

3-7-94

MRID No. 429053-01

DATA EVALUATION RECORD

- 1. **CHEMICAL:** MAT 7484.
Shaughnessey No. 129086.
- 2. **TEST MATERIAL:** ¹⁴C-MAT 7484 ([Ring-U-¹⁴C] O-[2-(1,1-dimethylethyl)-5-pyrimidinyl] O-ethyl O-(1-methylethyl) phosphorothioate); CAS No. 96182-53-5; Vial No. C-470; 97.7% active ingredient.
- 3. **STUDY TYPE:** 72-4. *Daphnia magna* Life-Cycle (21-day Renewal) Toxicity Test. Species Tested: Water Flea (*Daphnia magna*).
- 4. **CITATION:** Gagliano, G.G. 1993. Chronic Toxicity of ¹⁴C-MAT 7484 to the Waterflea (*Daphnia magna*) Under Static Renewal Conditions. Miles Report No. 106217. Prepared by Environmental Research Section, Miles Incorporated, Stilwell, KS. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 429053-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Date:

11/12/93

6. **APPROVED BY:**

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

Signature:

Date:

11/12/93

~~Henry T. Craven, M.S.~~
Supervisor, EEB/EFED
USEPA

Signature:

William Robert 2/9/94

Date:

3 7 94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a chronic toxicity study using *Daphnia magna*. The MATC, based on the most sensitive biological parameter, adult daphnid survival, was >11.0 ng ai/l and <19.3 ng ai/l, based on mean measured concentrations. The geometric mean MATC was 14.6 ng ai/l.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

36

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

- A. Test Animals:** First instar *Daphnia magna* (<24 hours old) used in this test were obtained from in-house cultures. The adults used as the source of the test daphnids were maintained under test conditions. They were fed algae (*Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*) and a trout chow, yeast, and cereal leaf suspension three times per week.
- B. Test System:** The test chambers were 250- or 1000-ml glass beakers containing approximately 200 or 900 ml of test solution, respectively. The beakers were randomly positioned in a water bath (20 ±1°C) under a 16-hour light/8-hour dark photoperiod. The intensity of the cool-white and Agro-lite fluorescent tubes was 50-55 footcandles. Thirty-minute transition periods between light and dark were used.

The dilution water was hard-blended water produced by mixing spring water with treated city water for a final hardness of 160-180 mg/l as CaCO₃. The water was screened weekly for residual chlorine and was aerated and UV sterilized prior to use. A chemical characterization of the water is presented in Table 1 (attached).

The test substance was dissolved in acetone. Aliquots of this solution were diluted in dilution water to prepare the test solutions. The test solutions were prepared with radiolabeled material only.

- C. Dosage:** Twenty-one-day, static-renewal, life-cycle test. Based on a preliminary test, five nominal concentrations [3, 6, 13, 25, and 50 ng active ingredient (ai)/l], a dilution water control, and a solvent control (0.5 ml acetone/l) were selected for the test.
- D. Design:** First instar daphnids were impartially selected and distributed to each of 10 test beakers per concentration. Seven 250-ml beakers contained one daphnid each for evaluation of daphnid reproduction and growth, and three 1-l beakers contained five daphnids each for evaluation of survival. The loading rate was approximately one daphnid per 200 ml of test solution for reproduction and one daphnid per 180 ml for survival. The test solutions were renewed every

Monday, Wednesday, and Friday. Survival of the parent daphnids was determined daily until the release of the first broods, after which observations for mortality, sublethal effects, and reproduction were made on solution renewal days. On day 21, the body lengths of the adult daphnids were measured to the nearest 0.1 mm using a dissecting microscope and stage micrometer. The dry weights of the adult daphnids in the reproduction chambers were determined by drying them at 60°C for at least 24 hours. The survival of the daphnids was monitored on the same schedule as the daphnids in the reproduction chambers.

The daphnids were fed algae (1.0×10^7 cells/l) once daily and 0.3 ml/l of a yeast, trout chow, and cereal leaf suspension was added to each chamber once weekly.

Temperature, dissolved oxygen concentration (DO), conductivity, total alkalinity, total hardness, and pH were measured in alternating replicates of the controls and the low, middle, and high treatment levels on days 0, 9, and 16 (new solutions) and 4, 9, 16, and 21 (old solutions). Hourly temperatures of a centrally-located test chamber were documented using a data logger.

Samples of the fresh test solutions were taken on days 0, 4, 11, and 18 to measure the amount of radioactivity using liquid scintillation counting. Old solutions were sampled on days 2, 7, 14, and 21. Samples of new and old 50 ng ai/l solutions were taken to determine the percentage of parent material using thin-layer chromatography.

- E. **Statistics:** Solvent and negative control data were compared using either Fisher's Exact Test or t-tests. Pooled data were used when the tests indicated no significant difference. All data sets were tested for normality and homogeneity of variance using Chi-square and Bartlett's tests, respectively. Survival data were analyzed using analysis of variance (ANOVA). The 21-day LC_{50} was determined using the binomial probability method. The number of young per adult reproductive day was calculated for each replicate and analyzed using ANOVA and Dunnett's test. The time (days) to first brood release and adult length and dry weight were analyzed using the same methods as the reproduction data. The responses of the exposed daphnids were compared to those of the pooled control data in all tests. Conclusions of statistical significance were based on a 95% confidence level.

12. **REPORTED RESULTS:** No undissolved material was observed in the treatment solutions. The results of the radio-analyses are presented in Table 2 (attached). The mean measured concentrations calculated from these data were 2.7, 5.2, 11.0, 19.3, and 38.9 ng ai/l. These values represent between 77 and 90% of nominal concentrations. The percentage of parent material ranged from 96 to 100% during the study (Appendix 2, Table 3, attached).

Daphnid survival in the two highest concentration treatment solutions was significantly reduced in comparison to the pooled control (Table 8, attached). Using binomial probability, the 21-day LC_{50} was determined to be 24.2 ng ai/l with a 95% confidence interval of 19.3-38.9 ng ai/l.

The number of days until release of the first brood in the pooled control and treatment solutions was similar (Table 9, attached). Mean time to first brood was 9.1 days for the pooled control and ranged from 9.1 to 10.4 days for the treatment groups. The number of young produced per adult per reproductive day in the pooled control and treatment solutions was similar (Table 9). Mean young per adult per reproductive day was 11.46 for the pooled control and ranged from 10.38 to 13.93 days for the treatment groups. All neonates appeared normal during the study.

The length and dry weight of adult daphnids were not significantly reduced in comparison to the pooled control length and dry weight (Table 10, attached).

The results of hourly measurements found that the test temperatures ranged from 19.1 to 20.8°C. The DO was 4 to 10 mg/l or 45 to 110% of saturation at 20°C. The pH values ranged from 7.4 to 8.1. Hardness and alkalinity were 160-180 and 116-131 mg/l as $CaCO_3$, respectively. Conductivity was 357-420 μ mhos/cm.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Based on the analyses of the above biological parameters, the no-observed-effect concentration (NOEC) was 11.0 ng ai/l and the lowest-observed-effect concentration (LOEC) was 19.3 ng ai/l. The maximum acceptable toxicant concentration (MATC) was 14.6 ng ai/l.

The results of this study compare favorably with the results from the previously submitted study (EPA MRID No. 420054-32) which defined the NOEC as 13 ng ai/l and the LOEC as 27 ng ai/l. The most sensitive endpoint was survival for both studies, and no effects on reproduction or growth were observed in either study.

Quality Assurance and Good Laboratory Practice (GLP) Certification statements were included in the report indicating adherence to EPA GLP Regulations.

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

- A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the SEP and ASTM guidelines, but deviated as follows:

The DO dropped below 50% of saturation during the last day of the test.

The daphnid lengths were measured to the nearest 0.1 mm at the end of the test. The SEP states that the daphnids should be measured to the nearest 0.01 mm.

The source of the test daphnids was not fully described. The SEP states that daphnids must be collected from adults which have had at least one brood and have been observed for at least 21 days.

- B. Statistical Analysis: To validate the author's statistical analyses, the reviewer used one-way ANOVA and Bonferroni's test to analyze the survival, time to first brood, number of reproductive days, number of young produced per female reproductive day, adult length, and adult dry weight data (see attached printouts). The survival data were arcsine square root transformed before the analysis. The results were similar to the author's. The most sensitive measure of the effect of the test material was survival, with an NOEC and LOEC of 11.0 and 19.3 ng ai/l, respectively. Using probit analysis, the reviewer obtained a slightly more conservative LC_{50} of 21.9 ng ai/l with a 95% confidence interval of 17.8-27.7 ng ai/l, respectively. The slope of the probit curve was 5.6.

- C. Discussion/Results: Since the DO dropped below 50% of saturation only during the last 24 hours of the study, the reviewer believes that this did not influence the outcome of the study overall. It was unclear how the number of offspring per female reproductive day was determined. Since there was only 1 adult in each reproduction vessel, total young should have been divided by number of reproductive days. However, the reviewer found that this was not the case. The reviewer recomputed the number of offspring per female reproductive day and submitted these data for analysis (Table 9).

This study is scientifically sound and meets the guideline requirements for a chronic toxicity study using *Daphnia magna*. The MATC, based on the most sensitive biological parameter, adult daphnid survival, was >11.0 ng ai/l and <19.3 ng ai/l, based on mean measured concentrations. The geometric mean MATC was 14.6 ng ai/l.

D. Adequacy of the Study:

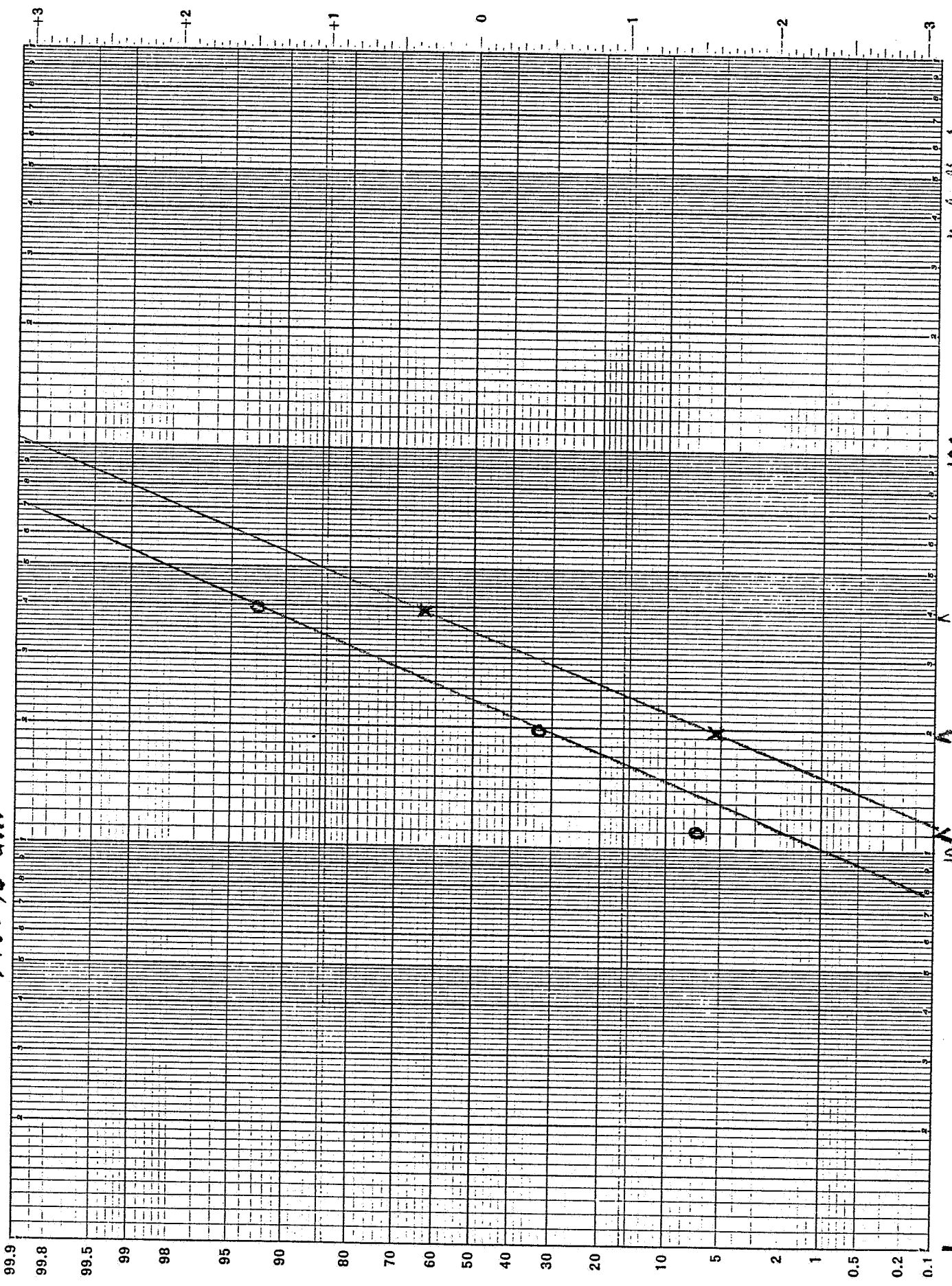
(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 10-19-93.

Phostebuspirum - *Daphnia magna* (21-Day Static Renewal)
97.7% a.i.



X - # of offspring

100

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Page _____ is not included in this copy.

Pages 8 through 15 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.
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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.

mossier MAT 7484 DAPHNIA MAGNA 10-18-93

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
38.9	15	14	93.33334	4.882813E-02
19.3	15	5	33.33334	15.08789
11	15	1	6.666667	4.882813E-02

THE BINOMIAL TEST SHOWS THAT 11 AND 38.9 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 22.99258

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.1353801	22.19592	18.09022	28.14498

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.207002	1	.5824373

SLOPE = 5.56086
95 PERCENT CONFIDENCE LIMITS = 3.030809 AND 8.09091

LC50 = 21.92427
95 PERCENT CONFIDENCE LIMITS = 17.77216 AND 27.70473

LC10 = 12.95815
95 PERCENT CONFIDENCE LIMITS = 8.035188 AND 16.26678

daphnia survival

File: dap

Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3.171	0.634	19.078
Within (Error)	15	0.499	0.033	
Total	20	3.670		

Critical F value = 2.90 (0.05,5,15)

Since F > Critical F REJECT Ho:All groups equal

daphnia survival

File: dap

Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	pooled control	1.441	1.000		
2	2.7	1.145	0.800	2.300	
3	5.2	1.219	0.867	1.728	
4	11	1.330	0.933	0.864	
5	19.3	0.966	0.667	3.684	*
6	38.9	0.241	0.067	9.312	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

daphnia survival

File: dap

Transform: ARC SINE(SQUARE ROOT(Y))

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	pooled control	6			
2	2.7	3	0.184	18.4	0.200
3	5.2	3	0.184	18.4	0.133
4	11	3	0.184	18.4	0.067
5	19.3	3	0.184	18.4	0.333
6	38.9	3	0.184	18.4	0.933

daphnia,time to 1st brood
 File: dap Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	11.908	2.382	2.058
Within (Error)	38	43.979	1.157	
Total	43	55.886		

Critical F value = 2.53 (0.05,5,30)
 Since F < Critical F FAIL TO REJECT Ho:All groups equal

daphnia time to 1st brood
 File: dap 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	pooled cont	11.917	11.917		
2	2.7	11.200	11.200	1.252	
3	5.2	11.143	11.143	1.512	
4	11	11.857	11.857	0.116	
5	19.3	10.833	10.833	2.014	
6	38.9	10.571	10.571	2.629	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=38,5)

daphnia time to 1st brood
 File: dap 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	pooled cont	12			
2	2.7	5	1.391	11.7	0.717
3	5.2	7	1.243	10.4	0.774
4	11	7	1.243	10.4	0.060
5	19.3	6	1.307	11.0	1.083
6	38.9	7	1.243	10.4	1.345

daphnia reproductive days
 File: dap Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	220.433	44.087	42.267
Within (Error)	39	40.679	1.043	
Total	44	261.111		

Critical F value = 2.53 (0.05,5,30)
 Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

daphnia reproductive days
 File: dap Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	pooled cont	12.917	12.917		
2	2.7	11.500	11.500	2.774	*
3	5.2	12.143	12.143	1.593	
4	11	12.857	12.857	0.123	
5	19.3	11.833	11.833	2.121	
6	38.9	6.429	6.429	13.358	*

Bonferroni T table value = 2.43 (1 Tailed Value, $P=0.05$, $df=39,5$)

daphnia reproductive days
 File: dap Transform: NO TRANSFORMATION

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	pooled cont	12			
2	2.7	6	1.239	9.6	1.417
3	5.2	7	1.178	9.1	0.774
4	11	7	1.178	9.1	0.060
5	19.3	6	1.239	9.6	1.083
6	38.9	7	1.178	9.1	6.488

young per reproductive day
 File: dap Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	289.795	57.959	7.064
Within (Error)	39	319.985	8.205	
Total	44	609.780		

Critical F value = 2.53 (0.05,5,30)
 Since F > Critical F REJECT Ho:All groups equal

young per reproductive day
 File: dap Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	15.433	15.433		
2	2.7	16.067	16.067	-0.442	
3	5.2	16.214	16.214	-0.573	
4	11	18.400	18.400	-2.178	
5	19.3	15.517	15.517	-0.058	
6	38.9	9.786	9.786	4.146	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=39,5)

young per reproductive day
 File: dap Transform: NO TRANSFORMATION

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	GRPS 1&2 POOLED	12			
2	2.7	6	3.475	22.5	-0.633
3	5.2	7	3.305	21.4	-0.781
4	11	7	3.305	21.4	-2.967
5	19.3	6	3.475	22.5	-0.083
6	38.9	7	3.305	21.4	5.648

daphnia length

File: dap

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.145	0.036	1.268
Within (Error)	33	0.945	0.029	
Total	37	1.091		

Critical F value = 2.69 (0.05,4,30)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

daphnia length

File: dap

Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	5.100	5.100		
2	2.7	5.233	5.233	-1.575	
3	5.2	5.143	5.143	-0.532	
4	11	5.200	5.200	-1.242	
5	19.3	5.050	5.050	0.591	

Bonferroni T table value = 2.35 (1 Tailed Value, P=0.05, df=33,4)

daphnia length

File: dap

Transform: NO TRANSFORMATION

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	GRPS 1&2 POOLED	12			
2	2.7	6	0.199	3.9	-0.133
3	5.2	7	0.189	3.7	-0.043
4	11	7	0.189	3.7	-0.100
5	19.3	6	0.199	3.9	0.050

daphnia dry weight

File: dap

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.354	0.089	0.897
Within (Error)	33	3.260	0.099	
Total	37	3.614		

Critical F value = 2.69 (0.05,4,30)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

daphnia dry weight

File: dap

Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.487	1.487		
2	2.7	1.248	1.248	1.522	
3	5.2	1.359	1.359	0.861	
4	11	1.363	1.363	0.829	
5	19.3	1.241	1.241	1.568	

Bonferroni T table value = 2.35 (1 Tailed Value, P=0.05, df=33,4)

daphnia dry weight

File: dap

Transform: NO TRANSFORMATION

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	GRPS 1&2 POOLED	12			
2	2.7	6	0.369	24.8	0.239
3	5.2	7	0.351	23.6	0.129
4	11	7	0.351	23.6	0.124
5	19.3	6	0.369	24.8	0.246

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