

US EPA ARCHIVE DOCUMENT

MRID No. 437457-01

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

- 1. **CHEMICAL:** Permethrin PC Code No.: 109701
- 2. **TEST MATERIAL:** ¹⁴C-Permethrin Purity: >98.6% radio purity

3. **CITATION:**

Authors: S.J. Kent, N.J. Williams, E. Gillings,
and D.S. Morris
Title: Permethrin: Chronic Toxicity to *Daphnia magna*

Study Completion Date: May 12, 1995

Laboratory: Zeneca Brixham Environmental Laboratory,
Brixham, Devon, U.K.

Sponsor: ZENECA Inc., Wilmington, DE

Laboratory Report ID: BL5443/B

MRID No.: 437457-01

DP Barcode: D218413

- 4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Associate Scientist
KBN Engineering and Applied Sciences, Inc.

Signature: *[Handwritten Signature]* Date: 2/23/96

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

Signature: *P. Kosalwat* Date: 2/23/96

- 5. **APPROVED BY:** *[Handwritten Signature]*

Signature: Date: 3/13/96

6. **STUDY PARAMETERS:**

Age of Test Organism: <24 hours
Definitive Test Duration: 21 days
Study Method: Flow-Through
Type of Concentrations: Mean Measured

*N. Cook
03/18/96*

- 7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater invertebrate life-cycle test using *Daphnia magna*.

Results Synopsis:

NOEC: 39 ng/L

LOEC: 84 ng/L

MATC: 57 ng/L



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LOEC's for specific effects:

- Neonates Produced: 84 ng/L
- Daphnid Survival: 340 ng/L
- Growth (weight): >340 ng/L
- Growth (length): 84 ng/L

8. ADEQUACY OF THE STUDY:

- A. Classification:** Core.
- B. Rationale:** Fulfills requirement.
- C. Repairability:** N/A.

9. GUIDELINE DEVIATIONS:

1. Several deviations from the EPA SEP were noted. However, the SEP was written for a static-renewal test. Therefore, the study design may not be practical when used in a flow-through test such as this.
2. Dechlorinated tap water was to prepare the reconstituted dilution water. However, the levels of residual and total chlorine were measured every weekday during the study and were less than 4 µg/L.
3. The pH (8.4-8.5) and total hardness (183-205 mg/L as CaCO₃) of the test solutions were slightly higher than recommended.

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>	In-house cultures of Brixham Environmental Laboratory.
<u>Parental Acclimation Conditions</u> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Parental cultures were maintained in a <i>Daphnia</i> culture medium at 20 ± 2°C.

Guideline Criteria	Reported Information
<u>Parental Acclimation Period</u> At least 21 days.	Daphnids were isolated and maintained as a single culture for at least 19±1 days prior to collection of neonates for testing.
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	Parental stock was 19±1 days old.
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Daphnids in each vessel were fed <i>Chlorella vulgaris</i> and "Frippak Booster®."
<u>Food Concentration</u> 5 mg/L (dry wt.) of synthetic food or 10 ⁸ cells/L of algae is recommended.	Daphnids in each vessel were fed twice daily with 1.25-1.75 ml of algal stock solution (containing 1.2 X 10 ⁸ cells/ml) and 57 µg of "Frippak Booster®."
Were daphnids in good health during acclimation period?	Yes.

B. Test System:

Guideline Criteria	Reported Information
<u>Test Water</u> Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).	Reconstituted dechlorinated tap water. The tap water was dechlorinated with sodium thiosulphate, UV sterilized, and filtered (activated carbon and 25- and 10-micron filters) prior to use.
<u>Water Temperature</u> 20°C ±2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.	Range: 19.4-20.6°C

Guideline Criteria	Reported Information
<p>pH 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.</p>	<p>Range: 8.4-8.5</p>
<p>Total Hardness 160 to 180 mg/L as CaCO₃ is recommended.</p>	<p>Range: 183-205 mg/L as CaCO₃</p>
<p>Dissolved Oxygen <u>Renewal</u>: must not drop below 50% for more than 48 hours. <u>Flow-through</u>: ≥ 60% throughout test.</p>	<p>≥92% of saturation at all time.</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>1. Borosilicate glass. 2. 1000-ml beakers containing 800 ml of test solutions.</p>
<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>	<p>Each test chamber had a hole cut into the side of the beaker which was covered with nylon mesh.</p>
<p>Type of Dilution System Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	<p>Continuous-flow diluter.</p>
<p>Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.</p>	<p>Approximately 15 mL/minute or 21.6 L/day.</p>

Guideline Criteria	Reported Information
<p><u>Aeration</u> Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	Aeration was used to mix the dilution water thoroughly prior to use.
<p><u>Photoperiod</u> 16 hours light, 8 hours dark.</p>	16 hours light, 8 hours dark with 20 minute dawn/dusk transition periods.
<p><u>Solvents</u> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.</p>	Solvent: Triethylene glycol Maximum conc.: 0.1 mL/L

C. Test Design:

Guideline Criteria	Reported Information
<p><u>Duration</u> 21 days</p>	21 days
<p><u>Nominal Concentrations</u> Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.</p>	Dilution water control, solvent control (0.1 mL triethylene glycol/L), and 5 test concentrations: 40, 80, 160, 320, and 640 ng/l.
<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>	40 daphnids/level; 4 test chambers with 10 daphnids each.
<p><u>Test organisms randomly or impartially assigned to test vessels?</u></p>	Yes.

Guideline Criteria	Reported Information
<p>Renewal 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>The test vessels were cleaned every Monday, Wednesday and Friday to remove algal growth.</p>
<p>Water Parameter Measurements 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.</p>	<p>D.O., pH, and temperature were measured in each test vessel on day 0 and twice weekly thereafter. Temperature was recorded hourly throughout the study in a dilution water control vessel.</p>
<p>Chemical Analysis Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.</p>	<p>Measured on days 0,1, and 2 in all vessels; and on days 4, 7, 11, 14, 18, and 21 in one replicate of each test concentration. On each sampling occasion, two test concentrations were sampled from 3-4 replicates. Samples were analyzed using liquid scintillation counting and thin layer chromatography.</p>

12. REPORTED RESULTS:

A. General Results:

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>Yes.</p>

Guideline Criteria	Reported Information
Control Mortality ≤ 30%	No control or solvent control mortality occurred during the study.
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 first-generation daphnids; - Number of young produced per female; - Length of surviving first-generation daphnids; - Dry weight of surviving first-generation daphnids; - Clinical observations.
Raw data included?	Yes.

Effects Data:

Toxicant Concentration (ng/L)		Percent Survival of Parent Daphnids	Number of Young per Female	Mean Total Length (mm)	Mean Dry Weight (mg)
Nominal	Measured				
Control	--	100% (40/40)*	70	4.0	690
Solvent Control	--	100% (40/40)	66	3.92	726
40	19	97.5% (39/40)	70	3.92	738
80	39	100% (40/40)	71	3.92	718
160	84	100% (40/40)	60	3.84	674
320	190	100% (40/40)	52	3.76	770
640	340	90% (36/40)	16	3.35	897

* = Number of surviving daphnids/total

Toxicity Observations: On Day 3, the adult daphnids in the highest test concentration appeared smaller in size than those in the dilution water control. On Day 5, the adults at 190 ng/L appeared smaller than those in the dilution water control. From Day 2, the daphnids in the highest test concentration were pale compared to those in the dilution water control.

B. Statistical Results:

Most sensitive endpoint: Length and number of young produced per female.

Endpoint	Method	NOEC (ng/L)	LOEC (ng/L)
Survival	Not analyzed.	-	-
Reproduction	ANOVA with Dunnett's test	39	84
Weight	ANOVA with Dunnett's test	≥340	>340
Length	ANOVA with Dunnett's test	39	84

13. VERIFICATION OF STATISTICAL RESULTS:

Most sensitive endpoint: The most sensitive endpoint was length and reproduction.

Endpoint	Method	NOEC (ng/L)	LOEC (ng/L)
Survival	Visual inspection	190	340
Reproduction	Williams' Test	39	84
Weight	ANOVA with Dunnett's test	340	>340
Length	ANOVA with Dunnett's test	39	84

14. REVIEWER'S COMMENTS: This study is scientifically sound, fulfills the guideline requirements for a daphnid life-cycle test and can be classified as CORE. Based on the most sensitive endpoints (length and reproduction), the NOEC and LOEC are 39 and 84 ng/L, respectively. The MATC is 57 ng/L.

Permethrin: Young per adult of D.magna
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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	9	10	9	0

Calculated Chi-Square goodness of fit test statistic = 5.2572
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Permethrin: Young per adult of D.magna
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Hartley's test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 7.86
Closest, conservative, Table H statistic = 216.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 3
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, Hartley's test may still be used as an approximate test (average df are used).

TITLE: Permethrin: Young per adult D.magna
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NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	69.0000	69.0000
1	GRPS 1&2 POOLED	2	65.0000	65.0000
1	GRPS 1&2 POOLED	3	60.0000	60.0000
1	GRPS 1&2 POOLED	4	68.0000	68.0000
1	GRPS 1&2 POOLED	5	69.0000	69.0000
1	GRPS 1&2 POOLED	6	67.0000	67.0000
1	GRPS 1&2 POOLED	7	69.0000	69.0000
1	GRPS 1&2 POOLED	8	73.0000	73.0000
2	19 ng/l	1	72.0000	72.0000
2	19 ng/l	2	74.0000	74.0000
2	19 ng/l	3	63.0000	63.0000
2	19 ng/l	4	70.0000	70.0000
3	39 ng/l	1	71.0000	71.0000
3	39 ng/l	2	73.0000	73.0000
3	39 ng/l	3	69.0000	69.0000
3	39 ng/l	4	72.0000	72.0000
4	84 ng/l	1	63.0000	63.0000
4	84 ng/l	2	60.0000	60.0000
4	84 ng/l	3	57.0000	57.0000
4	84 ng/l	4	61.0000	61.0000
5	190 ng/l	1	60.0000	60.0000
5	190 ng/l	2	63.0000	63.0000
5	190 ng/l	3	60.0000	60.0000
5	190 ng/l	4	63.0000	63.0000
6	340 ng/l	1	12.0000	12.0000
6	340 ng/l	2	18.0000	18.0000
6	340 ng/l	3	13.0000	13.0000
6	340 ng/l	4	22.0000	22.0000

Permethrin: Young per adult D.magna
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	GRPS 1&2 POOLED	8	60.000	73.000	67.500
2	19 ng/l	4	63.000	74.000	69.750
3	39 ng/l	4	69.000	73.000	71.250
4	84 ng/l	4	57.000	63.000	60.250
5	190 ng/l	4	60.000	63.000	61.500
6	340 ng/l	4	12.000	22.000	16.250

Permethrin: Young per adult D.magna

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	GRPS 1&2 POOLED	14.286	3.780	1.336	5.60
2	19 ng/l	22.917	4.787	2.394	6.86
3	39 ng/l	2.917	1.708	0.854	2.40
4	84 ng/l	6.250	2.500	1.250	4.15
5	190 ng/l	3.000	1.732	0.866	2.82
6	340 ng/l	21.583	4.646	2.323	28.59

Permethrin: Young per adult D.magna

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	67.500	67.500	69.000
2	19 ng/l	4	69.750	69.750	69.000
3	39 ng/l	4	71.250	71.250	69.000
4	84 ng/l	4	60.250	60.250	60.875
5	190 ng/l	4	61.500	61.500	60.875
6	340 ng/l	4	16.250	16.250	16.250

Permethrin: Young per adult D.magna

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	69.000				
19 ng/l	69.000	0.699		1.72	k= 1, v=22
39 ng/l	69.000	0.699		1.80	k= 2, v=22
84 ng/l	60.875	3.088	*	1.83	k= 3, v=22
190 ng/l	60.875	3.088	*	1.84	k= 4, v=22
340 ng/l	16.250	23.890	*	1.85	k= 5, v=22

s = 3.503

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

OBS	LEVEL	REP	LEN	WT	length in scale divisions weight in ug
1	CONTROL	1	47	733	
2	CONTROL	1	51	845	
3	CONTROL	1	47	654	
4	CONTROL	1	48	730	
5	CONTROL	1	46	750	
6	CONTROL	1	49	613	
7	CONTROL	1	46	671	
8	CONTROL	1	47	755	
9	CONTROL	1	51	575	
10	CONTROL	1	50	623	
11	CONTROL	2	50	522	
12	CONTROL	2	48	747	
13	CONTROL	2	50	630	
14	CONTROL	2	47	523	
15	CONTROL	2	50	706	
16	CONTROL	2	50	706	
17	CONTROL	2	46	614	
18	CONTROL	2	51	816	
19	CONTROL	2	49	783	
20	CONTROL	2	49	543	
21	CONTROL	3	49	577	
22	CONTROL	3	47	536	
23	CONTROL	3	47	674	
24	CONTROL	3	46	854	
25	CONTROL	3	51	637	
26	CONTROL	3	50	650	
27	CONTROL	3	48	733	
28	CONTROL	3	50	562	
29	CONTROL	3	46	677	
30	CONTROL	3	50	958	
31	CONTROL	4	50	833	
32	CONTROL	4	51	896	
33	CONTROL	4	47	600	
34	CONTROL	4	51	639	
35	CONTROL	4	46	570	
36	CONTROL	4	50	816	
37	CONTROL	4	50	575	
38	CONTROL	4	51	650	
39	CONTROL	4	49	908	
40	CONTROL	4	47	718	
41	SOL_CONT	1	47	770	
42	SOL_CONT	1	47	500	
43	SOL_CONT	1	50	733	
44	SOL_CONT	1	46	802	
45	SOL_CONT	1	49	699	
46	SOL_CONT	1	50	630	
47	SOL_CONT	1	48	866	
48	SOL_CONT	1	46	550	
49	SOL_CONT	1	46	929	
50	SOL_CONT	1	49	537	
51	SOL_CONT	2	46	781	
52	SOL_CONT	2	51	681	
53	SOL_CONT	2	50	603	
54	SOL_CONT	2	47	932	
55	SOL_CONT	2	47	707	
56	SOL_CONT	2	47	715	
57	SOL_CONT	2	50	874	
58	SOL_CONT	2	46	657	
59	SOL_CONT	2	51	736	
60	SOL_CONT	2	47	918	
61	SOL_CONT	3	47	.	
62	SOL_CONT	3	49	.	
63	SOL_CONT	3	48	.	
64	SOL_CONT	3	46	.	

OBS	LEVEL	REP	LEN	WT
65	SOL_CONT	3	48	.
66	SOL_CONT	3	49	736
67	SOL_CONT	3	47	905
68	SOL_CONT	3	50	831
69	SOL_CONT	3	49	810
70	SOL_CONT	3	50	520
71	SOL_CONT	4	45	588
72	SOL_CONT	4	50	866
73	SOL_CONT	4	49	379
74	SOL_CONT	4	48	702
75	SOL_CONT	4	48	630
76	SOL_CONT	4	49	794
77	SOL_CONT	4	50	873
78	SOL_CONT	4	50	622
79	SOL_CONT	4	49	690
80	SOL_CONT	4	48	832
81	TRT1	1	50	.
82	TRT1	1	47	762
83	TRT1	1	47	868
84	TRT1	1	48	581
85	TRT1	1	47	627
86	TRT1	1	47	583
87	TRT1	1	49	.
88	TRT1	1	48	.
89	TRT1	1	50	873
90	TRT1	1	51	738
91	TRT1	2	47	850
92	TRT1	2	51	615
93	TRT1	2	48	865
94	TRT1	2	46	475
95	TRT1	2	48	779
96	TRT1	2	49	683
97	TRT1	2	49	828
98	TRT1	2	51	765
99	TRT1	2	45	797
100	TRT1	2	51	620
101	TRT1	3	46	776
102	TRT1	3	48	696
103	TRT1	3	48	886
104	TRT1	3	50	728
105	TRT1	3	50	605
106	TRT1	3	46	703
107	TRT1	3	48	709
108	TRT1	3	45	604
109	TRT1	3	46	695
110	TRT1	3	48	875
111	TRT1	4	46	435
112	TRT1	4	50	797
113	TRT1	4	47	995
114	TRT1	4	50	837
115	TRT1	4	49	489
116	TRT1	4	47	473
117	TRT1	4	46	725
118	TRT1	4	46	657
119	TRT1	4	50	746
120	TRT2	1	47	787
121	TRT2	1	50	574
122	TRT2	1	46	726
123	TRT2	1	50	472
124	TRT2	1	48	865
125	TRT2	1	51	601
126	TRT2	1	47	471
127	TRT2	1	47	661
128	TRT2	1	50	588

OBS	LEVEL	REP	LEN	WT
129	TRT2	1	46	620
130	TRT2	2	47	687
131	TRT2	2	47	756
132	TRT2	2	49	654
133	TRT2	2	47	706
134	TRT2	2	47	680
135	TRT2	2	47	720
136	TRT2	2	50	694
137	TRT2	2	46	552
138	TRT2	2	50	582
139	TRT2	2	51	873
140	TRT2	3	47	770
141	TRT2	3	50	619
142	TRT2	3	46	704
143	TRT2	3	50	580
144	TRT2	3	50	850
145	TRT2	3	50	658
146	TRT2	3	51	846
147	TRT2	3	48	850
148	TRT2	3	48	697
149	TRT2	3	47	534
150	TRT2	4	50	795
151	TRT2	4	46	877
152	TRT2	4	48	745
153	TRT2	4	50	639
154	TRT2	4	51	742
155	TRT2	4	48	721
156	TRT2	4	48	991
157	TRT2	4	48	878
158	TRT2	4	49	.
159	TRT2	4	47	795
160	TRT3	1	47	715
161	TRT3	1	50	741
162	TRT3	1	50	977
163	TRT3	1	48	604
164	TRT3	1	46	.
165	TRT3	1	50	798
166	TRT3	1	48	533
167	TRT3	1	50	511
168	TRT3	1	47	598
169	TRT3	1	45	767
170	TRT3	2	45	647
171	TRT3	2	49	802
172	TRT3	2	46	638
173	TRT3	2	49	870
174	TRT3	2	50	614
175	TRT3	2	48	674
176	TRT3	2	44	756
177	TRT3	2	47	534
178	TRT3	2	48	514
179	TRT3	2	48	734
180	TRT3	3	45	790
181	TRT3	3	47	563
182	TRT3	3	45	619
183	TRT3	3	46	348
184	TRT3	3	45	613
185	TRT3	3	45	784
186	TRT3	3	45	702
187	TRT3	3	44	534
188	TRT3	3	48	709
189	TRT3	3	45	772
190	TRT3	4	47	745
191	TRT3	4	46	463
192	TRT3	4	46	656

OBS	LEVEL	REP	LEN	WT
193	TRT3	4	45	749
194	TRT3	4	51	740
195	TRT3	4	45	725
196	TRT3	4	44	708
197	TRT3	4	45	557
198	TRT3	4	46	493
199	TRT3	4	46	643
200	TRT4	1	46	703
201	TRT4	1	46	780
202	TRT4	1	47	699
203	TRT4	1	48	758
204	TRT4	1	44	726
205	TRT4	1	46	717
206	TRT4	1	45	759
207	TRT4	1	47	709
208	TRT4	1	47	709
209	TRT4	1	46	726
210	TRT4	2	49	746
211	TRT4	2	44	953
212	TRT4	2	46	951
213	TRT4	2	44	467
214	TRT4	2	47	789
215	TRT4	2	45	698
216	TRT4	2	47	746
217	TRT4	2	45	778
218	TRT4	2	46	847
219	TRT4	2	46	716
220	TRT4	3	46	944
221	TRT4	3	45	692
222	TRT4	3	45	702
223	TRT4	3	43	843
224	TRT4	3	49	846
225	TRT4	3	46	739
226	TRT4	3	47	747
227	TRT4	3	45	552
228	TRT4	3	47	691
229	TRT4	3	44	796
230	TRT4	4	49	623
231	TRT4	4	47	683
232	TRT4	4	46	739
233	TRT4	4	44	555
234	TRT4	4	47	744
235	TRT4	4	46	.
236	TRT4	4	47	783
237	TRT4	4	46	765
238	TRT4	4	46	711
239	TRT4	4	49	.
240	TRT5	1	42	758
241	TRT5	1	40	848
242	TRT5	1	41	511
243	TRT5	1	39	839
244	TRT5	1	41	912
245	TRT5	1	42	600
246	TRT5	1	41	666
247	TRT5	1	39	.
248	TRT5	2	43	.
249	TRT5	2	44	984
250	TRT5	2	44	906
251	TRT5	2	41	787
252	TRT5	2	40	.
253	TRT5	2	40	.
254	TRT5	2	40	913
255	TRT5	2	43	.
256	TRT5	2	42	792

Permethrin: Chronic Exposure to Daphnia magna
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OBS	LEVEL	REP	LEN	WT
257	TRT5	2	41	830
258	TRT5	3	40	828
259	TRT5	3	40	876
260	TRT5	3	40	898
261	TRT5	3	40	911
262	TRT5	3	44	.
263	TRT5	3	42	798
264	TRT5	3	40	590
265	TRT5	3	39	866
266	TRT5	3	40	.
267	TRT5	4	45	596
268	TRT5	4	41	598
269	TRT5	4	43	888
270	TRT5	4	45	545
271	TRT5	4	44	714
272	TRT5	4	42	.
273	TRT5	4	42	.
274	TRT5	4	41	.
275	TRT5	4	40	.

Permethrin: Chronic Exposure to Daphnia magna
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	LEVEL			
	CONTROL	SOL_CONT	TRT1	TRT2
	MEAN	MEAN	MEAN	MEAN
LEN	48.70	48.23	48.08	48.38
WT	690.05	725.66	715.00	706.67

(CONTINUED)

Permethrin: Chronic Exposure to Daphnia magna
07:14 Sunday, January 28, 1996

	LEVEL		
	TRT3	TRT4	TRT5
	MEAN	MEAN	MEAN
LEN	46.78	46.13	41.42
WT	665.13	740.32	778.16

Permethrin: Chronic Exposure to Daphnia magna
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LEVEL=CONTROL

Variable	N	Mean	Std Dev	CV
REP	40	2.500	1.132	45.291
LEN	40	48.700	1.786	3.667
WT	40	690.050	113.369	16.429

LEVEL=SOL_CONT

Variable	N	Mean	Std Dev	CV
REP	40	2.500	1.132	45.291
LEN	40	48.225	1.609	3.337
WT	35	725.657	136.564	18.819

LEVEL=TRT1

Variable	N	Mean	Std Dev	CV
REP	39	2.462	1.120	45.512
LEN	39	48.077	1.783	3.708
WT	36	715.000	132.586	18.543

LEVEL=TRT2

Variable	N	Mean	Std Dev	CV
REP	40	2.500	1.132	45.291
LEN	40	48.375	1.659	3.430
WT	39	706.667	120.478	17.049

LEVEL=TRT3

Variable	N	Mean	Std Dev	CV
REP	40	2.500	1.132	45.291
LEN	40	46.775	1.968	4.206
WT	39	665.128	124.092	18.657

LEVEL=TRT4

Variable	N	Mean	Std Dev	CV
REP	40	2.500	1.132	45.291
LEN	40	46.125	1.471	3.189
WT	38	740.316	97.667	13.193

Permethrin: Chronic Exposure to Daphnia magna
07:14 Sunday, January 28, 1996

LEVEL=TRT5

Variable	N	Mean	Std Dev	CV
REP	36	2.528	1.108	43.835
LEN	36	41.417	1.713	4.137
WT	25	778.160	136.353	17.522

Permethrin: Chronic Exposure to Daphnia magna

1. ANALYSIS OF Length

07:14 Sunday, January 28, 1996

General Linear Models Procedure
Class Level Information

Class Levels Values

LEVEL 7 CONTROL SOL_CONT TRT1 TRT2 TRT3 TRT4 TRT5

Number of observations in data set = 275

Permethrin: Chronic Exposure to Daphnia magna
1. ANALYSIS OF Length

07:14 Sunday, January 28, 1996

General Linear Models Procedure
Type I Estimable Functions for: LEVEL

Effect Coefficients

INTERCEPT	0
LEVEL CONTROL	L2
SOL_CONT	L3
TRT1	L4
TRT2	L5
TRT3	L6
TRT4	L7
TRT5	-L2-L3-L4-L5-L6-L7

Permethrin: Chronic Exposure to Daphnia magna
1. ANALYSIS OF Length

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General Linear Models Procedure

Dependent Variable: LEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1447.8862	241.3144	81.70	0.0001
Error	268	791.6192	2.9538		
Corrected Total	274	2239.5055			
R-Square		C.V.	Root MSE	LEN Mean	
	0.646521	3.665522	1.7187	46.887	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LEVEL	6	1447.8862	241.3144	81.70	0.0001

Permethrin: Chronic Exposure to Daphnia magna
1. ANALYSIS OF Length

07:14 Sunday, January 28, 1996

General Linear Models Procedure
Least Squares Means

LEVEL	LEN LSMEAN	LSMEAN Number
CONTROL	48.7000000	1
SOL_CONT	48.2250000	2
TRT1	48.0769231	3
TRT2	48.3750000	4

TRT3 46.7750000 5
TRT4 46.1250000 6
TRT5 41.4166667 7

Pr > |T| H0: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4	5	6	7
1	.	0.2175	0.1084	0.3985	0.0001	0.0001	0.0001
2	0.2175	.	0.7021	0.6966	0.0002	0.0001	0.0001
3	0.1084	0.7021	.	0.4416	0.0009	0.0001	0.0001
4	0.3985	0.6966	0.4416	.	0.0001	0.0001	0.0001
5	0.0001	0.0002	0.0009	0.0001	.	0.0919	0.0001
6	0.0001	0.0001	0.0001	0.0001	0.0919	.	0.0001
7	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	.

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

Permethrin: Chronic Exposure to Daphnia magna
1. ANALYSIS OF Length

07:14 Sunday, January 28, 1996

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: LEN

NOTE: This test controls the type I experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 268 MSE= 2.953803
Critical Value of Studentized Range= 4.201

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

LEVEL Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
CONTROL - TRT2	-0.8167	0.3250	1.4667
CONTROL - SOL_CONT	-0.6667	0.4750	1.6167
CONTROL - TRT1	-0.5259	0.6231	1.7721
CONTROL - TRT3	0.7833	1.9250	3.0667 ***
CONTROL - TRT4	1.4333	2.5750	3.7167 ***
CONTROL - TRT5	6.1103	7.2833	8.4563 ***
TRT2 - CONTROL	-1.4667	-0.3250	0.8167
TRT2 - SOL_CONT	-0.9917	0.1500	1.2917
TRT2 - TRT1	-0.8509	0.2981	1.4471
TRT2 - TRT3	0.4583	1.6000	2.7417 ***
TRT2 - TRT4	1.1083	2.2500	3.3917 ***
TRT2 - TRT5	5.7853	6.9583	8.1313 ***
SOL_CONT - CONTROL	-1.6167	-0.4750	0.6667
SOL_CONT - TRT2	-1.2917	-0.1500	0.9917
SOL_CONT - TRT1	-1.0009	0.1481	1.2971
SOL_CONT - TRT3	0.3083	1.4500	2.5917 ***
SOL_CONT - TRT4	0.9583	2.1000	3.2417 ***
SOL_CONT - TRT5	5.6353	6.8083	7.9813 ***
TRT1 - CONTROL	-1.7721	-0.6231	0.5259
TRT1 - TRT2	-1.4471	-0.2981	0.8509
TRT1 - SOL_CONT	-1.2971	-0.1481	1.0009
TRT1 - TRT3	0.1529	1.3019	2.4509 ***
TRT1 - TRT4	0.8029	1.9519	3.1009 ***
TRT1 - TRT5	5.4801	6.6603	7.8404 ***
TRT3 - CONTROL	-3.0667	-1.9250	-0.7833 ***

TRT3	- TRT2	-2.7417	-1.6000	-0.4583	***
TRT3	- SOL CONT	-2.5917	-1.4500	-0.3083	***
TRT3	- TRT1	-2.4509	-1.3019	-0.1529	***
TRT3	- TRT4	-0.4917	0.6500	1.7917	
TRT3	- TRT5	4.1853	5.3583	6.5313	***
TRT4	- CONTROL	-3.7167	-2.5750	-1.4333	***
TRT4	- TRT2	-3.3917	-2.2500	-1.1083	***
TRT4	- SOL CONT	-3.2417	-2.1000	-0.9583	***
TRT4	- TRT1	-3.1009	-1.9519	-0.8029	***
TRT4	- TRT3	-1.7917	-0.6500	0.4917	
TRT4	- TRT5	3.5353	4.7083	5.8813	***
TRT5	- CONTROL	-8.4563	-7.2833	-6.1103	***
TRT5	- TRT2	-8.1313	-6.9583	-5.7853	***
TRT5	- SOL CONT	-7.9813	-6.8083	-5.6353	***
TRT5	- TRT1	-7.8404	-6.6603	-5.4801	***
TRT5	- TRT3	-6.5313	-5.3583	-4.1853	***

Permethrin: Chronic Exposure to Daphnia magna
1. ANALYSIS OF Length

07:14 Sunday, January 28, 1996

General Linear Models Procedure

LEVEL Comparison	Simultaneous		Difference Between Means	Simultaneous	
	Lower Confidence Limit	Upper Confidence Limit		Lower Confidence Limit	Upper Confidence Limit
TRT5 - TRT4	-5.8813	-4.7083	-3.5353	***	

Permethrin: Chronic Exposure to Daphnia magna
1. ANALYSIS OF Length

07:14 Sunday, January 28, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: LEN

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

Alpha= 0.05 Confidence= 0.95 df= 268 MSE= 2.953803
Critical Value of Dunnett's T= 2.306

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

LEVEL Comparison	Simultaneous		Difference Between Means	Simultaneous	
	Lower Confidence Limit	Upper Confidence Limit		Lower Confidence Limit	Upper Confidence Limit
TRT2 - CONTROL	-1.2111	0.5611	-0.3250		
SOL CONT - CONTROL	-1.3611	0.4111	-0.4750		
TRT1 - CONTROL	-1.5149	0.2687	-0.6231		
TRT3 - CONTROL	-2.8111	-1.0389	-1.9250	***	
TRT4 - CONTROL	-3.4611	-1.6889	-2.5750	***	
TRT5 - CONTROL	-8.1938	-6.3729	-7.2833	***	

Permethrin: Chronic Exposure to Daphnia magna
2. ANALYSIS OF Weight

07:14 Sunday, January 28, 1996

General Linear Models Procedure

Class Level Information

Class	Levels	Values
LEVEL	7	CONTROL SOL_CONT TRT1 TRT2 TRT3 TRT4 TRT5

Number of observations in data set = 275

NOTE: Due to missing values, only 252 observations can be used in this analysis.

Permethrin: Chronic Exposure to Daphnia magna
2. ANALYSIS OF Weight

07:14 Sunday, January 28, 1996

General Linear Models Procedure
Type I Estimable Functions for: LEVEL

Effect Coefficients

INTERCEPT	0
LEVEL CONTROL	L2
SOL CONT	L3
TRT1	L4
TRT2	L5
TRT3	L6
TRT4	L7
TRT5	-L2-L3-L4-L5-L6-L7

Permethrin: Chronic Exposure to Daphnia magna
2. ANALYSIS OF Weight

07:14 Sunday, January 28, 1996

General Linear Models Procedure

Dependent Variable: WT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	252170.33	42028.39	2.79	0.0120
Error	245	3686478.38	15046.85		
Corrected Total	251	3938648.71			

R-Square	C.V.	Root MSE	WT Mean
0.064025	17.18980	122.67	713.60

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LEVEL	6	252170.33	42028.39	2.79	0.0120

Permethrin: Chronic Exposure to Daphnia magna
2. ANALYSIS OF Weight

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General Linear Models Procedure
Least Squares Means

LEVEL	WT LSMEAN	LSMEAN Number
CONTROL	690.050000	1
SOL_CONT	725.657143	2
TRT1	715.000000	3
TRT2	706.666667	4
TRT3	665.128205	5
TRT4	740.315789	6
TRT5	778.160000	7

Pr > |T| H0: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4	5	6	7
1	.	0.2110	0.3768	0.5478	0.3675	0.0717	0.0052
2	0.2110	.	0.7147	0.5067	0.0351	0.6105	0.1034
3	0.3768	0.7147	.	0.7691	0.0798	0.3758	0.0491
4	0.5478	0.5067	0.7691	.	0.1361	0.2300	0.0238
5	0.3675	0.0351	0.0798	0.1361	.	0.0077	0.0004
6	0.0717	0.6105	0.3758	0.2300	0.0077	.	0.2321
7	0.0052	0.1034	0.0491	0.0238	0.0004	0.2321	.

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

Permethrin: Chronic Exposure to Daphnia magna

2. ANALYSIS OF Weight

07:14 Sunday, January 28, 1996

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: WT

NOTE: This test controls the type I experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 245 MSE= 15046.85
Critical Value of Studentized Range= 4.204

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

LEVEL Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
TRT5 - TRT4	-56.07	37.84	131.76
TRT5 - SOL_CONT	-42.99	52.50	148.00
TRT5 - TRT1	-31.78	63.16	158.10
TRT5 - TRT2	-21.94	71.49	164.93
TRT5 - CONTROL	-4.87	88.11	181.09
TRT5 - TRT3	19.60	113.03	206.47 ****
TRT4 - TRT5	-131.76	-37.84	56.07
TRT4 - SOL_CONT	-70.78	14.66	100.10
TRT4 - TRT1	-59.50	25.32	110.13
TRT4 - TRT2	-49.48	33.65	116.78
TRT4 - CONTROL	-32.35	50.27	132.88
TRT4 - TRT3	-7.94	75.19	158.31
SOL_CONT - TRT5	-148.00	-52.50	42.99
SOL_CONT - TRT4	-100.10	-14.66	70.78
SOL_CONT - TRT1	-75.91	10.66	97.23
SOL_CONT - TRT2	-65.92	18.99	103.90
SOL_CONT - CONTROL	-48.80	35.61	120.02
SOL_CONT - TRT3	-24.38	60.53	145.44
TRT1 - TRT5	-158.10	-63.16	31.78

TRT1 - TRT4	-110.13	-25.32	59.50
TRT1 - SOL_CONT	-97.23	-10.66	75.91
TRT1 - TRT2	-75.95	8.33	92.62
TRT1 - CONTROL	-58.83	24.95	108.73
TRT1 - TRT3	-34.42	49.87	134.16
TRT2 - TRT5	-164.93	-71.49	21.94
TRT2 - TRT4	-116.78	-33.65	49.48
TRT2 - SOL_CONT	-103.90	-18.99	65.92
TRT2 - TRT1	-92.62	-8.33	75.95
TRT2 - CONTROL	-65.45	16.62	98.68
TRT2 - TRT3	-41.05	41.54	124.12
CONTROL - TRT5	-181.09	-88.11	4.87
CONTROL - TRT4	-132.88	-50.27	32.35
CONTROL - SOL_CONT	-120.02	-35.61	48.80
CONTROL - TRT1	-108.73	-24.95	58.83
CONTROL - TRT2	-98.68	-16.62	65.45
CONTROL - TRT3	-57.15	24.92	106.99
TRT3 - TRT5	-206.47	-113.03	-19.60 ***
TRT3 - TRT4	-158.31	-75.19	7.94
TRT3 - SOL_CONT	-145.44	-60.53	24.38
TRT3 - TRT1	-134.16	-49.87	34.42
TRT3 - TRT2	-124.12	-41.54	41.05

Permethrin: Chronic Exposure to Daphnia magna

2. ANALYSIS OF Weight

07:14 Sunday, January 28, 1996

General Linear Models Procedure

LEVEL Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
TRT3 - CONTROL	-106.99	-24.92	57.15

Permethrin: Chronic Exposure to Daphnia magna

2. ANALYSIS OF Weight

07:14 Sunday, January 28, 1996

General Linear Models Procedure

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

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

Alpha= 0.05 Confidence= 0.95 df= 245 MSE= 15046.85
Critical Value of Dunnnett's T= 2.317

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

LEVEL Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
TRT5 - CONTROL	15.65	88.11	160.57
TRT4 - CONTROL	-14.12	50.27	114.65
SOL_CONT - CONTROL	-30.18	35.61	101.39
TRT1 - CONTROL	-40.34	24.95	90.24
TRT2 - CONTROL	-47.34	16.62	80.58
TRT3 - CONTROL	-88.88	-24.92	39.04

