# DATA EVALUATION RECORD
# AQUATIC INVERTEBRATE LIFE CYCLE TEST
# § 72-4(c)

1. **CHEMICAL:** JAU6476-desthio

PC Code No.: 113961

2. **TEST MATERIAL:** JAU-6476 - Desthio (p. 13) Purity: 97.0%

Common name: JAU6476-desthio
Chemical:
IUPAC name: 2-(1-chlorcyclopropyl)-1-(2-chlophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol
CAS name: 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1, 2, 4-triazol-l-yl)-propan-2-ol
CAS No.: 120983-64-4
Synonyms: SXX0665

3. **CITATION:**

Authors: Blankinship, A.S., *et al.*

Title: Desthio JAU-6476: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*).

Study Completion Date: September 4, 2003

Laboratory: Wildlife International, Ltd.
8598 Commerce Drive
Easton, MD 21601

Sponsor: Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

Laboratory Report ID: 149A-130A

MRID No.: 46246030

DP Barcode: D303488

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

**Signature:** **Date:** 9/10/04

**APPROVED BY:** Gregory Hess, Staff Scientist, Dynamac Corporation

**Signature:** **Date:** 9/19/04

5. **APPROVED BY:** Kevin Costello, Geologist, OPP/EFED/ERB - IV

**Signature:** **Date:**

6. **SECONDARY REVIEW BY:**

Émilie Larivière, EAD, PMRA, HC
**Signature:** 9/9/05 **Date:** 9/9/05
Christopher J. Salice, ERB-IV, EFED, EPA
**Signature:** 8-23-05 **Date:** 8/23/05

7. **STUDY PARAMETERS:**

| | |
|---|---|
| **Scientific Name of Test Organisms:** | *Americamysis bahia*, formerly *Mysidopsis bahia* |
| **Age of Test Organism:** | Neonates (< 24 hours old) |
| **Definitive Test Duration** | 29 days |
| **Study Method:** | Flow-through |
| **Type of Concentrations:** | Mean-measured |

8. **CONCLUSIONS:**

In a 29-day life cycle test, *Americamysis bahia* neonates were exposed under flow-through conditions to JAU 6476 - Desthio (prothioconazole - desthio) at nominal concentrations of 0 (negative and solvent controls), 16, 32, 63, 125, and 250 ppb. Mean-measured concentrations were <10 (<LOQ, controls), 16, 32, 64, 128, and 252 ppb a.i., respectively.

Prior to sexual maturity and pairing, there were 60 mysids/level. At Day 14, up to 20 pair/level were isolated for individual matings. First-generation mysids were observed for mortality and signs of abnormal behavior once daily throughout the study. Once daily during the reproduction period, second-generation mysids were counted and apparently

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In a 29-day life cycle test, *Americamysis bahia* neonates were exposed under flow-through conditions to JAU 6476 - Desthio (prothioconazole - desthio) at nominal concentrations of 0 (negative and solvent controls), 16, 32, 63, 125, and 250 ppb. Mean-measured concentrations were <10 (<LOQ, controls), 16, 32, 64, 128, and 252 ppb a.i., respectively.

Prior to sexual maturity and pairing, there were 60 mysids/level. At Day 14, up to 20 pair/level were isolated for individual matings. First-generation mysids were observed for mortality and signs of abnormal behavior once daily throughout the study. Once daily during the reproduction period, second-generation mysids were counted and apparently discarded. Data endpoints included percent survival of first-generation mysids on Days 14 (at pairing) and 29 (study termination; combined sexes), the number of offspring per reproductive day, and total length and dry weight of surviving first-generation mysids (Day 29; combined sexes).

No treatment-related effect on survival was observed, either prior to or after pairing for reproduction. Prior to pairing (on Day 14), survival averaged 90 to 98% for all treatment and control groups. At study termination (on Day 29), survival averaged 88 to 95% for all treatment and control groups. The NOAEC for survival was 252 ppb a.i. Throughout the study, no treatment-related clinical signs of toxicity were observed, and the NOAEC for clinical signs of toxicity was 252 ppb a.i.

The day of first brood release was Day 17, and the median time of the first brood release for the negative and solvent controls was Day 22. There were no significant effects on young produced per reproductive day. The mean number of young produced per reproductive day averaged 0.592 and 0.573 for the negative and solvent control groups, respectively, and 0.527, 0.610, 0.615, 0.398, and 0.407 for the 16, 32, 64, 128, and 252 ppb a.i. test levels, respectively. Consequently, the NOAEC for reproduction was 64 ppb a.i.

since there was an apparent 30% reduction in the number of offspring produced per reproductive day at 128 ppb a.i. Although not statistically significant, a reproductive effect of this magnitude is likely to be biologically significant.

No treatment-related effects on growth were observed. Total body length of surviving mysids at study termination (Day 29) averaged 7.68 to 7.79 mm for all treatment and control groups, and dry weights averaged 0.815 to 0.863 mg for all treatment and control groups. The NOAEC for growth was 252 ppb a.i.

**Based on effects on reproductive success, the NOAEC and LOAEC are 64 and 128 ppb a.i., respectively.**

This study is scientifically sound. Although survival following pairing and terminal growth parameters should have been reported in terms of each gender, there were no apparent effects on these endpoints. Therefore, this study fulfills the guideline requirements for an aquatic invertebrate life-cycle toxicity test using *Americamysis bahia* (§72-4c), and is classified as **ACCEPTABLE**. In addition (though not required), offspring should have been observed daily for at least 4 days for survival, development, and behavior.

**Results Synopsis:**
**Survival (Day 14)**
NOAEC: 252 ppb a.i.
LOAEC: >252 ppb a.i.

**Survival (Day 29; combined sexes)**
NOAEC: 252 ppb a.i.
LOAEC: >252 ppb a.i.

**Reproduction (no. young/reproductive day)**
NOAEC: 64 ppb a.i.
LOAEC: 128 ppb a.i.

**Total length (combined sexes)**
NOAEC: 252 ppb a.i.
LOAEC: >252 ppb a.i.

**Dry weight (combined sexes**
NOAEC: 252 ppb a.i.
LOAEC: >252 ppb a.i.

Endpoint(s) affected: Reproduction

**9. ADEQUACY OF THE STUDY:**

**A. Classification:** ACCEPTABLE

**10. GUIDELINE DEVIATIONS:**

1. The health and/or mortality of the adult culture was not reported, and the parental stock were apparently not maintained separately from the brood stock.

2. The volume of the test chambers was 9 L, with a fill depth of approximately 7.2-7.7 cm (prior to sexual maturity). These chambers are significantly smaller than those recommended (20.25 L vessels with a water depth of 10 cm).

3. It was not specified if the test chambers were covered during the study.

4. Aeration was added to each test chamber after the mysids attained sexual maturity and were paired (p. 15) however, analytical recoveries were acceptable (≥88.2% of nominal) and aeration did not appear to have a detrimental effect on the test material concentrations during the exposure period.

5. The quantity of live brine shrimp fed to the mysids was not specified.

6. Daily survival data were not provided; survival data were summarized to include Days 0-14 (at pairing) and 14-28. In addition, following pairing, survival data should have been gender-specific.

7. Terminal dry weight and length measurements should have been gender-specific.

8. Survival, abnormal development, and aberrant behavior of second-generation mysids were observed for an unspecified period. Except for number present, survival and toxic effects of second-generation mysids were not addressed in the study.

**11. SUBMISSION PURPOSE:** This study was submitted to provide data on the chronic toxicity of JAU 6476 - Desthio (prothioconazole metabolite) to an estuarine/marine shrimp for the purpose of chemical registration of prothioconazole (NC).

**12. MATERIALS AND METHODS:**

**A. Test Organisms/Acclimation**

| Guideline Criteria | Reported Information |
|---|---|
| **Species**<br>An estuarine shrimp species, preferably *Americamysis bahia* | *Americamysis bahia*, formerly *Mysidopsis bahia* |
| **Source/Supplier** | Juveniles were collected from in-house laboratory cultures. |
| **Age at Beginning of Test**<br><24 hours old | <24 hours old |
| **Parental Acclimation**<br>Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. Mysids should be in good health. | Adult mysids were held in water from the same source as used during the test. The health of the adult mysids was not reported, and an isolated brood stock was apparently not maintained. |
| **Parental Acclimation Period**<br>At least 14 days | Continuous |
| **Brood Stock**<br>Test started with mysids from:<br>- one brood stock, or<br>- brood stock which has not obtained sexual maturity or had been maintained for >14 days in a laboratory with same food, water, temperature, and salinity used in the test. | At test initiation, juvenile mysids were collected from a culture stock that was maintained in the laboratory under the same conditions used in the definitive test. |

**B. Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Source of Dilution Water**<br>May be natural (sterilized and filtered) or a commercial mixture; water must be free of pollutants. | Natural seawater collected at Indian River Inlet, Delaware, was adjusted to 20‰ salinity using well water. The dilution water was aerated, filtered, and UV-sterilized prior to use.<br><br>Results of chemical characterization of the dilution water (July 2002) are provided in Appendix 2, pp. 37-38. |
| **Does water support test animals without observable signs of stress?** | Yes |
| **Water Temperature**<br>27°C for mysids<br>- At test termination, mean-measured temperature for each chamber should be within 1°C of selected test temperature.<br>- Must be within 3°C of the mean of the time-weighted averages.<br>- Must not differ by >2°C between chambers during the same interval. | Target: 25 ± 2°C<br>Actual range: 23.0-26.5°C<br><br>- All criteria were met. |
| **Salinity**<br>15-30 ‰<br>- The difference between highest and lowest measured salinities should be less than 5‰. | 20‰<br>- Criteria met. |
| **pH**<br>7.6-8.2 | 8.0-8.3 |
| **Dissolved Oxygen**<br>60-100% saturation | 5.8-7.5 mg/L (≥79% of saturation). |

| Guideline Criteria | Reported Information |
|---|---|
| **Photoperiod**<br>16-hr light/8-hr dark<br>(14-hr light/10-hr dark also acceptable) | 16 hours light, 8 hours dark, with 120-minute transition periods.<br><br>Measured light intensity was 40 lux over the surface of one representative test chamber at test initiation. |
| **Test Chambers**<br>1. Material:<br>All glass, No. 316 stainless steel, or perflorocarbon plastic<br>2. Size:<br>Typically 30 x 45 x 15 cm (20.25 L)<br>3. Fill depth:<br>10 cm<br><br>4. Were chambers identical and covered during the test? | 1. Glass<br><br>2. 9 L<br><br>3. 5 L and 7.2-7.7 cm depth (prior to sexual maturity); 7 L and 13.9-14.2 cm depth (after sexual maturity).<br><br>4. All chambers were identical; it was not specified if the chambers were covered. |
| **Test Compartments (within chambers)**<br>- 250-mL glass beakers with side cutouts covered with nylon mesh or stainless steel screen, or<br><br>- 90- or 140-mm id glass Petri dish bottoms with collars made of 200-250 µm mesh screen | Prior to sexual maturity, mysids were held in one compartment per replicate. Test compartments were 12-cm diameter x 19-cm height glass containers with nylon mesh screen attached to two holes on opposites sides.<br><br>Following sexual maturity, reproductive pairs (one pair per compartment) were placed in 6-cm glass petri dishes with sides on nylon mesh screen. |
| **Type of Dilution System**<br>Intermittent flow proportional diluters or continuous flow serial diluters should be used. | Continuous-flow diluter. |

| Guideline Criteria | Reported Information |
|---|---|
| **Toxicant Mixing**<br>1. Mixing chamber is recommended but not required; aeration should not be used for mixing.<br>2. If a mixing chamber was not employed, was it demonstrated that the test solution was completely mixed before introduction into the test system?<br>3. Was flow splitting accuracy within 10%? | 1. Mixing chambers were used to obtain the desired test concentrations.<br>2. The syringe pump was verified and the rotameters were calibrated prior to test initiation.<br>3. The flow rates were verified prior to the test and at weekly intervals thereafter. |
| **Flow Rate**<br>1. 5-10 volume additions per 24 hours.<br>2. Did the flow rate maintain the toxicant level and the DO at ≥60% of saturation?<br>3. Were the meter systems calibrated before study and checked twice daily during test period? | 1. 18 volume additions/24 hours prior to sexual maturity and 13 volume additions/24 hours after sexual maturity.<br>2. Yes.<br>3. Yes. |
| **Solvents**<br>- Acceptable solvents include triethylene glycol, methanol, acetone, and methanol.<br>- Solvent should not exceed 0.1 mL/L in a flow-through system. | Dimethylformamide (DMF), 0.05 mL/L. |
| **Aeration**<br>Dilution water should be vigorously aerated, but the test tanks should not be aerated. | The dilution water was aerated prior to use, and aeration was added to each test chamber after the mysids attained sexual maturity and were paired (p. 15). |

**C. Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Duration of the Test**<br>Approximately 28 days.<br><br>Was the test terminated within 7 days of the median time of first brood release in the controls? | 29 days<br><br>The study duration was adequate. The median time of first brood release was Day 22 for the negative and solvent controls (p. 19). |
| **Nominal Concentrations**<br>Negative control, a solvent control (when applicable), and at least five treatment levels, one of which must adversely affect a life stage and one must not affect any life stage. The dilution factor should not be >50%. | 0 (negative and solvent controls), 16, 32, 63, 125, and 250 ppb. |
| **Distribution**<br>Number of mysids before pairing:<br>Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/treatment level.<br><br>Number of mysids after pairing:<br>≥20 randomly selected pairs/treatment (excess males should be held in separate compartment in same treatment to replace paired males). | 60 mysids/level: 15 mysids/compartment, 1 compartment/aquarium, and 4 replicate test aquaria/level.<br><br>Up to 20 pair/level: 1 pair/compartment, up to 5 compartments/aquarium, and 4 replicate test aquaria/level.<br><br>Extra male mysids were pooled and placed in a separate compartment within the replicate; male mysids from this pool were used to replace dead males from the paired groups. Any immature mysids or extra females were discarded. |

| Guideline Criteria | Reported Information |
|---|---|
| **Pairing**<br>Should be conducted when most of the mysids are sexually mature, usually 10-14 days after test initiation. All pairing should occur on the same day. | Female and male adults were paired on Day 14 and reproduction was monitored through Day 29. |
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |
| **Were treatments randomly assigned to individual test chamber locations?** | Yes |
| **Feeding**<br>Mysids should be fed live brine shrimp nauplii at least once daily. 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended. | Mysids were fed 1-3 times/day (amount not specified) with live brine shrimp nauplii and supplemented occasionally with Algamac 2000. |
| **Counts**<br>Live adult mysids should be counted at initiation, at pairing, and daily after pairing.<br><br>Live young must be counted and removed daily.<br><br>Missing or impinged animals should be recorded. | Yes<br><br>Yes<br><br>None reported |
| **Controls**<br>Negative control and carrier control (when applicable) are required. | A negative (saltwater) control and solvent control were used. |

| Guideline Criteria | Reported Information |
|---|---|
| **Water Parameter Measurements**<br>1. Temperature should be monitored daily in one chamber and at least three times in all chambers.<br><br>2. Salinity should be measured daily in at least one test vessel.<br><br>3. pH should be measured at the beginning, the end, and at least weekly during the test in the control vessels and highest test level.<br><br>4. Dissolved oxygen must be measured at each concentration at least once a week. | 1. Temperature was measured in each chamber at the beginning and end of test, at weekly intervals, and continuously in one negative control.<br><br>2. Salinity was measured daily in alternate replicates of the negative control.<br><br>3. The pH was measured in alternating replicate test chambers at the beginning and end of the test and at weekly intervals during the test.<br><br>4. The DO was measured in alternating replicate test chambers at the beginning and end of the test and at weekly intervals up until sexual maturity of the mysids; thereafter, it was measured daily in alternating replicate aquaria. |
| **Chemical Analysis**<br>Toxicant concentration must be measured in one chamber at each toxicant level every week. | Toxicant concentration was measured from two alternating replicate chambers on Days 0, 7, 14, 21, and 29 (test termination). Analyses were performed using HPLC with UV (220 nm) detection. |

## 13. REPORTED RESULTS

### A. General Results

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes |
| **Chemical Analysis**<br>For all test groups,<br>a) the measured concentration of the test material should not be <50% of the time-weighted average measured concentration for >10% of the duration of the test, and<br>b) the measured concentration should not be >30% of the time-weighted average measured concentration for >5% of the duration of the test. | Mean-measured concentrations were <10.0 (LOQ, controls), 16, 32, 64, 128, and 252 ppb a.i. (Table 2, pp. 26-27).<br><br>Since samples were collected weekly, it could not be determined if criteria were met. However, in the concentrations verified, variability was low, with reviewer-calculated high-low ratios of 1.0-1.2. |
| **Controls**<br>- Survival of the paired first-generation controls must be ≥70%.<br>- ≥75% of the paired first-generation female controls produced young, or<br>- The average number of young produced by the first-generation female controls was ≥3. | - All criteria met. |

| Guideline Criteria | Reported Information |
|---|---|
| **Data Endpoints Must Include**<br>1. Survival of first-generation mysids, gender specified<br><br>2. Number of live young produced per female<br>3. Dry weight and length of each first generation mysid alive at the end of the test, gender specified<br><br>**Data Endpoints Should Also Include**<br>4. Incidence of morphological findings.<br><br>5. Survival, development, and behavior of second-generation mysids for at least 4 days. | 1. Criteria not met. Survival of first-generation mysids, but not gender-specified.<br>2. Criteria met. Number of live young produced per reproductive day.<br>3. Criteria not met. Dry weight and length of each first generation alive at end of test, but not gender specified.<br><br>4. Incident of morphological findings (none were reported).<br>5. Survival, development, and behavior of second-generation mysids was observed (observation duration not reported). |
| **Raw data must include**<br>1. Survival of first-generation mysids, gender specified<br>2. Number of live young produced per female<br>3. Terminal weight and length measurements, individual and gender specified | 1. Criteria not met. Survival was not gender specified.<br>2. Criteria met.<br><br>3. Criteria not met. Terminal growth was not gender specified. |

Effects Data

| Concentration (ppb a.i./L) | | Percent Mortality (ratio) | | Reproduction, Days 15-29 | | Growth, Day 29 | |
|---|---|---|---|---|---|---|---|
| Nominal | Mean Measured (% nominal) | Day 14 | Day 29 | Total No. of Young | Mean No. Young/No. Repro. Days | Mean Length, mm | Mean Dry Weight, mg |
| Control | <LOQ | 5 (3/60) | 6 (3/48) | 127 | 0.592 | 7.70 | 0.817 |
| Solvent Control | <LOQ | 2 (1/60) | 6 (3/47) | 149 | 0.573 | 7.79 | 0.861 |
| 16 | 16 (100) | 5 (3/60) | 9 (4/46) | 137 | 0.527 | 7.68 | 0.838 |
| 32 | 32 (100) | 8 (5/60) | 5 (2/38) | 144 | 0.610 | 7.78 | 0.852 |
| 63 | 64 (102) | 10 (6/60) | 12 (6/50) | 160 | 0.615 | 7.72 | 0.863 |
| 125 | 128 (102) | 8 (5/60) | 5 (2/37) | 91 | 0.398 | 7.75 | 0.853 |
| 250 | 252 (101) | 3 (2/60) | 10 (5/48) | 105 | 0.407 | 7.76 | 0.815 |

LOQ = 10.0 ppb a.i.

Toxicity Observations: No treatment-related effect on survival was observed, either prior to or after pairing for reproduction. Prior to pairing, survival averaged 90 to 98% for all treatment and control groups (assessed on Day 14, Table 7, p. 32). After pairing for reproduction, parental survival averaged 88 to 95% for all treatment and control groups (assessed on Day 29, Table 8, p. 33). The NOAEC for survival was 252 ppb a.i.

No treatment-related clinical signs of toxicity were observed (Appendices 4 and 5, pp. 51-58). Although several mysids appeared smaller in size than other mysids within the replicate, this effect was observed infrequently in mysids from the negative control, 16, 128, and 252 ppb a.i. treatment groups, and was therefore not considered to be the result of treatment (p. 20). The NOAEC for clinical signs of toxicity was 252 ppb a.i.

The day of first brood release was Day 17 (p. 21), and the median time of the first brood release for the negative and solvent controls was Day 22 (p. 19). Although not statistically-significant, a treatment-related effect on the number of young produced per reproductive day was observed at the 128 and 252 ppb a.i. treatment levels (pp. 21-22). The mean number of young produced per reproductive day averaged 0.592 and 0.573 for the negative and solvent control groups, respectively, and 0.527, 0.610, 0.615, 0.398, and 0.407 for the 16, 32, 64, 128, and 252 ppb a.i. test levels, respectively (Table 9, p. 34). Consequently, the NOAEC for reproduction was 64 ppb a.i. since there was approximately a 30% reduction in the number of offspring per reproductive day at 128 ppb a.i.

No treatment-related effects on growth were observed. Total body length of surviving mysids at study termination (Day 29) averaged 7.68 to 7.79 mm for all treatment and control groups, and dry weights averaged 0.815 to 0.863 mg for all treatment and control groups (Table 10, p. 35). The NOAEC for growth was 252 ppb a.i.

**B. Statistical Results:**

Statistical Method: Statistical analyses were performed on survival of the first-generation mysids (Days 14 and 29), the number of young produced per reproductive day, and the length and dry weight of each surviving first-generation mysid. The negative and solvent controls were compared with an appropriate test, and pooled for all subsequent statistical analyses. Survival data (discrete-variable data) were analyzed using the Chi-square and Fisher's Exact tests. Reproduction and growth data (continuous-variable data) were evaluated for normality using the Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. The treatment groups were compared to the pooled controls using ANOVA and Bonferroni's t-test. The MATC was the geometric mean of the NOAEC and LOAEC. The statistical analyses were calculated using TOXSTAT 3.5 or SAS 8.02 statistical software using mean-measured concentrations.

**Results Synopsis**

| Endpoint | Method | NOAEC | LOAEC | MATC |
|---|---|---|---|---|
| Survival (Day 14) | Chi-square and Fisher's Exact tests | 252 ppb a.i. | >252 ppb a.i. | ND |
| Survival (Day 29) | Chi-square and Fisher's Exact tests | 252 ppb a.i. | >252 ppb a.i. | ND |
| Reproduction (Offspring/repro. day) | ANOVA and Bonferroni's test | 64 ppb a.i. | 128 ppb a.i. | 91 ppb a.i. |
| Length (mm) | ANOVA and Bonferroni's test | 252 ppb a.i. | >252 ppb a.i. | ND |
| Dry Weight (mg) | ANOVA and Bonferroni's test | 252 ppb a.i. | >252 ppb a.i. | ND |

ND - Not determined.

Most sensitive endpoint(s): Reproduction (Number of offspring/repro. day)

## 14. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Endpoints assessed statistically in this study included percent survival (Days 14 and 29), reproduction (number of offspring per reproductive day), and growth (terminal length and dry weight). A t-test showed no significant differences between solvent and negative controls indicating no effects of the solvent. After confirming normality and homogeneity of variances all treatment data for each endpoint was compared to the solvent control using ANOVA and William's multiple comparisons test to determine any statistically significant treatment related effects. The above statistical analyses were performed via TOXSTAT statistical software. All toxicity values (NOAEC and LOAEC) were determined in terms of the mean-measured treatment concentrations. There was about a 30% reduction in the number of offspring per reproductive day at 128 ppb a.i., which was considered biologically significant although there it was not statistically different from control.

**Results Synopsis**

| Endpoint | Method | NOAEC | LOAEC |
|---|---|---|---|
| Survival (Day 14) | ANOVA and William's test | 252 ppb a.i. | >252 ppb a.i. |
| Survival (Day 29) | ANOVA and William's test | 252 ppb a.i. | >252 pb a.i. |
| Reproduction (Offspring/repro. day) | ANOVA and William's test | 64 ppb a.i. | 128 ppb a.i. |
| Total length (mm) | ANOVA and William's test | 252 ppb a.i. | >252 ppb a.i. |
| Dry Weight (mg) | ANOVA and William's test | 252 ppb a.i. | >252 ppb a.i. |

Most sensitive endpoint(s): Reproduction (Number of offspring/repro. day)

## 15. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors.

Daily survival and clinical effects data were provided in Appendices 4 and 5 (pp. 51-58); however, the survival data were not gender-specific following pairing. Similarly, terminal growth parameters were not provided in terms of each gender. However, there were no apparent effects on these parameters. Therefore, this study does fulfills guideline requirements for an aquatic invertebrate life-cycle toxicity test using *Mysidopsis bahia* (*Americamysis bahia*) [§72-4(c)]. This study is classifiedACCEPTABLE. In addition

(though not required), offspring should have been observed daily for at least 4 days for survival, development, and behavior.

Although the feeding rate for the mysids was not specified, the amount offered appeared to be sufficient and did not appear to have an adverse effect on any biological response (i.e., survival, growth, reproduction, lack of cannibalistic behavior).

In method validation (QC) samples run concurrently with the test sample analyses, recoveries of Desthio JAU-6476 (in acetonitrile) from filtered saltwater fortified at 15.0, 50.0, or 300 ppb averaged 98.6 ± 3.59% (Appendix 3.5, p. 44). Furthermore, no measurable levels of test material (<10.0 ppb a.i.) were found in matrix blank samples.

The study authors noted that the results of this study prompted additional toxicity testing to address the difference in toxicity shown in the 96-hour acute mysid study conducted in November 2001 (Wildlife Project No. 149A-129; MRID #: 46246017). In the 96-hour acute study, the $LC_{50}$ was 60 ppb a.i.; however, no treatment-related effects on survival were observed at the 16, 32, 64, 128, and 252 ppb a.i. level in the present life-cycle study (p. 22). Therefore, two additional acute tests, an exploratory non-GLP study and a GLP study, were conducted in the fall of 2001 (details provided in Appendix 9, pp. 77-105). The $LC_{50}$ of the non-GLP acute study was >2000 ppb (nominal), and the $LC_{50}$ of the GLP acute study was >1009 ppb a.i. (mean-measured). These data suggest that the results from the first acute test were not repeatable (p. 22-23). The more recent acute tests are consistent with the toxicity observed in the life-cycle test, and the reasons for the differences observed between the earlier and later acute studies could not be determined. It was noted that these tests were run with different batches of saltwater, food, and organisms.

This study was performed according to U.S. EPA (40 CFR 160, 1989), OECD and JMAFF Good Laboratory Practice Standards. A Quality Assurance Statement was provided.

## 16. REFERENCES:

**U.S. Environmental Protection Agency.** 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.1350: *Mysid Chronic Toxicity Test.*

**ASTM Standard E1191-90.** 1991. *Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids,* American Society for Testing and Materials.

**West, Inc. and D.D. Gulley.** 1996. TOXSTAT Version 3.5, Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

**SAS Institute, Inc.** 2001. The SAS System for Windows. Release 8.02. Cary, North Carolina.

## 17. RESULTS OF STATISTICAL VERIFICATION:

**Percent Survival of Juvenile Mysids (Day 14)**
File: 6030sad Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 186.357 | 37.271 | 1.130 |
| Within (Error) | 22 | 725.500 | 32.977 | |
| Total | 27 | 911.857 | | |

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Percent Survival of Juvenile Mysids (Day 14)
File: 6030sad Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 96.500 | 96.500 | | |
| 2 | 16 | 94.750 | 94.750 | 0.498 | |
| 3 | 32 | 91.750 | 91.750 | 1.351 | |
| 4 | 64 | 90.000 | 90.000 | 1.848 | |
| 5 | 128 | 91.500 | 91.500 | 1.422 | |
| 6 | 252 | 96.500 | 96.500 | 0.000 | |

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

**Percent Survival (Day 29)**
File: 6030s2d Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 150.804 | 30.161 | 0.688 |
| Within (Error) | 22 | 963.875 | 43.813 | |
| Total | 27 | 1114.679 | | |

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Percent Survival (Day 29)

File: 6030s2d Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 94.125 | 94.125 | | |
| 2 | 16 | 92.000 | 92.000 | 0.524 | |
| 3 | 32 | 94.750 | 94.750 | -0.154 | |
| 4 | 64 | 88.250 | 88.250 | 1.449 | |
| 5 | 128 | 93.750 | 93.750 | 0.093 | |
| 6 | 252 | 89.750 | 89.750 | 1.079 | |

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

**Number of young per reproductive day**
File: 6030rd Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 0.207 | 0.041 | 2.278 |
| Within (Error) | 22 | 0.394 | 0.018 | |
| Total | 27 | 0.600 | | |

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Number of young per redproductive day
File: 6030rd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 0.583 | 0.583 | | |
| 2 | 16 | 0.527 | 0.527 | 0.677 | |
| 3 | 32 | 0.610 | 0.610 | -0.327 | |
| 4 | 64 | 0.615 | 0.615 | -0.397 | |
| 5 | 128 | 0.398 | 0.398 | 2.250 | |
| 6 | 252 | 0.408 | 0.408 | 2.135 | |

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

**Terminal adult length (mm)**
File: 6030ld Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 0.025 | 0.005 | 0.227 |
| Within (Error) | 22 | 0.486 | 0.022 | |
| Total | 27 | 0.510 | | |

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Terminal adult length (mm)
File: 6030ld Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 7.746 | 7.746 | | |
| 2 | 16 | 7.683 | 7.683 | 0.702 | |
| 3 | 32 | 7.785 | 7.785 | -0.427 | |
| 4 | 64 | 7.725 | 7.725 | 0.234 | |
| 5 | 128 | 7.755 | 7.755 | -0.096 | |
| 6 | 252 | 7.760 | 7.760 | -0.151 | |

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

**Terminal adult dry weight (mg)**
File: 6030wd Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 0.006 | 0.001 | 0.250 |
| Within (Error) | 22 | 0.084 | 0.004 | |
| Total | 27 | 0.089 | | |

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Terminal adult dry weight (mg)
File: 6030wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 0.839 | 0.839 | | |
| 2 | 16 | 0.837 | 0.837 | 0.036 | |
| 3 | 32 | 0.852 | 0.852 | -0.352 | |
| 4 | 64 | 0.863 | 0.863 | -0.610 | |
| 5 | 128 | 0.853 | 0.853 | -0.358 | |
| 6 | 252 | 0.815 | 0.815 | 0.623 | |

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Statistical re-analysis of young/reproductive dat, non-pooled controls

prothio desthio mysid young/repro day
File: mysrep.dat Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 0.190 | 0.038 | 2.045 |
| Within (Error) | 18 | 0.335 | 0.019 | |
| Total | 23 | 0.526 | | |

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho: All equal

prothio desthio mysid young/repro day
File: mysrep.dat Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | solv control | 0.574 | 0.574 | | |
| 2 | 16 | 0.527 | 0.527 | 0.479 | |
| 3 | 32 | 0.610 | 0.610 | -0.376 | |
| 4 | 64 | 0.615 | 0.615 | -0.435 | |
| 5 | 128 | 0.398 | 0.398 | 1.819 | |
| 6 | 252 | 0.408 | 0.408 | 1.720 | |

Bonferroni t table value = 2.55 (1 Tailed Value, P=0.05, df=18,5)

**Data Evaluation Report on the Acute Toxicity of the transformation product JAU6476-desthio to Marine Invertebrates- Acute (Crustacean)**
PMRA Submission Number 2004-0843 EPA MRID Number 46246030

**EAD Assessment of USEPA DER**

Reviewer: Émilie Larivière (#1269); PMRA Date: September 9, 2005

**PMRA Submission Number:** 2004-0843

**Study Type:** Non-Target Marine Invertebrates - Chronic (Crustacean)

Blankinship, A.S., T.Z. Kendall and H.O. Krueger. 2003. Desthio-JAU6476: a flow-through life-cycle toxicity test with the saltwater mysid (*Mysidopsis bahia*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory ID 149A-130A. Study submitted by Bayer CropScience, RTP, NC. Bayer Report 200485. September 4, 2003.

PMRA DATA CODE: 9.4.5
EPA DP Barcode: D303488
OECD Data Point: IIA 8.3.2
EPA MRID: 46246017
EPA Guideline: §72-4(C); 850.1350

**Company Code:** BCZ
**Active Code:** PRB
**Use Site Category:** 7, 13, 14
**EPA PC Code:** 113961

**EAD Executive Summary:**

In a 29-day life cycle test, *Americamysis bahia* neonates were exposed under flow-through conditions to the transformation product JAU6476-desthio (purity: 97%) at nominal concentrations of 0 (negative and solvent controls), 16, 32, 63, 125, and 250 µg JAU6476-desthio/L. Mean measured concentrations were <10 (<LOQ, controls), 16, 32, 64, 128, and 252 µg JAU6476-desthio/L, respectively. This study was conducted following U.S. EPA OPPTS Guideline 850.1350 and ASTM Standard E1191-90, and was in compliance with U.S. EPA (40 CFR Part 160), OECD, and Japan MAFF Good Laboratory Practice Regulations.

Prior to sexual maturity and pairing, there were 60 mysids/level. At Day 14, up to 20 pair/level

were isolated for individual matings. First-generation mysids were observed for mortality and signs of abnormal behaviour once daily throughout the study. Once daily during the reproduction period, second-generation mysids were counted and apparently discarded. Data endpoints included percent survival of first-generation mysids on Days 14 (at pairing) and 29 (study termination; combined sexes), the number of offspring per reproductive day, and total length and dry weight of surviving first-generation mysids (Day 29; combined sexes).

No treatment-related effect on survival was observed, either prior to or after pairing for reproduction. Prior to pairing (on Day 14), survival averaged 90 to 98% for all treatment and control groups. At study termination (on Day 29), survival averaged 88 to 95% for all treatment and control groups. The NOEC for survival was 252 µg JAU6476-desthio/L. Throughout the study, no treatment-related clinical signs of toxicity were observed, and the NOEC for clinical signs of toxicity was 252 µg JAU6476-desthio/L.

The day of first brood release was Day 17, and the median time of the first brood release for the negative and solvent controls was Day 22. The mean number of young produced per reproductive day averaged 0.592 and 0.573 for the negative and solvent control groups, respectively, and 0.527, 0.610, 0.615, 0.398, and 0.407 for the 16, 32, 64, 128, and 252 ppb a.i. test levels, respectively. The NOEC for reproduction was 64 µg JAU6476-desthio/L since there was an apparent 30% reduction in the number of offspring produced per reproductive day at 128 µg JAU6476-desthio/L. Although not statistically significant, a reproductive effect of this magnitude is likely to be biologically significant.

No treatment-related effects on growth were observed. Total body length of surviving mysids at study termination (Day 29) averaged 7.68 to 7.79 mm for all treatment and control groups, and dry weights averaged 0.815 to 0.863 mg for all treatment and control groups. The NOEC for growth was 252 µg JAU6476-desthio/L.

**Based on effects on reproductive success, the NOEC and LOEC are 64 and 128 µg JAU6476-desthio/L, respectively.**

**Results Synopsis:**
**Survival (Day 14)**
NOEC: 252 µg JAU6476-desthio/L
LOEC: >252 µg JAU6476-desthio/L

**Survival (Day 29; combined sexes)**
NOEC: 252 µg JAU6476-desthio/L
LOEC: >252 µg JAU6476-desthio/L

**Reproduction (no. young/reproductive day)**

NOEC: 64 µg JAU6476-desthio/L
LOEC: 128 µg JAU6476-desthio/L

**Total length (combined sexes)**
NOEC: 252 µg JAU6476-desthio/L
LOEC: >252 µg JAU6476-desthio/L

**Dry weight (combined sexes)**
NOEC: 252 µg JAU6476-desthio/L
LOEC: >252 µg JAU6476-desthio/L

Endpoint(s) affected: Reproduction

**EAD Evaluator Comments:**

1. The appropriate PMRA information (PMRA Submission Number, PMRA Data Code, PMRA company code, PMRA active ingredient code, PMRA use site category, OECD data point) was added to the PMRA review portion of the DER. The PMRA Submission Number was added to the Header of the DER. Information on the chemical name (IUPAC name, CAS name and synonym) available from the study report, the PMRA Chemistry review and other sources of information was added at the beginning of the DER. The name of the EAD secondary reviewer was added to the front portion of the DER and the sections were renumbered accordingly.

2. The study authors claim that the sex of each surviving first-generation was confirmed at test termination (p. 19), but no data on sex determination were provided.

3. The EAD reviewer reviewed the study results and statistical analyses of the EPA reviewer and feels the results are acceptable. The reviewer did not feel that redoing statistical analyses would produce different results/conclusions, as therefore agrees with the conclusions of the EPA reviewer.

**Study Acceptability:**
This study is scientifically sound. Although survival following pairing and terminal growth parameters should have been reported in terms of each gender, there were no apparent effects on these endpoints. Therefore, this study fulfills the guideline requirements for an aquatic invertebrate life-cycle toxicity test using *Americamysis bahia* (§72-4c), and is classified as **ACCEPTABLE**. In addition (though not required), offspring should have been observed daily for at least 4 days for survival, development, and behaviour.