

US EPA ARCHIVE DOCUMENT

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:**

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Signature: *Andrew Bryceland*
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Date:

6. **APPROVED BY:**

Ann Stavola, Supervisory Biologist
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Signature: *Ann Stavola*
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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.

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:**

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Date: 3/2/94
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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
EC₅₀ was 4.5 µg ai/l.
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 $20 \pm 1^\circ\text{C}$. 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 $\mu\text{mhos/cm}$, 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 $20 \pm 1^\circ\text{C}$ 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 $\mu\text{g ai/l}$) were selected for this study. A dilution water control and a solvent control were also included. The solvent control contained 17 $\mu\text{l/l}$ 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*; 4×10^7 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.

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 $p \leq 0.01$.

EC₅₀ 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 $\mu\text{g ai/l}$ (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 $\mu\text{g ai/l}$. Due to the variability in measurements obtained between replicate control vessels on a given day (i.e., 0.54 and 3.4 $\mu\text{g ai/l}$), 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 $\mu\text{g ai/l}$) was significantly reduced when compared to that of the pooled control (Table 3, attached). Based on survival data, the 21-day EC₅₀ (95% confidence interval) was 4.5 (3.9-5.2) $\mu\text{g ai/l}$.

Mean body length and the number of offspring produced per female at 2.6 $\mu\text{g ai/l}$ 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 $\mu\text{g ai/l}$ 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 $\mu\text{mhos/cm}$, 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 CaCO₃, 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 EC₅₀ 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 $>0.63 \mu\text{g ai/l}$ 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.

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 EC₅₀ 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.

Fifteen 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.
 - Description of quality control procedures.
 - Identity of the source of product ingredients.
 - Sales or other commercial/financial information.
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 - The product confidential statement of formula.
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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.

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
Bartlett's 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	mean measured conc	1	0.9000	1.2490
2	0.63	2	1.0000	1.4120
2		3	1.0000	1.4120
2		4	1.0000	1.4120
3		1	0.9000	1.2490
3	1.5	2	1.0000	1.4120
3		3	0.9000	1.2490
3		4	1.0000	1.4120
4	2.6	1	0.7000	0.9912
4		2	1.0000	1.4120
4		3	1.0000	1.4120
4		4	0.9000	1.2490
5	5.8	1	0.5000	0.7854
5		2	0.1000	0.3218
5		3	0.5000	0.7854
5		4	0.3000	0.5796
6	12.0	1	0.0000	0.1588
6		2	0.0000	0.1588
6		3	0.0000	0.1588
6		4	0.0000	0.1588

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	16.00	10.00	4.00	
3	1.5	1.5	14.00	10.00	4.00	
4	2.6	3.0	14.00	10.00	4.00	
5	5.8	6.0	10.00	10.00	4.00	*
6	12.0	12.0	10.00	10.00	4.00	*

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	1.8000
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

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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

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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

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	6
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						0.0000

BONFERRONI ADJUSTMENT.
 MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5	6
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	1.0000
6						1.0000

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ANOVA on Lengths
 LEVELS ENCOUNTERED DURING PROCESSING ARE:
 TRT

REP	1.0000	2.0000	3.0000	4.0000	5.0000	6.0000
	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

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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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Bartlett's 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).

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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	MEAN measured conc. 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
	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

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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.