

MRID No. 437380-01

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

- 1. **CHEMICAL:** Disulfoton PC Code No.: 032501
- 2. **TEST MATERIAL:** <sup>14</sup>C-Di-Syston sulfone Purity: 99.3%

3. **CITATION:**

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

Study Completion Date: June 26, 1995  
Laboratory: Bayer Corporation, Stilwell, KS  
Sponsor: Bayer Corporation, Kansas City, MO  
Laboratory Report ID: DI840701  
MRID No.: 437380-01  
DP Barcode: Not reported

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

Signature:  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: Date: 7/9/99

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 (length)

NOEC: 0.14 ppb ai      LOEC: 0.27 ppb ai      MATC: 0.19 ppb ai



LOEC's for specific endpoints:

Neonates Produced: 1.12 ppb ai  
 Time to First Brood: 2.42 ppb ai  
 Daphnid Survival: 2.42 ppb ai  
 Length: 0.27 ppb ai  
 Dry Weight: 1.12 ppb ai

8. ADEQUACY OF THE STUDY:A. Classification: CoreB. Rationale: N/AC. Repairability: N/A9. GUIDELINE DEVIATIONS: No deviations of major importance 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.	16 days

Guideline Criteria	Reported Information
<b>Food</b> 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
<b>Food Concentration</b> 5 mg/L (dry wt.) of synthetic food or $10^8$ cells/L of algae is recommended.	$1 \times 10^7$ cells/L 2-4 times daily, and 12 mg/L suspension daily
<b>Were daphnids in good health during acclimation period?</b>	Yes

**B. Test System:**

Guideline Criteria	Reported Information
<b>Test Water</b> Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details)	Hard blended water was comprised of soft process water and spring water. The water was particle filtered, carbon filtered, aerated, and UV sterilized.
<b>Water Temperature</b> $20^\circ\text{C} \pm 2^\circ\text{C}$ . Must not deviate from $20^\circ\text{C}$ by more than $5^\circ\text{C}$ for more than 48 hours.	$19.4-20.5^\circ\text{C}$
<b>pH</b> 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.	7.6-8.0
<b>Total Hardness</b> 160 to 180 mg/L as $\text{CaCO}_3$ is recommended.	164-172 mg/L as $\text{CaCO}_3$
<b>Dissolved Oxygen</b> <u>Renewal</u> : Must not drop below 50% for more than 48 hours. <u>Flow-through</u> : $\geq 60\%$ throughout test.	$\geq 56\%$ of saturation during the test

Guideline Criteria	Reported Information
<p><b><u>Test Vessels or Compartments</u></b>            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</p> <p>1-L beakers filled with 900 mL of solution</p>
<p><b><u>Covers</u></b>  <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>	<p>Side drains of the beakers were covered with 40-mesh nylon screen.</p>
<p><b><u>Type of Dilution System</u></b>            Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	<p>Intermittent-flow proportional diluter</p>
<p><b><u>Renewal Rate</u></b>            Three times weekly.</p>	<p>8.5 volume additions per day</p>
<p><b><u>Aeration</u></b>            Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	<p>Solutions not aerated</p>
<p><b><u>Photoperiod</u></b>            16 hours light, 8 hours dark</p>	<p>16 hours light, 8 hours dark</p>
<p><b><u>Solvents</u></b>            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: acetone            Maximum conc.: 91 <math>\mu</math>L/L</p>

**C. Test Design:**

Guideline Criteria	Reported Information
<u>Duration</u> 21 days	21 days
<u>Nominal Concentrations</u> Control(s) and at least 5 test concentrations; dilution factor not less than 50%.	Dilution water and solvent controls and five treatment concentrations: 0.16, 0.31, 0.63, 1.25, and 2.5 $\mu\text{g ai/L}$
<u>Number of Test Organisms</u> 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.	40 daphnids/level, four replicates per level
<u>Test organisms randomly or impartially assigned to test vessels?</u>	Randomly distributed
<u>Renewal</u> Parent daphnids in all beakers must be transferred to containers with fresh test solution (< 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).	N/A
<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.	DO was measured in alternating replicates weekly.  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.  Temperature was measured daily in one chamber of each group and hourly in one centrally-located chamber.

Guideline Criteria	Reported Information
<p><b><u>Chemical Analysis</u></b>            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 1.25 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
<p><b>Quality assurance and GLP compliance statements were included in the report?</b></p>	<p>Yes</p>
<p><b>Percent Recovery of Chemical</b>            Percent of nominal,            Procedural recovery,            Limit of quantitation (LOQ)</p>	<p>86-97%,            Procedural recovery of 90-104%,            LOQ = 0.002 ppb</p>
<p><b><u>Control Mortality</u></b>            ≤ 30%</p>	<p>Control: 2%            Solvent Control: 10%</p>
<p><b>Did daphnids in each control produce at least 40 young after 21 days?</b></p>	<p>Yes</p>
<p><b>Were no ephippia produced in any of the controls?</b></p>	<p>Not reported</p>
<p><b><u>Data Endpoints</u></b>            - 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.</p>	<p>-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.</p>
<p><b>Raw data included?</b></p>	<p>Yes</p>

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	8.3	8.48	4.60	1.161
Sol. Con.	<0.01 (N/A)	90	9.3	8.13	4.40	0.745
0.16	0.14 (7%)	95	9.0	7.50	4.31	0.547
0.31	0.27 (4%)	93	9.0	8.04	4.29	0.633
0.63	0.54 (4%)	88	9.3	7.89	4.25	0.672
1.25	1.12 (4%)	85	9.0	6.25	4.05	0.348
2.50	2.42 (1%)	0	10.0	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 for survival and length and to the solvent control for reproduction and dry weight.

Endpoint	Method	NOEC (ppb ai)	LOEC (ppb ai)
Survival	ANOVA and Dunnett's test	1.12	2.42
Reproduction	"	0.54	1.12
Length	"	0.54	1.12
Dry weight	"	0.54	1.12

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	1.12	2.42
Reproduction	"	0.54	1.12
Time to first brood	"	1.12	2.42
Length	Bonferroni's test	0.14	0.27
Dry weight	"	0.54	1.12

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 (length), the NOEC and LOEC are 0.19 and 0.27 ppb ai, respectively. The geometric mean MATC is 0.19 ppb ai.



Daphnid survival

File: dam 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	1.260	1.299
2	0.14 ppb ai	4	0.950	1.331	1.299
3	0.27 ppb ai	4	0.925	1.307	1.299
4	0.54 ppb ai	4	0.875	1.219	1.219
5	1.12 ppb ai	4	0.850	1.190	1.190
6	2.42 ppb ai	4	0.000	0.159	0.159

Daphnid survival

File: dam 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.	1.299				
0.14 ppb ai	1.299	0.367		1.73	k= 1, v=18
0.27 ppb ai	1.299	0.367		1.82	k= 2, v=18
0.54 ppb ai	1.219	0.380		1.85	k= 3, v=18
1.12 ppb ai	1.190	0.650		1.86	k= 4, v=18
2.42 ppb ai	0.159	10.256	*	1.87	k= 5, v=18

s = 0.152

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

*NOEC = 1.12 ppb ai*  
*LOEC = 2.42 ppb ai*

Daphnid time to first brood

File: dam 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	9.250	9.250	9.083
2	0.14 ppb ai	4	9.000	9.000	9.083
3	0.27 ppb ai	4	9.000	9.000	9.083
4	0.54 ppb ai	4	9.250	9.250	9.125
5	1.12 ppb ai	4	9.000	9.000	9.125
6	2.42 ppb ai	4	10.000	10.000	10.000

Daphnid time to first brood

File: dam 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.	9.083				
0.14 ppb ai	9.083	0.817		1.73	k= 1, v=18
0.27 ppb ai	9.083	0.817		1.82	k= 2, v=18
0.54 ppb ai	9.125	0.612		1.85	k= 3, v=18
1.12 ppb ai	9.125	0.612		1.86	k= 4, v=18
2.42 ppb ai	10.000	3.674	*	1.87	k= 5, v=18

s = 0.289

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

*NOEC = 1.12 ppb ai*

*LOEC = 2.42 ppb ai*

Young per adult reproductive day  
 File: dam 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	8.150	8.150	8.150
2	0.14 ppb ai	4	7.500	7.500	7.800
3	0.27 ppb ai	4	8.025	8.025	7.800
4	0.54 ppb ai	4	7.875	7.875	7.800
5	1.12 ppb ai	4	6.275	6.275	6.275

Young per adult reproductive day  
 File: dam 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.	8.150				
0.14 ppb ai	7.800	0.391		1.75	k= 1, v=15
0.27 ppb ai	7.800	0.391		1.84	k= 2, v=15
0.54 ppb ai	7.800	0.391		1.87	k= 3, v=15
1.12 ppb ai	6.275	2.097	*	1.88	k= 4, v=15

s = 1.265

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

*NOEC = 0.54 ppb ai*

*LOEC = 1.12 ppb ai*

Daphnia length

File: dam

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	4.400	4.400	4.400
2	0.14 ppb ai	4	4.313	4.313	4.313
3	0.27 ppb ai	4	4.290	4.290	4.290
4	0.54 ppb ai	4	4.255	4.255	4.255
5	1.12 ppb ai	4	4.052	4.052	4.052

Daphnia length

File: dam

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.	4.400				
0.14 ppb ai	4.313	0.839		1.75	k= 1, v=15
0.27 ppb ai	4.290	1.055		1.84	k= 2, v=15
0.54 ppb ai	4.255	1.391		1.87	k= 3, v=15
1.12 ppb ai	4.052	3.333	*	1.88	k= 4, v=15

s = 0.147

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

*NOEC = 0.54 ppb ai*

*LOEC = 1.12 ppb ai*

Daphnia dry weight

File: dam Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	4	0.745	-0.149	-0.149
2	0.14 ppb ai	4	0.552	-0.259	-0.217
3	0.27 ppb ai	4	0.633	-0.216	-0.217
4	0.54 ppb ai	4	0.672	-0.175	-0.217
5	1.12 ppb ai	4	0.349	-0.459	-0.459

Daphnia dry weight

File: dam Transform: LOG BASE 10(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.	-0.149				
0.14 ppb ai	-0.217	0.974		1.75	k= 1, v=15
0.27 ppb ai	-0.217	0.974		1.84	k= 2, v=15
0.54 ppb ai	-0.217	0.974		1.87	k= 3, v=15
1.12 ppb ai	-0.459	4.453	*	1.88	k= 4, v=15

s = 0.098

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

*NOEC = 0.54 ppb ai*

*LOEC = 1.12 ppb ai*

TRT=1

--- Sol. Con. ---

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	36	4.4194444	0.2423922	4.0000000	4.9000000
WEIGHT	36	0.7565556	0.2792418	0.2260000	1.3520000

TRT=2

= 0.14 ppb ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	38	4.3131579	0.1298045	4.0000000	4.5000000
WEIGHT	38	0.5534737	0.0923023	0.2660000	0.7060000

TRT=3

= 0.27 ppb ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	38	4.2947368	0.1505797	4.0000000	4.7000000
WEIGHT	38	0.6453158	0.1992257	0.2020000	1.0410000

TRT=4

= 0.54 ppb ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	35	4.2542857	0.1482106	4.0000000	4.5000000
WEIGHT	35	0.6695714	0.1009479	0.4420000	0.8280000

TRT=5

= 1.12 ppb ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	35	4.0457143	0.1633165	3.8000000	4.4000000
WEIGHT	35	0.3508286	0.0851903	0.1730000	0.5490000

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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

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General Linear Models Procedure

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.8359422	0.4051346	14.09	0.0001
Error	174	5.0027941	0.0287517		
Corrected Total	181	7.8387363			
R-Square		C.V.	Root MSE	LENGTH Mean	
		0.361786	0.1696	4.2676	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	4	2.662647	0.665662	23.18	0.0001
REP	3	0.1696775	0.0565592	1.97	0.1207
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	4	2.7025054	0.6756264	23.50	0.0001
REP	3	0.1696775	0.0565592	1.97	0.1207

General Linear Models Procedure

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	4.5614025	0.6516289	28.87	0.0001
Error	174	3.9271839	0.0225700		
Corrected Total	181	8.4885864			

R-Square C.V. Root MSE WEIGHT Mean

0.537357 25.19948 0.1502 0.5962

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	4	3.3824083	0.8456021	37.47	0.0001
REP	3	1.1785942	0.3929981	17.41	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	4	3.4181796	0.8545449	37.86	0.0001
REP	3	1.1789942	0.3929981	17.41	0.0001

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General Linear Models Procedure  
 Least Squares Means

TRT	LENGTH LSMEAN	Pr >  T  H0: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5
1	4.41639913						
2	4.3160041	1	0.0108	0.0022	0.0001	0.0001	0.0001
3	4.29396394	2	0.0022	0.5968	0.2967	0.0001	0.0001
4	4.25254121	3	0.0001	0.1191	0.2967	0.0001	0.0001
5	4.04143537	4	0.0001	0.0001	0.0001	0.0001	0.0001

TRT	WEIGHT LSMEAN	Pr >  T  1/j	H0: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5
1	0.75239585	1	0.0001	0.0018	0.0310	0.0001	0.0001	0.0001
2	0.55544407	2	0.0001	0.0134	0.0009	0.0001	0.0001	0.0001
3	0.64165207	3	0.0018	0.0134	0.3480	0.0001	0.0001	0.0001
4	0.67480405	4	0.0310	0.0009	0.3480	0.0001	0.0001	0.0001
5	0.34639961	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

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

Di-System Sulfone - DAPHNIA CHRONIC TEST  
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General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: LENGTH

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

Alpha= 0.05 Confidence= 0.95 df= 174 MSE= 0.028752  
Critical Value of T= 2.84327

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

TRT Comparison	Simultaneous		Difference Between Means	Simultaneous		Significance
	Lower Limit	Upper Limit		Lower Limit	Upper Limit	
1 - 2	-0.00584	0.21842	0.10629	0.21842	***	
1 - 3	0.01258	0.23684	0.12471	0.23684	***	
1 - 4	0.05071	0.27960	0.15116	0.27960	***	
1 - 5	0.25929	0.48817	0.37573	0.48817	***	
2 - 1	-0.21842	0.00584	-0.10629	0.00584	***	
2 - 3	-0.09218	0.12903	0.01842	0.12903	***	
2 - 4	-0.05408	0.17182	0.05887	0.17182	***	
2 - 5	0.15449	0.38039	0.26744	0.38039	***	
3 - 1	-0.23684	-0.01258	-0.12471	-0.01258	***	
3 - 2	-0.12903	0.09218	-0.01842	0.09218	***	
3 - 4	-0.07250	0.15340	0.04045	0.15340	***	
3 - 5	0.13607	0.36197	0.24902	0.36197	***	
4 - 1	-0.27960	-0.05071	-0.16516	-0.05071	***	
4 - 2	-0.17182	0.05408	-0.05887	0.05408	***	
4 - 3	-0.15340	0.07250	-0.04045	0.07250	***	
4 - 5	0.09332	0.32382	0.20857	0.32382	***	
5 - 1	-0.48817	-0.25929	-0.37373	-0.25929	***	
5 - 2	-0.38039	-0.15449	-0.26744	-0.15449	***	
5 - 3	-0.36197	-0.13607	-0.24902	-0.13607	***	
5 - 4	-0.32382	-0.09332	-0.20857	-0.09332	***	

Di-System Sulfone - DAPHNIA CHRONIC TEST  
14:52 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= 0.02257  
Critical Value of T= 2.84327

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

TRT Comparison	Simultaneous		Difference Between Means	Simultaneous		Significance
	Lower Limit	Upper Limit		Lower Limit	Upper Limit	
1 - 4	-0.01441	0.18838	0.08698	0.18838	***	
1 - 3	0.01189	0.21059	0.11124	0.21059	***	
1 - 2	0.10373	0.30243	0.20308	0.30243	***	
1 - 5	0.30433	0.40573	0.40573	0.50712	***	
4 - 1	-0.18838	-0.01441	-0.08698	-0.01441	***	
4 - 3	-0.07582	0.12433	0.02426	0.12433	***	
4 - 2	0.01602	0.11610	0.11610	0.21617	***	
4 - 5	0.21663	0.42085	0.31874	0.42085	***	
3 - 1	-0.21059	-0.11124	-0.11124	-0.01189	***	
3 - 4	-0.12433	-0.02426	-0.02426	0.07582	***	
3 - 2	-0.00615	0.09184	0.09184	0.18984	***	
3 - 5	0.19441	0.29449	0.29449	0.39456	***	
2 - 1	-0.30243	-0.20308	-0.20308	-0.10373	***	
2 - 4	-0.21617	-0.11610	-0.11610	-0.01602	***	
2 - 3	-0.18984	-0.09184	-0.09184	0.00615	***	
2 - 5	0.10257	0.20265	0.20265	0.30272	***	
5 - 1	-0.50712	-0.40573	-0.40573	-0.30433	***	
5 - 4	-0.42085	-0.31874	-0.31874	-0.21663	***	
5 - 3	-0.39456	-0.29449	-0.29449	-0.19441	***	
5 - 2	-0.30272	-0.20265	-0.20265	-0.10257	***	

\*\*\*3 Not considered  
treatment -  
related since  
treatment 4 value  
was higher than  
treatments 2 & 3.  
values.

Di-System Sulfone - DAPHNIA CHRONIC TEST  
14:52 Wednesday, June 30, 1999

General Linear Models Procedure

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

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

Alpha= 0.05 Confidence= 0.95 df= 174 MSE= 0.028752  
Critical Value of Dunnett's T= 2.175

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

TRT Comparison	Simultaneous		Difference Between Means	Simultaneous		Significance
	Lower Limit	Upper Limit		Lower Limit	Upper Limit	
2 - 1	-0.19207	-0.10629	-0.10629	-0.02051	***	
3 - 1	-0.21049	-0.12471	-0.12471	-0.03893	***	
4 - 1	-0.25271	-0.16516	-0.16516	-0.07761	***	
5 - 1	-0.46128	-0.37373	-0.37373	-0.28618	***	

Di-System Sulfone - DAPHNIA CHRONIC TEST  
14:52 Wednesday, June 30, 1999

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= 0.02257  
Critical Value of Dunnett's T= 2.175

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

TRT Comparison	Simultaneous		Difference		Simultaneous	
	Lower Limit	Upper Limit	Between Means	Confidence Limit	Lower Limit	Upper Limit
4 - 1	-0.16455	-0.08698	-0.00941	***	-0.00941	***
3 - 1	-0.18724	-0.11124	-0.03524	***	-0.03524	***
2 - 1	-0.27908	-0.20308	-0.12708	***	-0.12708	***
5 - 1	-0.48330	-0.40573	-0.32816	***	-0.32816	***

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
14:52 Wednesday, June 30, 1999

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  
14:52 Wednesday, June 30, 1999

General Linear Models Procedure

pendent Variable:	LENGTH	DF	Sum of Squares	Mean Square	F Value	Pr > F
urce		19	5.6061172	0.2950588	21.41	0.0001
del		162	2.2326190	0.0137816		
ror		181	7.8387363			
orrected Total						

R-Square	C.V.	Root MSE	LENGTH Mean
0.715181	2.750856	0.1174	4.2676

urce	DF	Type I SS	Mean Square	F Value	Pr > F
P	3	0.1334368	0.0444789	3.23	0.0241
T	4	2.7025054	0.6756264	49.02	0.0001
P*TRT	12	2.7701750	0.2308479	16.75	0.0001
urce	DF	Type III SS	Mean Square	F Value	Pr > F
P	3	0.1669235	0.0556412	4.04	0.0084
T	4	2.3026346	0.5756586	41.77	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	2.3026346	0.5756586	2.49	0.0987

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

Dependent Variable: WEIGHT	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	19	7.0542236	0.3712749	41.93	0.0001
Model					
Error	162	1.4343628	0.0088541		
Corrected Total	181	8.4885864			

R-Square	C.V.	Root MSE	WEIGHT Mean
0.831025	15.78329	0.0941	0.5962

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	3	1.1432229	0.3810743	43.04	0.0001
TRT	4	3.4181796	0.8545449	96.51	0.0001
REP*TRT	12	2.4928211	0.2077351	23.46	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	3	1.1251043	0.3750348	42.36	0.0001
TRT	4	3.2040628	0.8010157	90.47	0.0001
REP*TRT	12	2.4928211	0.2077351	23.46	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	3.2040628	0.8010157	3.86	0.0307

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
14:52 Wednesday, June 30, 1999

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  H0: LSMEAN(i)=LSMEAN(j)
1	4.39937500	1 0.4537 0.3469 0.2267 0.0107
2	4.31250000	2 0.4537 0.8378 0.6093 0.0404
3	4.28937500	3 0.3469 0.8378 0.7549 0.0586
4	4.25326389	4 0.2267 0.6093 0.7549 0.1081
5	4.05223214	5 0.0107 0.0404 0.0586 0.1081

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

TRT	WEIGHT	Pr >  T  H0: LSMEAN(i)=LSMEAN(j)
-----	--------	----------------------------------



	1/j	2	3	4	5
1	0.74476875	0.0950	0.3144	0.5164	0.0034
2	0.55190833	0.0950	0.4552	0.2839	0.0828
3	0.63285000	0.3144	0.4552	0.7212	0.0215
4	0.67204306	0.5164	0.2839	0.7212	0.0122
5	0.34846607	0.0034	0.0215	0.0122	

E: 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

Dunnnett'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= 0.230848  
Critical Value of Dunnnett's T= 2.409

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
2 - 1	-0.3754	-0.1063	-0.1247	0.1629	0.1444
3 - 1	-0.3939	-0.1247	-0.1652	0.1095	0.0990
4 - 1	-0.4399	-0.1652	-0.3737	-0.0990	****
5 - 1	-0.6484	-0.3737			

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
14:52 Wednesday, June 30, 1999

General Linear Models Procedure

Dunnnett'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= 0.207735  
Critical Value of Dunnnett's T= 2.409

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	-0.3476	-0.0870	-0.1112	0.1736	0.1441
3 - 1	-0.3666	-0.1112	-0.2031	0.0522	0.0522
2 - 1	-0.4584	-0.2031	-0.4057	-0.1451	****
5 - 1	-0.6663	-0.4057			