

US EPA ARCHIVE DOCUMENT

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

1. CHEMICAL: PIRATE™; AC 303,630 PC Code No.: 129093

2. TEST MATERIAL: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile

Purity: 94.5%

3. CITATION

Authors: Davis, Jay W. and Wisk, Joseph D.
Title: Chronic Toxicity of ¹⁴C-AC 303,630 During the Complete Life-Cycle of *Daphnia magna* Under Flow-Through Test Conditions

Study Completion Date: September 28, 1994

Laboratory: Toxikon Environmental Sciences, 106 Coastal Way, Jupiter, Florida 33477

Sponsor: American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ 08543-0400

Laboratory Report ID: J9201014

MRID No.: 434928-22

DP Barcode: D210808

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

Signature: *William Evans* Date: 6/21/96

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

Signature: *Ann Stavola* Date: 10/8/96

6. STUDY PARAMETERS

Age of Test Organism: <24 hours old at test initiation

Definitive Test Duration: 21 days

Study Method: Flow-through

Type of Concentrations: Mean measured

7. CONCLUSIONS: Although the dissolved oxygen (DO) was between 34% to 48% saturation on day 7 in all test concentrations, DO levels ranged from 42% to 72% saturation on days 14 and 21. However, true toxicological effects based on survival, reproduction, weight, and length first appeared at the 7.7

$\mu\text{g/L}$ level on day 14 of the study. It is therefore likely that effects observed in this study are due to test substance rather than the borderline DO levels. This study has therefore been classified as Core with a calculated MATC of $5.24 \mu\text{g/L}$ based on the survival, reproduction, weight, and length of *Daphnia magna*. The NOEC was $3.57 \mu\text{g/L}$.

Results Synopsis

NOEC: $3.57 \mu\text{g ai/L}$ LOEC: $7.7 \mu\text{g ai/L}$

LOEC's for specific effects

Egg Production: $7.7 \mu\text{g ai/L}$ Larvae Survival: $7.7 \mu\text{g ai/L}$ Growth (length or weight): $7.7 \mu\text{g ai/L}$

8. ADEQUACY OF THE STUDY

- A. Classification: Core
- B. Rationale: See 7. above.
- C. Repairability: N/A

9. GUIDELINE DEVIATIONS

1. Parental acclimation period was not mentioned. Guidelines recommend at least 21 days.
2. There was no mention of the age of parental stock at the beginning of the acclimation period. Guidelines recommend at least 10 - 12 days.
3. The recommended pH is 7.6 to 8.0 and must not deviate by more than one unit for more than 48 hours. The pH ranged from 7.2 to 7.8 throughout the test.
4. Hardness of 160 to 180 mg/L as CaCO_3 is recommended. Hardness was measured as 90 - 100 mg/L as CaCO_3 .
5. Dissolved Oxygen measured in a flow-through test must be $\geq 60\%$ throughout the test. D.O. measurements ranged from 34% to 95% during the study.
6. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. Alkalinity, hardness, and conductance were measured only in the control at test initiation and termination.
7. Water 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. Water temperature was monitored hourly in the dilution control and water bath temperature was monitored continuously.

10 **SUBMISSION PURPOSE:** Data requirement for the registration for use on cotton.

11. **MATERIALS AND METHODS**

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
<u>Species</u> <i>Daphnia magna</i>	The species tested was <i>Daphnia magna</i>
<u>Source</u>	Toxikon Environmental Sciences. Cultures originally received from U.S. EPA, Duluth, Minnesota in October 1989.
<u>Parental Acclimation Conditions</u> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Parental stock isolated and maintained prior to testing, then re-isolated in dilution water.
<u>Parental Acclimation Period</u> At least 21 days.	Not mentioned.
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	There was no mentioned of the age of parental stock at the beginning of the acclimation period.
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Green alga and mixture of cereal leaves.
<u>Food Concentration</u> 5 mg/L (dry wt.) of synthetic food or 10^8 cells/L of algae is recommended.	5 g/L dried cereal leaves. Also 2×10^8 cells/mL. (2×10^5 cells/mL)

Guideline Criteria	Reported Information
Were daphnids in good health during acclimation period?	Not reported. Data was not provided concerning the acclimation period.

B. Test System

Guideline Criteria	Reported Information
<p>Test Water Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).</p>	Jupiter, Florida town water aerated, filtered, and re-aerated by an air stone.
<p>Water Temperature 20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.</p>	Target: 20°C Range: 20.2 to 21.2°C
<p>pH 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.</p>	7.2 to 7.8
<p>Total Hardness 160 to 180 mg/L as CaCO₃ is recommended.</p>	Hardness was measured as 90 - 100 mg/L as CaCO ₃ .
<p>Dissolved Oxygen <u>Renewal</u>: must not drop below 50% for more than 48 hours. <u>Flow-through</u>: ≥ 60% throughout test.</p>	DO levels remained > 50% saturation on all weekly measurement days other than day 7. DO levels ranged from 34% to 48% saturation on day 7 in all test concentrations. Control replicates were all ≥ 89% saturation.
<p>Test Vessels or Compartments 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.</p>	Glass test vessels measured 5.1cm x 7.0 cm x 10 cm and each test vessel contained 300 mL test solution.

Guideline Criteria	Reported Information
<p><u>Covers</u> Renewal: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.</p>	<p>Test compartments were covered with 355 μm mesh Nitex screening.</p>
<p><u>Type of Dilution System</u> Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	<p>Intermittent flow proportional vacuum-siphon diluter was used</p>
<p><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.</p>	<p>21 volume additions per replicate were provided every 24 hours.</p>
<p><u>Aeration</u> Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	<p>Dilution water was aerated.</p>
<p><u>Photoperiod</u> 16 hours light, 8 hours dark.</p>	<p>16 hours light, 8 hours dark.</p>
<p><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 dimethylformamide, triethylene glycol, methanol, acetone and ethanol.</p>	<p>Solvent: Dimethylformamide (DMF) Maximum conc.: 0.024 mL of DMF per L.</p>

C. Test Design

Guideline Criteria	Reported Information
<p><u>Duration</u> 21 days</p>	<p>21 days</p>

Guideline Criteria	Reported Information
<p><u>Nominal Concentrations</u> Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.</p>	<p>A dilution water and solvent control and 6 test concentrations each 50% greater than the last.</p>
<p><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.</p>	<p>40 daphnids/treatment level. 10 daphnids distributed to each of four replicates.</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Yes</p>
<p><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).</p>	<p>N/A</p>
<p><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.</p>	<p>1&2. Dissolved oxygen and pH measured on days 0, 7, 14, and 21. Alkalinity, hardness, and conductivity were measured the control at test initiation and termination. 3. Water temperature was monitored hourly in the dilution control. Water bath temperature was monitored continuously.</p>
<p><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.</p>	<p>Measured concentration of test substance was performed on days 0, 6, 14, and 21. Measurements done as ¹⁴C- AC 303,630 equivalents in test solutions.</p>

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%	Control mortality ≤ 30%
Did daphnids in each control produce at least 40 young after 21 days?	Yes
Were no ephippia produced in any of the controls?	Yes
<u>Data Endpoints</u> - 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 adult female, - Dry weight and length of each first generation daphnid alive at the end of the test, - No other observations noted.
Raw data included?	Yes

Effects Data

Toxicant Concentration (µg/L)		No. (%) Dead or Immobile (21 Days)	Young per Female per Repro. Day	Total Length (mm)	Dry Weight (mg)
Nominal	Measured				
Control	<0.089	5 (12.5%)	5.5	4.09	0.75
Solvent Control	<0.089	4 (10%)	9.1	4.30	0.92
0.33	0.278	5 (12.5%)	9.2	4.43	0.93
0.65	0.448	4 (10%)	8.1	4.39	0.86

Toxicant Concentration (µg/L)		No. (%) Dead or Immobile (21 Days)	Young per Female per Repro. Day	Total Length (mm)	Dry Weight (mg)
Nominal	Measured				
1.3	0.987	3 (7.5%)	9.0	4.39	0.95
2.5	1.88	4 (10%)	8.4	4.33	0.94
5.00	3.57	4 (10%)	7.6	4.21	0.91
10.0	7.7	24 (60%)	4.5	3.85	0.56

Toxicity Observations: No abnormalities in behavior or physical appearance were noted in the report.

B. Statistical Results

Most sensitive endpoint: Survival, Reproduction, and Length

Endpoint	Method	NOEC	LOEC	MATC
Survival	ANOVA, t-Test, Dunnett	3.57µg/L	7.7µg/L	5.24µg/L
Reproduction	ANOVA, t-Test, Dunnett	3.57µg/L	7.7µg/L	5.24µg/L
Weight	ANOVA, t-Test, Dunnett	Not observed	Not observed	Not observed
Length	ANOVA, t-Test, Dunnett	3.57µg/L	7.7µg/L	5.24µg/L

13. VERIFICATION OF STATISTICAL RESULTS

Most sensitive endpoint: Survival, reproduction, weight, and length.

Endpoint	Method	NOEC	LOEC	MATC
Survival	ANOVA (Dunnetts, Williams)	3.57µg/L	7.7µg/L	5.24µg/L
Reproduction	ANOVA (Dunnetts, Williams)	3.57µg/L	7.7µg/L	5.24µg/L

Endpoint	Method	NOEC	LOEC	MATC
Weight	ANOVA (Dunnetts, Williams)	3.57 μ g/L	7.7 μ g/L	5.24 μ g/L
Length	ANOVA (Dunnetts, Williams)	3.57 μ g/L	7.7 μ g/L	5.24 μ g/L

14. **REVIEWER'S COMMENTS:** Dissolved Oxygen measured in a flow-through test must be \geq 60% throughout the test. D.O. measurements ranged from 34% to 48% saturation on day 7 of the study. Although the dissolved oxygen (DO) was between 34% to 48% saturation on day 7 in all test concentrations, DO levels ranged from 42% to 72% saturation on days 14 and 21. However, true toxicological effects based on survival, reproduction, weight, and length first appeared at the 7.7 μ g/L level on day 14 of the study. It is therefore likely that effects observed in this study are due to test substance rather than the borderline DO levels. This study has therefore been classified as Core with a calculated MATC of 5.24 μ g/L based on the survival, reproduction, weight, and length of *Daphnia magna*. The NOEC was 3.57 μ g/L.

TITLE: pirate Daphnia chronic length
 FILE: piratelgn
 TRANSFORM: NO TRANSFORM

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	9.0000	9.0000
1	solvent control	2	10.1000	10.1000
1	solvent control	3	8.2000	8.2000
1	solvent control	4	8.9000	8.9000
2	0.278	1	9.7000	9.7000
2	0.278	2	9.4000	9.4000
2	0.278	3	8.3000	8.3000
2	0.278	4	9.4000	9.4000
3	0.448	1	8.3000	8.3000
3	0.448	2	7.1000	7.1000
3	0.448	3	8.5000	8.5000
3	0.448	4	8.3000	8.3000
4	0.987	1	8.5000	8.5000
4	0.987	2	9.7000	9.7000
4	0.987	3	8.5000	8.5000
4	0.987	4	9.6000	9.6000
5	1.88	1	8.5000	8.5000
5	1.88	2	8.5000	8.5000
5	1.88	3	7.6000	7.6000
5	1.88	4	9.2000	9.2000
6	3.57	1	7.9000	7.9000
6	3.57	2	7.6000	7.6000
6	3.57	3	7.4000	7.4000
6	3.57	4	7.5000	7.5000
7	7.7	1	4.3000	4.3000
7	7.7	2	5.0000	5.0000
7	7.7	3	3.6000	3.6000
7	7.7	4	8.0000	8.0000

pirate Daphnia chronic length
 File: piratelgn Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	solvent control	4	8.200	10.100	9.050
2	0.278	4	8.300	9.700	9.200
3	0.448	4	7.100	8.500	8.050
4	0.987	4	8.500	9.700	9.075
5	1.88	4	7.600	9.200	8.450
6	3.57	4	7.400	7.900	7.600
7	7.7	4	3.600	8.000	5.225

pirate Daphnia chronic length
 File: piratelgn Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	solvent control	0.617	0.785	0.393
2	0.278	0.380	0.616	0.308
3	0.448	0.410	0.640	0.320
4	0.987	0.443	0.665	0.333
5	1.88	0.430	0.656	0.328
6	3.57	0.047	0.216	0.108
7	7.7	3.749	1.936	0.968

pirate Daphnia chronic length
 File: piratelgn Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	46.814	7.802	8.988
Within (Error)	21	18.225	0.868	
Total	27	65.039		

Critical F value = 2.57 (0.05,6,21)
 Since F > Critical F REJECT Ho:All groups equal

pirate Daphnia chronic length
 File: piratelgn 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	solvent control	9.050	9.050		
2	0.278	9.200	9.200	-0.228	
3	0.448	8.050	8.050	1.518	
4	0.987	9.075	9.075	-0.038	
5	1.88	8.450	8.450	0.911	
6	3.57	7.600	7.600	2.201	
7	7.7	5.225	5.225	5.806	*

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

pirate Daphnia chronic length

11

File: piratelgn 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	solvent control	4			
2	0.278	4	1.621	17.9	-0.150
3	0.448	4	1.621	17.9	1.000
4	0.987	4	1.621	17.9	-0.025
5	1.88	4	1.621	17.9	0.600
6	3.57	4	1.621	17.9	1.450
7	7.7	4	1.621	17.9	3.825

pirate Daphnia chronic length
File: piratelgn Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	46.814	7.802	8.988
Within (Error)	21	18.225	0.868	
Total	27	65.039		

Critical F value = 2.57 (0.05,6,21)
Since F > Critical F REJECT Ho:All groups equal

pirate Daphnia chronic length
File: piratelgn 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	solvent control	9.050	9.050		
2	0.278	9.200	9.200	-0.228	
3	0.448	8.050	8.050	1.518	
4	0.987	9.075	9.075	-0.038	
5	1.88	8.450	8.450	0.911	
6	3.57	7.600	7.600	2.201	
7	7.7	5.225	5.225	5.806	*

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

pirate Daphnia chronic length
File: piratelgn 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	solvent control	4			
2	0.278	4	1.714	18.9	-0.150
3	0.448	4	1.714	18.9	1.000
4	0.987	4	1.714	18.9	-0.025
5	1.88	4	1.714	18.9	0.600
6	3.57	4	1.714	18.9	1.450
7	7.7	4	1.714	18.9	3.825

pirate Daphnia chronic length

File: piratelgn

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WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	4	9.050	9.050	9.125
2	0.278	4	9.200	9.200	9.125
3	0.448	4	8.050	8.050	8.563
4	0.987	4	9.075	9.075	8.563
5	1.88	4	8.450	8.450	8.450
6	3.57	4	7.600	7.600	7.600
7	7.7	4	5.225	5.225	5.225

pirate Daphnia chronic length

File: piratelgn

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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 control	9.125				
0.278	9.125	0.114		1.72	k= 1, v=21
0.448	8.563	0.740		1.80	k= 2, v=21
0.987	8.563	0.740		1.83	k= 3, v=21
1.88	8.450	0.911		1.84	k= 4, v=21
3.57	7.600	2.201	*	1.85	k= 5, v=21
7.7	5.225	5.807	*	1.85	k= 6, v=21

s = 0.932

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

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1	solvent control	2	10.1000	10.1000
1	solvent control	3	8.2000	8.2000
1	solvent control	4	8.9000	8.9000
2	0.278	1	9.7000	9.7000
2	0.278	2	9.4000	9.4000
2	0.278	3	8.3000	8.3000
2	0.278	4	9.4000	9.4000
3	0.448	1	8.3000	8.3000
3	0.448	2	7.1000	7.1000
3	0.448	3	8.5000	8.5000
3	0.448	4	8.3000	8.3000
4	0.987	1	8.5000	8.5000
4	0.987	2	9.7000	9.7000
4	0.987	3	8.5000	8.5000
4	0.987	4	9.6000	9.6000
5	1.88	1	8.5000	8.5000
5	1.88	2	8.5000	8.5000
5	1.88	3	7.6000	7.6000
5	1.88	4	9.2000	9.2000
6	3.57	1	7.9000	7.9000
6	3.57	2	7.6000	7.6000
6	3.57	3	7.4000	7.4000
6	3.57	4	7.5000	7.5000
7	7.7	1	4.3000	4.3000
7	7.7	2	5.0000	5.0000
7	7.7	3	3.6000	3.6000
7	7.7	4	8.0000	8.0000

pirate Daphnia chronic length
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

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5	1.88	4	7.600	9.200	8.450
6	3.57	4	7.400	7.900	7.600
7	7.7	4	3.600	8.000	5.225

pirate Daphnia chronic length
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
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4	0.987	0.443	0.665	0.333
5	1.88	0.430	0.656	0.328
6	3.57	0.047	0.216	0.108
7	7.7	3.749	1.936	0.968

pirate Daphnia chronic length
 File: piratelgn Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	46.814	7.802	8.988
Within (Error)	21	18.225	0.868	
Total	27	65.039		

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

pirate Daphnia chronic length
 File: piratelgn 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
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2	0.278	9.200	9.200	-0.228	
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5	1.88	8.450	8.450	0.911	
6	3.57	7.600	7.600	2.201	
7	7.7	5.225	5.225	5.806	*

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

pirate Daphnia chronic length

File: piratelgn 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	solvent control	4			
2	0.278	4	1.621	17.9	-0.150
3	0.448	4	1.621	17.9	1.000
4	0.987	4	1.621	17.9	-0.025
5	1.88	4	1.621	17.9	0.600
6	3.57	4	1.621	17.9	1.450
7	7.7	4	1.621	17.9	3.825

pirate Daphnia chronic length
File: piratelgn Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	46.814	7.802	8.988
Within (Error)	21	18.225	0.868	
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Critical F value = 2.57 (0.05, 6, 21)
Since F > Critical F REJECT Ho: All groups equal

pirate Daphnia chronic length
File: piratelgn 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	solvent control	9.050	9.050		
2	0.278	9.200	9.200	-0.228	
3	0.448	8.050	8.050	1.518	
4	0.987	9.075	9.075	-0.038	
5	1.88	8.450	8.450	0.911	
6	3.57	7.600	7.600	2.201	
7	7.7	5.225	5.225	5.806	*

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

pirate Daphnia chronic length
File: piratelgn Transform: NO TRANSFORM

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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	solvent control	4			
2	0.278	4	1.714	18.9	-0.150
3	0.448	4	1.714	18.9	1.000
4	0.987	4	1.714	18.9	-0.025
5	1.88	4	1.714	18.9	0.600
6	3.57	4	1.714	18.9	1.450
7	7.7	4	1.714	18.9	3.825

pirate Daphnia chronic length

File: piratelgn

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	4	9.050	9.050	9.125
2	0.278	4	9.200	9.200	9.125
3	0.448	4	8.050	8.050	8.563
4	0.987	4	9.075	9.075	8.563
5	1.88	4	8.450	8.450	8.450
6	3.57	4	7.600	7.600	7.600
7	7.7	4	5.225	5.225	5.225

pirate Daphnia chronic length

File: piratelgn

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 control	9.125				
0.278	9.125	0.114		1.72	k= 1, v=21
0.448	8.563	0.740		1.80	k= 2, v=21
0.987	8.563	0.740		1.83	k= 3, v=21
1.88	8.450	0.911		1.84	k= 4, v=21
3.57	7.600	2.201	*	1.85	k= 5, v=21
7.7	5.225	5.807	*	1.85	k= 6, v=21

s = 0.932

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

TITLE: pirate Daphnia chronic length
 FILE: C:\TOXSTAT\PIRATELG.
 TRANSFORM: NO TRANSFORM

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	4.1700	4.1700
1	solvent control	2	4.4500	4.4500
1	solvent control	3	4.2400	4.2400
1	solvent control	4	4.3200	4.3200
2	0.278	1	4.3900	4.3900
2	0.278	2	4.4200	4.4200
2	0.278	3	4.4600	4.4600
2	0.278	4	4.4800	4.4800
3	0.448	1	4.3600	4.3600
3	0.448	2	4.4000	4.4000
3	0.448	3	4.3800	4.3800
3	0.448	4	4.4200	4.4200
4	0.987	1	4.3700	4.3700
4	0.987	2	4.3900	4.3900
4	0.987	3	4.5000	4.5000
4	0.987	4	4.2800	4.2800
5	1.88	1	4.3500	4.3500
5	1.88	2	4.2400	4.2400
5	1.88	3	4.4000	4.4000
5	1.88	4	4.3500	4.3500
6	3.57	1	4.1700	4.1700
6	3.57	2	4.2800	4.2800
6	3.57	3	4.1900	4.1900
6	3.57	4	4.2200	4.2200
7	7.7	1	3.9200	3.9200
7	7.7	2	3.8700	3.8700
7	7.7	3	3.7200	3.7200
7	7.7	4	4.2400	4.2400

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	solvent control	4	4.170	4.450	4.295
2	0.278	4	4.390	4.480	4.438
3	0.448	4	4.360	4.420	4.390
4	0.987	4	4.280	4.500	4.385
5	1.88	4	4.240	4.400	4.335
6	3.57	4	4.170	4.280	4.215
7	7.7	4	3.720	4.240	3.938

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	solvent control	0.014	0.120	0.060
2	0.278	0.002	0.040	0.020
3	0.448	0.001	0.026	0.013
4	0.987	0.008	0.090	0.045
5	1.88	0.005	0.068	0.034
6	3.57	0.002	0.048	0.024
7	7.7	0.048	0.219	0.109

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.690	0.115	10.455
Within (Error)	21	0.239	0.011	
Total	27	0.929		

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

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

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 control	4.295	4.295		
2	0.278	4.438	4.438	-1.921	
3	0.448	4.390	4.390	-1.281	
4	0.987	4.385	4.385	-1.214	
5	1.88	4.335	4.335	-0.539	
6	3.57	4.215	4.215	1.079	
7	7.7	3.938	3.938	4.821	*

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

pirate Daphnia chronic length
File: C:\TOXSTAT\PIRATELG.

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	solvent control	4			
2	0.278	4	0.182	4.2	-0.143
3	0.448	4	0.182	4.2	-0.095
4	0.987	4	0.182	4.2	-0.090
5	1.88	4	0.182	4.2	-0.040
6	3.57	4	0.182	4.2	0.080
7	7.7	4	0.182	4.2	0.357

pirate Daphnia chronic length
File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.690	0.115	10.455
Within (Error)	21	0.239	0.011	
Total	27	0.929		

Critical F value = 2.57 (0.05,6,21)
Since F > Critical F REJECT Ho:All groups equal

pirate Daphnia chronic length
File: C:\TOXSTAT\PIRATELG.

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	solvent control	4.295	4.295		
2	0.278	4.438	4.438	-1.921	
3	0.448	4.390	4.390	-1.281	
4	0.987	4.385	4.385	-1.214	
5	1.88	4.335	4.335	-0.539	
6	3.57	4.215	4.215	1.079	
7	7.7	3.938	3.938	4.821	*

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

pirate Daphnia chronic length

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File: C:\TOXSTAT\PIRATELG.

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	solvent control	4			
2	0.278	4			
3	0.448	4	0.193	4.5	-0.143
4	0.987	4	0.193	4.5	-0.095
5	1.88	4	0.193	4.5	-0.090
6	3.57	4	0.193	4.5	-0.040
7	7.7	4	0.193	4.5	0.080
					0.357

pirate Daphnia chronic length

File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	4	4.295	4.295	4.377
2	0.278	4	4.438	4.438	4.377
3	0.448	4	4.390	4.390	4.377
4	0.987	4	4.385	4.385	4.377
5	1.88	4	4.335	4.335	4.335
6	3.57	4	4.215	4.215	4.215
7	7.7	4	3.938	3.938	3.938

pirate Daphnia chronic length

File: C:\TOXSTAT\PIRATELG.

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 control	4.377				
0.278	4.377	1.085		1.72	k= 1, v=21
0.448	4.377	1.085		1.80	k= 2, v=21
0.987	4.377	1.085		1.83	k= 3, v=21
1.88	4.335	0.530		1.84	k= 4, v=21
3.57	4.215	1.061		1.85	k= 5, v=21
7.7	3.938	4.739	*	1.85	k= 6, v=21

s = 0.107

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

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TITLE: pirate Daphnia chronic length
 FILE: C:\TOXSTAT\PIRATELG.
 TRANSFORM: NO TRANSFORM

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	4.1700	4.1700
1	solvent control	2	4.4500	4.4500
1	solvent control	3	4.2400	4.2400
1	solvent control	4	4.3200	4.3200
2	0.278	1	4.3900	4.3900
2	0.278	2	4.4200	4.4200
2	0.278	3	4.4600	4.4600
2	0.278	4	4.4800	4.4800
3	0.448	1	4.3600	4.3600
3	0.448	2	4.4000	4.4000
3	0.448	3	4.3800	4.3800
3	0.448	4	4.4200	4.4200
4	0.987	1	4.3700	4.3700
4	0.987	2	4.3900	4.3900
4	0.987	3	4.5000	4.5000
4	0.987	4	4.2800	4.2800
5	1.88	1	4.3500	4.3500
5	1.88	2	4.2400	4.2400
5	1.88	3	4.4000	4.4000
5	1.88	4	4.3500	4.3500
6	3.57	1	4.1700	4.1700
6	3.57	2	4.2800	4.2800
6	3.57	3	4.1900	4.1900
6	3.57	4	4.2200	4.2200
7	7.7	1	3.9200	3.9200
7	7.7	2	3.8700	3.8700
7	7.7	3	3.7200	3.7200
7	7.7	4	4.2400	4.2400

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	solvent control	4	4.170	4.450	4.295
2	0.278	4	4.390	4.480	4.438
3	0.448	4	4.360	4.420	4.390
4	0.987	4	4.280	4.500	4.385
5	1.88	4	4.240	4.400	4.335
6	3.57	4	4.170	4.280	4.215
7	7.7	4	3.720	4.240	3.938

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	solvent control	0.014	0.120	0.060
2	0.278	0.002	0.040	0.020
3	0.448	0.001	0.026	0.013
4	0.987	0.008	0.090	0.045
5	1.88	0.005	0.068	0.034
6	3.57	0.002	0.048	0.024
7	7.7	0.048	0.219	0.109

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.690	0.115	10.455
Within (Error)	21	0.239	0.011	
Total	27	0.929		

Critical F value = 2.57 (0.05, 6, 21)
 Since F > Critical F REJECT Ho: All groups equal

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

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	solvent control	4.295	4.295		
2	0.278	4.438	4.438	-1.921	
3	0.448	4.390	4.390	-1.281	
4	0.987	4.385	4.385	-1.214	
5	1.88	4.335	4.335	-0.539	
6	3.57	4.215	4.215	1.079	
7	7.7	3.938	3.938	4.821	*

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

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pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

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	solvent control	4			
2	0.278	4	0.182	4.2	-0.143
3	0.448	4	0.182	4.2	-0.095
4	0.987	4	0.182	4.2	-0.090
5	1.88	4	0.182	4.2	-0.040
6	3.57	4	0.182	4.2	0.080
7	7.7	4	0.182	4.2	0.357

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.690	0.115	10.455
Within (Error)	21	0.239	0.011	
Total	27	0.929		

Critical F value = 2.57 (0.05, 6, 21)
 Since F > Critical F REJECT Ho: All groups equal

pirate Daphnia chronic length
 File: C:\TOXSTAT\PIRATELG.

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	solvent control	4.295	4.295		
2	0.278	4.438	4.438	-1.921	
3	0.448	4.390	4.390	-1.281	
4	0.987	4.385	4.385	-1.214	
5	1.88	4.335	4.335	-0.539	
6	3.57	4.215	4.215	1.079	
7	7.7	3.938	3.938	4.821	*

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

pirate Daphnia chronic length

24

File: C:\TOXSTAT\PIRATELG.

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	solvent control	4			
2	0.278	4	0.193	4.5	-0.143
3	0.448	4	0.193	4.5	-0.095
4	0.987	4	0.193	4.5	-0.090
5	1.88	4	0.193	4.5	-0.040
6	3.57	4	0.193	4.5	0.080
7	7.7	4	0.193	4.5	0.357

pirate Daphnia chronic length

File: C:\TOXSTAT\PIRATELG.

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	4	4.295	4.295	4.377
2	0.278	4	4.438	4.438	4.377
3	0.448	4	4.390	4.390	4.377
4	0.987	4	4.385	4.385	4.377
5	1.88	4	4.335	4.335	4.335
6	3.57	4	4.215	4.215	4.215
7	7.7	4	3.938	3.938	3.938

pirate Daphnia chronic length

File: C:\TOXSTAT\PIRATELG.

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 control	4.377				
0.278	4.377	1.085		1.72	k= 1, v=21
0.448	4.377	1.085		1.80	k= 2, v=21
0.987	4.377	1.085		1.83	k= 3, v=21
1.88	4.335	0.530		1.84	k= 4, v=21
3.57	4.215	1.061		1.85	k= 5, v=21
7.7	3.938	4.739	*	1.85	k= 6, v=21

S = 0.107

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

25