

US EPA ARCHIVE DOCUMENT

MRID No. 442448-02

**DATA EVALUATION RECORD
ESTUARINE FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4**

1. **CHEMICAL:** Diazinon PC Code No.: 057801

2. **TEST MATERIAL:** Diazinon MG Purity: 87.3%

3. **CITATION:**

Author: Joseph V. Sousa
Title: Diazinon - Chronic Toxicity to
Sheepshead Minnow, (*Cyprinodon
variegatus*), Under Flow-Through
Conditions

Study Completion Date: March 18, 1997

Laboratory: Springborn Laboratories, Inc., Wareham,
MA

Laboratory Report ID: 97-2-6887

Sponsor: Novartis Crop Protection, Inc.,
Greensboro, NC

MRID No.: 442448-02

DP Barcode: D240486

4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist,
Golder Associates Inc.

Signature: *Karl Bullock* **Date:** 3/4/99

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

Signature: *P. Kosalwat* **Date:** 3/4/99

5. **APPROVED BY:**

Signature: *Diana A. Dwyer* **Date:** 5/6/99

6. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an estuarine fish early life-stage test. Based on the most sensitive endpoints (length, dry weight, wet weight), the NOEC and LOEC were 4.3 and 8.0 ppb ai, respectively. The geometric-mean MATC was determined to be 5.9 ppb ai.

7. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core.

B. **Rationale:** N/A



C. Repairability: N/A

8. MAJOR GUIDELINE DEVIATIONS:

1. The test consisted of only 2 true replicates; 4 replicates are recommended.
2. The concentration of solvent in the solvent control is unclear.

9. MATERIALS AND METHODS

A. Biological System

| Guideline Criteria | Reported Information |
|---|---|
| Species: An estuarine fish species, preferably a silversides species or sheepshead minnow (<i>Cyprinodon variegatus</i>). | <i>Cyprinodon variegatus</i> |
| Source | Embryos were obtained from Aquatic BioSystems, Inc., Ft. Collins, CO. Fertilization success was estimated to be 76%. |
| Age at beginning of test: Embryos 2 to 24 hours old. | Embryos \leq 24 hours old. |
| Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure. | 60 embryos per incubator cup; 1 cup per replicate vessel; 2 replicate vessels per treatment or control. Post hatch exposure: On test day 7, surviving larvae (35-48 fish per replicate, 73-89 fish per treatment) were placed into their respective exposure aquaria. |
| Post Hatch: % of embryos that produce live fry must be \geq 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup. | 60-68% of embryos in the control produced live fry. |

| Guideline Criteria | Reported Information |
|--|---|
| Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32. | Fish were fed live brine shrimp nauplii <i>ad libitum</i> 3 times daily until the day prior to test termination when food was withheld. |
| Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching. | Survival of embryos and larvae was recorded daily. |
| Controls: Avg. survival at end of test must be $\geq 80\%$. Survival in any control chamber must not be $< 70\%$. | 100 and 97% survival in the negative and solvent controls, respectively, at test termination. |
| Controls: Negative control and carrier control (when applicable) are required. | Negative and solvent control. |

Comments: Only two replicates per treatment level were used in this test.

B. Physical System

| Guideline Criteria | Reported Information |
|--|---|
| Test Water: 1) May be natural (sterilized and filtered) or a commercial mixture; 2) Natural seawater should have weekly range of salinity less than 6‰, monthly pH range less than 0.8 pH units; 3) Salinity should be ≥ 15 parts per thousand; 4) Water must be free of pollutants. | 1) Natural filtered seawater. 2) pH range of 7.4-7.9 during test. 3) Salinity range of 30-33‰ during test. 4) No significant levels of pollutants were detected. |
| Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For sheepshead minnow, either 25°C or 30°C is recommended. | Mean: 25°C Range: 23-26°C |
| Photoperiod: Recommend 16L/8D. | 16 hours light/8 hours dark. |

| Guideline Criteria | Reported Information |
|---|--|
| <p>Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.</p> | <p>An intermittent-flow proportional diluter was used. The test consisted of a dilution water control, solvent control, and 5 concentrations with a dilution factor of 0.5.</p> |
| <p>Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.</p> | <p>1) A mixing chamber was used. 2) Aeration was not used in mixing. 3) No undissolved test material was observed. 4) Not reported.</p> |
| <p>Test Vessels: All glass or glass with stainless steel frame.</p> | <p>Test vessels were 39 X 20 X 25 cm and contained approximately 15 L of exposure solution.</p> |
| <p>Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</p> | <p>Embryo cups were constructed using glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex® mesh screen bottoms.</p> |
| <p>Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain D.O. at above 75% of saturation and maintain the toxicant level.</p> | <p>6.3 volume additions every 24 hours; 90% replacement in 8 hours.</p> |
| <p>Aeration: Dilution water should be aerated to insure D.O. concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</p> | <p>D.O. \geq76% (5.2 mg/L) of saturation at all times.</p> |

C. Chemical System

| Guideline Criteria | Reported Information |
|---|--|
| <p>Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <p>- Toxicant conc. must be measured in one tank at each toxicant level every week.</p> <p>- One concentration must adversely affect a life stage and one concentration must not affect any life stage.</p> | <p>- Dilution water control, solvent control, and five nominal concentrations (3.8, 7.5, 15, 30, and 60 $\mu\text{g ai/L}$).</p> <p>- Toxicant concentrations were measured in both replicates on days 0 and 6, and in alternate replicates on days 13, 20, 27, and 34 from both controls and treatments.</p> <p>- NOEC and LOEC were achieved.</p> |
| <p>Other Variables:</p> <p>1) D.O. must be measured at each conc. at least once a week;</p> <p>2) Natural seawater must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range <0.8 pH units.</p> | <p>1) D.O., pH, temperature, and salinity was measured daily in each aquarium.</p> <p>2) Salinity and pH did not fluctuate beyond recommended levels.</p> |
| <p>Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p> | <p>The concentration of solvent in all exposure aquaria was 18 $\mu\text{L acetone/L}$, with the exception of the solvent control, which was not reported.</p> |

Comments: Mean measured concentrations ranged from 89 to 110% of nominal concentrations and were consistent between each replicate. Limit of detection = 0.38 - 0.88 $\mu\text{g ai/L}$. Spike recoveries = 88.4 - 110%.

10. REPORTED RESULTS

| Guideline Criteria | Reported Information |
|---|--|
| Data Endpoints must include: <ul style="list-style-type: none"> - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if appropriate); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs. | All appropriate data endpoints listed were reported. |
| Raw data included? (Y/N) | Yes |

Effects Data

| Toxicant Concentration ($\mu\text{g ai/L}$) | | Mean Percent Hatch | Percent Post-Hatch Survival (28 days) | Total Length (mm) | Wet Weight (g) | Dry Weight (g) |
|---|--------------------|--------------------|---------------------------------------|-------------------|-------------------|--------------------|
| Nominal | Mean Measured (SD) | | | | | |
| Control | <0.88 | 68 | 100 | 24.5 | 0.25 | 0.075 |
| Solvent Control | <0.88 | 64 | 97 | 24.5 | 0.24 | 0.069 |
| 3.8 | 4.3 (0.51) | 73 | 91 | 24.3 | 0.24 | 0.065 |
| 7.5 | 8.0 (0.81) | 61 | 97 | 23.5 ^a | 0.22 ^a | 0.053 ^a |
| 15 | 13 (0.62) | 74 | 92 | 22.2 ^a | 0.19 ^a | 0.047 ^a |
| 30 | 28 (2.6) | 66 | 97 | 21.7 ^a | 0.19 ^a | 0.048 ^a |
| 60 | 56 (6.1) | 63 | 93 | 19.4 ^a | 0.14 ^a | 0.032 ^a |

^a Significantly reduced when compared to the solvent control ($p < 0.05$).

Comments: Hatching was completed by day 7.

Toxicity Observations: No sublethal effects were reported.

Statistical Results:

Statistical Method: Williams' test with response comparisons made between the treatment concentrations and the pooled control (percentage data were arc sine square-root transformed prior to analysis). Analyses were performed using the mean organism response in each treatment group rather than individual response values.

NOEC: 4.3 $\mu\text{g ai/L}$

LOEC: 8.0 $\mu\text{g ai/L}$

MATC: 5.9 $\mu\text{g ai/L}$

Most sensitive endpoints: Growth (dry weight)

11. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: Bonferroni's Test and Dunnett's Test were used for continuous data (length and weight) and Williams' Test was used for survival and hatching success (percentage data were arc sine square-root transformed prior to analysis). Analyses were performed using individual response values; comparisons were made against the solvent control.

NOEC: 4.3 ppb ai

LOEC: 8.0 ppb ai

MATC: 5.9 ppb ai

Most sensitive endpoint: Growth (length, wet weight, and dry weight)

- 12. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an estuarine fish early life-stage test. Based on mean measured concentrations and the most sensitive endpoints (length, wet weight, and dry weight), the NOEC and LOEC for sheepshead minnows exposed to diazinon were determined to be 4.3 and 8.0 ppb ai, respectively. The geometric mean MATC was determined to be 5.9 ppb ai. This study is classified as **Core**.

Diazinon: Chronic Sheepshead - % Hatch

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Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|-----------------|---|---------------|------------------|-----------------|
| 1 | Solvent Control | 2 | 0.640 | 0.928 | 0.980 |
| 2 | 4.3 | 2 | 0.735 | 1.033 | 0.980 |
| 3 | 8.0 | 2 | 0.605 | 0.891 | 0.964 |
| 4 | 13 | 2 | 0.740 | 1.036 | 0.964 |
| 5 | 28 | 2 | 0.655 | 0.945 | 0.945 |
| 6 | 56 | 2 | 0.625 | 0.912 | 0.912 |

Diazinon: Chronic Sheepshead - % Hatch

File: 44244802

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|-----------------|-----------------|----------------|-----------|----------------|--------------------|
| Solvent Control | 0.980 | | | | |
| 4.3 | 0.980 | 0.710 | | 1.94 | k= 1, v= 6 |
| 8.0 | 0.964 | 0.483 | | 2.06 | k= 2, v= 6 |
| 13 | 0.964 | 0.483 | | 2.10 | k= 3, v= 6 |
| 28 | 0.945 | 0.233 | | 2.12 | k= 4, v= 6 |
| 56 | 0.912 | 0.209 | | 2.13 | k= 5, v= 6 |

s = 0.074

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

Diazinon: Chronic Sheepshead - % Larval Survival
 File: 44244802 Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|-----------------|---|---------------|------------------|-----------------|
| 1 | Solvent Control | 2 | 0.975 | 1.418 | 1.418 |
| 2 | 4.3 | 2 | 0.905 | 1.287 | 1.353 |
| 3 | 8.0 | 2 | 0.975 | 1.418 | 1.353 |
| 4 | 13 | 2 | 0.920 | 1.289 | 1.348 |
| 5 | 28 | 2 | 0.970 | 1.407 | 1.348 |
| 6 | 56 | 2 | 0.935 | 1.313 | 1.313 |

Diazinon: Chronic Sheepshead - % Larval Survival
 File: 44244802 Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|-----------------|-----------------|----------------|-----------|----------------|--------------------|
| Solvent Control | 1.418 | | | | |
| 4.3 | 1.353 | 0.560 | | 1.94 | k= 1, v= 6 |
| 8.0 | 1.353 | 0.560 | | 2.06 | k= 2, v= 6 |
| 13 | 1.348 | 0.600 | | 2.10 | k= 3, v= 6 |
| 28 | 1.348 | 0.600 | | 2.12 | k= 4, v= 6 |
| 56 | 1.313 | 0.900 | | 2.13 | k= 5, v= 6 |

s = 0.117

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

Diazinon: Sheepshead Minnow Chronic Toxicity
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TRT=I

| Variable | N | Mean | Std Dev | Minimum | Maximum |
|----------|----|------------|-----------|------------|------------|
| LEN | 80 | 24.3065000 | 1.5226876 | 21.5300000 | 28.0700000 |
| WETWT | 80 | 0.2416125 | 0.0483645 | 0.1593000 | 0.3893000 |
| DRYWT | 80 | 0.0649075 | 0.0132465 | 0.0433000 | 0.1119000 |

TRT=II

| Variable | N | Mean | Std Dev | Minimum | Maximum |
|----------|----|------------|-----------|------------|------------|
| LEN | 71 | 23.4915493 | 1.4317398 | 20.0000000 | 26.6100000 |
| WETWT | 71 | 0.2152056 | 0.0466005 | 0.1087000 | 0.3342000 |
| DRYWT | 71 | 0.0528310 | 0.0126220 | 0.0228000 | 0.0839000 |

TRT=III

| Variable | N | Mean | Std Dev | Minimum | Maximum |
|----------|----|------------|-----------|------------|------------|
| LEN | 82 | 22.2391463 | 2.4694270 | 16.7400000 | 26.8100000 |
| WETWT | 82 | 0.1926305 | 0.0671733 | 0.0645000 | 0.3318000 |
| DRYWT | 82 | 0.0472512 | 0.0182231 | 0.0140000 | 0.0859000 |

TRT=IV

| Variable | N | Mean | Std Dev | Minimum | Maximum |
|----------|----|------------|-----------|------------|------------|
| LEN | 77 | 21.6735065 | 2.1053508 | 17.9500000 | 26.3400000 |
| WETWT | 77 | 0.1922091 | 0.0546759 | 0.0926000 | 0.3487000 |
| DRYWT | 77 | 0.0477442 | 0.0137998 | 0.0215000 | 0.0827000 |

TRT=Solvent

| Variable | N | Mean | Std Dev | Minimum | Maximum |
|----------|----|------------|-----------|------------|------------|
| LEN | 75 | 24.4464000 | 1.5726373 | 21.2900000 | 27.9900000 |
| WETWT | 75 | 0.2383413 | 0.0498894 | 0.1483000 | 0.3532000 |
| DRYWT | 75 | 0.0685800 | 0.0151210 | 0.0413000 | 0.1013000 |

TRT=V

| Variable | N | Mean | Std Dev | Minimum | Maximum |
|----------|----|------------|-----------|------------|------------|
| LEN | 70 | 19.4084286 | 2.3780559 | 14.6800000 | 24.9800000 |
| WETWT | 70 | 0.1415643 | 0.0528605 | 0.0518000 | 0.2933000 |
| DRYWT | 70 | 0.0315886 | 0.0134791 | 0.0102000 | 0.0753000 |

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General Linear Models Procedure
Class Level Information

| Class | Levels | Values |
|-------|--------|--------|
| | | |

TRT 6 I II III IV Solvent V
REP 2 A B

Number of observations in data set = 455

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General Linear Models Procedure

Dependent Variable: LEN

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|-----------------|-----|----------------|-------------|---------|--------|
| Model | 5 | 1334.4703 | 266.8941 | 69.24 | 0.0001 |
| Error | 449 | 1730.6942 | 3.8546 | | |
| Corrected Total | 454 | 3065.1645 | | | |

| R-Square | C.V. | Root MSE | LEN Mean |
|----------|----------|----------|----------|
| 0.435367 | 8.675397 | 1.9633 | 22.631 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|--------|----|-------------|-------------|---------|--------|
| TRT | 5 | 1334.4703 | 266.8941 | 69.24 | 0.0001 |
| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
| TRT | 5 | 1334.4703 | 266.8941 | 69.24 | 0.0001 |

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General Linear Models Procedure

Dependent Variable: WETWT

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|-----------------|-----|----------------|-------------|---------|--------|
| Model | 5 | 0.5046577 | 0.1009315 | 34.69 | 0.0001 |
| Error | 449 | 1.3064784 | 0.0029098 | | |
| Corrected Total | 454 | 1.8111360 | | | |

| R-Square | C.V. | Root MSE | WETWT Mean |
|----------|----------|----------|------------|
| 0.278641 | 26.39401 | 0.0539 | 0.2044 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|--------|----|-------------|-------------|---------|--------|
| TRT | 5 | 0.5046577 | 0.1009315 | 34.69 | 0.0001 |
| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
| TRT | 5 | 0.5046577 | 0.1009315 | 34.69 | 0.0001 |

Diazinon: Sheepshead Minnow Chronic Toxicity
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General Linear Models Procedure

Dependent Variable: DRYWT

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|-----------------|----------|----------------|-------------|------------|--------|
| Model | 5 | 0.0663235 | 0.0132647 | 62.14 | 0.0001 |
| Error | 449 | 0.0958419 | 0.0002135 | | |
| Corrected Total | 454 | 0.1621654 | | | |
| | R-Square | C.V. | Root MSE | DRYWT Mean | |
| | 0.408987 | 27.87354 | 0.0146 | 0.0524 | |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|--------|----|-------------|-------------|---------|--------|
| TRT | 5 | 0.0663235 | 0.0132647 | 62.14 | 0.0001 |
| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
| TRT | 5 | 0.0663235 | 0.0132647 | 62.14 | 0.0001 |

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General Linear Models Procedure
Least Squares Means

| TRT | LEN LSMEAN | LSMEAN Number |
|---------|------------|---------------|
| I | 24.3065000 | 1 |
| II | 23.4915493 | 2 |
| III | 22.2391463 | 3 |
| IV | 21.6735065 | 4 |
| Solvent | 24.4464000 | 5 |
| V | 19.4084286 | 6 |

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

| i/j | 1 | 2 | 3 | 4 | 5 | 6 |
|-----|--------|--------|--------|--------|--------|--------|
| 1 | . | 0.0112 | 0.0001 | 0.0001 | 0.6577 | 0.0001 |
| 2 | 0.0112 | . | 0.0001 | 0.0001 | 0.0035 | 0.0001 |
| 3 | 0.0001 | 0.0001 | . | 0.0701 | 0.0001 | 0.0001 |
| 4 | 0.0001 | 0.0001 | 0.0701 | . | 0.0001 | 0.0001 |
| 5 | 0.6577 | 0.0035 | 0.0001 | 0.0001 | . | 0.0001 |
| 6 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | . |

| TRT | WETWT LSMEAN | LSMEAN Number |
|---------|--------------|---------------|
| I | 0.24161250 | 1 |
| II | 0.21520563 | 2 |
| III | 0.19263049 | 3 |
| IV | 0.19220909 | 4 |
| Solvent | 0.23834133 | 5 |
| V | 0.14156429 | 6 |

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

| i/j | 1 | 2 | 3 | 4 | 5 | 6 |
|-----|--------|--------|--------|--------|--------|--------|
| 1 | . | 0.0028 | 0.0001 | 0.0001 | 0.7061 | 0.0001 |
| 2 | 0.0028 | . | 0.0101 | 0.0099 | 0.0099 | 0.0001 |
| 3 | 0.0001 | 0.0101 | . | 0.9608 | 0.0001 | 0.0001 |
| 4 | 0.0001 | 0.0099 | 0.9608 | . | 0.0001 | 0.0001 |
| 5 | 0.7061 | 0.0099 | 0.0001 | 0.0001 | . | 0.0001 |

6 0.0001 0.0001 0.0001 0.0001

| TRT | DRYWT LSMEAN | LSMEAN Number |
|---------|--------------|---------------|
| I | 0.06490750 | 1 |
| II | 0.05283099 | 2 |
| III | 0.04725122 | 3 |
| IV | 0.04774416 | 4 |
| Solvent | 0.06858000 | 5 |
| V | 0.03158857 | 6 |

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

| i/j | 1 | 2 | 3 | 4 | 5 | 6 |
|-----|--------|--------|--------|--------|--------|--------|
| 1 | . | 0.0001 | 0.0001 | 0.0001 | 0.1185 | 0.0001 |
| 2 | 0.0001 | . | 0.0189 | 0.0349 | 0.0001 | 0.0001 |
| 3 | 0.0001 | 0.0189 | . | 0.8317 | 0.0001 | 0.0001 |
| 4 | 0.0001 | 0.0349 | 0.8317 | . | 0.0001 | 0.0001 |
| 5 | 0.1185 | 0.0001 | 0.0001 | 0.0001 | . | 0.0001 |
| 6 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | . |

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General Linear Models Procedure
Least Squares Means

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

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General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: LEN

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

Alpha = 0.05 Confidence = 0.95 df = 449 MSE = 3.854553
Critical Value of T = 2.95099

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

| TRT Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | |
|----------------|-------------------------------------|--------------------------|-------------------------------------|-----|
| Solvent - I | -0.7913 | 0.1399 | 1.0711 | |
| Solvent - II | -0.0045 | 0.9549 | 1.9142 | |
| Solvent - III | -1.2816 | 2.2073 | 3.1329 | *** |
| Solvent - IV | 1.8330 | 2.7729 | 3.7128 | *** |
| Solvent - V | 4.0751 | 5.0380 | 6.0008 | *** |
| I - Solvent | -1.0711 | -0.1399 | 0.7913 | |
| I - II | -0.1297 | 0.8150 | 1.7596 | |
| I - III | 1.1569 | 2.0674 | 2.9778 | *** |
| I - IV | 1.7081 | 2.6330 | 3.5579 | *** |
| I - V | 3.9499 | 4.8981 | 5.8463 | *** |
| II - Solvent | -1.9142 | -0.9549 | 0.0045 | |
| II - I | -1.7596 | -0.8150 | 0.1297 | |
| II - III | 0.3132 | 1.2524 | 2.1916 | *** |
| II - IV | 0.8648 | 1.8180 | 2.7713 | *** |

| TRT Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | Significance |
|----------------|-------------------------------------|--------------------------|-------------------------------------|--------------|
| I - I | -3.1073 | 4.0831 | 5.0590 | *** |
| III - I | -3.1329 | -2.2073 | -1.2816 | *** |
| III - II | -2.9778 | -2.0674 | -1.1569 | *** |
| III - III | -2.1916 | -1.2524 | -0.3132 | *** |
| III - IV | -0.3538 | 0.5656 | 1.4850 | *** |
| III - V | 1.8879 | 2.8307 | 3.7735 | *** |
| IV - Solvent | -3.7128 | -2.7729 | -1.8330 | *** |
| I - I | -3.5579 | -2.6330 | -1.7081 | *** |
| IV - II | -2.7713 | -1.8180 | -0.8648 | *** |
| IV - III | -1.4850 | -0.5656 | 0.3538 | *** |
| IV - V | 1.3083 | 2.2651 | 3.2219 | *** |
| V - Solvent | -6.0008 | -5.0380 | -4.0751 | *** |
| V - I | -5.8463 | -4.8981 | -3.9499 | *** |
| V - II | -5.0590 | -4.0831 | -3.1073 | *** |
| V - III | -3.7735 | -2.8307 | -1.8879 | *** |
| V - IV | -3.2219 | -2.2651 | -1.3083 | *** |

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General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: METWT

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

Alpha= 0.05 Confidence= 0.95 df= 449 MSE= 0.00291
Critical Value of T= 2.95099

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

| TRT Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | Significance |
|----------------|-------------------------------------|--------------------------|-------------------------------------|--------------|
| IV - I | -0.025682 | -0.000421 | 0.024839 | *** |
| IV - II | 0.024357 | 0.050645 | 0.076933 | *** |
| V - I | -0.126101 | -0.100048 | -0.073996 | *** |
| V - Solvent | -0.123252 | -0.096777 | -0.070523 | *** |
| V - II | -0.100453 | -0.073641 | -0.046829 | *** |
| V - III | -0.076970 | -0.051066 | -0.025163 | *** |
| V - IV | -0.076933 | -0.050645 | -0.024357 | *** |

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General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: DRYWT

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

Alpha= 0.05 Confidence= 0.95 df= 449 MSE= 0.000213
Critical Value of T= 2.95099

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

| TRT Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | Significance |
|----------------|-------------------------------------|--------------------------|-------------------------------------|--------------|
| Solvent - I | -0.003257 | 0.003672 | 0.010602 | *** |
| Solvent - II | 0.008610 | 0.015749 | 0.022888 | *** |
| Solvent - IV | 0.013841 | 0.020836 | 0.027831 | *** |
| Solvent - III | 0.014440 | 0.021329 | 0.028217 | *** |
| Solvent - V | 0.029826 | 0.033319 | 0.044157 | *** |
| I - Solvent | -0.010602 | -0.003672 | 0.003257 | *** |
| I - II | 0.005047 | 0.012077 | 0.019106 | *** |
| I - IV | 0.010280 | 0.017163 | 0.024046 | *** |
| I - III | 0.010881 | 0.017656 | 0.024432 | *** |
| I - V | 0.026263 | 0.033319 | 0.040375 | *** |
| II - Solvent | -0.022888 | -0.015749 | -0.008610 | *** |
| II - I | -0.019106 | -0.012077 | -0.005047 | *** |
| II - IV | -0.002007 | 0.005087 | 0.012181 | *** |
| II - III | -0.001410 | 0.005580 | 0.012569 | *** |
| II - V | 0.013980 | 0.021242 | 0.028504 | *** |
| IV - Solvent | -0.027831 | -0.020836 | -0.013841 | *** |
| IV - I | -0.024046 | -0.017163 | -0.010280 | *** |
| IV - II | -0.012181 | -0.005087 | 0.002007 | *** |
| IV - III | -0.006349 | 0.000493 | 0.007335 | *** |
| IV - V | 0.009035 | 0.016156 | 0.023276 | *** |
| III - Solvent | -0.028217 | -0.021329 | -0.014440 | *** |
| III - I | -0.024432 | -0.017656 | -0.010881 | *** |
| III - II | -0.012569 | -0.005580 | 0.001410 | *** |
| III - IV | -0.007335 | -0.000493 | 0.006349 | *** |
| III - V | 0.008647 | 0.015663 | 0.022679 | *** |
| V - Solvent | -0.044157 | -0.036691 | -0.029826 | *** |
| V - I | -0.040375 | -0.033319 | -0.026263 | *** |
| V - II | -0.028504 | -0.021242 | -0.013980 | *** |
| V - III | -0.023276 | -0.016156 | -0.009035 | *** |
| V - IV | -0.022679 | -0.015663 | -0.008647 | *** |

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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= 449 MSE= 3.854553
Critical Value of Dunnett's T= 2.239

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

| TRT Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | |
|----------------|-------------------------------------|--------------------------|-------------------------------------|-----|
| I - Solvent | -0.8466 | -0.1399 | 0.5668 | |
| II - Solvent | -1.6829 | -0.9549 | -0.2268 | *** |
| III - Solvent | -2.9097 | -2.2073 | -1.5048 | *** |
| IV - Solvent | -3.4862 | -2.7729 | -2.0596 | *** |
| V - Solvent | -5.7686 | -5.0380 | -4.3073 | *** |

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General Linear Models Procedure

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

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

Alpha= 0.05 Confidence= 0.95 df= 449 MSE= 0.00291
Critical Value of Dunnett's T= 2.239

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

| TRT Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | |
|----------------|-------------------------------------|--------------------------|-------------------------------------|-----|
| I - Solvent | -0.016144 | 0.003271 | 0.022687 | |
| II - Solvent | -0.043138 | -0.023136 | -0.003133 | *** |
| III - Solvent | -0.065012 | -0.045711 | -0.026410 | *** |
| IV - Solvent | -0.065730 | -0.046132 | -0.026534 | *** |
| V - Solvent | -0.116853 | -0.096777 | -0.076702 | *** |

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General Linear Models Procedure

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

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

Alpha= 0.05 Confidence= 0.95 df= 449 MSE= 0.000213
Critical Value of Dunnett's T= 2.239

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

Simultaneous Simultaneous

| TRT Comparison | Lower Confidence Limit | Difference Between Means | Upper Confidence Limit | |
|----------------|------------------------|--------------------------|------------------------|-----|
| I - Solvent | -0.008931 | -0.003672 | 0.001586 | |
| II - Solvent | -0.021167 | -0.015749 | -0.010331 | *** |
| IV - Solvent | -0.026144 | -0.020836 | -0.015528 | *** |
| III - Solvent | -0.026556 | -0.021329 | -0.016101 | *** |
| V - Solvent | -0.042429 | -0.036991 | -0.031554 | *** |