

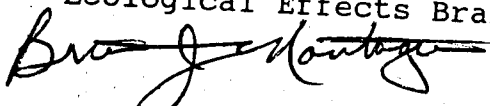
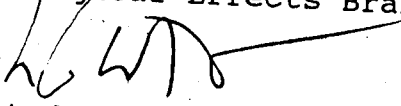
US EPA ARCHIVE DOCUMENT

(3-8-96)

DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4(A)

- 1. CHEMICAL: Alachlor Shaughnessey #:090501
- 2. TEST MATERIAL: Alachlor technical Purity:
- 3. CITATION:

Authors: Rhodes, Jon E. and Michelle Muckerman
Title: Early Life-Stage Toxicity of Alachlor to the Rainbow Trout (*Onchorhynchus mykiss*) Under Flow-Through Conditions
Study Completion Date: 6/20/95
Duration of Exposure: 96 days
Laboratory: ABC Laboratories, Columbia, Mo.
Laboratory Report ID: ABC # 42187
Sponsor: Monsanto Chemical Company
MRID No.: 438626-01
Barcode: D221890

- 4. REVIEWED BY: Brian Montague, Fisheries Biologist
Ecological Effects Branch, EFED
Signature:  Date: 3/7/96
- 5. APPROVED BY: Les Fouart PhD, Section Supervisor
Ecological Effects Branch, EFED (7507C)
Signature:  Date: 3/8/96

6. CONCLUSIONS: The study has shown Alachlor to be highly toxic to rainbow trout when chronically exposed from eggs to 60 days post hatch. Growth (both length and weight) were significantly affected. Survival was also affected at higher dosage levels. No effects were observed on hatch success or time to hatch (avg 36 days all levels). Exophthalmia was noted at 1.63 mg ai/L in some fry as was a 3 day delay in time to swim-up of larvae.

| Parameter Effected | LOEC | NOEC |
|-------------------------|-----------|-----------|
| A. Growth (length) | 0.388 PPM | 0.187 PPM |
| B. Growth (wet wt.) | 0.388 PPM | 0.187 PPM |
| C. Posthatch Srv. (60D) | 1.63 PPM | 0.809 PPM |

- 7. ADEQUACY OF THE STUDY:
 - A. Classification: Core
 - B. Rationale: Study was conducted according to EPA accepted methodology and study director's conclusions are supported by the Agency's independent statistical analysis.
 - C. Reparability: N.A.

8. **MAJOR GUIDELINE DEVIATIONS:** No major guideline deviations were noted. A finer mesh screen is recommended for embryo cups in Agency guidelines. At termination it was discovered that one control fish was missing and that the dilution control had mysteriously gained a new occupant. Since the controls were pooled in the laboratory analysis this would have been accounted for in the overall comparisons.

9. **MATERIALS AND METHODS:**

A. **Biological System:**

| Guideline Criteria | Reported Information |
|--|--|
| Species: | Onchorynchus mykiss |
| Source | Unfertilized eggs obtained from Mt. Lassen Trout Farms on March 15, 1995. |
| Age at beginning of test: Embryos 2 to 24 hours old. | Eggs were fertilized 1 hour before intro into incubation cups. |
| Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure. | 30 embryos/cup 4 replicate cups/test level (50 embryos used in dilution control cups) |
| Post Hatch: % Viability and Hatch Success | 76-90% Viable Eggs-Controls 93-100% Egghatch-All levels |
| Feeding: Feed Schedule and Types of Feed utilized. | 12 Days Posthatch-live brine shrimp. 21 Days posthatch-salmon starter added to diet. Finally starter pellets were fed. Feeding-2-3 times per day minimum. Food withheld 24 hours prior to termination. |
| Counts: Posthatch Fry Counts and Observation Frequency | Daily Counts and observations of fry mortality were made. |
| Controls Survival: Mean and Individual Replicate | Control and Solvent Control Survival: Mean=98.7% Min. Replicate=>93% |
| Solvent Controls: | Solvent Control employed |

Comments:

B. Physical System:

| Guideline Criteria | Reported Information |
|--|---|
| <p>Test Water: Source Filtration procedures Hardness Range pH Range</p> | <p>Well water subjected to reverse osmosis and then blended with unfiltered well water. Subsequent blend then polypropene cartridge filtered and UV sterilized. Hardness:136-152 mg/L as CaCO₃ pH: 7.94-8.36</p> |
| <p>Test Temperature Ranges:</p> | <p>Replicate Means=10.1-10.5⁰C Overall Range=9.5-11.0⁰C</p> |
| <p>Photoperiod and Intensity:</p> | <p>Embryos-darkness until 1 week post hatch. Thereafter, all aquaria at 16D/8N photoperiod at 37-49 footcandle intensity</p> |
| <p>Dosing Apparatus: Type and description of construction materials</p> | <p>2-L proportional diluter (Mount and Brungs) with introduction of test solution to each test exposure chamber. Dilution factor=0.5. Construction: Plate glass, silicon adhesive, glass delivery tubing.</p> |
| <p>Toxicant Mixing:</p> | <p>Flow splitting and mixing cells divided each aliquot before delivery to test aquaria where they were split again. Pretest and day 95 volumetric measurement were made: <10% variation.</p> |
| <p>Test Vessels:Description and materials</p> | <p>Glass aquaria divided in half with a glass partition. Individual chambers measured 15.8x30.4 cm with 24.3 cm depth. Volume-11.7 L. Screened side drains on each chamber. Top screens after swim-up.</p> |
| <p>Embryo Cups: Description and materials.</p> | <p>Glass jars-9 cm diameter-with ends covered with 16 mesh Nytex screen. Suspended on steel wire and oscillated by motor driven rocker arm.</p> |

| Guideline Criteria | Reported Information |
|---|---|
| Flow Rate: Rate and volume replacements per day. | Initial Rate:95.5 L/replicate/day with an 8.2 volume replacement rate per day. This was gradually increased to 138 L/rep/Day as biomass load increased. |
| Bioload Factor | 0.189 g/L/day at study termin. |
| Aeration: | Not described |

Comments: No comments.

C. Chemical System:

| Guideline Criteria | Reported Information |
|---|---|
| <p>Concentrations: Nominal concentration spread:</p> <p>Toxicant concentration measurement regime:</p> | <p>-0.11, 0.23, 0.45, 0.90, 1.8 mg ai/L with solvent & dilution controls.</p> <p>-10 ml concentration samples from 2 of 4 replicates were composited on alternate sample days and analyzed using GL Chromatography with ECD detection. Sample days were days -6, 0, 1, 7, and weekly thereafter.</p> |
| <p>Water Quality Measurement:</p> <p>2) Freshwater parameters in a control and one concentration must be analyzed once a week.</p> | <p>DO: Measured alternately in 2 of 4 reps(each level) on days 0, 1, 7 and weekly thereafter.</p> <p>Temperature: measured days 0, 1, 7 and weekly thereafter in 2 of 4 replicates-each conc. Continuous temp. meas-one replicate centrally located.</p> <p>Conductivity, Hardness, Alkalinity and pH: Day 0 and weekly thereafter in 1 replicate of control, 0.11, 0.45, and 1.8 test levels.</p> |
| Solvents: | <p>Stock Solutions: 7.452 g alachlor in 100 ml DMF</p> <p>Highest Conc. 0.025 ml DMF/L</p> |

Comments:

10. REPORTED RESULTS:

| Guideline Criteria | Reported Information |
|---|---|
| Data Endpoints Biological Endpoints Monitored during this study : | 1. % Egg Hatch Success 2. Time to Hatch 3. Viability 3. 35 Day and 60 Day Hatch Survival 4. 35D and 60D Length 5. 60D blotted wet weight 6. Time to Swimup 7. Behavioral and physical aberrations. |
| Raw data : | Raw data is included with report |

Effects Data:All data below is based on mean of 4 replicates per concentration level.

| Toxicant Conc. ($\mu\text{g/L}$) 1 | | % Hatch | Time to Hatch | 35D Survival | 60D Survival | Total Length (mm) | | Wet wt (gm) |
|--------------------------------------|--------|---------|---------------|--------------|--------------|-------------------|------|-------------|
| Nom. | M.Meas | % | Days | % | % | 35D | 60D | 60D |
| Ctrl | | 100 | 36 | 98.3 | 98.3 | 33.9 | 47.3 | 1.641 |
| Solv | | 100 | 35 | 100 | 98.3 | 34.1 | 47.8 | 1.677 |
| 0.11 | 0.0945 | 100 | 36 | 100 | 100 | 33.9 | 47.4 | 1.647 |
| 0.23 | 0.187 | 100 | 36 | 100 | 98.3 | 33.9 | 46.9 | 1.586 |
| 0.45 | 0.388 | 100 | 37 | 100 | 98.3 | 33.0 | 45.8 | 1.460 |
| 0.90 | 0.809 | 98.3 | 36 | 96.7 | 93.3 | 33.1 | 44.8 | 1.400 |
| 1.8 | 1.63 | 100 | 37 | 96.7 | 88.3 | 29.3 | 35.2 | 0.651 |

Toxicity Observations: In the 1.63 mg/L concentration fish were observed resting on the bottom, displaying irregular respiration, and exophthalmia. They were not noted in other concentration levels. Time to swimup was delayed in the 1.63 mg ai/L test concentration by 3 days over the lower concentrations and controls(52 days vs.48-49 days).

Statistical Results:

Statistical Method: Contingency tables for proportional scale data . Untransformed ANOVA and one tailed Dunnet's multiple comparison. SAS with $p \leq 0.05$.

NOEC: 0.187 mg ai/L

LOEC: 0.388 mg ai/L

MATC: 0.269

Most sensitive endpoint: Growth

Comments: None

11. Reviewer's Statistical Results:

Statistical Method: Dunnett's, Bonferroni T, and William's Test using Toxstat.

NOEL: 0.187 PPM

LOEC: 0.388 PPM

MATC: N.D.

Most sensitive endpoint: Growth, 60 Day length and weight

Comments:

12. COMPLETION OF ONE-LINER FOR STUDY: Yes

Alachlor Rainbow Trout ELS 60D Length
 File: AlacrBT.len Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|-----|-----------------|---|--------|--------|--------|
| 1 | Solvent Control | 4 | 46.800 | 48.200 | 47.800 |
| 2 | Dilution Contro | 4 | 46.100 | 48.400 | 47.375 |
| 3 | 0.0945 | 4 | 46.400 | 48.400 | 47.425 |
| 4 | 0.187 | 4 | 46.800 | 47.200 | 46.950 |
| 5 | 0.388 | 4 | 44.700 | 46.800 | 45.775 |
| 6 | 0.809 | 4 | 44.300 | 45.900 | 44.800 |
| 7 | 1.63 | 4 | 34.700 | 35.700 | 35.250 |

Alachlor Rainbow Trout ELS 60D Length
 File: AlacrBT.len Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM |
|-----|-----------------|----------|-------|-------|
| 1 | Solvent Control | 0.447 | 0.668 | 0.334 |
| 2 | Dilution Contro | 1.143 | 1.069 | 0.534 |
| 3 | 0.0945 | 0.749 | 0.866 | 0.433 |
| 4 | 0.187 | 0.037 | 0.191 | 0.096 |
| 5 | 0.388 | 0.842 | 0.918 | 0.459 |
| 6 | 0.809 | 0.553 | 0.744 | 0.372 |
| 7 | 1.63 | 0.230 | 0.480 | 0.240 |

Alachlor Rainbow Trout ELS 60D Length
 File: AlacrBT.len Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|---------|--------|---------|
| Between | 6 | 475.387 | 79.231 | 138.516 |
| Within (Error) | 21 | 12.002 | 0.572 | |
| Total | 27 | 487.390 | | |

Critical F value = 2.57 (0.05,6,21)
 Since F > Critical F REJECT Ho:All groups equal

Alachlor Rainbow Trout ELS 60D Length

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|-----------------|------------------|-----------------------------------|--------|-----|
| 1 | Solvent Control | 47.800 | 47.800 | | |
| 2 | Dilution Contro | 47.375 | 47.375 | 0.795 | |
| 3 | 0.0945 | 47.425 | 47.425 | 0.701 | |
| 4 | 0.187 | 46.950 | 46.950 | 1.589 | |
| 5 | 0.388 | 45.775 | 45.775 | 3.787 | * |
| 6 | 0.809 | 44.800 | 44.800 | 5.610 | * |
| 7 | 1.63 | 35.250 | 35.250 | 23.467 | * |

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

Alachlor Rainbow Trout ELS 60D Length

File: AlacrBT.len

Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|-----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1 | Solvent Control | 4 | | | |
| 2 | Dilution Contro | 4 | 1.316 | 2.8 | 0.425 |
| 3 | 0.0945 | 4 | 1.316 | 2.8 | 0.375 |
| 4 | 0.187 | 4 | 1.316 | 2.8 | 0.850 |
| 5 | 0.388 | 4 | 1.316 | 2.8 | 2.025 |
| 6 | 0.809 | 4 | 1.316 | 2.8 | 3.000 |
| 7 | 1.63 | 4 | 1.316 | 2.8 | 12.550 |

Alachlor Rainbow Trout ELS 60D Length

File: AlacrBT.len

Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|---------|--------|---------|
| Between | 6 | 475.387 | 79.231 | 138.516 |
| Within (Error) | 21 | 12.002 | 0.572 | |
| Total | 27 | 487.390 | | |

Critical F value = 2.57 (0.05,6,21)

Since F > Critical F REJECT Ho:All groups equal

Alachlor Rainbow Trout ELS 60D Length

File: AlacrBT.len

Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|-----------------|------------------|-----------------------------------|--------|-----|
| 1 | Solvent Control | 47.800 | 47.800 | | |
| 2 | Dilution Contro | 47.375 | 47.375 | 0.795 | |
| 3 | 0.0945 | 47.425 | 47.425 | 0.701 | |
| 4 | 0.187 | 46.950 | 46.950 | 1.589 | |
| 5 | 0.388 | 45.775 | 45.775 | 3.787 | * |
| 6 | 0.809 | 44.800 | 44.800 | 5.610 | * |
| 7 | 1.63 | 35.250 | 35.250 | 23.467 | * |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=21,6)

Alachlor Rainbow Trout ELS 60D Length

File: AlacrBT.len Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|-----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1 | Solvent Control | 4 | | | |
| 2 | Dilution Contro | 4 | 1.392 | 2.9 | 0.425 |
| 3 | 0.0945 | 4 | 1.392 | 2.9 | 0.375 |
| 4 | 0.187 | 4 | 1.392 | 2.9 | 0.850 |
| 5 | 0.388 | 4 | 1.392 | 2.9 | 2.025 |
| 6 | 0.809 | 4 | 1.392 | 2.9 | 3.000 |
| 7 | 1.63 | 4 | 1.392 | 2.9 | 12.550 |

Alachlor Rainbow Trout ELS 60D Length

File: AlacrBT.len Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|-----------------|---|---------------|------------------|-----------------|
| 1 | Solvent Control | 4 | 47.800 | 47.800 | 47.800 |
| 2 | Dilution Contro | 4 | 47.375 | 47.375 | 47.400 |
| 3 | 0.0945 | 4 | 47.425 | 47.425 | 47.400 |
| 4 | 0.187 | 4 | 46.950 | 46.950 | 46.950 |
| 5 | 0.388 | 4 | 45.775 | 45.775 | 45.775 |
| 6 | 0.809 | 4 | 44.800 | 44.800 | 44.800 |
| 7 | 1.63 | 4 | 35.250 | 35.250 | 35.250 |

Alachlor Rainbow Trout ELS 60D Length

File: AlacrBT.len Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|-----------------|--------------------|-------------------|--------------|-------------------|-----------------------|
| Solvent Control | 47.800 | | | | |
| Dilution Contro | 47.400 | 0.748 | | 1.72 | k= 1, v=21 |
| 0.0945 | 47.400 | 0.748 | | 1.80 | k= 2, v=21 |
| 0.187 | 46.950 | 1.590 | | 1.83 | k= 3, v=21 |
| 0.388 | 45.775 | 3.788 | * | 1.84 | k= 4, v=21 |
| 0.809 | 44.800 | 5.612 | * | 1.85 | k= 5, v=21 |
| 1.63 | 35.250 | 23.476 | * | 1.85 | k= 6, v=21 |

s = 0.756

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

Alachlor Rainbow Trout 60D Mean Wet Wt
File: AlacRBT.wwt Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|-----|----------------|---|-------|-------|-------|
| 1 | Solvent C | 4 | 1.562 | 1.722 | 1.677 |
| 2 | Dilution C | 4 | 1.498 | 1.759 | 1.644 |
| 3 | 0.0945 | 4 | 1.568 | 1.743 | 1.648 |
| 4 | 0.187 | 4 | 1.533 | 1.661 | 1.587 |
| 5 | 0.388 | 4 | 1.343 | 1.589 | 1.458 |
| 6 | 0.809 | 4 | 1.345 | 1.467 | 1.396 |
| 7 | 1.63 | 4 | 0.618 | 0.682 | 0.652 |

Alachlor Rainbow Trout 60D Mean Wet Wt
File: AlacRBT.wwt Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM |
|-----|----------------|----------|-------|-------|
| 1 | Solvent C | 0.006 | 0.077 | 0.038 |
| 2 | Dilution C | 0.015 | 0.122 | 0.061 |
| 3 | 0.0945 | 0.008 | 0.091 | 0.045 |
| 4 | 0.187 | 0.004 | 0.060 | 0.030 |
| 5 | 0.388 | 0.012 | 0.108 | 0.054 |
| 6 | 0.809 | 0.003 | 0.051 | 0.026 |
| 7 | 1.63 | 0.001 | 0.027 | 0.014 |

Alachlor Rainbow Trout 60D Mean Wet Wt
File: AlacRBT.wwt Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|-------|-------|--------|
| Between | 6 | 3.145 | 0.524 | 74.857 |
| Within (Error) | 21 | 0.143 | 0.007 | |
| Total | 27 | 3.288 | | |

Critical F value = 2.57 (0.05,6,21)
Since $F > \text{Critical } F$ REJECT H_0 :All groups equal

Alachlor Rainbow Trout 60D Mean Wet Wt

DUNNETTS TEST

TABLE 1 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|----------------|------------------|-----------------------------------|--------|-----|
| 1 | Solvent C | 1.677 | 1.677 | | |
| 2 | Dilution C | 1.644 | 1.644 | 0.562 | |
| 3 | 0.0945 | 1.648 | 1.648 | 0.503 | |
| 4 | 0.187 | 1.587 | 1.587 | 1.534 | |
| 5 | 0.388 | 1.458 | 1.458 | 3.702 | * |
| 6 | 0.809 | 1.396 | 1.396 | 4.754 | * |
| 7 | 1.63 | 0.652 | 0.652 | 17.338 | * |

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

Alachlor Rainbow Trout 60D Mean Wet Wt

File: AlacrBT.wwt

Transform: NO TRANSFORM

DUNNETTS TEST

TABLE 2 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1 | Solvent C | 4 | | | |
| 2 | Dilution C | 4 | 0.146 | 8.7 | 0.033 |
| 3 | 0.0945 | 4 | 0.146 | 8.7 | 0.030 |
| 4 | 0.187 | 4 | 0.146 | 8.7 | 0.091 |
| 5 | 0.388 | 4 | 0.146 | 8.7 | 0.219 |
| 6 | 0.809 | 4 | 0.146 | 8.7 | 0.281 |
| 7 | 1.63 | 4 | 0.146 | 8.7 | 1.026 |

Alachlor Rainbow Trout 60D Mean Wet Wt

File: AlacrBT.wwt

Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|-------|-------|--------|
| Between | 6 | 3.145 | 0.524 | 74.857 |
| Within (Error) | 21 | 0.143 | 0.007 | |
| Total | 27 | 3.288 | | |

Critical F value = 2.57 (0.05,6,21)

Since F > Critical F REJECT Ho:All groups equal

Alachlor Rainbow Trout 60D Mean Wet Wt

File: AlacrBT.wwt

Transform: NO TRANSFORM

BONFERRONI T-TEST

- TABLE 1 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|----------------|------------------|-----------------------------------|--------|-----|
| 1 | Solvent C | 1.677 | 1.677 | | |
| 2 | Dilution C | 1.644 | 1.644 | 0.562 | |
| 3 | 0.0945 | 1.648 | 1.648 | 0.503 | |
| 4 | 0.187 | 1.587 | 1.587 | 1.534 | |
| 5 | 0.388 | 1.458 | 1.458 | 3.702 | * |
| 6 | 0.809 | 1.396 | 1.396 | 4.754 | * |
| 7 | 1.63 | 0.652 | 0.652 | 17.338 | * |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=21,6)

Alachlor Rainbow Trout 60D Mean Wet Wt

File: AlacrBT.wwt

Transform: NO TRANSFORM

BONFERRONI T-TEST

- TABLE 2 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1 | Solvent C | 4 | | | |
| 2 | Dilution C | 4 | 0.154 | 9.2 | 0.033 |
| 3 | 0.0945 | 4 | 0.154 | 9.2 | 0.030 |
| 4 | 0.187 | 4 | 0.154 | 9.2 | 0.091 |
| 5 | 0.388 | 4 | 0.154 | 9.2 | 0.219 |
| 6 | 0.809 | 4 | 0.154 | 9.2 | 0.281 |
| 7 | 1.63 | 4 | 0.154 | 9.2 | 1.026 |

Alachlor Rainbow Trout 60D Mean Wet Wt

File: AlacrBT.wwt

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|----------------|---|---------------|------------------|-----------------|
| 1 | Solvent C | 4 | 1.677 | 1.677 | 1.677 |
| 2 | Dilution C | 4 | 1.644 | 1.644 | 1.646 |
| 3 | 0.0945 | 4 | 1.648 | 1.648 | 1.646 |
| 4 | 0.187 | 4 | 1.587 | 1.587 | 1.587 |
| 5 | 0.388 | 4 | 1.458 | 1.458 | 1.458 |
| 6 | 0.809 | 4 | 1.396 | 1.396 | 1.396 |
| 7 | 1.63 | 4 | 0.652 | 0.652 | 0.652 |

Alachlor Rainbow Trout 60D Mean Wet Wt

File: AlacrBT.wwt

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|----------------|--------------------|-------------------|--------------|-------------------|-----------------------|
| Solvent C | 1.677 | | | | |
| Dilution C | 1.646 | 0.541 | | 1.72 | k= 1, v=21 |
| 0.0945 | 1.646 | 0.541 | | 1.80 | k= 2, v=21 |
| 0.187 | 1.587 | 1.557 | | 1.83 | k= 3, v=21 |
| 0.388 | 1.458 | 3.759 | * | 1.84 | k= 4, v=21 |
| 0.809 | 1.396 | 4.827 | * | 1.85 | k= 5, v=21 |
| 1.63 | 0.652 | 17.604 | * | 1.85 | k= 6, v=21 |

s = 0.082

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

Alachlor ELS Rainbow Trt 60D Posthatch
 File: Alachsrv.60D Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|-----|-----------------|---|--------|--------|--------|
| 1 | Solvent Control | 4 | 14.000 | 15.000 | 14.750 |
| 2 | Dilution C | 4 | 14.000 | 15.000 | 14.750 |
| 3 | 0.0945 | 4 | 15.000 | 15.000 | 15.000 |
| 4 | 0.187 | 4 | 14.000 | 15.000 | 14.750 |
| 5 | 0.388 | 4 | 14.000 | 15.000 | 14.750 |
| 6 | 0.809 | 4 | 12.000 | 15.000 | 14.000 |
| 7 | 1.63 | 4 | 12.000 | 14.000 | 13.250 |

Alachlor ELS Rainbow Trt 60D Posthatch
 File: Alachsrv.60D Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM |
|-----|-----------------|----------|-------|-------|
| 1 | Solvent Control | 0.250 | 0.500 | 0.250 |
| 2 | Dilution C | 0.250 | 0.500 | 0.250 |
| 3 | 0.0945 | 0.000 | 0.000 | 0.000 |
| 4 | 0.187 | 0.250 | 0.500 | 0.250 |
| 5 | 0.388 | 0.250 | 0.500 | 0.250 |
| 6 | 0.809 | 2.000 | 1.414 | 0.707 |
| 7 | 1.63 | 0.917 | 0.957 | 0.479 |

Alachlor ELS Rainbow Trt 60D Posthatch
 File: Alachsrv.60D Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|--------|-------|-------|
| Between | 6 | 9.214 | 1.536 | 2.743 |
| Within (Error) | 21 | 11.750 | 0.560 | |
| Total | 27 | 20.964 | | |

Critical F value = 2.57 (0.05,6,21)
 Since F > Critical F REJECT Ho:All groups equal

Alachlor ELS Rainbow Trt 60D Posthatch

DUNNETTS TEST

- TABLE 1 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|-----------------|------------------|-----------------------------------|--------|-----|
| 1 | Solvent Control | 14.750 | 14.750 | | |
| 2 | Dilution C | 14.750 | 14.750 | 0.000 | |
| 3 | 0.0945 | 15.000 | 15.000 | -0.472 | |
| 4 | 0.187 | 14.750 | 14.750 | 0.000 | |
| 5 | 0.388 | 14.750 | 14.750 | 0.000 | |
| 6 | 0.809 | 14.000 | 14.000 | 1.417 | |
| 7 | 1.63 | 13.250 | 13.250 | 2.835 | * |

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

Alachlor ELS Rainbow Trt 60D Posthatch

File: Alachsrv.60D

Transform: NO TRANSFORM

DUNNETTS TEST

- TABLE 2 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|-----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1 | Solvent Control | 4 | | | |
| 2 | Dilution C | 4 | 1.302 | 8.8 | 0.000 |
| 3 | 0.0945 | 4 | 1.302 | 8.8 | -0.250 |
| 4 | 0.187 | 4 | 1.302 | 8.8 | 0.000 |
| 5 | 0.388 | 4 | 1.302 | 8.8 | 0.000 |
| 6 | 0.809 | 4 | 1.302 | 8.8 | 0.750 |
| 7 | 1.63 | 4 | 1.302 | 8.8 | 1.500 |

Alachlor ELS Rainbow Trt 60D Posthatch

File: Alachsrv.60D

Transform: NO TRANSFORM

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|--------|-------|-------|
| Between | 6 | 9.214 | 1.536 | 2.743 |
| Within (Error) | 21 | 11.750 | 0.560 | |
| Total | 27 | 20.964 | | |

Critical F value = 2.57 (0.05,6,21)

Since F > Critical F REJECT Ho:All groups equal

Alachlor ELS Rainbow Trt 60D Posthatch

File: Alachsrv.60D

Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2

Ho: Control < Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|-----------------|------------------|-----------------------------------|--------|-----|
| 1 | Solvent Control | 14.750 | 14.750 | | |
| 2 | Dilution C | 14.750 | 14.750 | 0.000 | |
| 3 | 0.0945 | 15.000 | 15.000 | -0.472 | |
| 4 | 0.187 | 14.750 | 14.750 | 0.000 | |
| 5 | 0.388 | 14.750 | 14.750 | 0.000 | |
| 6 | 0.809 | 14.000 | 14.000 | 1.417 | |
| 7 | 1.63 | 13.250 | 13.250 | 2.835 | * |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=21,6)

Alachlor ELS Rainbow Trt 60D Posthatch

File: Alachsrv.60D Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2

Ho: Control < Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|-----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1 | Solvent Control | 4 | | | |
| 2 | Dilution C | 4 | 1.377 | 9.3 | 0.000 |
| 3 | 0.0945 | 4 | 1.377 | 9.3 | -0.250 |
| 4 | 0.187 | 4 | 1.377 | 9.3 | 0.000 |
| 5 | 0.388 | 4 | 1.377 | 9.3 | 0.000 |
| 6 | 0.809 | 4 | 1.377 | 9.3 | 0.750 |
| 7 | 1.63 | 4 | 1.377 | 9.3 | 1.500 |

Alachlor ELS Rainbow Trt 60D Posthatch

File: Alachsrv.60D Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|-----------------|---|---------------|------------------|-----------------|
| 1 | Solvent Control | 4 | 14.750 | 14.750 | 14.833 |
| 2 | Dilution C | 4 | 14.750 | 14.750 | 14.833 |
| 3 | 0.0945 | 4 | 15.000 | 15.000 | 14.833 |
| 4 | 0.187 | 4 | 14.750 | 14.750 | 14.750 |
| 5 | 0.388 | 4 | 14.750 | 14.750 | 14.750 |
| 6 | 0.809 | 4 | 14.000 | 14.000 | 14.000 |
| 7 | 1.63 | 4 | 13.250 | 13.250 | 13.250 |

Alachlor ELS Rainbow Trt 60D Posthatch

File: Alachsrv.60D Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|-----------------|-----------------|----------------|-----------|----------------|--------------------|
| Solvent Control | 14.833 | | | | |
| Dilution C | 14.833 | 0.158 | | 1.72 | k= 1, v=2. |
| 0.0945 | 14.833 | 0.158 | | 1.80 | k= 2, v=2. |
| 0.187 | 14.750 | 0.000 | | 1.83 | k= 3, v=2. |
| 0.388 | 14.750 | 0.000 | | 1.84 | k= 4, v=2. |
| 0.809 | 14.000 | 1.418 | | 1.85 | k= 5, v=2. |
| 1.63 | 13.250 | 2.836 | * | 1.85 | k= 6, v=2. |

s = 0.748

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

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