3-29-94

MRID No. 429186-72

DATA EVALUATION RECORD

1. **CHEMICAL:** Fipronil and derivatives (M&B 46030). Shaughnessey No. 129121.

2. **TEST MATERIAL:** M&B 46136; Batch No. WAB 487; Ref No. 42; 99.2% active ingredient; a white powder.

3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Test. Species Tested: *Daphnia magna*.

4. **CITATION:** McNamara, P.C. 1992. (M&B 46136) - Chronic Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through Conditions. SLI Report No. 91-8-3886. Study conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 429186-72.

5. **REVIEWED BY:**

Andrew C. Bryceland, Fishery Biologist **Signature:** 3/1/94
Review Section 5
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) **Date:**

6. **APPROVED BY:**

Ann Stavola, Supervisory Biologist **Signature:**
Review Section 5
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) **Date:** 3/29/94

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a daphnid life-cycle test. Both the dilution water control and the solvent control were contaminated with high levels of the test material throughout the test period.

8. **RECOMMENDATIONS:**

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** *Daphnia magna* were obtained from populations cultured at the testing facility. The cultures were maintained in fortified well water at 20 ±1°C. The daphnids were fed daily a combination of

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5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
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KBN Engineering and
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Date: 3/2/94

6. **APPROVED BY:**

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Signature: P. Kosalwat
Date: 3/2/94

James J. Goodyear, Ph.D.
Project Officer, EEB/EFED
USEPA

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Date:

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a daphnid life-cycle test. Both the dilution water control and the solvent control were contaminated with high levels of the test material throughout the test period. The MATC of M&B 46136 for *Daphnia magna* could not be determined since reproduction was significantly reduced at all test levels. The 21-day EC_{50} was 4.5 µg ai/l.

8. **RECOMMENDATIONS:**

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

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11. MATERIALS AND METHODS:

A. Test Animals: *Daphnia magna* were obtained from populations cultured at the testing facility. The cultures were maintained in fortified well water at 20 ±1°C. The daphnids were fed daily a combination of green alga (*Ankistrodesmus falcatus*) and a trout food suspension.

B. Test System: The test system was an intermittent-flow proportional diluter with a 50% dilution factor. The test vessels were 1.6-l glass battery jars with a solution volume of 1.4 l. Test solutions drained through two 2-cm holes located 15 cm from the bottom of each jar. The drains were covered with Nitex® 40-mesh screen. The diluter delivered test solution to each vessel at an approximate rate of 6 volume replacements per day. The 90% replacement time was approximately 11 hours.

The dilution water, which was the same as the culture water, was fortified well water. The dilution water had a pH range of 7.9-8.3, a specific conductivity range of 400-500 µmhos/cm, and total hardness and alkalinity ranges of 160-180 and 110-130 mg/l as $CaCO_3$, respectively. The fortified water was filtered through a resin column and a carbon filter prior to use.

Sixteen hours of light at an intensity of 50-85 footcandles were provided each day. Sudden transitions from light to dark and vice versa were avoided. Test temperature was maintained at approximately 20 ±1°C by a water bath.

A diluter stock solution (0.70 mg ai/ml) was prepared by diluting 0.0352 g of test material with acetone to a final volume of 50 ml.

C. Dosage: Twenty-one-day, flow-through test. Based on the results of preliminary testing, five nominal test concentrations (0.75, 1.5, 3.0, 6.0, and 12 µg ai/l) were selected for this study. A dilution water control and a solvent control were also included. The solvent control contained 17 µl/l of acetone, the highest solvent concentration used in any exposure solution.

D. Design: Ten daphnids (≤24 hours old) were impartially selected and distributed to each of four test vessels per treatment (i.e., 40 daphnids/treatment).

The daphnids were fed 2.0 ml of trout food (5 mg/ml), 3.0 ml of green alga (*Ankistrodesmus falcatus*; 4 x 10^7 cells/ml) suspension, and 0.5 ml of Selco® (0.6 mg/ml) two to three times daily. The jars were brushed and the solutions filtered through fine-mesh nets twice weekly.

Daphnid survival was determined on days 1, 2, 4, 7, 10, 13, 14, 17, 20, and 21. The offspring produced were counted and discarded on days 7, 10, 13, 14, 17, 20, and 21. At test termination, the total body length and dry weight of each surviving adult was recorded.

Dissolved oxygen concentration (DO), pH, and temperature were measured once a week in every test vessel. The DO was also measured every weekday in alternate replicate vessels of each group. Temperature was measured daily in alternate replicate vessels of each group and monitored continuously with a max/min thermometer in one vessel of the 1.5 µg ai/l (nominal) group. Total hardness, alkalinity, specific conductivity, and pH were measured weekly in alternate replicate vessels of each group.

Water samples were collected from the midpoint of two replicate vessels of each group on test days 0, 8, 14, and 21. In addition, samples were collected and analyzed on day 17 to verify exposure concentrations. All samples were analyzed for M&B 46136 using high pressure liquid chromatography.

E. **Statistics:** The percentage survival data were arcsine square-root transformed before analysis. A Student's T-test demonstrated that control and solvent control responses for dry weight and survival were statistically similar; therefore, the pooled control data were used to assess significant treatment effects for these parameters. Length and reproduction data in the dilution water control and the solvent control were shown to be different from one another; therefore, the treatment data for these parameters were compared to the solvent control data.

Survival, reproduction, and growth data were normally distributed (Shapiro-Wilks test); therefore, Williams' test was used to assess exposure-level effects. If daphnid survival in any treatment level was significantly affected, growth and reproduction data for that level were excluded from further statistical analysis.

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All analyses were performed using the mean organism response in each replicate vessel rather than individual responses. The level of significance was set at $p \leq 0.05$ for all analyses except the Shapiro-Wilks test which was $p \leq 0.01$.

EC_{50} values were determined using the computer program by Stephan (1977, 1982) or empirically.

12. **REPORTED RESULTS:** Throughout the 21 day exposure period, no visible sign of undissolved test material was observed in any test vessel or in the diluter apparatus. Mean measured concentrations were 0.63, 1.5, 2.6, 5.8, and 12 µg ai/l (Table 2, attached). The average coefficient of variation was 16%. "On days 0, 8, 17 and 21, the control and solvent control vessels had measured concentrations of M&B 46136 that ranged from 0.22 to 7.2 µg ai/l. Due to the variability in measurements obtained between replicate control vessels on a given day (i.e., 0.54 and 3.4 µg ai/l), the confirmation of proper diluter functioning during these sampling intervals, and no adverse effects observed in the control vessels, it is believed that these measured concentrations are not representative of the concentration of test material in the control vessels, rather they are an artifact resulting from the extraction and processing of the samples for analysis."

Survival and reproductive rates for the control groups exceeded the minimum EPA guideline requirements of 70% survival and 40 offspring/female. Survival in the two highest test concentrations (5.8 and 12 µg ai/l) was significantly reduced when compared to that of the pooled control (Table 3, attached). Based on survival data, the 21-day EC_{50} (95% confidence interval) was 4.5 (3.9-5.2) µg ai/l.

Mean body length and the number of offspring produced per female at 2.6 µg ai/l were statistically reduced when compared to those of the solvent control (Tables 4 and 5, attached). Mean dry weight at 1.5 and 2.6 µg ai/l was significantly reduced when compared to the pooled control data (Table 5, attached).

During the study, the test solutions had a pH of 7.9-8.3, a specific conductance of 500 µmhos/cm, a mean DO range of 7.8-8.2 mg/l, a continuous temperature reading of 18-23°C, and a mean total hardness and alkalinity of 170 and 110-120 mg/l as $CaCO_3$, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Based on the observed adverse effect of M&B 46136 on daphnid dry weight, the maximum acceptable toxicant concentration (MATC) of this test material to *Daphnia magna* was estimated to be >0.63 and <1.5 µg ai/l (geometric mean MATC = 0.97 µg ai/l).

A GLP compliance statement and a quality assurance 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 Good Laboratory Practice Regulations (40 CFR Part 160) except in the case of stability, characterization and verification of test substance identity.

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; therefore, the SEP for the *Daphnia magna* static-renewal test was used as a general guidance. Study weaknesses were as follows:

Both control solutions appeared to be contaminated with high levels of test material (0.35-7.2 µg ai/l in the control and 0.22-3.6 µg ai/l in the solvent control) on days 0, 8, and 21 (Table 2, attached). The author claimed that these detected concentrations were an artifact resulting from the extraction and processing of the samples for analysis. If the artifact could result in such high levels of measured concentrations, then all measured concentrations are questionable.

Raw survival and water quality data were not included in the report. In addition, the results of the chemical analyses conducted on day 17 were not included in the report. All raw data must be submitted with the report.

The author states that the survival and reproduction rates of control daphnids met "the standard criteria established by the U.S. EPA (1985) under FIFRA Guidelines #72-4." The literature cited shows only a reference to the SEP for acute toxicity test for estuarine and marine organisms (1985). This is a discrepancy in the report.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to verify the author's 21-day EC_{50} value and obtained similar results (page 1 of

printouts, attached). The reviewer analyzed the survival data using Toxstat® and Steel's Many-One Rank test, and obtained the same results as the author (page 6 of printouts, attached).

Weight and length data were analyzed using a two-way analysis of variance (ANOVA) and Bonferroni's comparison test. Both weight and length were adversely affected at concentrations >0.63 µg ai/l when compared to the dilution water control and to the solvent control (pages 11 and 12 of printouts, attached).

The author evaluated the effects of the test material on reproduction using average number of young produced per female. Since there was more than one female per test chamber, and reproduction did not start on the same day and was not monitored on a daily basis, the appropriate endpoint for reproduction is the number of young per female reproductive day, rather than number of young per female. Raw survival data were not included in the report, therefore the reviewer was unable to determine the number of young per female reproductive day. Therefore, the reviewer analyzed the reproduction data using mean number of young per female like the author did. However, the treatments were compared to the dilution water control, since not all treatment solutions contained the same amount of solvent as that in the solvent control and the solvent used had an adverse effect on daphnids. Reproduction data were analyzed using Toxstat® and Williams' test (page 20 of printouts, attached). The results demonstrated a significant reduction in reproduction at all test levels when compared to the control.

Length and weight data were individually measured; however, the author analyzed these data using the mean value of each replicate. When mean values are used, the variation that exists within each replicate is ignored. Individual measurements should have been used.

The author excluded from statistical analysis those treatments which showed effects on survival. Length data for these treatment levels should have been included in the analysis since they were part of the experiment and could have contributed to the experimental error in the ANOVA. Furthermore, excluding this data from statistical analysis would make it appear as if only survival was affected at these treatment levels.

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C. **Discussion/Results:** This study is not scientifically sound and does not meet the guideline requirements for a daphnid life-cycle test. Both the dilution water control and the solvent control were contaminated with high levels of the test material throughout the test period. Based on the reviewer's results, the MATC of M&B 46136 for *Daphnia magna* could not be determined since reproduction was significantly reduced at all test levels when compared to the dilution water control. The 21-day EC_{50} was 4.5 µg ai/l.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** Both the dilution water control and the solvent control were contaminated with high levels of the test material throughout the test period.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes; 3 February 1994.

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Fipanil Review

Page _____ is not included in this copy.

Pages 9 through 12 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

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_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Rosemary Graham Mora M&B 46136 Daphnia magna

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
12	40	40	100	0
5.8	40	26	65	0
2.6	40	4	10	0
1.5	40	2	5	0
.63	40	1	2.5	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.749848

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	3.064288E-02	4.507749	3.935787	5.202022

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.70143	11.75908	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.760911
95 PERCENT CONFIDENCE LIMITS =-1.144779 AND 8.6666

LC50 = 4.380417
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 2.012942
95 PERCENT CONFIDENCE LIMITS = 0 AND 4.909776

MB 46136: Survival of Exposed D. magna
File: 42918672.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	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	5	13	6	0

Calculated Chi-Square goodness of fit test statistic = 4.9364
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

4

MB 46136: Survival of Exposed D. magna
File: 42918672.sur Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.310

W = 0.887

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

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

MB 46136: Survival of Exposed D. magna
File: 42918672.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.

TITLE: MB 46136: Survival of Exposed D. magna
FILE: 42918672.sur
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.4120
1	Control	2	1.0000	1.4120
1	Control	3	1.0000	1.4120
1	Control	4	1.0000	1.4120
2	0.75	1	0.9000	1.2490
2	0.75	2	1.0000	1.4120
2	0.75	3	1.0000	1.4120
2	0.75	4	1.0000	1.4120
3	1.5	1	0.9000	1.2490
3	1.5	2	1.0000	1.4120
3	1.5	3	0.9000	1.2490
3	1.5	4	1.0000	1.4120
4	3.0	1	0.7000	0.9912
4	3.0	2	1.0000	1.4120
4	3.0	3	1.0000	1.4120
4	3.0	4	0.9000	1.2490
5	6.0	1	0.5000	0.7854
5	6.0	2	0.1000	0.3218
5	6.0	3	0.5000	0.7854
5	6.0	4	0.3000	0.5796
6	12.0	1	0.0000	0.1588
6	12.0	2	0.0000	0.1588
6	12.0	3	0.0000	0.1588
6	12.0	4	0.0000	0.1588

mean measured conc
0.63
1.5
2.6
5.8
12.0

MB 46136: Survival of Exposed D. magna
File: 42918672.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	Control		1.412				
2	0.63	0.75	1.371	16.00	10.00	4.00	
3	1.5	1.5	1.331	14.00	10.00	4.00	
4	2.6	3.0	1.266	14.00	10.00	4.00	
5	5.8	6.0	0.618	10.00	10.00	4.00	*
6	12.0	12.0	0.159	10.00	10.00	4.00	*

(mean measured conc. / nominal conc.)

Critical values use k = 5, are 1 tailed, and alpha = 0.05

TRT 1 = CONTROL
TRT 2 = Solvent Control
TRT 3 = 0.75 ug ai/l nominal (0.63 ug ai/l mean measured)
TRT 4 = 1.5 ug ai/l nominal (1.5 ug ai/l mean measured)
TRT 5 = 3.0 ug ai/l nominal (2.6 ug ai/l mean measured)
TRT 6 = 6.0 ug ai/l nominal (5.8 ug ai/l mean measured)

		TRT	REP	LENGTH	WEIGHT
CASE	1	1.0000	1.0000	4.9000	1.9800
CASE	2	1.0000	1.0000	5.2000	2.0300
CASE	3	1.0000	1.0000	5.1000	1.5800
CASE	4	1.0000	1.0000	5.3000	1.9700
CASE	5	1.0000	1.0000	5.3000	1.2200
CASE	6	1.0000	1.0000	4.7000	0.6500
CASE	7	1.0000	1.0000	5.3000	1.8900
CASE	8	1.0000	1.0000	5.2000	1.1700
CASE	9	1.0000	1.0000	5.0000	1.5500
CASE	10	1.0000	1.0000	5.1000	1.5900
CASE	11	1.0000	2.0000	5.4000	2.1900
CASE	12	1.0000	2.0000	5.0000	1.4700
CASE	13	1.0000	2.0000	5.4000	2.2800
CASE	14	1.0000	2.0000	5.3000	1.9400
CASE	15	1.0000	2.0000	5.3000	1.9600
CASE	16	1.0000	2.0000	5.1000	1.6900
CASE	17	1.0000	2.0000	5.4000	2.1300
CASE	18	1.0000	2.0000	5.3000	2.1500
CASE	19	1.0000	2.0000	5.2000	1.7400
CASE	20	1.0000	2.0000	5.4000	2.0400
CASE	21	1.0000	3.0000	5.4000	2.0000
CASE	22	1.0000	3.0000	5.2000	2.1800
CASE	23	1.0000	3.0000	5.2000	2.1400
CASE	24	1.0000	3.0000	5.2000	1.9800
CASE	25	1.0000	3.0000	5.1000	1.9600
CASE	26	1.0000	3.0000	5.2000	2.1000
CASE	27	1.0000	3.0000	5.3000	2.0900
CASE	28	1.0000	3.0000	5.2000	1.9900
CASE	29	1.0000	3.0000	5.3000	2.2100
CASE	30	1.0000	3.0000	5.5000	2.1800
CASE	31	1.0000	4.0000	5.3000	1.8800
CASE	32	1.0000	4.0000	5.5000	2.1700
CASE	33	1.0000	4.0000	5.2000	1.9100
CASE	34	1.0000	4.0000	5.5000	2.1300
CASE	35	1.0000	4.0000	5.2000	1.8700
CASE	36	1.0000	4.0000	5.3000	2.0700
CASE	37	1.0000	4.0000	5.2000	1.9500
CASE	38	1.0000	4.0000	5.3000	1.9900
CASE	39	1.0000	4.0000	5.3000	2.0200
CASE	40	1.0000	4.0000	5.5000	2.2600
CASE	41	2.0000	1.0000	5.0000	1.3800
CASE	42	2.0000	1.0000	5.1000	1.9800
CASE	43	2.0000	1.0000	4.9000	1.7900
CASE	44	2.0000	1.0000	4.9000	1.0800
CASE	45	2.0000	1.0000	5.1000	2.0100
CASE	46	2.0000	1.0000	5.0000	1.9600
CASE	47	2.0000	1.0000	5.0000	1.8200
CASE	48	2.0000	1.0000	5.1000	1.9200
CASE	49	2.0000	1.0000	5.1000	2.0200
CASE	50	2.0000	2.0000	5.0000	1.8600
CASE	51	2.0000	2.0000	5.0000	1.6700
CASE	52	2.0000	2.0000	5.3000	2.0300
CASE	53	2.0000	2.0000	5.0000	1.8600
CASE	54	2.0000	2.0000	5.4000	2.1600
CASE	55	2.0000	2.0000	5.0000	1.8300
CASE	56	2.0000	2.0000	5.2000	1.9600

CASE	57	2.0000	3.0000	5.4000	2.0800
CASE	58	2.0000	3.0000	5.0000	1.8300
CASE	59	2.0000	3.0000	5.4000	2.0400
CASE	60	2.0000	3.0000	4.9000	1.7300
CASE	61	2.0000	3.0000	4.9000	1.8400
CASE	62	2.0000	3.0000	5.0000	1.5800
CASE	63	2.0000	3.0000	5.0000	1.8000
CASE	64	2.0000	3.0000	5.0000	1.9100
CASE	65	2.0000	3.0000	5.3000	2.0900
CASE	66	2.0000	4.0000	5.3000	2.0300
CASE	67	2.0000	4.0000	5.3000	2.0200
CASE	68	2.0000	4.0000	5.1000	1.9700
CASE	69	2.0000	4.0000	5.1000	1.9200
CASE	70	2.0000	4.0000	5.2000	1.9400
CASE	71	2.0000	4.0000	5.0000	1.8800
CASE	72	2.0000	4.0000	5.1000	1.5700
CASE	73	2.0000	4.0000	4.9000	2.0400
CASE	74	2.0000	4.0000	5.2000	2.0600
CASE	75	2.0000	4.0000	5.0000	1.7900
CASE	76	3.0000	1.0000	5.2000	1.8800
CASE	77	3.0000	1.0000	5.4000	2.0400
CASE	78	3.0000	1.0000	5.2000	1.8400
CASE	79	3.0000	1.0000	5.1000	1.8400
CASE	80	3.0000	1.0000	5.1000	1.8400
CASE	81	3.0000	1.0000	5.3000	1.8900
CASE	82	3.0000	1.0000	5.3000	1.9900
CASE	83	3.0000	1.0000	5.3000	1.8200
CASE	84	3.0000	1.0000	5.4000	1.8200
CASE	85	3.0000	2.0000	5.1000	1.7900
CASE	86	3.0000	2.0000	5.2000	1.7000
CASE	87	3.0000	2.0000	5.4000	1.9600
CASE	88	3.0000	2.0000	5.4000	2.0300
CASE	89	3.0000	2.0000	5.4000	1.7400
CASE	90	3.0000	2.0000	5.3000	1.9400
CASE	91	3.0000	2.0000	5.3000	2.0700
CASE	92	3.0000	2.0000	5.2000	2.0000
CASE	93	3.0000	2.0000	5.4000	2.0800
CASE	94	3.0000	2.0000	5.2000	1.9100
CASE	95	3.0000	3.0000	5.5000	2.1100
CASE	96	3.0000	3.0000	5.3000	2.1700
CASE	97	3.0000	3.0000	5.2000	1.7600
CASE	98	3.0000	3.0000	5.5000	2.1800
CASE	99	3.0000	3.0000	5.3000	2.0800
CASE	100	3.0000	3.0000	5.3000	2.0400
CASE	101	3.0000	3.0000	5.3000	1.7200
CASE	102	3.0000	3.0000	4.9000	1.8800
CASE	103	3.0000	3.0000	5.4000	2.0600
CASE	104	3.0000	3.0000	5.2000	2.1200
CASE	105	3.0000	4.0000	4.9000	1.8800
CASE	106	3.0000	4.0000	5.2000	1.8400
CASE	107	3.0000	4.0000	5.0000	1.7900
CASE	108	3.0000	4.0000	5.1000	1.3100
CASE	109	3.0000	4.0000	5.1000	2.0600
CASE	110	3.0000	4.0000	5.2000	1.9200
CASE	111	3.0000	4.0000	5.0000	1.7900
CASE	112	3.0000	4.0000	5.2000	1.8000
CASE	113	3.0000	4.0000	5.3000	1.9100
CASE	114	3.0000	4.0000	5.2000	1.4100
CASE	115	4.0000	1.0000	5.1000	1.7400
CASE	116	4.0000	1.0000	5.0000	1.6900
CASE	117	4.0000	1.0000	5.2000	1.7900
CASE	118	4.0000	1.0000	5.1000	1.5300
CASE	119	4.0000	1.0000	5.0000	1.5400
CASE	120	4.0000	1.0000	4.9000	1.7600
CASE	121	4.0000	1.0000	5.1000	1.6900
CASE	122	4.0000	1.0000	4.9000	1.5400

CASE	123	4.0000	1.0000	4.9000	1.6700
CASE	124	4.0000	2.0000	5.1000	1.8100
CASE	125	4.0000	2.0000	5.0000	1.7800
CASE	126	4.0000	2.0000	4.9000	1.6700
CASE	127	4.0000	2.0000	4.9000	1.6100
CASE	128	4.0000	2.0000	5.1000	1.6300
CASE	129	4.0000	2.0000	5.0000	1.8200
CASE	130	4.0000	2.0000	5.0000	1.6200
CASE	131	4.0000	2.0000	4.8000	1.7800
CASE	132	4.0000	2.0000	4.9000	1.5200
CASE	133	4.0000	2.0000	5.0000	1.9200
CASE	134	4.0000	3.0000	4.4000	1.1800
CASE	135	4.0000	3.0000	4.8000	1.2400
CASE	136	4.0000	3.0000	4.9000	1.3800
CASE	137	4.0000	3.0000	4.9000	1.4100
CASE	138	4.0000	3.0000	4.8000	1.5800
CASE	139	4.0000	3.0000	4.7000	1.4500
CASE	140	4.0000	3.0000	5.0000	1.4200
CASE	141	4.0000	3.0000	4.8000	1.3200
CASE	142	4.0000	3.0000	4.7000	1.1900
CASE	143	4.0000	4.0000	5.1000	1.7700
CASE	144	4.0000	4.0000	5.2000	1.8700
CASE	145	4.0000	4.0000	5.1000	1.8800
CASE	146	4.0000	4.0000	5.3000	1.9600
CASE	147	4.0000	4.0000	5.1000	2.0400
CASE	148	4.0000	4.0000	5.2000	1.9800
CASE	149	4.0000	4.0000	5.2000	1.6900
CASE	150	4.0000	4.0000	5.3000	1.2100
CASE	151	4.0000	4.0000	5.0000	1.4200
CASE	152	4.0000	4.0000	5.2000	1.8900
CASE	153	5.0000	1.0000	4.8000	1.4100
CASE	154	5.0000	1.0000	4.8000	1.4500
CASE	155	5.0000	1.0000	4.7000	1.3200
CASE	156	5.0000	1.0000	4.8000	1.5200
CASE	157	5.0000	1.0000	4.7000	1.1500
CASE	158	5.0000	1.0000	4.8000	1.3600
CASE	159	5.0000	1.0000	4.5000	1.1700
CASE	160	5.0000	2.0000	4.7000	1.4100
CASE	161	5.0000	2.0000	4.5000	1.3000
CASE	162	5.0000	2.0000	4.3000	1.2100
CASE	163	5.0000	2.0000	4.6000	1.2100
CASE	164	5.0000	2.0000	4.5000	1.2200
CASE	165	5.0000	2.0000	4.5000	1.1900
CASE	166	5.0000	2.0000	4.3000	0.7100
CASE	167	5.0000	2.0000	4.5000	1.2100
CASE	168	5.0000	2.0000	4.5000	1.2400
CASE	169	5.0000	2.0000	4.4000	1.2700
CASE	170	5.0000	3.0000	4.7000	1.3400
CASE	171	5.0000	3.0000	4.7000	1.2500
CASE	172	5.0000	3.0000	4.8000	1.3800
CASE	173	5.0000	3.0000	4.6000	1.5000
CASE	174	5.0000	3.0000	4.7000	1.3100
CASE	175	5.0000	3.0000	4.7000	1.3400
CASE	176	5.0000	3.0000	4.6000	1.3900
CASE	177	5.0000	3.0000	4.8000	1.5900
CASE	178	5.0000	3.0000	4.6000	1.5700
CASE	179	5.0000	3.0000	4.5000	1.3900
CASE	180	5.0000	4.0000	4.3000	1.1200
CASE	181	5.0000	4.0000	4.5000	1.1500
CASE	182	5.0000	4.0000	4.4000	1.2200
CASE	183	5.0000	4.0000	4.2000	0.8300
CASE	184	5.0000	4.0000	4.6000	1.4100
CASE	185	5.0000	4.0000	4.5000	0.9700
CASE	186	5.0000	4.0000	4.6000	1.2100
CASE	187	5.0000	4.0000	4.2000	1.0300
CASE	188	5.0000	4.0000	4.4000	1.2600

CASE	189	6.0000	1.0000	4.0000	0.2300
CASE	190	6.0000	1.0000	4.3000	0.9500
CASE	191	6.0000	1.0000	4.2000	0.8400
CASE	192	6.0000	1.0000	3.7000	0.3900
CASE	193	6.0000	1.0000	3.9000	0.4300
CASE	194	6.0000	2.0000	3.6000	0.6700
CASE	195	6.0000	3.0000	3.8000	0.3900
CASE	196	6.0000	3.0000	4.0000	0.7000
CASE	197	6.0000	3.0000	3.7000	0.5700
CASE	198	6.0000	3.0000	4.0000	0.2400
CASE	199	6.0000	3.0000	4.1000	0.7400
CASE	200	6.0000	4.0000	3.5000	0.6300
CASE	201	6.0000	4.0000	3.9000	0.5500
CASE	202	6.0000	4.0000	3.6000	0.1600

22

ANOVA on Weights
LEVELS ENCOUNTERED DURING PROCESSING ARE:
TRT
1.0000 2.0000 3.0000 4.0000 5.0000 6.0000
REP
1.0000 2.0000 3.0000 4.0000

DEP VAR: WEIGHT N: 202 MULTIPLE R: 0.903 SQUARED MULTIPLE R: 0.816

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	22.9212	5	4.5842	109.9293	0.0000
REP	0.2058	3	0.0686	1.6450	0.1807
TRT*REP	3.1963	15	0.2131	5.1098	0.0000
ERROR	7.4229	178	0.0417		

DURBIN-WATSON D STATISTIC 2.233
FIRST ORDER AUTOCORRELATION -.134

Post-hoc pairwise comparison of weight/Bonferroni.
USING LEAST SQUARES MEANS.
POST HOC TEST OF WEIGHT

MATRIX OF PAIRWISE MEAN DIFFERENCES:

	1	2	3	4	5
1	0.0000				
2	-0.0367	0.0000			
3	-0.0093	0.0274	0.0000		
4	-0.2824	-0.2457	-0.2731	0.0000	
5	-0.6384	-0.6017	-0.6291	-0.3560	0.0000
6	-1.3543	-1.3176	-1.3450	-1.0719	-0.7159

	6
6	0.0000

BONFERRONI ADJUSTMENT.
MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	1.0000	1.0000			
3	1.0000	1.0000	1.0000		
4	0.0000	0.0000	0.0000	1.0000	
5	0.0000	0.0000	0.0000	0.0000	1.0000
6	0.0000	0.0000	0.0000	0.0000	0.0000

	6
6	1.0000

ANOVA on Lengths
LEVELS ENCOUNTERED DURING PROCESSING ARE:
TRT

1.0000	2.0000	3.0000	4.0000	5.0000	6.0000

REP

1.0000	2.0000	3.0000	4.0000

DEP VAR: LENGTH N: 202 MULTIPLE R: 0.948 SQUARED MULTIPLE R: 0.900

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	24.8539	5	4.9708	251.6477	0.0000
REP	0.0932	3	0.0311	1.5723	0.1977
TRT*REP	2.2368	15	0.1491	7.5493	0.0000
ERROR	3.5160	178	0.0198		

DURBIN-WATSON D STATISTIC 2.354
FIRST ORDER AUTOCORRELATION -.184

Post-hoc pairwise comparison of length/Bonferroni.
USING LEAST SQUARES MEANS.
POST HOC TEST OF LENGTH

MATRIX OF PAIRWISE MEAN DIFFERENCES:

	1	2	3	4	5
1	0.0000				
2	-0.1673	0.0000			
3	-0.0211	0.1462	0.0000		
4	-0.2750	-0.1077	-0.2539	0.0000	
5	-0.6876	-0.5203	-0.6665	-0.4126	0.0000
6	-1.4583	-1.2910	-1.4372	-1.1833	-0.7708

	6
6	0.0000

BONFERRONI ADJUSTMENT.
MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	0.0000	1.0000			
3	1.0000	0.0002	1.0000		
4	0.0000	0.0204	0.0000	1.0000	
5	0.0000	0.0000	0.0000	0.0000	1.0000
6	0.0000	0.0000	0.0000	0.0000	0.0000

	6
6	1.0000

THE FOLLOWING RESULTS ARE FOR:
TRT = 1.0000
TOTAL OBSERVATIONS: 40

	WEIGHT	LENGTH
N OF CASES	40	40
MINIMUM	0.6500	4.7000
MAXIMUM	2.2800	5.6000
MEAN	1.9075	5.2600
STANDARD DEV	0.3303	0.1692

THE FOLLOWING RESULTS ARE FOR:
TRT = 2.0000

TOTAL OBSERVATIONS: 35

	WEIGHT	LENGTH
N OF CASES	35	35
MINIMUM	1.0800	4.9000
MAXIMUM	2.1600	5.4000
MEAN	1.8700	5.0914
STANDARD DEV	0.2141	0.1522

THE FOLLOWING RESULTS ARE FOR:
TRT = 3.0000

TOTAL OBSERVATIONS: 39

	WEIGHT	LENGTH
N OF CASES	39	39
MINIMUM	1.3100	4.9000
MAXIMUM	2.1800	5.5000
MEAN	1.8985	5.2385
STANDARD DEV	0.1810	0.1462

THE FOLLOWING RESULTS ARE FOR:
TRT = 4.0000

TOTAL OBSERVATIONS: 38

	WEIGHT	LENGTH
N OF CASES	38	38
MINIMUM	1.1800	4.4000
MAXIMUM	2.0400	5.3000
MEAN	1.6313	4.9895
STANDARD DEV	0.2305	0.1828

THE FOLLOWING RESULTS ARE FOR:
TRT = 5.0000
TOTAL OBSERVATIONS: 36

	WEIGHT	LENGTH
N OF CASES	36	36
MINIMUM	0.7100	4.2000
MAXIMUM	1.5900	4.8000
MEAN	1.2669	4.5639
STANDARD DEV	0.1856	0.1743

THE FOLLOWING RESULTS ARE FOR:
TRT = 6.0000
TOTAL OBSERVATIONS: 14

	WEIGHT	LENGTH
N OF CASES	14	14
MINIMUM	0.1600	3.5000
MAXIMUM	0.9500	4.3000
MEAN	0.5350	3.8786
STANDARD DEV	0.2381	0.2392

SUMMARY STATISTICS FOR WEIGHT

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 19.5096 DF= 5 PROBABILITY = 0.0015

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	29.4256	5	5.8851	105.1815	0.0000
WITHIN GROUPS	10.9666	196	0.0560		

SUMMARY STATISTICS FOR LENGTH

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 6.5312 DF= 5 PROBABILITY = 0.2579

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	29.2797	5	5.8559	199.3287	0.0000
WITHIN GROUPS	5.7582	196	0.0294		

KOLMOGOROV-SMIRNOV ONE SAMPLE TEST USING STANDARD NORMAL DISTRIBUTION

VARIABLE	N-OF-CASES	MAXDIF	PROBABILITY (2-TAIL)
LENGTH	202.0000	0.9998	0.0000
WEIGHT	202.0000	0.7710	0.0000

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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	1.340	4.840	7.640	4.840	1.340
OBSERVED	0	6	6	8	0

Calculated Chi-Square goodness of fit test statistic = 5.3732
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

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Shapiro Wilks test for normality

D = 3895.750

W = 0.924

Critical W (P = 0.05) (n = 20) = 0.905
Critical W (P = 0.01) (n = 20) = 0.868

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

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Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 3.98
Closest, conservative, Table H statistic = 151.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 5, df (# reps-1) = 3
Actual values ==> R (# groups) = 5, 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, the Hartley test may still be used as an approximate test (average df are used).

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Bartletts test for homogeneity of variance

Calculated B statistic = 1.86
Table Chi-square value = 13.28 (alpha = 0.01)
Table Chi-square value = 9.49 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 4

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: M&B 46136: Reproduction of Exposed D. magna
FILE: 42918672.rep
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 5

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	201.0000	201.0000
1	Control	2	217.0000	217.0000
1	Control	3	203.0000	203.0000
1	Control	4	190.0000	190.0000
2	0.63	1	184.0000	184.0000
2	0.63	2	178.0000	178.0000
2	0.63	3	178.0000	178.0000
2	0.63	4	137.0000	137.0000
3	1.5	1	125.0000	125.0000
3	1.5	2	133.0000	133.0000
	1.5	3	108.0000	108.0000
3	1.5	4	93.0000	93.0000
4	2.6	1	116.0000	116.0000
4	2.6	2	80.0000	80.0000
4	2.6	3	110.0000	110.0000
4	2.6	4	93.0000	93.0000
5	5.8	1	44.0000	44.0000
5	5.8	2	26.0000	26.0000
5	5.8	3	22.0000	22.0000
5	5.8	4	41.0000	41.0000

mean measured conc.

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	202.750	202.750	202.750
2	0.63	4	169.250	169.250	169.250
3	1.5	4	114.750	114.750	114.750
4	2.6	4	99.750	99.750	99.750
5	5.8	4	33.250	33.250	33.250

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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
Control	202.750				
0.63	169.250	2.940	*	1.75	k= 1, v=15
1.5	114.750	7.722	*	1.84	k= 2, v=15
2.6	99.750	9.039	*	1.87	k= 3, v=15
5.8	33.250	14.874	*	1.88	k= 4, v=15

s = 16.116
Note: df used for table values are approximate when v > 20.