MRID No. 420817-07

DATA EVALUATION RECORD

1. **CHEMICAL:** Methidathion.
Shaughnessey No. 100301.

2. **TEST MATERIAL:** Methidathion Technical; Lot No. FL-890331; 96.1% active ingredient; a colorless waxy solid.

3. **STUDY TYPE:** Freshwater Invertebrate Life-Cycle Flow-Through Test. Species Tested: *Daphnia magna*

4. **CITATION:** Putt, A.E. 1991. Methidathion Technical - The Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions. Report No. 91-8-3881. Study conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Agricultural Division, Greensboro, NC. EPA MRID No. 420817-07.

5. **REVIEWED BY:**

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7. **CONCLUSIONS:** This study is ~~not~~ scientifically sound and does ~~not~~ fulfill the guideline requirements for a daphnid life-cycle test. The measured concentrations varied greatly during the test, therefore, the actual concentrations to which the organisms were exposed are unknown. The MATC of Methidathion Technical for *Daphnia magna* was ~~>0.72 and <1.3 µg a.i./l (geometric mean MATC = 0.97 µg a.i./l)~~. MATC > 0.66 < 1.13 ugai/L

8. **RECOMMENDATIONS:** See Attached revisions BJ. Montague

9. **BACKGROUND:** Study was originally graded invalid - reevaluation based on the final 3 measurement only exclude 0 hr.

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A. MATC revised downward study is acceptable

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A. **Test Animals:** *Daphnia magna* were obtained from populations cultured at the testing facility. The cultures were maintained in a temperature controlled water bath (20 ±1°C). A photoperiod of 16 hours of light and 8 hours of darkness with a light intensity of 50-65 footcandles was provided. The culture water was the same as that used for dilution water. The daphnids were fed a combination of green alga (*Ankistrodesmus falcatus*) and a trout food suspension once daily.

B. **Test System:** The flow-through test system was an intermittent-flow proportional diluter with a 50% dilution factor. The diluter delivered five nominal concentrations of test material, a dilution water control, and a solvent control to the test vessels. The diluter delivered water to each vessel at an average rate of approximately 6 volume replacements per day. The time for 90% solution replacement was approximately 9 hours.

Test vessels were 1.6-l glass battery jars. Each jar drained through two 2-cm holes which were covered with a Nitex® 40-mesh screen and located 15 cm from the bottom of the test vessel. The volume of test solutions was maintained at 1.4 l.

Sixteen hours of light at an intensity of 18-50 footcandles (194-538 lux) were provided each day. The study was conducted in an air-temperature controlled room which was designed to maintained the test temperature at 20 ±1°C.

The dilution (and culture) water was fortified well water which was filtered through a resin column and a carbon filter to remove potential organic contaminants. Total hardness and alkalinity ranges of the dilution water were 160-180 and 110-130 mg/l as $CaCO_3$, respectively. The pH ranged from 7.9 to 8.3 and the specific conductivity ranged from 400 to 600 µmhos/cm.

A primary stock solution (17.5 mg a.i./ml) was prepared by dissolving 0.091 g (0.0875 g as active ingredient) of test material with triethylene glycol (TEG) to a volume of 5 ml. A 1.0 ml aliquot of this solution was combined with TEG to a final volume of 100 ml to prepare a secondary stock solution (0.175 mg a.i./ml). The diluter system delivered 0.0066 ml of this secondary stock solution and 386 ml of dilution water to the diluter mixing chamber. This mixture provided

the highest treatment level and subsequent dilutions of this concentration provided the remaining test levels.

C. **Dosage:** Twenty-one-day, life-cycle, flow-through test. Nominal test concentrations selected based on results of a preliminary study were 0.19, 0.38, 0.75, 1.5, and 3.0 µg a.i./l. In addition, a dilution water control and solvent control (17 µl TEG/l) were included.

D. **Design:** Ten daphnids (≤24 hours old) were impartially selected and distributed to each of four replicate exposure vessels (i.e., 40 daphnids/concentration) of the Methidathion Technical concentrations and controls.

The daphnids were fed 2.0 ml of trout food (5 mg/ml), 3.0 ml of algal suspension (*Ankistrodesmus falcatus*; 4 x 10^7 cells/ml), and 0.5 ml of Selco® (a commercial mixture of proteins and fatty acids, 0.6 mg/ml) three times daily on weekdays and twice daily on weekends and holidays. Test vessels were brushed to remove algal growth and the solutions filtered through a fine mesh net at least twice a week.

Adult survival was determined on days 1, 2, 4, 7, 10, 13, 14, 17, 20, and 21. Offspring production was noted on days 7, 10, 13, 14, 17, 20, and 21. The offspring were discarded after counting. At test termination, total body length and dry weight of all surviving adults were recorded.

Dissolved oxygen (DO) concentration was measured every weekday in one replicate vessel of each test level and control. Temperature was measured daily in one replicate vessel of each treatment and control and continuously throughout the test period in the C replicate of the 3.0 µg/l solution. Total hardness, alkalinity, specific conductance, and pH were measured weekly in one replicate vessel of each test level and control. The DO concentration, pH, and temperature were also measured once a week in all replicate vessels of each treatment group and control.

Water samples were collected from the midpoint of alternating replicate vessels (A/B or C/D) of the four highest test concentrations and composite samples from either replicates A and B or C and D of the lowest concentration and controls on days 0, 7, 14, and 21. The samples were analyzed for Methidathion using gas chromatography.

E. **Statistics:** The percentage survival data were transformed (arcsine square-root percentage) before analysis. Survival and reproduction data were normally distributed (Shapiro-Wilks test); therefore, Williams' test was used to assess exposure-level effects of these parameters. Growth data were not normally distributed (Shapiro-Wilds test) or homogeneous (Bartlett's test); therefore, Kruskal-Wallis test was used. T-test showed no significant difference between control and solvent control data; therefore, survival, reproduction, and growth data were compared to pooled control data. Analyses were performed using the mean organism response in each replicate vessel. All statistical analyses were considered significant at $p \leq 0.05$.

EC_{50} values were determined using a computer program modified from the program of C. Stephan (Peltier, 1985).

12. **REPORTED RESULTS:** No visible sign of undissolved test material was evident in the diluter system or any test vessel. The mean measured concentrations established in the test solutions were 0.13, 0.36, 0.72, 1.3, and 2.5 µg a.i./l (Table 2, attached). "Analyses of the treatment solutions resulted in mean measured concentrations of Methidathion Technical were generally consistent between replicate vessels and sampling intervals."

The survival and reproductive rates for control groups exceeded the minimum EPA guideline requirements of 70% survival and 40 offspring/female. Survival ranged from 93 to 98% in the controls and from 90 to 98% in the three lowest concentrations, and 0 to 3% in the highest concentrations (Table 3, attached). Survival was significantly reduced at 1.3 and 2.5 µg a.i./l when compared to that of the pooled control data. The two highest test concentrations were excluded from the analyses for growth and reproduction since there was a significant effect on survival. Reproduction at 0.13-0.72 µg a.i./l was statistically similar to that of the pooled control (Table 6, attached). Mean total body length and mean dry weight of daphnids at 0.13, 0.36, and 0.72 µg a.i./l were not significantly reduced when compared to those of the pooled control organisms (Tables 8 and 9, attached).

The 21-day EC_{50} (95% confidence interval) for immobilization was 0.79 (0.66-0.94) µg a.i./l at test termination using the moving average angle analysis.

During the study, pH was 7.9-8.4; mean total hardness and alkalinity were 170-180 and 120 mg/l as $CaCO_3$, respectively; specific conductance was 500 µmhos/cm; and the mean DO concentration was 8.3-8.6 mg/l. The daily temperatures ranged from 19 to 21°C and the continuous temperature ranged from 18 to 21°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on the adverse effect observed on daphnid survival, the No-Observed Effect Concentration (NOEC) for this study was determined to be 0.72 µg a.i./l and the Lowest Observed Effect Concentration (LOEC) was determined to be 1.3 µg a.i./l. Analyses of these data determined the Maximum Acceptable Toxicant Concentration (MATC) of Methidathion Technical to *D. magna* to be >0.72 µg a.i./l and <1.3 µg a.i./l (Geometric Mean MATC = 0.97 µg a.i./l)."

A Good Laboratory Practices (GLP) Compliance Statement was included in the report indicating that the data and report prepared for this study were produced and compiled in accordance with all pertinent EPA GLP Regulations (40 CFR, Part 160) except in the case of stability, characterization and verification of test substance identity. This statement was signed by the Study Director.

A Quality Assurance Unit Statement was included and signed by a representative of the Regulatory Affairs and Quality Assurance Unit of the preforming laboratory.

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

A. **Test Procedure:** An SEP for *Daphnia* chronic flow-through studies is not available at this time, thus ASTM recommended procedures were used in this data validation process. The test procedure was in accordance with ASTM, except for the following deviations:

The highest measured concentration at 0.19, 0.38, 1.5, and 3.0 µg a.i./l nominal concentrations was more than twice that of the lowest measured concentration at the same level (Table 2, attached). According to the guidelines, such variation in test concentration is unacceptable.

Raw data for water quality parameters were not presented in the report. All raw data for each biological endpoint and for physical and chemical parameters measured during the test must always be submitted.

The author did not indicate the selection criteria (i.e., brood number) for organisms used in the test.

Test chambers were 1.6-l beakers containing 1.4 l of test solution; 2-l beakers containing 1.5 l of test solution are recommended.

The report did not indicate whether the dilution water was intensely aerated prior to addition of the test material as recommended in the guidelines. However, the range of DO throughout the test period is acceptable.

The diluter system should operate for at least two days prior to test initiation as recommended; the pretest operation period was not reported.

The report does not indicate whether treatments were randomly assigned to test chambers as required in the guidelines.

Daphnid length was measured to the nearest 0.1 mm; it should be measured to the nearest 0.01 mm.

A 15- to 30-minute dawn and dusk simulation period was not employed in this study.

B. **Statistical Analysis:** The survival data (arcsine square-root transformed) were not homogeneous according to Hartley and Bartlett's tests (printout, attached), therefore, a non-parametric test (Steels Many-One Rank test) was used to analyze the survival data. The results showed that survival was statistically reduced at the two highest test levels (printout, attached).

A two-way ANOVA with Bonferroni's comparison test was performed on each growth endpoint. Length and weight were not significantly reduced at any test level (printouts, attached). These results are similar to those presented by the author. However, it should be noted that the author used replicate means to determine the effects of the test material on the test organisms. When mean values are used, the variation that exists within each replicate is ignored.

It is unclear whether immobilized offspring were included in the total number of alive offspring (Table 6, attached). The reviewer conducted statistical analysis with the assumption that they had not been included. The author evaluated the effects of the test

material on reproduction using average number of young produced per female. Since reproduction was observed every 1-3 days (not daily), the appropriate endpoint is number of young per female reproduction day, not the number of young per female. The reviewer determined that the number of young per female reproductive day was not homogeneous using Hartley and Bartlett's tests (printout, attached), therefore, the data were analyzed using Steels Many-One Rank test. The highest test concentration was significantly different from that of the control.

C. **Discussion/Results:** This study is ~~not~~ scientifically sound and does ~~not~~ meet the guideline requirements for a life-cycle toxicity study using freshwater invertebrates. The measured concentrations varied greatly during the test. The highest measured value at four test levels (0.13, 0.36, 1.3, and 2.5 µg a.i./l) was more than twice the lowest at the same level. Therefore, the actual test concentrations to which the organisms were exposed are unknown.

The MATC of Methidathion Technical for *Daphnia magna* was ~~>0.72 and <1.3 µg a.i.~~/l (geometric mean MATC = 0.97 µg a.i./l). Based on Day 7, 14, and 21 day meas. Concentrations MATC ~~<0.96~~ >0.96 < 1.13 ug/l

D. **Adequacy of the Study:**

(1) **Classification:** ~~Invalid.~~ Core B. Montague

(2) **Rationale:** The measured concentrations varied greatly during the test, therefore, the actual test concentrations to which the organisms were exposed are ~~unknown~~ based on the final 3 measurements. The 0 hour meas. were not used.

(3) **Repairability:** ~~No~~ repaired

15. **COMPLETION OF ONE-LINER:** Yes, February 24, 1992.

Sem 11/12/98

Table 2. Concentrations of Methidathion Technical measured in the exposure solutions during the 21-day chronic test with daphnids (*Daphnia magna*).

Nominal Concentration (µg A.I./L)	Measured Concentration (µg A.I./L) Day 0[a]	Day 7[b]	Day 14[a]	Day 21[b]	Mean[c]
3.0	3.6	1.9	2.4	2.2	2.5(0.77)
	3.6	1.8	2.9	1.6	
1.5	1.6	0.96	1.3	<0.38	1.3(0.28)
	1.5	0.99	1.4	0.99	
0.75	0.92	0.55	0.70	0.73	0.72(0.14)
	0.89	0.62	0.77	0.58	
0.38	0.49	0.26	0.37	0.31	0.36(0.11)
	0.53	0.32	0.41	0.22	
0.19	0.18	0.11	0.15	0.10	0.13(0.043)
	0.19	0.073	0.14	0.088	
Control	<0.088	<0.034	<0.074	<0.059	
	<0.088	<0.034	<0.074	<0.059	
Solvent Control	<0.088	<0.034	<0.74	<0.059	
	<0.088	<0.034	<0.074	<0.059	
QC #1[e]	0.309[f]	0.267	0.291	0.200	
	(0.250)	(0.250)	(0.250)	(0.250)	
QC #2	1.10	1.06	0.923	1.07	
	(1.00)	(1.00)	(1.00)	(1.00)	
QC #3	4.71	4.30	4.43	4.47	
	(4.00)	(4.00)	(4.00)	(4.00)	

a Samples were removed from replicates A and B. Samples removed from 0.19 µg A.I./L, solvent and dilution water control solutions were a composite of A, B, C and D replicates.

b Samples were removed from replicates C and D. Samples removed from 0.19 µg A.I./L, solvent and dilution water control solutions were a composite of A, B, C and D replicates.

c Mean measured concentrations are presented with standard deviations in parentheses and were calculated using actual (unrounded) analytical results and not with the rounded (two significant figures) values presented in this table.

d Error was assumed during the extraction of this analytical sample. Value was not included in calculation of the mean.

e QC = Quality Control sample.

f Nominal fortified concentration for each QC sample is presented in parentheses.

Table 3. **Cumulative mean percentage survival of parental daphnids (*Daphnia magna*) during the 21-day chronic exposure to Methidathion Technical.**

Cumulative Percent Survival

Mean Measured Concentration (µg A.I./L)	Mean Cumulative Percent Survival (SD)[a]				
	Day 1	Day 2	Day 4	Day 7	Day 10
2.5	100(0)	100(0)	100(0)	10(0)	0(0)
1.3	100(0)	98(5)	98(5)	98(5)	98(5)
0.72	100(0)	100(0)	100(0)	98(5)	98(5)
0.36	100(0)	100(0)	98(5)	98(5)	98(5)
0.13	100(0)	100(0)	100(0)	100(0)	100(0)
Control	100(0)	100(0)	100(0)	100(0)	98(5)
Solvent Control	100(0)	100(0)	100(0)	100(0)	100(0)
Pooled Control	100(0)	100(0)	100(0)	100(0)	99(4)

[a] SD = standard deviation.

Table 3. Continued.

Cumulative Percent Survival

Mean Measured Concentration (µg A.I./L)	Mean Cumulative Percent Survival (SD)[a]				
	Day 13	Day 14	Day 17	Day 20	Day 21
2.5	0(0)	0(0)	0(0)	0(0)	0(0)[b]
1.3	13(15)	5(6)	3(5)	3(5)	3(5)[b]
0.72	95(6)	95(6)	93(5)	90(8)	90(8)
0.36	98(5)	98(5)	93(10)	93(10)	93(10)
0.13	100(0)	100(0)	98(5)	98(5)	98(5)
Control	95(6)	95(6)	95(6)	93(10)	93(10)
Solvent Control	100(0)	100(0)	100(0)	98(5)	98(5)
Pooled Control	98(5)	98(5)	98(5)	95(8)	95(8)

[a] SD = standard deviation.
[b] Significantly different ($p \leq 0.05$) as compared to the pooled control organisms.

Table 6. **Total number of offspring alive and total number of offspring immobilized at each observation interval after first brood release during the 21-day chronic exposure of daphnids (*Daphnia magna*) to Methidathion Technical.**

Mean Measured Concentration (µg A.I./L)		Number of Offspring Alive (# Offspring Immobilized) Day: 7	10	13	14	17	20	21	Total
2.5	A	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	B	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	C	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	D	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	Total	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
1.3[a]	A	0(0)	1(1)	368(0)	0(0)	0(0)	0(0)	0(0)	369(1)
	B	0(0)	0(0)	301(0)	14(0)	48(0)	0(0)	0(0)	363(0)
	C	0(0)	0(0)	185(0)	0(0)	0(0)	0(0)	0(0)	185(0)
	D	0(0)	2(0)	158(0)	0(5)	35(0)	24(0)	6(0)	225(5)
	Total	0(0)	3(1)	1012(0)	14(5)	83(0)	24(0)	6(0)	1142(6)
0.72[b]	A	0(0)	0(0)	279(0)	42(0)	305(0)	0(0)	64(0)	690(0)
	B	0(0)	0(0)	292(0)	117(0)	229(0)	269(0)	155(0)	1062(0)
	C	0(0)	1(2)	290(0)	33(0)	220(0)	399(0)	186(0)	1129(2)
	D	0(0)	0(0)	259(0)	170(0)	381(0)	389(0)	365(0)	1564(0)
	Total	0(0)	1(2)	1120(0)	362(0)	1135(0)	1057(0)	770(0)	4445(2)
0.36[c]	A	0(0)	0(0)	179(0)	109(0)	406(0)	496(0)	606(0)	1796(0)
	B	0(0)	0(0)	0(0)	28(0)	173(0)	362(0)	236(0)	799(0)
	C	0(0)	0(0)	267(0)	131(0)	355(0)	510(0)	540(0)	1803(0)
	D	0(0)	0(0)	69(0)	3(0)	531(0)	401(0)	59(0)	1063(0)
	Total	0(0)	0(0)	515(0)	271(0)	1465(0)	1769(0)	1441(0)	5461(0)
0.13[d]	A	0(0)	0(0)	0(0)	0(0)	373(0)	287(0)	66(0)	726(0)
	B	0(0)	0(0)	86(0)	75(0)	479(0)	632(0)	459(0)	1731(0
	C	0(0)	0(0)	262(0)	112(0)	471(0)	641(0)	607(0)	2093(0)
	D	0(0)	0(0)	69(0)	108(0)	422(0)	409(0)	493(0)	1501(0)
	Total	0(0)	0(0)	417(0)	295(0)	1745(0)	1969(0)	1625(0)	6051(0)
Control[a]	A	0(0)	0(0)	54(0)	1(4)	53(0)	367(0)	245(0)	720(4)
	B	0(0)	1(0)	252(0)	56(0)	422(0)	455(0)	240(0)	1426(0)
	C	0(0)	0(0)	243(0)	240(0)	374(0)	533(0)	641(0)	2031(0)
	D	0(0)	0(0)	243(0)	74(0)	403(0)	417(0)	389(0)	1526(0)
	Total	0(0)	1(0)	792(0)	371(4)	1252(0)	1772(0)	1515(0)	5703(4)
Solvent[e] Control	A	0(0)	4(0)	53(0)	3(0)	0(0)	0(0)	40(0)	100(0)
	B	0(0)	2(3)	328(0)	60(17)	456(0)	433(0)	269(0)	1548(20)
	C	0(0)	0(0)	310(0)	213(0)	325(0)	563(0)	620(0)	2031(0)
	D	0(0)	5(1)	17(0)	83(0)	453(0)	551(0)	674(0)	1783(1)
	Total	0(0)	11(4)	708(0)	359(17)	1234(0)	1547(0)	1603(0)	5462(21)
Pooled Control		0	6	750	365	1243	1660	1559	5583

[a] First brood release was observed on test day 9.
[b] First brood release was observed on test day 10.
[c] First brood release was observed on test day 12.
[d] First brood release was observed on test day 11.
[e] First brood release was observed on test day 8.

Table 8. **Individual organism length measurements made at the termination of the 21-day chronic toxicity test with daphnids (*Daphnia magna*) and Methidathion Technical. All measurements are expressed as millimeters (mm).**

	Mean Measured Concentration (µg A.I./L)						
Observation	Ctl	S Ctl	0.13	0.36	0.72	1.3	2.5
1	5.2	4.8	5.1	5.2	5.2	4.6	
2	4.7	4.5	4.5	5.5	5.2		
3	4.8	4.6	4.9	5.4	4.9		
4	4.6	5.1	4.6	5.3	5.3		
5	4.7	4.4	4.9	5.4	4.9		
6	5.3	4.5	4.6	5.5	5.1		
7	4.7	5.0	4.5	5.5	5.1		
8	5.3	4.8	4.7	5.5	5.2		
9	5.2	4.7	4.6	5.4	4.9		
10	4.7	4.5	4.6	5.4	5.0		
11	5.3	5.4	5.3	5.3	5.4		
12	5.2	5.4	5.4	5.1	5.1		
13	5.4	5.6	5.4	5.3	5.4		
14	5.4	5.3	5.4	5.4	5.2		
15	5.4	5.0	5.1	5.3	5.2		
16	5.3	5.4	5.4	5.4	5.3		
17	5.4	5.5	5.4	5.3	5.2		
18	5.3	5.3	5.3	5.2	5.1		
19	5.2	5.5	5.3	5.2	5.2		
20	5.4	5.4	5.3	5.5	5.1		
21	5.3	5.4	5.3	5.3	5.5		
22	5.2	5.3	5.5	5.5	4.8		
23	5.4	5.1	5.4	5.4	5.4		
24	5.4	5.4	5.5	5.2	4.9		
25	5.4	5.4	5.5	5.5	5.4		
26	5.1	5.5	5.4	5.4	5.5		
27	5.2	5.3	5.7	5.3	5.1		
28	5.1	5.2	5.6	5.2	5.3		
29	5.5	5.3	5.2	5.2	5.0		
30	5.5	5.4	4.0	5.2	5.2		
31	5.3	5.4	5.2	5.4	5.0		
32	5.4	5.3	5.3	5.4	5.1		
33	5.4	5.4	5.2	5.3	5.2		
34	5.2	5.4	5.4	5.2	5.3		
35	5.4	5.3	5.0	5.1	5.2		
36	5.4	5.4	5.1	5.5	5.1		
37	5.4	5.4	5.5	5.0			
38		5.4	5.2				
39		5.1	5.3				
40							
Mean	5.22	5.18	5.14	5.33	5.17		
S.D	0.25	0.33	0.38	0.13	0.17		
N	37	39	39	37	36		

Table 9. **Individual organism weight measurements made at the termination of the 21-day chronic toxicity test with daphnids (*Daphnia magna*) and Methidathion Technical. All measurements are expressed as milligrams (mg).**

	Mean Measured Concentration (µg A.I./L)						
Observation	Ctl	S Ctl	0.13	0.36	0.72	1.3	2.5
1	1.18	0.76	1.32	1.91	1.76	0.97	
2	0.87	1.40	1.42	1.48	1.79		
3	0.86	1.39	1.90	1.34	1.88		
4	0.71	1.48	1.41	1.19	1.29		
5	1.60	1.21	1.89	1.33	1.96		
6	1.01	1.43	1.43	1.56	1.79		
7	1.52	1.67	1.36	1.53	2.05		
8	0.76	1.24	1.48	1.42	1.87		
9	1.60	1.17	1.51	1.40	1.59		
10	0.92	0.90	1.53	1.43	0.78		
11	1.30	1.61	1.37	1.25	1.24		
12	1.49	2.84	1.35	1.93	1.34		
13	1.54	1.55	2.24	1.34	1.18		
14	2.19	1.75	1.44	1.51	1.25		
15	1.39	1.45	1.52	1.32	1.46		
16	2.11	2.27	1.29	1.43	1.26		
17	2.21	1.51	1.51	1.39	1.27		
18	2.29	2.36	1.41	2.00	1.55		
19	1.41	1.25	1.74	1.70	1.89		
20	1.38	1.34	1.50	1.57	1.01		
21	1.61	1.47	1.44	1.49	1.35		
22	1.40	1.50	1.48	1.56	1.72		
23	1.64	1.33	1.48	1.50	1.06		
24	1.57	1.47	1.43	1.47	1.39		
25	1.46	1.47	1.53	1.70	1.46		
26	1.11	1.63	1.48	1.34	1.44		
27	1.47	1.50	2.18	1.47	1.91		
28	1.52	1.36	1.57	1.37	1.37		
29	1.50	1.70	1.42	1.57	1.43		
30	1.52	1.58	1.17	1.70	1.56		
31	1.42	1.43	1.29	1.39	1.38		
32	1.47	1.50	1.41	1.43	1.30		
33	1.50	1.53	1.62	2.16	1.24		
34	1.04	1.55	1.72	2.06	1.42		
35	1.44	1.39	2.00	1.68	1.17		
36	1.34	1.62	1.97	1.56	1.57		
37	1.67	1.30	1.63	1.23			
38		1.43	1.44				
39		2.05	1.39				
40							
Mean	1.43	1.52	1.55	1.53	1.47		
S.D.	0.37	0.36	0.24	0.23	0.30		
N	37	39	39	37	36		

Mehidathion: survial of exposed daphnids
File: B:\METHI.SUR Transform: ARC SINE(SQUARE ROOT(Y))

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	5	18	5	0

Calculated Chi-Square goodness of fit test statistic = 9.6707
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Mehidathion: survial of exposed daphnids
File: B:\METHI.SUR Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.872

W = 0.807

Critical W (P = 0.05) (n = 28) = 0.924
Critical W (P = 0.01) (n = 28) = 0.896

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Mehidathion: survial of exposed daphnids
File: B:\METHI.SUR Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Mehidathion: # young/female reproductive day
File: B:\METHI.REP Transform: NO TRANSFORMATION

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	8	13	7	0

alculated Chi-Square goodness of fit test statistic = 4.4768
able Chi-Square value (alpha = 0.01) = 13.277

ata PASS normality test. Continue analysis.

ehidathion: # young/female reproductive day
ile: B:\METHI.REP Transform: NO TRANSFORMATION

hapiro Wilks test for normality

= 323.673

= 0.949

ritical W (P = 0.05) (n = 28) = 0.924
ritical W (P = 0.01) (n = 28) = 0.896

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

ehidathion: # young/female reproductive day
ile: B:\METHI.REP Transform: NO TRANSFORMATION

artley test for homogeneity of variance
artletts test for homogeneity of variance

hese two tests can not be performed because at least one group has
ero variance.

ata FAIL to meet homogeneity of variance assumption.
dditional transformations are useless.

TITLE: Mehidathion: # young/female reproductive day
FILE: b:methi.rep
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Solvent control	1	0.7000	0.7000
1	Solvent control	2	11.5000	11.5000
1	Solvent control	3	14.5000	14.5000
1	Solvent control	4	12.7000	12.7000
2	Control	1	5.2000	5.2000
2	Control	2	11.7000	11.7000
2	Control	3	14.5000	14.5000
2	Control	4	11.9000	11.9000
3	0.13 ug a.i./l	1	5.2000	5.2000
3	0.13 ug a.i./l	2	12.4000	12.4000
3	0.13 ug a.i./l	3	15.0000	15.0000
3	0.13 ug a.i./l	4	11.3000	11.3000
4	0.36 ug a.i./l	1	12.8000	12.8000
4	0.36 ug a.i./l	2	6.3000	6.3000
4	0.36 ug a.i./l	3	14.3000	14.3000
4	0.36 ug a.i./l	4	7.6000	7.6000
5	0.72 ug a.i./l	1	5.7000	5.7000
5	0.72 ug a.i./l	2	7.6000	7.6000
5	0.72 ug a.i./l	3	8.8000	8.8000
5	0.72 ug a.i./l	4	11.8000	11.8000
6	1.3 ug a.i./l	1	13.7000	13.7000
6	1.3 ug a.i./l	2	9.1000	9.1000
6	1.3 ug a.i./l	3	6.2000	6.2000
6	1.3 ug a.i./l	4	5.2000	5.2000
7	2.5 ug a.i./l	1	0.0000	0.0000
7	2.5 ug a.i./l	2	0.0000	0.0000
7	2.5 ug a.i./l	3	0.0000	0.0000
7	2.5 ug a.i./l	4	0.0000	0.0000

Mehidathion: # young/female reproductive day
File: b:methi.rep Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Solvent control	9.850				
2	Control	10.825	18.50	10.00	4.00	
3	0.13 ug a.i./l	10.975	18.00	10.00	4.00	
4	0.36 ug a.i./l	10.250	18.00	10.00	4.00	
5	0.72 ug a.i./l	8.475	15.00	10.00	4.00	
6	1.3 ug a.i./l	8.550	16.00	10.00	4.00	
7	2.5 ug a.i./l	0.000	10.00	10.00	4.00	*

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

TITLE: Mehidathion: survial of exposed daphnids
FILE: b:methi.sur

TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Solvent control	1	1.0000	1.4120
1	Solvent control	2	0.9000	1.2490
1	Solvent control	3	1.0000	1.4120
1	Solvent control	4	1.0000	1.4120
2	Control	1	1.0000	1.4120
2	Control	2	0.8000	1.1071
2	Control	3	1.0000	1.4120
2	Control	4	0.9000	1.2490
3	0.13 ug a.i./l	1	1.0000	1.4120
3	0.13 ug a.i./l	2	1.0000	1.4120
3	0.13 ug a.i./l	3	1.0000	1.4120
3	0.13 ug a.i./l	4	0.9000	1.2490
4	0.36 ug a.i./l	1	0.1000	0.3218
4	0.36 ug a.i./l	2	0.8000	1.1071
4	0.36 ug a.i./l	3	0.9000	1.2490
4	0.36 ug a.i./l	4	1.0000	1.4120
5	0.72 ug a.i./l	1	0.8000	1.1071
5	0.72 ug a.i./l	2	1.0000	1.4120
5	0.72 ug a.i./l	3	0.9000	1.2490
5	0.72 ug a.i./l	4	0.9000	1.2490
6	1.3 ug a.i./l	1	0.0000	0.1588
6	1.3 ug a.i./l	2	0.0000	0.1588
6	1.3 ug a.i./l	3	0.0000	0.1588
6	1.3 ug a.i./l	4	0.1000	0.3218
7	2.5 ug a.i./l	1	0.0000	0.1588
7	2.5 ug a.i./l	2	0.0000	0.1588
7	2.5 ug a.i./l	3	0.0000	0.1588
7	2.5 ug a.i./l	4	0.0000	0.1588

Mehidathion: survial of exposed daphnids
File: b:methi.sur Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	7.044	1.174	28.261
Within (Error)	21	0.872	0.042	
Total	27	7.917		

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

Mehidathion: survial of exposed daphnids
File: b:methi.sur 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	Solvent control	1.371	0.975		
2	Control	1.295	0.925	0.529	

3	0.13 ug a.i./l	1.371	0.975	0.000	
4	0.36 ug a.i./l	1.022	0.700	2.420	
5	0.72 ug a.i./l	1.254	0.900	0.812	
6	1.3 ug a.i./l	0.200	0.025	8.130	*
7	2.5 ug a.i./l	0.159	0.000	8.413	*

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

Mehidathion: survial of exposed daphnids
File: b:methi.sur 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	Solvent control	4			
2	Control	4	0.256	26.3	0.050
3	0.13 ug a.i./l	4	0.256	26.3	0.000
4	0.36 ug a.i./l	4	0.256	26.3	0.275
5	0.72 ug a.i./l	4	0.256	26.3	0.075
6	1.3 ug a.i./l	4	0.256	26.3	0.950
7	2.5 ug a.i./l	4	0.256	26.3	0.975

Mehidathion: survial of exposed daphnids
File: b:methi.sur Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Solvent control	1.371				
2	Control	1.295	15.50	10.00	4.00	
3	0.13 ug a.i./l	1.371	18.00	10.00	4.00	
4	0.36 ug a.i./l	1.022	13.00	10.00	4.00	
5	0.72 ug a.i./l	1.254	13.50	10.00	4.00	
6	1.3 ug a.i./l	0.200	10.00	10.00	4.00	*
7	2.5 ug a.i./l	0.159	10.00	10.00	4.00	*

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

Analysis of Variance METHIDATHION Date: 02-21-1992

FILTER: None

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors: C	R	N	Mean	S.D.
*	*	188	5.2080	0.2775
1	*	39	5.1821	0.3324
2	*	37	5.2189	0.2526
3	*	39	5.1462	0.3776
4	*	37	5.3297	0.1331
5	*	36	5.1667	0.1740
*	1	48	4.9604	0.3413
*	2	45	5.3000	0.1414
*	3	48	5.3000	0.2577
*	4	47	5.2787	0.1444
1	1	10	4.6900	0.2331
1	2	9	5.3778	0.1716
1	3	10	5.3300	0.1160
1	4	10	5.3500	0.0972
2	1	10	4.9200	0.2898
2	2	8	5.3375	0.0744
2	3	10	5.2700	0.1252
2	4	9	5.3889	0.0928
3	1	10	4.7000	0.2000
3	2	10	5.3300	0.0949
3	3	10	5.3200	0.4803
3	4	9	5.2444	0.1509
4	1	10	5.4100	0.0994
4	2	8	5.2875	0.0991
4	3	9	5.3667	0.1225
4	4	10	5.2500	0.1509
5	1	8	5.1125	0.1458
5	2	10	5.1800	0.1619
5	3	9	5.2111	0.2571
5	4	9	5.1556	0.1130

Fmax for testing homogeneity of between subjects variances: 41.67
Number of variances= 20 df per variance= 8.

Analysis of Variance Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	187	14.3980			
C (CONC)	4	0.7896	0.1974	5.407	0.0004
R (REP)	3	4.0593	1.3531	37.065	0.0000
CR	12	3.4161	0.2847	7.798	0.0000
Subj w Groups	168	6.1330	0.0365		

Post-hoc tests for factor C (CONC)

Level	Mean
1	5.182
2	5.219
3	5.146
4	5.330
5	5.167

Comparison	Bon-ferroni
1 < 2	
1 > 3	
1 < 4	0.0096
1 > 5	
2 > 3	
2 < 4	
2 > 5	
3 < 4	0.0005
3 < 5	
4 > 5	0.0037

Post-hoc tests for factor R (REP)

Level	Mean
1	4.960
2	5.300
3	5.300
4	5.279

Comparison	Bon-ferroni
1 < 2	0.0000
1 < 3	0.0000
1 < 4	0.0000
2 > 3	
2 > 4	
3 > 4	

Analysis of Variance METHIDATHION Date: 02-21-1992

FILTER: None

N's, means and standard deviations based on dependent variable: WEIGHT

* Indicates statistics are collapsed over this factor

Factors: C	R	N	Mean	S.D.
*	*	188	1.5021	0.3066
1	*	39	1.5228	0.3626
2	*	37	1.4335	0.3745
3	*	39	1.5456	0.2416
4	*	37	1.5327	0.2316
5	*	36	1.4717	0.2955
*	1	48	1.4148	0.3377
*	2	45	1.5904	0.4018
*	3	48	1.4912	0.2016
*	4	47	1.5179	0.2319
1	1	10	1.2650	0.2733
1	2	9	1.8433	0.5255
1	3	10	1.4770	0.1194
1	4	10	1.5380	0.2039
2	1	10	1.1030	0.3498
2	2	8	1.8150	0.4203
2	3	10	1.4590	0.1529
2	4	9	1.4333	0.1723
3	1	10	1.5250	0.2051
3	2	10	1.5370	0.2763
3	3	10	1.5187	0.2562
3	4	9	1.6078	0.2535
4	1	10	1.4590	0.1909
4	2	8	1.5213	0.2851
4	3	9	1.5333	0.1151
4	4	10	1.6150	0.2990
5	1	8	1.7988	0.2272
5	2	10	1.2920	0.2277
5	3	9	1.4700	0.3229
5	4	9	1.3822	0.1336

Fmax for testing homogeneity of between subjects variances: 20.84
Number of variances= 20 df per variance= 8.

Analysis of Variance Dependent variable: WEIGHT

Source	df	SS (H)	MSS	F	P
Between Subjects	187	17.5817			
C (CONC)	4	0.3325	0.0831	1.168	0.3243
R (REP)	3	0.7365	0.2455	3.451	0.0178
CR	12	4.5600	0.3800	5.341	0.0000
Subj w Groups	168	11.9527	0.0711		

Post-hoc tests for factor C (CONC)

Level	Mean
1	1.523
2	1.434
3	1.546
4	1.533
5	1.472

Comparison	Bon-ferroni
1 > 2	
1 < 3	
1 < 4	
1 > 5	
2 < 3	
2 < 4	
2 < 5	
3 > 4	
3 > 5	
4 > 5	

Post-hoc tests for factor R (REP)

Level	Mean
1	1.415
2	1.590
3	1.491
4	1.518

Comparison	Bon-ferroni
1 < 2	0.0108
1 < 3	
1 < 4	
2 > 3	
2 > 4	
3 < 4	

Data listing METHIDATHION Date: 02-21-1992

FILTER: None

Obs.	CONC	REP	LENGTH	WEIGHT
1	1	1.0	4.8	0.760
2	1	1.0	4.5	1.400
3	1	1.0	4.6	1.390
4	1	1.0	5.1	1.480
5	1	1.0	4.4	1.210
6	1	1.0	4.5	1.430
7	1	1.0	5.0	1.670
8	1	1.0	4.8	1.240
9	1	1.0	4.7	1.170
10	1	1.0	4.5	0.900
11	1	2.0	5.4	1.610
12	1	2.0	5.4	2.840
13	1	2.0	5.6	1.550
14	1	2.0	5.3	1.750
15	1	2.0	5.0	1.450
16	1	2.0	5.4	2.270
17	1	2.0	5.5	1.510
18	1	2.0	5.3	2.360
19	1	2.0	5.5	1.250
20	1	3.0	5.4	1.340
21	1	3.0	5.4	1.470
22	1	3.0	5.3	1.500
23	1	3.0	5.1	1.330
24	1	3.0	5.4	1.470
25	1	3.0	5.4	1.470
26	1	3.0	5.5	1.630
27	1	3.0	5.3	1.500
28	1	3.0	5.2	1.360
29	1	3.0	5.3	1.700
30	1	4.0	5.4	1.580
31	1	4.0	5.4	1.430
32	1	4.0	5.3	1.500
33	1	4.0	5.4	1.530
34	1	4.0	5.4	1.550
35	1	4.0	5.3	1.390
36	1	4.0	5.4	1.620
37	1	4.0	5.4	1.300
38	1	4.0	5.4	1.430
39	1	4.0	5.1	2.050
40	2	1.0	5.2	1.180
41	2	1.0	4.7	0.870
42	2	1.0	4.8	0.860
43	2	1.0	4.6	0.710
44	2	1.0	4.7	1.600
45	2	1.0	5.3	1.010
46	2	1.0	4.7	1.520
47	2	1.0	5.3	0.760
48	2	1.0	5.2	1.600
49	2	1.0	4.7	0.920
50	2	2.0	5.3	1.300
51	2	2.0	5.2	1.490
52	2	2.0	5.4	1.540
53	2	2.0	5.4	2.190
54	2	2.0	5.4	1.390
55	2	2.0	5.3	2.110

Data listing METHIDATHION Date: 02-21-1992

FILTER: None

Obs.	CONC	REP	LENGTH	WEIGHT
56	2	2.0	5.4	2.210
57	2	2.0	5.3	2.290
58	2	3.0	5.2	1.410
59	2	3.0	5.4	1.380
60	2	3.0	5.3	1.610
61	2	3.0	5.2	1.400
62	2	3.0	5.4	1.640
63	2	3.0	5.4	1.590
64	2	3.0	5.4	1.460
65	2	3.0	5.1	1.110
66	2	3.0	5.2	1.470
67	2	3.0	5.1	1.520
68	2	4.0	5.5	1.500
69	2	4.0	5.5	1.520
70	2	4.0	5.3	1.420
71	2	4.0	5.4	1.470
72	2	4.0	5.4	1.500
73	2	4.0	5.2	1.040
74	2	4.0	5.4	1.440
75	2	4.0	5.4	1.340
76	2	4.0	5.4	1.670
77	3	1.0	5.1	1.320
78	3	1.0	4.5	1.420
79	3	1.0	4.9	1.900
80	3	1.0	4.6	1.410
81	3	1.0	4.9	1.890
82	3	1.0	4.6	1.430
83	3	1.0	4.5	1.360
84	3	1.0	4.7	1.480
85	3	1.0	4.6	1.510
86	3	1.0	4.6	1.530
87	3	2.0	5.3	1.370
88	3	2.0	5.4	1.350
89	3	2.0	5.4	2.240
90	3	2.0	5.4	1.440
91	3	2.0	5.1	1.520
92	3	2.0	5.4	1.290
93	3	2.0	5.4	1.510
94	3	2.0	5.3	1.410
95	3	2.0	5.3	1.740
96	3	2.0	5.3	1.500
97	3	3.0	5.3	1.440
98	3	3.0	5.5	1.480
99	3	3.0	5.4	1.480
100	3	3.0	5.5	1.430
101	3	3.0	5.5	1.530
102	3	3.0	5.4	1.480
103	3	3.0	5.7	2.180
104	3	3.0	5.6	1.577
105	3	3.0	5.3	1.420
106	3	3.0	4.0	1.170
107	3	4.0	5.2	1.290
108	3	4.0	5.3	1.410
109	3	4.0	5.2	1.620
110	3	4.0	5.4	1.720

Data listing METHIDATHION Date: 02-21-1992

FILTER: None

Obs.	CONC	REP	LENGTH	WEIGHT
111	3	4.0	5.0	2.000
112	3	4.0	5.1	1.970
113	3	4.0	5.5	1.630
114	3	4.0	5.2	1.440
115	3	4.0	5.3	1.390
116	4	1.0	5.2	1.910
117	4	1.0	5.5	1.480
118	4	1.0	5.4	1.340
119	4	1.0	5.3	1.190
120	4	1.0	5.4	1.330
121	4	1.0	5.5	1.560
122	4	1.0	5.5	1.530
123	4	1.0	5.5	1.420
124	4	1.0	5.4	1.400
125	4	1.0	5.4	1.430
126	4	2.0	5.3	1.250
127	4	2.0	5.1	1.930
128	4	2.0	5.3	1.340
129	4	2.0	5.4	1.510
130	4	2.0	5.3	1.320
131	4	2.0	5.4	1.430
132	4	2.0	5.3	1.390
133	4	2.0	5.2	2.000
134	4	3.0	5.2	1.700
135	4	3.0	5.5	1.570
136	4	3.0	5.3	1.490
137	4	3.0	5.5	1.560
138	4	3.0	5.4	1.500
139	4	3.0	5.2	1.470
140	4	3.0	5.5	1.700
141	4	3.0	5.4	1.340
142	4	3.0	5.3	1.470
143	4	4.0	5.2	1.370
144	4	4.0	5.2	1.570
145	4	4.0	5.2	1.700
146	4	4.0	5.4	1.390
147	4	4.0	5.4	1.430
148	4	4.0	5.3	2.160
149	4	4.0	5.2	2.060
150	4	4.0	5.1	1.680
151	4	4.0	5.5	1.560
152	4	4.0	5.0	1.230
153	5	1.0	5.2	1.760
154	5	1.0	5.2	1.790
155	5	1.0	4.9	1.880
156	5	1.0	5.3	1.290
157	5	1.0	4.9	1.960
158	5	1.0	5.1	1.790
159	5	1.0	5.1	2.050
160	5	1.0	5.2	1.870
161	5	2.0	4.9	1.590
162	5	2.0	5.0	0.780
163	5	2.0	5.4	1.240
164	5	2.0	5.1	1.340
165	5	2.0	5.4	1.180

Data listing METHIDATHION Date: 02-21-1992

FILTER: None

Obs.	CONC	REP	LENGTH	WEIGHT
166	5	2.0	5.2	1.250
167	5	2.0	5.2	1.460
168	5	2.0	5.3	1.260
169	5	2.0	5.2	1.270
170	5	2.0	5.1	1.550
171	5	3.0	5.2	1.890
172	5	3.0	5.1	1.010
173	5	3.0	5.5	1.350
174	5	3.0	4.8	1.720
175	5	3.0	5.4	1.060
176	5	3.0	4.9	1.390
177	5	3.0	5.4	1.460
178	5	3.0	5.5	1.440
179	5	3.0	5.1	1.910
180	5	4.0	5.3	1.370
181	5	4.0	5.0	1.430
182	5	4.0	5.2	1.560
183	5	4.0	5.0	1.380
184	5	4.0	5.1	1.300
185	5	4.0	5.2	1.240
186	5	4.0	5.3	1.420
187	5	4.0	5.2	1.170
188	5	4.0	5.1	1.570