

MRID No. 437380-02

DATA EVALUATION RECORD
§ 72-4 - AQUATIC INVERTEBRATE LIFE CYCLE TEST

1. **CHEMICAL:** Disulfoton PC Code No.: 032501

2. **TEST MATERIAL:** ¹⁴C-Di-Syston sulfoxide Purity: 98.9%

3. **CITATION:**

Author: L.M. Bowers
Title: Chronic Toxicity of ¹⁴C-Di-Syston Sulfoxide to the Waterflea (*Daphnia magna*) Under Flow Through Conditions

Study Completion Date: June 15, 1995

Laboratory: Bayer Corporation, Stilwell, KS

Sponsor: Bayer Corporation, Kansas City, MO

Laboratory Report ID: DI840702

MRID No.: 437380-02

DP Barcode: Not reported

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist
Golder Associates Inc.

Signature: *Mark Mossler*

Date: 7/7/99

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
Golder Associates Inc.

Signature: *P. Kosalwat*

Date: 7/7/99

5. **APPROVED BY:**

Signature: *Henry Thoren*

Date: 7/12/99

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.

Results Synopsis: Most sensitive endpoint: growth

NOEC: 1.53 ppb ai LOEC: 2.97 ppb ai MATC: 2.13 ppb ai



LOEC's for specific endpoints:

Neonates Produced: >2.97 ppb ai
 Daphnid Survival: 6.49 ppb ai
 Time to First Brood: >6.49 ppb ai
 Length: 2.97 ppb ai
 Dry Weight: 2.97 ppb ai

8. ADEQUACY OF THE STUDY:A. **Classification:** CoreB. **Rationale:** N/AC. **Repairability:** N/A9. **GUIDELINE DEVIATIONS:** No deviations were noted.**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> Laboratory, commercial, or wild stock.	In-house cultures
<u>Parental Acclimation Conditions</u> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Held at 20 ±2°C in dilution water
<u>Parental Acclimation Period</u> At least 21 days.	Continuous
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	12 days
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Mixture of <i>Selenastrum capricornutum</i> and/or <i>Ankistrodesmus falcatus</i> , supplemented with yeast/trout chow/cereal leaf suspension

Guideline Criteria	Reported Information
<p>Food Concentration 5 mg/L (dry wt.) of synthetic food or 10^8 cells/L of algae is recommended.</p>	<p>1×10^7 cells/L 2-4 times daily, and 10 mg/L suspension daily</p>
<p>Were daphnids in good health during acclimation period?</p>	<p>Yes</p>

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>	<p>Hard blended water was comprised of soft process water and spring water. The water was particle filtered, carbon filtered, aerated, and UV sterilized.</p>
<p>Water Temperature $20^\circ\text{C} \pm 2^\circ\text{C}$. Must not deviate from 20°C by more than 5°C for more than 48 hours.</p>	<p>19.4-21.0°C</p>
<p>pH 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.</p>	<p>7.8-8.1</p>
<p>Total Hardness 160 to 180 mg/L as CaCO_3 is recommended.</p>	<p>164-188 mg/L as CaCO_3</p>
<p>Dissolved Oxygen <u>Renewal</u>: Must not drop below 50% for more than 48 hours. <u>Flow-through</u>: $\geq 60\%$ throughout test.</p>	<p>$\geq 65\%$ of saturation during the test</p>
<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>	<p>Glass 1-L beakers filled with 900 mL of solution</p>

Guideline Criteria	Reported Information
<p>Covers <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.</p>	Side drains of the beakers were covered with 40-mesh nylon screen.
<p>Type of Dilution System Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	Intermittent-flow proportional diluter
<p>Renewal Rate Three times weekly.</p>	9.5 volume additions per day
<p>Aeration Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	Solutions not aerated
<p>Photoperiod 16 hours light, 8 hours dark</p>	16 hours light, 8 hours dark
<p>Solvents 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>	Solvent: acetone Maximum conc.: 91 µL/L

C. Test Design:

Guideline Criteria	Reported Information
<p>Duration 21 days</p>	21 days
<p>Nominal Concentrations Control(s) and at least 5 test concentrations; dilution factor not less than 50%.</p>	Dilution water and solvent controls and five treatment concentrations: 0.38, 0.75, 1.5, 3.0, and 6.0 µg ai/L

Guideline Criteria	Reported Information
<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/level, four replicates per level</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Randomly distributed</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></p> <ol style="list-style-type: none"> 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>DO was measured in alternating replicates weekly.</p> <p>pH, alkalinity, hardness, and conductivity were measured in alternating replicates of both control groups and the low, middle, and high concentrations groups on the same schedule as DO.</p> <p>Temperature was measured daily in one chamber of each group and hourly in one centrally-located chamber.</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>Samples of solutions collected on days 0, 7, 14, and 21 were analyzed by LSC. A sample of the 6.0 µg ai/L solution was assayed by TLC to determine the percentage of parent material.</p>

12. REPORTED RESULTS:**A. General Results:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Percent Recovery of Chemical Percent of nominal, Procedural recovery, Limit of quantitation (LOQ)	99-108%, Procedural recovery of 91-98%, LOQ = 0.02 ppb
Control Mortality ≤ 30%	Control: 2% Solvent Control: 10%
Did daphnids in each control produce at least 40 young after 21 days?	Yes
Were no ephippia produced in any of the controls?	Not reported
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 parental daphnids, -Total number of offspring per replicate and number of offspring per female reproductive day, -Time to first brood, -Individual length and dry weight of surviving first-generation daphnids.
Raw data included?	Yes

Effects Data

Toxicant Concentration (ppb ai)		Average Terminal Survival (%)	Avg. Time to 1st Brood (days)	Average Young per Adult Repro. Day	Avg. Adult Length (mm)	Avg. Adult Dry Weight (mg)
Nominal	Measured* (RSD)					
Con.	<0.01 (N/A)	98	9.3	7.56	4.51	1.052
Sol. Con.	<0.01 (N/A)	90	9.5	6.63	4.51	0.855
0.38	0.38 (11%)	93	9.3	6.79	4.37	0.781
0.75	0.75 (8%)	90	9.0	6.95	4.42	0.877
1.5	1.53 (7%)	95	9.0	7.32	4.51	0.871
3.0	2.97 (6%)	89	9.0	5.70	4.25	0.662
6.0	6.49 (7%)	7.5	9.5	N/A	N/A	N/A

*Results of the TLC analysis confirmed that the compound was present entirely in the parent form.

Toxicity Observations: No abnormal observations were reported.

B. Statistical Results: Comparison was made to the pooled control data for all parameters.

Endpoint	Method	NOEC (ppb ai)	LOEC (ppb ai)
Survival	ANOVA and Dunnett's test	2.97	6.49
Reproduction	"	1.53	2.97
Length	"	1.53	2.97
Dry weight	"	1.53	2.97

Toxicant Concentration (ppb ai)		Average Terminal Survival (%)	Avg. Time to 1st Brood (days)	Average Young per Adult Repro. Day	Avg. Adult Length (mm)	Avg. Adult Dry Weight (mg)
Nominal	Measured (RSD)					
1.5	1.53 (7%)	95	9.0	7.32	4.51	0.871
3.0	2.97 (6%)	89	9.0	5.70	4.25	0.662
6.0	6.49 (7%)	7.5	9.5	N/A	N/A	N/A

*Results of the TLC analysis confirmed that the compound was present entirely in the parent form.

Toxicity Observations: No abnormal observations were reported.

B. Statistical Results: Comparison was made to the pooled control data for all parameters.

Endpoint	Method	NOEC (ppb ai)	LOEC (ppb ai)
Survival	ANOVA and Dunnett's test	2.97	6.49
Reproduction	"	1.53	2.97
Length	"	1.53	2.97
Dry weight	"	1.53	2.97

13. VERIFICATION OF STATISTICAL RESULTS: All comparisons were made to the solvent control data.

Endpoint	Method	NOEC (ppb ai)	LOEC (ppb ai)
Survival	Williams' test	2.97	6.49
Reproduction	"	2.97	>2.97*
Time to first brood	"	6.49	>6.49

Endpoint	Method	NOEC (ppb ai)	LOEC (ppb ai)
Length	Bonferroni's test	1.53	2.97
Dry weight	"	1.53	2.97

*6.49 ppb ai treatment level data were not included in the statistical analysis due to significant mortality.

14. **REVIEWER'S COMMENTS:** This study is scientifically sound, fulfills the requirements for a daphnid life-cycle test, and can be classified as **Core**. Based on the most sensitive endpoint (growth), the NOEC and LOEC are 1.53 and 2.97 ppb ai, respectively. The geometric mean MATC is 2.13 ppb ai.

Daphnia survival

File: dam2

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	4	0.900	1.254	1.284
2	0.38 ppb ai	4	0.925	1.290	1.284
3	0.75 ppb ai	4	0.900	1.254	1.284
4	1.53 ppb ai	4	0.950	1.336	1.284
5	2.97 ppb ai	4	0.875	1.225	1.225
6	6.49 ppb ai	4	0.075	0.281	0.281

Daphnia survival

File: dam2

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	1.284				
0.38 ppb ai	1.284	0.324		1.73	k= 1, v=18
0.75 ppb ai	1.284	0.324		1.82	k= 2, v=18
1.53 ppb ai	1.284	0.324		1.85	k= 3, v=18
2.97 ppb ai	1.225	0.321		1.86	k= 4, v=18
6.49 ppb ai	0.281	10.778	*	1.87	k= 5, v=18

s = 0.128

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

NOEC = 2.97 ppb ai

LOEC = 6.49 ppb ai

Daphnia time to first brood
 File: dam2 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	4	9.500	9.500	9.150
2	0.38 ppb ai	4	9.250	9.250	9.150
3	0.75 ppb ai	4	9.000	9.000	9.150
4	1.53 ppb ai	4	9.000	9.000	9.150
5	2.97 ppb ai	4	9.000	9.000	9.150
6	6.49 ppb ai	4	9.500	9.500	9.500

Daphnia time to first brood
 File: dam2 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	9.150				
0.38 ppb ai	9.150	1.266		1.73	k= 1, v=18
0.75 ppb ai	9.150	1.266		1.82	k= 2, v=18
1.53 ppb ai	9.150	1.266		1.85	k= 3, v=18
2.97 ppb ai	9.150	1.266		1.86	k= 4, v=18
6.49 ppb ai	9.500	0.000		1.87	k= 5, v=18

s = 0.391

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

6.49
 NOEC = ~~2.97~~ ppb ai
 LOEC > 6.49
 ATC
 7/12/99

Daphnia reproduction

File: dam2

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	4	6.650	6.650	6.931
2	0.38 ppb ai	4	6.800	6.800	6.931
3	0.75 ppb ai	4	6.925	6.925	6.931
4	1.53 ppb ai	4	7.350	7.350	6.931
5	2.97 ppb ai	4	5.700	5.700	5.700

Daphnia reproduction

File: dam2

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	6.931				
0.38 ppb ai	6.931	0.475		1.75	k= 1, v=15
0.75 ppb ai	6.931	0.475		1.84	k= 2, v=15
1.53 ppb ai	6.931	0.475		1.87	k= 3, v=15
2.97 ppb ai	5.700	1.604		1.88	k= 4, v=15

s = 0.838

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

NOEC = 2.97 ppb ai

TRT=1
 ~ Sol. Cont.

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	36	4.5083333	0.2394935	3.6000000	4.9000000
WEIGHT	36	0.8560278	0.1618520	0.3470000	1.0860000

TRT=2
 ~ 0.38 ppb ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	37	4.3729730	0.1880435	3.6000000	4.7000000
WEIGHT	37	0.7817297	0.1487536	0.2120000	0.9450000

TRT=3
 ~ 0.75 ppb ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	36	4.4250000	0.1338976	4.0000000	4.7000000
WEIGHT	36	0.8764167	0.1842231	0.4280000	1.2040000

TRT=4
 ~ 1.53 ppb ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	38	4.5078947	0.1806618	4.1000000	4.8000000
WEIGHT	38	0.8666316	0.1329510	0.5430000	1.0220000

TRT=5
 ~ 2.97 ppb ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	35	4.2457143	0.1335992	3.9000000	4.5000000
WEIGHT	35	0.6656286	0.0830390	0.4740000	0.8210000

Di-System Sulfoxide - DAPHNIA CHRONIC TEST
 13:22 Wednesday, June 30, 1999
 General Linear Models Procedure
 Class Level Information

Class	Levels	Values
TRT	5	1 2 3 4 5
REP	4	1 2 3 4

Number of observations in data set = 182

Di-System Sulfoxide - DAPHNIA CHRONIC TEST
 13:22 Wednesday, June 30, 1999
 General Linear Models Procedure

Dependent Variable: LENGTH

File:A:\dam2.sas Page 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.4663679	0.3523383	12.34	0.0001
Error	174	4.9692914	0.0285591		
Corrected Total	181	7.4356593			

R-Square 0.331695
 C.V. 3.828831
 Root MSE 0.1690
 LENGTH Mean 4.4137

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	4	1.7131976	0.4282994	15.00	0.0001
REP	3	0.7531703	0.2510568	8.79	0.0001

Di-System Sulfoxide - DAPHNIA CHRONIC TEST
 13:22 Wednesday, June 30, 1999
 General Linear Models Procedure

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1.3952765	0.1993252	9.88	0.0001
Error	174	3.5104385	0.0201749		
Corrected Total	181	4.9057150			

R-Square 0.284419
 C.V. 17.52361
 Root MSE 0.1420
 WEIGHT Mean 0.8106

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	4	1.1159649	0.2789912	13.83	0.0001
REP	3	0.2793115	0.0931038	4.61	0.0039

Di-System Sulfoxide - DAPHNIA CHRONIC TEST
 13:22 Wednesday, June 30, 1999
 General Linear Models Procedure
 Least Squares Means

TRT	LENGTH LSMEAN	PR > T HO: LSMEAN(i)=LSMEAN(j)	i/j	1	2	3	4	5
1	4.50585142	1	0.0009	0.0390	0.9629	0.0001		
2	4.37261809	2	0.0009	0.2049	0.0007	0.0026		
3	4.42299385	3	0.0390	0.2049	0.0327	0.0001		
4	4.50768439	4	0.9629	0.0007	0.0327	0.0001		
5	4.25070574	5	0.0001	0.0026	0.0001	0.0001		

TRT	WEIGHT	Pr > T	H0: LSMEAN(i)=LSMEAN(j)	4	5
	LSMEAN	i/j	1 2 3		
1	0.85698540	1	0.0222	0.5208	0.7538
2	0.78022828	2	0.0222	0.0036	0.0001
3	0.87853183	3	0.5208	0.0087	0.0007
4	0.86736223	4	0.7538	0.7359	0.0001
5	0.56405524	5	0.0001	0.0001	0.0001

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

Di-Syston Sulfoxide - DAPHNIA CHRONIC TEST
13:22 Wednesday, June 30, 1999

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= 174 MSE= 0.028559
Critical Value of T= 2.84327

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

TRT Comparison	Simultaneous Lower Confidence Limit		Difference Between Means	Simultaneous Upper Confidence Limit	
	Lower Confidence Limit	Upper Confidence Limit		Lower Confidence Limit	Upper Confidence Limit
1 - 4	-0.1132	0.00044	0.00044	0.11219	
1 - 3	-0.02992	0.08333	0.19659	0.19659	****
1 - 2	0.02287	0.13536	0.24785	0.24785	****
1 - 5	0.14856	0.26262	0.26262	0.37668	****
4 - 1	-0.11219	-0.00044	-0.00044	0.11132	
4 - 3	-0.02886	0.08289	0.19465	0.19465	****
4 - 2	0.02395	0.13492	0.16451	0.24590	****
4 - 5	0.14961	0.26218	0.26218	0.37475	****
3 - 1	-0.19659	-0.08333	-0.08333	0.02992	****
3 - 4	-0.19465	-0.08289	-0.08289	0.02886	****
3 - 2	-0.06046	0.05203	0.16451	0.16451	****
3 - 5	0.06523	0.17929	0.17929	0.29335	****
2 - 1	-0.24785	-0.13536	-0.13536	-0.02287	****
2 - 4	-0.24590	-0.13492	-0.13492	-0.02395	****
2 - 3	-0.16451	-0.05203	-0.05203	0.06046	****
2 - 5	0.01396	0.12726	0.12726	0.24056	****
5 - 1	-0.37668	-0.26262	-0.26262	-0.14856	****
5 - 4	-0.37475	-0.26218	-0.26218	-0.14961	****
5 - 3	-0.29335	-0.17929	-0.17929	-0.06523	****
5 - 2	-0.24056	-0.12726	-0.12726	-0.01396	****

Di-Syston Sulfoxide - DAPHNIA CHRONIC TEST
13:22 Wednesday, June 30, 1999

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= 174 MSE= 0.020175
Critical Value of T= 2.84327

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

TRT Comparison	Simultaneous Lower Confidence Limit		Difference Between Means	Simultaneous Upper Confidence Limit	
	Lower Confidence Limit	Upper Confidence Limit		Lower Confidence Limit	Upper Confidence Limit
3 - 4	-0.08414	0.00979	0.00979	0.10371	
3 - 1	-0.07480	0.02039	0.02039	0.11558	****
3 - 2	0.00014	0.09469	0.09469	0.18923	****
3 - 5	0.11492	0.21079	0.21079	0.30665	****
4 - 3	-0.10371	-0.00979	-0.00979	0.08414	
4 - 1	-0.08332	0.01060	0.01060	0.10453	
4 - 2	-0.00837	0.08490	0.08490	0.17818	
4 - 5	0.10639	0.20100	0.20100	0.29562	****
1 - 3	-0.11558	-0.02039	-0.02039	0.07480	
1 - 4	-0.10453	-0.01060	-0.01060	0.08332	
1 - 2	-0.02025	0.07430	0.07430	0.16884	****
1 - 5	0.09453	0.19040	0.19040	0.28627	****
2 - 3	-0.18923	-0.09469	-0.09469	-0.00014	****
2 - 4	-0.17818	-0.08490	-0.08490	0.00837	****
2 - 1	-0.16884	-0.07430	-0.07430	0.02025	****
2 - 5	0.02088	0.11610	0.11610	0.21133	****
5 - 3	-0.30665	-0.21079	-0.21079	-0.11492	****
5 - 4	-0.29562	-0.20100	-0.20100	-0.10639	****
5 - 1	-0.28627	-0.19040	-0.19040	-0.09453	****
5 - 2	-0.21133	-0.11610	-0.11610	-0.02088	****

Di-Syston Sulfoxide - DAPHNIA CHRONIC TEST
13:22 Wednesday, June 30, 1999

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= 174 MSE= 0.028559
Critical Value of Dunnett's T= 2.175

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

TRT Comparison	Simultaneous Lower Confidence Limit		Difference Between Means	Simultaneous Upper Confidence Limit	
	Lower Confidence Limit	Upper Confidence Limit		Lower Confidence Limit	Upper Confidence Limit
4 - 1	-0.08593	-0.00044	-0.00044	0.08505	
3 - 1	-0.16997	-0.08333	-0.08333	0.00331	****
2 - 1	-0.22141	-0.13536	-0.13536	-0.04931	****
5 - 1	-0.34988	-0.26262	-0.26262	-0.17536	****

Di-Syston Sulfoxide - DAPHNIA CHRONIC TEST
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not considered treatment-related

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= 174 MSE= 0.020175
Critical Value of Dunnett's T= 2.175

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

TRT Comparison	Simultaneous Lower Confidence Limit		Difference Between Means		Simultaneous Upper Confidence Limit	
	Lower Limit	Upper Limit	Mean	Standard Error	Mean	Standard Error
3 - 1	-0.05243	0.02039	0.02039	0.09321	0.09321	0.09321
4 - 1	-0.06125	0.01060	0.01060	0.08246	0.08246	0.08246
2 - 1	-0.14662	-0.07430	-0.07430	-0.00197	-0.00197	***
5 - 1	-0.26374	-0.19040	-0.19040	-0.11706	-0.11706	***

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM
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General Linear Models Procedure
Class Level Information

Class	Levels	Values
REP	4	1 2 3 4
TRT	5	1 2 3 4 5

Number of observations in data set = 182

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM
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General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	3.5746712	0.1881406	7.89	0.0001
Error	162	3.8609881	0.0238333		
Corrected Total	181	7.4356593			

R-Square 0.480747
C.V. 3.497722
Root MSE 0.1544
LENGTH Mean 4.4137

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	3	0.8380506	0.2793502	11.72	0.0001
TRT	4	1.6283173	0.4070793	17.08	0.0001
REP*TRT	12	1.1083033	0.0923586	3.88	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	3	0.6721791	0.2240597	9.40	0.0001
TRT	4	1.6741528	0.4185332	17.56	0.0001

General Linear Models Procedure

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	4	1.6741528	0.4185332	4.53	0.0184

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM
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General Linear Models Procedure

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	3.0374643	0.1598665	13.86	0.0001
Error	162	1.8682507	0.0115324		
Corrected Total	181	4.9057150			

R-Square 0.619169
C.V. 13.24883
Root MSE 0.1074
WEIGHT Mean 0.8106

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	3	0.2494382	0.0831461	7.21	0.0001
TRT	4	1.1458382	0.2864596	24.84	0.0001
REP*TRT	12	1.6421878	0.1368490	11.87	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	3	0.3053214	0.1017738	8.83	0.0001
TRT	4	1.2264502	0.3066126	26.59	0.0001
REP*TRT	12	1.6421878	0.1368490	11.87	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	4	1.2264502	0.3066126	2.24	0.1255

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM
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General Linear Models Procedure
Least Squares Means

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

TRT	LENGTH LSMEAN	Pr > T 1/j	H0: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5
1	4.50597222	1	0.0846	0.2810	0.9962	0.0036		
2	4.37194444	2	0.0846	0.4722	0.0818	0.1026		
3	4.42486111	3	0.2810	0.4722	0.2772	0.0288		
4	4.50562500	4	0.9962	0.0818	0.2772	0.0034		
5	4.24468254	5	0.0036	0.1026	0.0288	0.0034		

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

TRT	WEIGHT	Pr > T 1/j	H0: LSMEAN(i)=LSMEAN(j)
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	LSMEAN	i/j	1	2	3	4	5
1	0.86021250	1	0.3792	0.7647	0.9060	0.0442	
2	0.78096944	2	0.3792	0.2453	0.3160	0.1985	
3	0.88699514	3	0.7647	0.2453	0.8528	0.0254	
4	0.87062500	4	0.9060	0.3160	0.8528	0.0338	
5	0.66165556	5	0.0442	0.1985	0.0254	0.0338	

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
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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= 12 MSE= 0.092359
Critical Value of Dunnett's T= 2.408

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

TRT Comparison	Simultaneous Lower Confidence Limit		Difference Between Means		Simultaneous Upper Confidence Limit	
	4 - 1	-0.17068	-0.00044	-0.00044	0.16980	0.16980
3 - 1	-0.25586	-0.08333	-0.08333	0.08919	0.08919	
2 - 1	-0.30671	-0.13536	-0.13536	0.03599	0.03599	
5 - 1	-0.45637	-0.26262	-0.26262	-0.08887	-0.08887	***

ANALYSIS USING TRT*REP INTERACTION AS THE ERROR TERM
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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= 12 MSE= 0.136849
Critical Value of Dunnett's T= 2.408

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

TRT Comparison	Simultaneous Lower Confidence Limit		Difference Between Means		Simultaneous Upper Confidence Limit	
	3 - 1	-0.18962	0.02039	0.02039	0.23039	0.23039
4 - 1	-0.19662	0.01060	0.01060	0.21783	0.21783	
2 - 1	-0.28288	-0.07430	-0.07430	0.13428	0.13428	
5 - 1	-0.40190	-0.19040	-0.19040	0.02110	0.02110	