***
cont - I	-0.24285	-0.11730	0.00824	
cont - solv	-0.21443	-0.08888	0.03666	
cont - III	-0.15879	-0.03407	0.09065	
cont - II	-0.11217	0.01175	0.13567	
cont - IV	0.06163	0.18717	0.31272	***
II - I	-0.25460	-0.12905	-0.00351	***
II - solv	-0.22618	-0.10063	0.02491	
II - III	-0.17054	-0.04582	0.07890	
II - cont	-0.13567	-0.01175	0.11217	
II - IV	0.04988	0.17542	0.30097	***
IV - I	-0.43162	-0.30447	-0.17733	***
IV - solv	-0.40320	-0.27605	-0.14891	***
IV - III	-0.34757	-0.22124	-0.09492	***

IV - cont	-0.31272	-0.18717	-0.06163	***
IV - II	-0.30097	-0.17542	-0.04988	***

ETHOPROP TECHNICAL - DAPHNIA CHRONIC TEST

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General Linear Models Procedure

Dunnett's One-tailed T tests for variable: LENGTH

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 224 MSE= 0.029516
Critical Value of Dunnett's T= 2.245

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
I - solv	-0.09374	-0.00526	0.08321	
III - solv	-0.22671	-0.13880	-0.05089	***
cont - solv	-0.24552	-0.15816	-0.07080	***
II - solv	-0.27802	-0.19066	-0.10330	***
IV - solv	-0.42795	-0.33947	-0.25100	***

ETHOPROP TECHNICAL - DAPHNIA CHRONIC TEST

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General Linear Models Procedure

Dunnett's One-tailed T tests for variable: WEIGHT

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 224 MSE= 0.03489
Critical Value of Dunnett's T= 2.245

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
I - solv	-0.06777	0.02842	0.12461	
III - solv	-0.15039	-0.05481	0.04076	
cont - solv	-0.18386	-0.08888	0.00610	
II - solv	-0.19561	-0.10063	-0.00565	***
IV - solv	-0.37225	-0.27605	-0.17986	***

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM

14:40 Wednesday, July 24, 1996

General Linear Models Procedure
Class Level Information

Class	Levels	Values
REP	4	a b c d
TRT	6	I II III IV cont solv

Number of observations in data set = 233

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM

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General Linear Models Procedure

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	6.7727727	0.2944684	14.66	0.0001
Error	209	4.1975278	0.0200839		
Corrected Total	232	10.9703004			
R-Square		C.V.	Root MSE	LENGTH Mean	
	0.617373	3.315946	0.1417	4.2738	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	3	1.3504075	0.4501358	22.41	0.0001
TRT	5	3.0082288	0.6016458	29.96	0.0001
REP*TRT	15	2.4141363	0.1609424	8.01	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	3	1.2579061	0.4193020	20.88	0.0001
TRT	5	3.1809702	0.6361940	31.68	0.0001
REP*TRT	15	2.4141363	0.1609424	8.01	0.0001

Tests of Hypotheses using the Type III MS for REP*TRT as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	3.1809702	0.6361940	3.95	0.0174

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM

14:40 Wednesday, July 24, 1996

General Linear Models Procedure

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	5.2265968	0.2272433	8.81	0.0001
Error	209	5.3903611	0.0257912		
Corrected Total	232	10.6169579			
R-Square		C.V.	Root MSE	WEIGHT Mean	
	0.492288	22.13485	0.1606	0.7255	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	3	0.6425727	0.2141909	8.30	0.0001
TRT	5	2.1589167	0.4317833	16.74	0.0001
REP*TRT	15	2.4251075	0.1616738	6.27	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
--------	----	-------------	-------------	---------	--------

		3	0.5517625	0.1839208	7.13	0.0001
REP		3	0.5517625	0.1839208	7.13	0.0001
TRT		5	2.3010061	0.4602012	17.84	0.0001
REP*TRT		15	2.4251075	0.1616738	6.27	0.0001

Tests of Hypotheses using the Type III MS for REP*TRT as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	2.3010061	0.4602012	2.85	0.0530

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM

14:40 Wednesday, July 24, 1996

General Linear Models Procedure
Least Squares MeansStandard Errors and Probabilities calculated using the Type III MS for
REP*TRT as an Error term

TRT	LENGTH LSMEAN	Pr > T i/j	HO: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5	6
I	4.41861111	1	0.0477	0.1307	0.0020	0.0922	0.9746		
II	4.22250000	2	0.0477	0.5901	0.1237	0.7222	0.0511		
III	4.27222222	3	0.1307	0.5901	0.0471	0.8513	0.1387		
IV	4.07416667	4	0.0020	0.1237	0.0471	0.0653	0.0021		
cont	4.25500000	5	0.0922	0.7222	0.8513	0.0653	0.0982		
solv	4.41562500	6	0.9746	0.0511	0.1387	0.0021	0.0982		

Standard Errors and Probabilities calculated using the Type III MS for
REP*TRT as an Error term

TRT	WEIGHT LSMEAN	Pr > T i/j	HO: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5	6
I	0.84291667	1	0.1567	0.3437	0.0041	0.1932	0.7620		
II	0.70700000	2	0.1567	0.6173	0.0723	0.8978	0.2579		
III	0.75319444	3	0.3437	0.6173	0.0285	0.7089	0.5158		
IV	0.53077778	4	0.0041	0.0723	0.0285	0.0570	0.0079		
cont	0.71875000	5	0.1932	0.8978	0.7089	0.0570	0.3115		
solv	0.81437500	6	0.7620	0.2579	0.5158	0.0079	0.3115		

NOTE: To ensure overall protection level, only probabilities associated
with pre-planned comparisons should be used.

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM

14:40 Wednesday, July 24, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: LENGTH

NOTE: This tests controls the type I experimentwise error for
comparisons of all treatments against a control.Alpha= 0.05 Confidence= 0.95 df= 15 MSE= 0.160942
Critical Value of Dunnett's T= 2.442

Comparisons significant at the 0.05 level are indicated by '****'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
-------------------	--	--------------------------------	--

		-0.22998	-0.00526	0.21945
I	- solv	-0.22998	-0.00526	0.21945
III	- solv	-0.36207	-0.13880	0.08447
cont	- solv	-0.38004	-0.15816	0.06373
II	- solv	-0.41254	-0.19066	0.03123
IV	- solv	-0.56419	-0.33947	-0.11476

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM

14:40 Wednesday, July 24, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: WEIGHT

NOTE: This tests controls the type I experimentwise error for
comparisons of all treatments against a control.Alpha= 0.05 Confidence= 0.95 df= 15 MSE= 0.161674
Critical Value of Dunnett's T= 2.442

Comparisons significant at the 0.05 level are indicated by '****'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
I - solv	-0.19680	0.02842	0.25364
III - solv	-0.27858	-0.05481	0.16896
cont - solv	-0.31127	-0.08888	0.13351
II - solv	-0.32302	-0.10063	0.12176
IV - solv	-0.50127	-0.27605	-0.05083

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

MAX FEKEN ETHOPROP DAPHIA MAGNA 07-17-96

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
23	40	40	100	0
11	40	1	2.5	0
5.7	40	1	2.5	0
2.4	40	0	0	0
.8	40	2	5	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 15.41236

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	2.795019E-02	14.98532	13.48423	16.93273

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
11	82.49003	329.6963	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.893339
95 PERCENT CONFIDENCE LIMITS = -23.38513 AND 29.17181

LC50 = 15.29142
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 5.565558
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

ETHOPROP TECHNICAL - TOTAL OFFSPRING

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WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	4	706.000	706.000	762.688
2	0.80	4	762.500	762.500	762.688
3	2.4	4	754.000	754.000	762.688
4	5.7	4	828.250	828.250	762.688
5	11	4	662.750	662.750	662.750
6	23	4	214.500	214.500	214.500

ETHOPROP TECHNICAL - TOTAL OFFSPRING

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WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT	762.688				
0.80	762.688	0.498		1.73	k= 1, v=18
2.4	762.688	0.498		1.82	k= 2, v=18
5.7	762.688	0.498		1.85	k= 3, v=18
11	662.750	0.380		1.86	k= 4, v=18
23	214.500	4.317	*	1.87	k= 5, v=18

s = 161.004

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

ethroprop chronic daphnid *reproduction*

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Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	10	10	8	0

Calculated Chi-Square goodness of fit test statistic = 5.5524

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

ethroprop chronic daphnid *reproduction*

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Shapiro Wilks test for normality

D = 502633.000

W = 0.903

Critical W (P = 0.05) (n = 28) = 0.924

Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

ethroprop chronic daphnid *Reproduction*

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Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 43.35

Closest, conservative, Table H statistic = 216.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 3

Actual values ==> R (# groups) = 7, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

ethroprop chronic daphnid *Reproduction*
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Bartlett's test for homogeneity of variance

Calculated B statistic = 10.63
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

```

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/lf{load for}bd
/ap{astore pop}bd
/ad{array def}bd
/P{pop pop pop pop}bd
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TITLE: ethroprop chronic daphnid *Reproduction*
 FILE: C:\TOXSTAT\ETHOPROP.
 TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent	1	618.0000	618.0000
1	solvent	2	819.0000	819.0000
1	solvent	3	738.0000	738.0000
1	solvent	4	649.0000	649.0000
2	control	1	641.0000	641.0000
2	control	2	756.0000	756.0000
2	control	3	585.0000	585.0000
2	control	4	823.0000	823.0000
3	0.80	1	649.0000	649.0000
3	0.80	2	1000.0000	1000.0000
3	0.80	3	791.0000	791.0000
3	0.80	4	610.0000	610.0000
4	2.4	1	912.0000	912.0000
4	2.4	2	638.0000	638.0000
4	2.4	3	794.0000	794.0000
4	2.4	4	717.0000	717.0000
5	5.7	1	632.0000	632.0000
5	5.7	2	693.0000	693.0000
5	5.7	3	1275.0000	1275.0000
5	5.7	4	713.0000	713.0000
6	11	1	642.0000	642.0000
6	11	2	814.0000	814.0000

6	11	3	576.0000	576.0000
6	11	4	619.0000	619.0000
7	23	1	206.0000	206.0000
7	23	2	279.0000	279.0000
7	23	3	172.0000	172.0000
7	23	4	201.0000	201.0000

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	solvent	4	618.000	819.000	706.000
2	control	4	585.000	823.000	701.250
3	0.80	4	610.000	1000.000	762.500
4	2.4	4	638.000	912.000	765.250
5	5.7	4	632.000	1275.000	828.250
6	11	4	576.000	814.000	662.750
7	23	4	172.000	279.000	214.500

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	solvent	8262.000	90.896	45.448
2	control	11654.917	107.958	53.979
3	0.80	31119.000	176.406	88.203
4	2.4	13627.583	116.737	58.369
5	5.7	89891.583	299.819	149.910
6	11	10915.583	104.478	52.239
7	23	2073.667	45.538	22.769

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1008508.857	168084.810	7.023
Within (Error)	21	502633.000	23934.905	
Total	27	1511141.857		

Critical F value = 2.57 (0.05,6,21)
 Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent	706.000	706.000		
2	control	701.250	701.250	0.043	
3	0.80	762.500	762.500	-0.516	
4	2.4	765.250	765.250	-0.542	
5	5.7	828.250	828.250	-1.118	
6	11	662.750	662.750	0.395	
7	23	214.500	214.500	4.493	*

Dunnett table value = 2.46 (1 Tailed Value, $P=0.05$, $df=20,6$)

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent	4			
2	control	4	269.114	38.1	4.750
3	0.80	4	269.114	38.1	-56.500
4	2.4	4	269.114	38.1	-59.250
5	5.7	4	269.114	38.1	-122.250
6	11	4	269.114	38.1	43.250
7	23	4	269.114	38.1	491.500

ethroprop chronic daphnid
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1008508.857	168084.810	7.023
Within (Error)	21	502633.000	23934.905	
Total	27	1511141.857		

Critical F value = 2.57 (0.05,6,21)
 Since $F > \text{Critical } F$ REJECT H_0 :All groups equal

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 H_0 :Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent	706.000	706.000		
2	control	701.250	701.250	0.043	
3	0.80	762.500	762.500	-0.516	
4	2.4	765.250	765.250	-0.542	
5	5.7	828.250	828.250	-1.118	
6	11	662.750	662.750	0.395	
7	23	214.500	214.500	4.493	*

Bonferroni T table value = 2.60 (1 Tailed Value, $P=0.05$, $df=21,6$)

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2 H_0 :Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent	4			
2	control	4	284.648	40.3	4.750
3	0.80	4	284.648	40.3	-56.500
4	2.4	4	284.648	40.3	-59.250
5	5.7	4	284.648	40.3	-122.250
6	11	4	284.648	40.3	43.250
7	23	4	284.648	40.3	491.500

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1008508.857	168084.810	7.023
Within (Error)	21	502633.000	23934.905	
Total	27	1511141.857		

Critical F value = 2.57 (0.05,6,21)
 Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP							
				0	0	0	0	0	0	0	0
				7	6	2	1	3	4	5	
7	23	214.500	214.500	\							
6	11	662.750	662.750	*	\						
2	control	701.250	701.250	*	.	\					
1	solvent	706.000	706.000	*	.	.	\				
3	0.80	762.500	762.500	*	.	.	.	\			
4	2.4	765.250	765.250	*	.	.	.	.	\		
5	5.7	828.250	828.250	*	.	.	.	.	.	\	

* = significant difference (p=0.05)
 Tukey value (7,21) = 4.62

. = no significant difference
 s = 23934.905

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent	4	706.000	706.000	752.650
2	control	4	701.250	701.250	752.650
3	0.80	4	762.500	762.500	752.650
4	2.4	4	765.250	765.250	752.650
5	5.7	4	828.250	828.250	752.650
6	11	4	662.750	662.750	662.750
7	23	4	214.500	214.500	214.500

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. 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
solvent	752.650				
control	752.650	0.426		1.72	k= 1, v=21
0.80	752.650	0.426		1.80	k= 2, v=21
2.4	752.650	0.426		1.83	k= 3, v=21
5.7	752.650	0.426		1.84	k= 4, v=21
11	662.750	0.395		1.85	k= 5, v=21

23 214.500 4.493 * 1.85 k= 6, v=21

s = 154.709

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

ethroprop chronic daphnid *Reproduction*
File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	solvent	706.000				
2	control	701.250	18.00	10.00	4.00	
3	0.80	762.500	18.50	10.00	4.00	
4	2.4	765.250	20.00	10.00	4.00	
5	5.7	828.250	19.00	10.00	4.00	
6	11	662.750	15.00	10.00	4.00	
7	23	214.500	10.00	10.00	4.00	*

Critical values use k = 6, are 1 tailed, and alpha = 0.05

ethroprop chronic daphnid *Reproduction*
File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	solvent	706.000				
2	control	701.250	18.00	None	4	
3	0.80	762.500	18.50	None	4	
4	2.4	765.250	20.00	None	4	
5	5.7	828.250	19.00	None	4	
6	11	662.750	15.00	None	4	
7	23	214.500	10.00	None	4	

Critical values use k = 6, are 1 tailed, and alpha = 0.05

ethroprop chronic daphnid *Reproduction*
File: C:\TOXSTAT\ETHOPROP. Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	solvent	706.000	706.000	65.500
2	control	701.250	701.250	63.000
3	0.80	762.500	762.500	69.500
4	2.4	765.250	765.250	77.000

5	5.7	828.250	828.250	71.000
6	11	662.750	662.750	50.000
7	23	214.500	214.500	10.000

Calculated H Value = 11.499 Critical H Value Table = 12.590
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

ethroprop chronic daphnid *Reproduction*
 File: C:\TOXSTAT\ETHOPROP. 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	0	0
				7	6	2	1	3	4	5	
7	23	214.500	214.500	\							
6	11	662.750	662.750	.	\						
2	control	701.250	701.250	.	.	\					
1	solvent	706.000	706.000	.	.	.	\				
3	0.80	762.500	762.500	.	.	.	.	\			
4	2.4	765.250	765.250	.	.	.	.	.	\		
5	5.7	828.250	828.250	.	.	.	.	.	.	\	

* = significant difference (p=0.05)
 Table q value (0.05,7) = 3.038

. = no significant difference
 SE = 5.816