# DATA EVALUATION RECORD
# MIDGE CHRONIC TOXICITY STUDY
## Non Guideline (OPPTS 850.1790)

1. **CHEMICAL:** Pyraclostrobin — PC Code No.: 099100

2. **TEST MATERIAL:** BAS 500 F (batch 27882/191/C) $^{14}$C-labelled (batch 579-1201) — Purity: 97.1% radiochemical purity at test initiation: 95.1%

3. **CITATION:**

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Title: Effects of BAS 500 F on the Development of Sediment Dwelling Larvae of *Chironomus riparius* in a Water-Sediment System

Study Completion Date: January 20, 2000

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5. **APPROVED BY:** Lewis Ross Brown, Biologist, EFED/ERB-I

**Signature:** Lewis R Brown **Date:** 2/10/05

## 6. STUDY PARAMETERS:

| | |
|---|---|
| **Age of Test Organism:** | 1st Instar, < 3 days old |
| **Definitive Test Duration:** | 28 days |
| **Study Method:** | Static |
| **Type of Concentrations:** | Nominal and mean measured |

## 7. CONCLUSIONS:

The 28-day chronic toxicity of BAS 500 F (Pyraclostrobin) to the midge, *Chironomus riparius*, was studied under static conditions in water-spiked exposures. The nominal test concentrations were 0 (negative and solvent controls), 0.020, 0.040, 0.080, 0.160 and 0.320 ppm. BAS 500F rapidly dissipates and degrades in the water phase, and radiolabeled test substance was used at the nominal 0.080 and 0.320 ppm test concentrations to determine the fate of the test material in overlying water, pore water, and sediment on days 1, 7, and 28; non-radiolabeled test material, analyzed at test initiation only, was used at the other three nominal concentrations (0.020, 0.040, and 0.160 ppm). Based on these labeled and non-labeled analytical determinations, the mean-measured concentrations of BAS 500 F at test initiation in the overlying water were 0.021, 0.040, 0.080, 0.166, and 0.315 ppm. Sediment concentrations were shown to be highest (82.4 and 81.7 %TAR) at day 28 and pore water concentrations of BAS 500 F were negligible (≤0.1 %TAR) during the 28-day study. Endpoints assessed were the total number of emerged midges and emergence and development rates (combined sexes); survival and growth were not assessed in this study.

Development rate was the most sensitive endpoint, exhibiting significant reductions from the pooled control at the three highest test concentrations (0.080, 0.166, and 0.315 ppm; overlying measured concentrations at test initiation). Total emergence and emergence rate were adversely affected at the 0.166 and 0.315 ppm test levels. No endpoint exhibited reductions which exceeded 50%, so $EC_{50}$ values were visually determined to be >0.315 ppm (overlying water); >0.261 ppm (28-day sediment concentration). Based on reductions in development rate, the NOAEC for this study was 0.040 ppm and the LOAEC was 0.080 ppm.

This study was designed to follow guideline OPPTS 850.1790 (Public Draft), EPA-712-C-96-354 (April 1996), and does not fulfill any currently-approved U.S. EPA SEP guideline. This study is scientifically sound, but it is only considered to provide Supplemental data because the sediment was not spiked with test material (for a toxicity study with sediment-dwelling organisms) and sediment concentrations were not analyzed at every nominal level during the study (particularly at day 28, which was shown to be the day of maximum sediment exposure to BAS 500F). As a result, toxicity values can only be expressed for the

measured overlying water concentrations at test initiation and not for the 28-day measured sediment concentrations. This study only provides supplemental information on the 28-day toxicity of BAS 500 F (a.i. Pyraclostrobin) to the sediment-dwelling midge, *Chironomus riparius* . This study is classified as **SUPPLEMENTAL**

**Results Synopsis:**

Sediment Concentrations

| | |
|---|---|
| $LC_{50}$ mortality: N/A | 95% C.I.: N/A |
| $EC_{50}$ growth: N/A | 95% C.I.: N/A |
| $EC_{50}$ emergence: >0.261 ppm | 95% C.I.: N/A |
| $EC_{50}$ development: >0.261 ppm | 95% C.I.: N/A |
| | |
| NOAEC (Mortality): N/A | Probit Slope (Morality): N/A |
| NOAEC (Growth): N/A | Probit Slope (Growth): N/A |
| NOAEC (Emergence): 0.066 ppm (d28 measured) | Probit Slope (Emergence): N/A |
| NOAEC (Development): 0.040 ppm (nominal) | Probit Slope (Development): N/A |

Overlying Water Concentrations

| | |
|---|---|
| $LC_{50}$ mortality: N/A | 95% C.I.: N/A |
| $EC_{50}$ growth: N/A | 95% C.I.: N/A |
| $EC_{50}$ emergence: >0.315 ppm | 95% C.I.: N/A |
| $EC_{50}$ development: >0.315 ppm | 95% C.I.: N/A |
| | |
| NOAEC (Mortality): N/A | Probit Slope (Morality): N/A |
| NOAEC (Growth): N/A | Probit Slope (Growth): N/A |
| NOAEC (Emergence): 0.080 ppm (d1 measured) | Probit Slope (Emergence): N/A |
| NOAEC (Development): 0.040 ppm (d1 measured) | Probit Slope (Development): N/A |

Pore Water Concentrations (test substance not detected in this phase)

| | |
|---|---|
| $LC_{50}$ mortality: N/A | 95% C.I.: N/A |
| $EC_{50}$ growth: N/A | 95% C.I.: N/A |
| $EC_{50}$ emergence: N/A | 95% C.I.: N/A |
| $EC_{50}$ development: N/A | 95% C.I.: N/A |
| | |
| NOAEC (Mortality): N/A | Probit Slope (Morality): N/A |
| NOAEC (Growth): N/A | Probit Slope (Growth): N/A |
| NOAEC (Emergence): N/A | Probit Slope (Emergence): N/A |
| NOAEC (Development): N/A | Probit Slope (Development): N/A |

**Endpoints affected:** Development rate (most sensitive), emergence rate and total

emergence

## 8. ADEQUACY OF THE STUDY:

**A. Classification:** SUPPLEMENTAL

**B. Rationale:** This study was designed to provide toxicity data for a sediment-dwelling midge; however, the test material was not measured at every nominal level in the sediment phase, so some endpoints cannot be expressed based on actual sediment exposure concentrations.

**C. Repairability:** None. Another study should be conducted in which the sediment is spiked with test material (rather than the overlying water) and analytically determined over the course of the study to express toxicity values as actual exposure concentrations for larvae of this sediment-dwelling midge.

## 9. GUIDELINE DEVIATIONS:

The following sources were used as guidance in evaluating this study, and deviations from these guidance documents are listed below:

U.S. EPA. 1996. Ecological Effects Test Guidelines, OPPTS 850.1735 & 850.1790 (Public Draft), EPA-712-C-96-354. April 1996.

U.S. EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates. Office of Research and Development and Office of Water, Washington, D.C. EPA/600/R-99/064. March 2000.

1. The EPA-recommended (USEPA 2000) ash-free dry weight (recommended to reduce the influence of ingesting different sediment grain sizes on weight for *C. tentans*) was not determined.

2. Pre-test mortality was not reported. It was not reported if the water used supported test animals without observable signs of stress; however, there was >90% adult emergence in the control group.

3. The water temperature (18.6-22.3°C) was lower than recommended (22-24°C) and it was measured 5 times during the test in 3 different vessels. Guidelines recommend that temperature should be monitored at least hourly throughout the test in one test

chamber, and near the beginning, middle and end of the test in all test chambers.

4. The pH of the overlying water ranged from 7.24-10.2, which substantially deviates from the guideline requirements of not more than 0.4 pH unit deviations.

5. Light intensity during the study (700-1600 lux) was higher than recommended (100-1000 lux).

6. The sediment characterization analyses for TOC, total volatile sulfides, particle size distribution, and water holding capacity were not reported

7. Sediments were not analyzed for BOD, COD, Eh, total inorganic carbon, total volatile solids, acid volatile sulfides, metals, oil and grease, and petroleum hydrocarbons. These analyses are suggested in the guidance documents.

8. The overlying, pore water and sediment concentrations were not determined for all treatment groups (only 80 and 320 ppb were measured by test termination).

**10.** **SUBMISSION PURPOSE:** This study was submitted to provide information on the toxicity of BAS 500 F (Pyraclostrobin) to sediment-dwelling chironomids for the purpose of pesticide registration.

## 11. MATERIALS AND METHODS:

### A. Test Organisms

| Guideline Criteria | Reported Information |
|---|---|
| **Species** Chironomus tentans *Other species which can be used are Hyalella azteca, Chironomus riparius, Daphnia sp., Ceriodaphnia sp.* (Specific criteria for these species are not listed in this report) | *Chironomus riparius* |
| **Life Stage** Second to third instar larvae (about 10 d old larvae with at least 50% at third instar. | 1st instar, < 3 days old. |
| **Supplier** Brood stock can be obtained from laboratory, commercial, or government sources.(Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.) | From in house culture |
| **All organisms from the same source?** | Yes. |

### B. Source/Acclimation

| Guideline Criteria | Reported Information |
|---|---|
| **Acclimation Period** Brood stock must be acclimated to culture water gradually from transport water to 100% culture water; water temperature exchange rate not to exceed 2°C within 24 hr; Avoid unnecessary stress, crowding and rapid temperature and water quality changes. | Fresh egg masses were collected on 11-June-99 and transferred to glass crystalizing dishes containing M4 (Elendt medium) water to which a small amount of algae was added as a feed starter. Hatched larvae from these egg masses (< 3 days old) were taken with Pasteur pipette in groups of about 5 and added to the test vessels. |

| Guideline Criteria | Reported Information |
|---|---|
| **Feeding**<br>Feeding should begin on day 0 and continue through day 9 unless food is not being eaten. | TetraMin was finely ground and suspended in M4 water, Chironomus larvae were fed approx. 1 mg TetraMin/larvae/day on each working day (reduced amounts were added after larvae started emerging) |
| **Pretest Mortality**<br>A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test). | Not reported., Chironomus larvae were placed in the test vessels containing dilution water 24 hours prior to treatment (test intiation) |

**C. Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Source of dilution water (Overlying water) and sediment**<br>Soft reconstituted water or water from a natural source, **not** de-chlorinated tap water.<br>[Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details)]. | Overlying water was from the same source as the culture water (reconstituted water, M4 according to Elendt), medium prepared with ultrapure deionized water that was continuously aerated; Table 2, p. 14).<br><br>The sediment was prepared in the laboratory by combining 69% quartz sand (particle size ≥ 80% 0.063 - 0.2 mm), 10% sphagnum peat, 20% Kaolin (kaolinite content ≥ 30%) and 1% $CaCO_3$ |
| **Does water support test animals without observable signs of stress?** | No reported, although the test specimens were from in house culture. |
| **Quality Of Water**<br>If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L | No problems were reported. |

| Guideline Criteria | Reported Information |
|---|---|
| **Water Temperature**<br>23°C ± 1°C. Daily mean test temperature Must not deviate more than ±1°C and instantaneous temperature must be within ±. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers. | Mean test water temperature was maintained at 20.8 ± 1.4°C, and was measured every 7 days in the negative control and 0.04 mg a.i./L (Table 7, p. 27). Temperature ranged from 18.6 - 22.3°C throughout the study period. |
| **pH**<br>Not specified, but should be appropriate to the test species and should not deviate more than 0.4 pH units. | Overlying water ranged from 7.4 to 10.2 by test end. 7.76-8.09 at test initiation. |
| **Dissolved Oxygen**<br>Should be measured at the beginning and end of short term tests. DO should be >40 percent and <100 percent saturation. | DO ranged from 5.4 - 10.2 mg/L, and was measured every 7 days in every replicate vessel (Table 7, p. 27). |
| **Total Hardness**<br>Prefer 40 - 200 mg/L as $CaCO_3$. | Overlying water at test initiation, 2.45 mmol/L, alkalinity not determined; (p. 13). |
| **Conductivity**<br>Not specified, but should be amenable to the test species. | Reported as 683 µS/cm at test intitiation; (p. 13). |
| **Sediment Characterization**<br>All sediment must be characterized for: pH, organic carbon content (TOC), total volatile sulfides, particle size distribution (% sand, silt, clay), and percent water content. | pH: M4-medium reported to be 8.32<br>TOC: Not reported<br>Total volatile sulfides: Not reported<br>Particle size distribution: 69% quartz sand (particle size ≥ 80% 0.063 - 0.2 mm), 10% sphagnum peat, 20% Kaolin (kaolinite content ≥ 30%) and 1% $CaCO_3$ Water holding capacity: Not reported |

| Guideline Criteria | Reported Information |
|---|---|
| **Additional Sediment Analysis**<br>BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis. | Not reported |
| **Laboratory Spiked Sediment**<br>Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures. | The sediment was not spiked, but rather the test chemical (labeled and non-labeled) was applied to the overlying water. The test substance, BAS 500 F (batch 27882/191/C) and $^{14}$C-labelled (batch 579-1201) were adequately characterized, 97.09 and 95.1% purity, respectively (p. 15). |
| **Stock Solutions**<br>Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required. | The test material was dissolved in acetone. Negative (dilution water) and solvent (acetone, 0.10 mL/L) controls were used in the test. |
| **Test Concentrations For Spiked Sediment**<br>For LC50 calculation, test concentrations should bracket the predicted LC50; Sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer. | Not applicable, as the sediment was not spiked. Test concentrations for the overlying water-spike were selected based upon a range-finding study in order to define the $EC_{50}$ (pp. 8-9).<br><br>Applications were made to the overlying water, not the sediment. Aliquots of the stock solution were applied just below the water surface, and the dilution water was gently mixed. |

| Guideline Criteria | Reported Information |
|---|---|
| **Test Aquaria**<br>1. Material: Glass or stainless steel or perfluorocarbon plastics.<br>2. Size: 300 ml high-form lipless beakers containing 100ml of sediment and 175 ml of overlying water. | 1. Glass beakers<br><br>2. 2 L (tall form) containing a 2-cm layer of sediment and 16.5 cm of overlying water. The volume of water was 1.8 L. |
| **Covers**<br>Static: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen. | Test vessels covered by glass plates and later exchanged for a plastic foil cover to avoid escape of the emerging midges. |
| **Type of Dilution System**<br>Must provide reproducible supply of toxicant. | N/A - Static system. |
| **Flow Rate**<br>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period. | N/A - Static system. |
| **Aeration**<br>Dilution water should be vigorously aerated so that dissolved oxygen in the overlying water remains above 40% saturation. In static systems, overlying water may be gently aerated through a 1-mL pipet located not closer than 2 cm from the sediment surface; Test organisms should not added 12 to 24h; Water quality characteristics should be measured before test organisms are added. | Aeration was provided through glass Pasteur pipette ~3 cm above the sediment layer. During addition of the larvae and for approx. 24 h afterwards the aeration was stopped. |

| Guideline Criteria | Reported Information |
|---|---|
| **Photoperiod**<br>16 hours light, 8 hours dark with a 15-30 min transition period and illuminance of about 100 to 1000 lux. | 16 hours light, 8 hours dark. Light intensity averaged 700-1600 lux. |
| **Solvents**<br>Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used. | Acetone, 0.10 mL/L |

**D. Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Sediment Into Test Chambers**<br>One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be throughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment | Test containers were prepared with sediment and overlying water 6 days prior to treatment (p. 17). The sediment was carefully overlaid (without mixing the sediment) with 1.8 L of dilution water. |
| **Renewal of Overlying Water:**<br>Renewal is required and flow rates should not differ by more than 10% in any two test chambers and should begin on day -1. | None performed. |
| **Placing Organisms in Test Chambers:**<br>Should be handled as little as possible and introduced into overlying water below the air-water interface. | One day prior to treatment, 1st instar larvae were added to the test vessels in groups of about 5 before transfer to the individual vessels in a random way using a pipette. |
| **Range Finding Test** | The definitive test concentrations were determined based on a range finding study. The results of the range finding study were not reported. |
| **Monitoring the test**<br>All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance. | Visual assessments (behavioral, mortalities and emergence) were made at least on each working day. The number, time and sex of emerged adults were recorded and removed from the vessels. |

| Guideline Criteria | Reported Information |
|---|---|
| **Nominal Concentrations of Definitive Test**<br>Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used. | 0 (negative and solvent controls), 0.02, 0.04, 0.08, 0.16, and 0.32 mg a.i./L.<br><br>The 0.08 and 0.32 mg a.i./L test concentrations received radio-labeled test compound in order to assess potential metabolites occurring in the sediment. Additional vessels were established 1 and 7 days after test initiation for pore water and sediment analysis at the 0.08 and 0.32 mg a.i./L test concentration. However, the sediment analysis after 28 days was conducted in the test vessel used for biological assessments (p. 17). |
| **Number of Test Organisms**<br>10 organisms per test chamber are recommended. 8 replicates per treatment should be used. | 25 larvae/replicate with three replicates per treatment level and 2 replicates for the negative control and 4 replicates for the solvent control. |
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |
| **Feeding**<br>Midges in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin® suspension daily. A drop in d.o. level below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until d.o. levels increase. | TetraMin was finely ground and suspended in M4 water, Chironomus larvae were fed approx. 1 mg TetraMin/larvae/day (i.e. 25 mg/vessel/day) on each working day (reduced amounts were added after larvae started emerging) No drop in DO was observed (Table 7, p. 27) |

| Guideline Criteria | Reported Information |
|---|---|
| **Water Parameter Measurements** Overlying Water Quality should measure conductivity, hardness, pH, alkalinity, and ammonia in all treatments at beginning and end of a test and should not vary by more than 50% within a treatment during the test. | DO and pH were measured every 7 days in each replicate vessel, temperature was measured in both negative control replicate vessels and one replicate vessel for the 0.04 mg a.i./L treatment concentration. Conductivity, hardness and alkalinity of the medium were measured at test initiation. Conductivity and hardness were also measured at test initiation in the overlying water. |
| **Chemical Analysis** Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution. | Pooled samples from the nominal test concentrations of 0.02, 0.04 and 0.16 mg a.i./L were analyzed at test initiation for BAS 500 F (non radio-labeled) via HPLC and UV detection. LSC-measurements were used to determine the measured concentrations of BAS 500 F (radio-labeled) in the 0.08 and 0.32 mg a.i./L test concentrations for overlying water and sediment (days 0, 1, 7 and 28) and overlying water (0, 2, 7, 21 and 28) in various replicate vessels. (Table 13, p. 33 and Appendix 5, p. 36) |

## 12. REPORTED RESULTS:

### A. General Results

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes. |

| Guideline Criteria | Reported Information |
|---|---|
| **Control Mortality**<br>Must be ≤ 30% in the sediment at end of the test. | Mortality was not measured, but these data could be inferred by the reviewer from the total emergence data:<br><br>Negative control: 0% (51/50)<br>Solvent control: 5% (5/100)<br>(Table 12, p. 30). |

| Guideline Criteria | Reported Information |
|---|---|
| **Percent Recovery of Chemical:**<br>1) % of nominal; | 1. In overlying water at 0.02, 0.04, 0.08 0.16 and 0.32 mg a.i./L: 98.5 - 105.7% on Day 0 (Table 3, p. 19).<br><br>Results of radio-labeled analysis for the 0.08 mg a.i./L nominal treatment level included: 99.6, 74.2 and 8.4% TAR (Total Applied Radioactivity) by days 0, 2 and 21, respectively, for the overlying water; 10.1, 74.7 and 73.2% TAR by days 1, 7 and 28, respectively, for the sediment extracts; and 0.0076, 0.0229 and 0.0521% TAR by days 1, 7 and 28 for pore water (Table 17, p. 41).<br><br>Results of radio-labeled analysis for the 0.32 mg a.i./L nominal treatment level included: 98.5, 55.1 and 8.2% TAR by days 0, 2 and 21, respectively, for the overlying water; 26.4, 69.1 and 70.6% TAR by days 1, 7 and 28, respectively, for the sediment extracts; and 0.0057, 0.0276 and 0.0224% TAR by days 1, 7 and 28 for pore water (Table 18, p. 42). |
| 2) Procedural recovery; | 2. Correlation coefficients were >0.999 for the linearity of the test procedure (p. 32) |
| 3) Limit of quantitation (LOQ) | 3. LOQ = 0.05 µg a.i./L for the 0.02, 0.04 and 0.16 mg a.i./L nominal concentrations analytical results. An LOQ for the radio-labeled nominal concentrations, 0.08 and 0.32 mg a.i./L was not reported. |

| Guideline Criteria | Reported Information |
|---|---|
| **Data Endpoints**<br>- Survival of Larvae<br>- Ash-free dry weight (AFDW) should be determined by pooling all living organisms from a replicate and drying to a constant weight (e.g. 60°C for 24 h) | - Number emerged per sex<br>- Emergence rate (combined sexes)<br>- Development rate (combined sexes) |
| **Raw data included?** | Yes |

Effects Data

| Toxicant Concentration | | | | Cumulative Number Dead[2] | Mean Dry Weight per midge (mg) |
|---|---|---|---|---|---|
| Nominal (ppm) | Measured | | | | |
| | Sediment d28 ppm[1] | Pore Water d28 ppm | Overlying Water d1 ppm | | |
| Control | ND | ND | ND | ND | ND |
| Solvent Control | ND | ND | ND | 5 (5%) | ND |
| 0.02 | ND | ND | 0.02 | 5 (7%) | ND |
| 0.04 | ND | ND | 0.04 | 5 (7%) | ND |
| 0.08* | 0.066 | ND (0.1% TAR) | 0.08 | 7 (9%) | ND |
| 0.16 | ND | ND | 0.16 | 15 (20%) | ND |
| 0.32* | 0.261 | ND (0% TAR) | 0.32 | 33 (44%) | ND |

ND - Not determined

[1] extractable + residual radioactive residues

[2] The cumulative number dead and (%) mortality were calculated from emergence data by the reviewer

* Measured concentrations were calculated by the reviewer based on the % Total Applied Radioactivity.

| Nominal Concentrations (ppm) | No. of emerged midges[1] | Mean Emergence Rate (%) | | | Mean Development Time (days) | Mean Development Rate (1/days) |
|---|---|---|---|---|---|---|
| | | Total | Male | Female | | |
| Control | 51/50[2] | 102[2] | ND | ND | NR | 0.0734 |
| Solvent Control | 95/100 | 95 | ND | ND | NR | 0.0773 |
| 0.02 | 70/75 | 93 | ND | ND | NR | 0.0735 |
| 0.04 | 70/75 | 93 | ND | ND | NR | 0.0744 |
| 0.08 | 68/75 | 91 | ND | ND | NR | 0.0686 |
| 0.16 | 60/75 | 80 | ND | ND | NR | 0.0646 |
| 0.32 | 42/75 | 66 | ND | ND | NR | 0.0562 |

[1] Observations were made from two and four replicates for the negative and solvent controls, respectively, and three replicates for each treatment group, with 25 midges/replicate (Table 4, p. 24).

[2] The number of midges per treatment was reported as 50, however, 51 midges emerged from the negative control.
* Treatment-related reduction.
ND - Not determined because the number of male and female midges was not controlled at test initiation.
NR- Not reported, the reviewer was unable to determine the mean development time from the reported data.

Other Significant Results: None reported

**B. Statistical Results**

Method: Endpoints assessed included the number of emerged midges, the emergence rate (combined sexes), and the development rate. Calculations were performed using TOXSTAT statistical software; individual vessels were considered as replicates, and nominal concentrations were used for all calculations. Emergence rate data were first acrsin-transformed prior to statistical analysis to meet the assumptions of ANOVA and Dunnett's test (p. 28). Un-transformed development rate data were statistically analyzed via ANOVA, Dunnet's and William's tests, the later of which was reported to be more conservative. For both endpoints, treatment groups were compared to the pooled control group. The total number of midges emerged were analyzed statistically to establish the $EC_{10}$ and $EC_{50}$ values and associated 95% C.I. via probit analysis.

Nominal Concentrations in the Overlying Water
$LC_{50}$ (mortality): Not determined
$EC_{50}$ (growth): Not determined

**Emergence Rate**
$EC_{50}$: 0.377 ppm 95% C.I.: 0.302-0.451 ppm
Probit Slope: Not reported
$EC_{10}$: 0.129 ppm 95% C.I.: 0.107-0.151 ppm
Probit Slope: Not reported
NOAEC: 0.080 ppm
LOAEC: 0.160 ppm

**Development Rate**
$EC_{50}$: 0.377 ppm 95% C.I.: 0.302-0.451 ppm
Probit Slope: Not reported
NOAEC: 0.040 ppm
LOAEC: 0.080 ppm

## 13. VERIFICATION OF STATISTICAL RESULTS:

Method: The NOAEC and LOAEC values were determined for total emergence, emergence rate, and development rate using the initial overlying water concentrations and the 28-day sediment concentrations which were provided (concentrations were not analytically determined for all test concentrations in sediment by day 28, the day of maximum test substance exposure). Because the number of male and female organisms was not controlled at test initiation, it was not appropriate to analyze emergence separately by sex. For all endpoints, the solvent control group was compared to the negative control group using a Student's t-test and, upon detecting no significant differences, the two groups were pooled for comparison to the treatment groups. After confirming normality and homogeneity of variances, NOAEC and LOAEC values were determined with ANOVA and William's multiple comparisons test for all endpoints via TOXSTAT software. Because reductions did not exceed 50% for any endpoint, $EC_{50}$ values could be visually determined.

Nominal Concentrations in the Overlying Water:

| PARAMETER | RESULT |
|---|---|
| $EC_{50}$ total emergence (95% C.I.)<br>$EC_{50}$ emergence rate (95% C.I.)<br>$EC_{50}$ development rate (95% C.I.) | >0.315 ppm (visual)<br>>0.315 ppm (visual)<br>>0.315 ppm (visual) |
| Binomial Test<br>$LC_{50}$ mortality (95% C.I.)<br>$EC_{50}$ growth (95% C.I.)<br>$EC_{50}$ total emergence (95% C.I.)<br>$EC_{50}$ emergence rate (95% C.I.)<br>$EC_{50}$ development rate (95% C.I.) | N/A |
| Moving Average Angle Test<br>$LC_{50}$ mortality (95% C.I.)<br>$EC_{50}$ growth (95% C.I.)<br>$EC_{50}$ total emergence (95% C.I.)<br>$EC_{50}$ emergence rate (95% C.I.)<br>$EC_{50}$ development rate (95% C.I.) | N/A |
| Probit Test<br>$LC_{50}$ mortality (95% C.I.)<br>$EC_{50}$ growth (95% C.I.)<br>$EC_{50}$ total emergence (95% C.I.)<br>$EC_{50}$ emergence rate (95% C.I.)<br>$EC_{50}$ development rate (95% C.I.) | N/A |

| PARAMETER | RESULT |
|---|---|
| Probit Slope:<br>Mortality<br>Growth<br>Total emergence<br>Emergence rate<br>Development rate | N/A |
| NOAEC:<br>Mortality<br>Growth<br>Total emergence<br>Emergence rate<br>Development rate | <br>N/A<br>N/A<br>0.080 ppm<br>0.080 ppm<br>0.040 ppm |
| LOAEC:<br>Mortality<br>Growth<br>Total emergence<br>Emergence rate<br>Development rate | <br>N/A<br>N/A<br>0.166 ppm<br>0.166 ppm<br>0.080 ppm |

## 14. REVIEWER'S COMMENTS:

Results of the reviewer's statistical verification were identical to those of the study author; development was the most sensitive endpoint and reductions did not exceed 50% for any endpoint. Based on development and the initial measured overlying water concentrations, the NOAEC was 0.040 ppm; however, this study was conducted with a sediment-dwelling midge and sediment concentrations were not measured at every treatment level. Furthermore, the test substance was applied to the water instead of directly to the sediment. As a result, this study is classified as SUPPLEMENTAL. The reviewer recommends that another study be conducted in which the sediment is spiked with the test material and the sediment test concentrations are analytically determined at all test levels.

## 15. REFERENCES: None reported

## APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

**total emerged (m+f)**

total emerged
File: 6705e Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 255.738 | 51.148 | 9.188 |
| Within (Error) | 15 | 83.500 | 5.567 | |
| Total | 20 | 339.238 | | |

Critical F value = 2.90 (0.05,5,15)
Since F > Critical F REJECT Ho:All groups equal

total emerged
File: 6705e 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 | 24.500 | 24.500 | | |
| 2 | 20 | 23.667 | 23.667 | 0.499 | |
| 3 | 40 | 23.333 | 23.333 | 0.699 | |
| 4 | 80 | 22.667 | 22.667 | 1.099 | |
| 5 | 160 | 20.000 | 20.000 | 2.697 | * |
| 6 | 320 | 14.000 | 14.000 | 6.294 | * |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

total emerged
File: 6705e Transform: NO TRANSFORMATION

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 | GRPS 1&2 POOLED | 6 | | | |
| 2 | 20 | 3 | 4.343 | 17.7 | 0.833 |
| 3 | 40 | 3 | 4.343 | 17.7 | 1.167 |
| 4 | 80 | 3 | 4.343 | 17.7 | 1.833 |
| 5 | 160 | 3 | 4.343 | 17.7 | 4.500 |
| 6 | 320 | 3 | 4.343 | 17.7 | 10.500 |

total emerged
File: 6705e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|---|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 6 | 24.500 | 24.500 | 24.500 |
| 2 | 20 | 3 | 23.667 | 23.667 | 23.667 |
| 3 | 40 | 3 | 23.333 | 23.333 | 23.333 |
| 4 | 80 | 3 | 22.667 | 22.667 | 22.667 |
| 5 | 160 | 3 | 20.000 | 20.000 | 20.000 |
| 6 | 320 | 3 | 14.000 | 14.000 | 14.000 |

total emerged
File: 6705e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|---|---|---|---|---|---|
| GRPS 1&2 POOLED | 24.500 | | | | |
| 20 | 23.667 | 0.500 | | 1.75 | k= 1, v=15 |
| 40 | 23.333 | 0.699 | | 1.84 | k= 2, v=15 |
| 80 | 22.667 | 1.099 | | 1.87 | k= 3, v=15 |
| 160 | 20.000 | 2.697 | * | 1.88 | k= 4, v=15 |
| 320 | 14.000 | 6.294 | * | 1.89 | k= 5, v=15 |

s = 2.359
Note: df used for table values are approximate when v > 20.

**Emergence rate (m + f)**

emergence rate, BAS 500 F
File: 6705ed Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 0.375 | 0.075 | 9.375 |
| Within (Error) | 15 | 0.125 | 0.008 | |
| Total | 20 | 0.500 | | |

Critical F value = 2.90 (0.05,5,15)
Since F > Critical F REJECT Ho:All groups equal

emergence rate, BAS 500 F
File: 6705ed 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 | 0.967 | 0.967 | | |
| 2 | 0.02 | 0.933 | 0.933 | 0.527 | |
| 3 | 0.04 | 0.933 | 0.933 | 0.527 | |
| 4 | 0.08 | 0.907 | 0.907 | 0.949 | |
| 5 | 0.16 | 0.800 | 0.800 | 2.635 | * |
| 6 | 0.32 | 0.567 | 0.567 | 6.325 | * |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

emergence rate, BAS 500 F
File: 6705ed Transform: NO TRANSFORMATION

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 | GRPS 1&2 POOLED | 6 | | | |
| 2 | 0.02 | 3 | 0.165 | 17.0 | 0.033 |
| 3 | 0.04 | 3 | 0.165 | 17.0 | 0.033 |
| 4 | 0.08 | 3 | 0.165 | 17.0 | 0.060 |
| 5 | 0.16 | 3 | 0.165 | 17.0 | 0.167 |
| 6 | 0.32 | 3 | 0.165 | 17.0 | 0.400 |

emergence rate, BAS 500 F
File: 6705ed Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|---|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 6 | 0.967 | 0.967 | 0.967 |
| 2 | 0.02 | 3 | 0.933 | 0.933 | 0.933 |
| 3 | 0.04 | 3 | 0.933 | 0.933 | 0.933 |
| 4 | 0.08 | 3 | 0.907 | 0.907 | 0.907 |
| 5 | 0.16 | 3 | 0.800 | 0.800 | 0.800 |
| 6 | 0.32 | 3 | 0.567 | 0.567 | 0.567 |

emergence rate, BAS 500 F
File: 6705ed Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|---|---|---|---|---|---|
| GRPS 1&2 POOLED | 0.967 | | | | |
| 0.02 | 0.933 | 0.517 | | 1.75 | k= 1, v=15 |

| | | | | | |
|---|---|---|---|---|---|
| 0.04 | 0.933 | 0.517 | | 1.84 | k= 2, v=15 |
| 0.08 | 0.907 | 0.930 | | 1.87 | k= 3, v=15 |
| 0.16 | 0.800 | 2.584 | * | 1.88 | k= 4, v=15 |
| 0.32 | 0.567 | 6.202 | * | 1.89 | k= 5, v=15 |

s = 0.091
Note: df used for table values are approximate when v > 20.

**Development Rate (m + F)**

development rate, BAS 500 F
File: 6705dx Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 9.751 | 1.950 | 9.375 |
| Within (Error) | 15 | 3.115 | 0.208 | |
| Total | 20 | 12.866 | | |

Critical F value = 2.90 (0.05,5,15)
Since F > Critical F REJECT Ho:All groups equal

development rate, BAS 500 F
File: 6705dx 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 | 7.598 | 7.598 | | |
| 2 | 0.02 | 7.350 | 7.350 | 0.770 | |
| 3 | 0.04 | 7.440 | 7.440 | 0.491 | |
| 4 | 0.08 | 6.860 | 6.860 | 2.289 | |
| 5 | 0.16 | 6.457 | 6.457 | 3.540 | * |
| 6 | 0.32 | 5.620 | 5.620 | 6.135 | * |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

development rate, BAS 500 F
File: 6705dx Transform: NO TRANSFORMATION

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 | GRPS 1&2 POOLED | 6 | | | |
| 2 | 0.02 | 3 | 0.839 | 11.0 | 0.248 |
| 3 | 0.04 | 3 | 0.839 | 11.0 | 0.158 |
| 4 | 0.08 | 3 | 0.839 | 11.0 | 0.738 |

| | | | | | |
|---|---|---|---|---|---|
| 5 | 0.16 | 3 | 0.839 | 11.0 | 1.142 |
| 6 | 0.32 | 3 | 0.839 | 11.0 | 1.978 |

development rate, BAS 500 F
File: 6705dx Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|---|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 6 | 7.598 | 7.598 | 7.598 |
| 2 | 0.02 | 3 | 7.350 | 7.350 | 7.395 |
| 3 | 0.04 | 3 | 7.440 | 7.440 | 7.395 |
| 4 | 0.08 | 3 | 6.860 | 6.860 | 6.860 |
| 5 | 0.16 | 3 | 6.457 | 6.457 | 6.457 |
| 6 | 0.32 | 3 | 5.620 | 5.620 | 5.620 |

development rate, BAS 500 F
File: 6705dx Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|---|---|---|---|---|---|
| GRPS 1&2 POOLED | 7.598 | | | | |
| 0.02 | 7.395 | 0.631 | | 1.75 | k= 1, v=15 |
| 0.04 | 7.395 | 0.631 | | 1.84 | k= 2, v=15 |
| 0.08 | 6.860 | 2.291 | * | 1.87 | k= 3, v=15 |
| 0.16 | 6.457 | 3.543 | * | 1.88 | k= 4, v=15 |
| 0.32 | 5.620 | 6.140 | * | 1.89 | k= 5, v=15 |

s = 0.456
Note: df used for table values are approximate when v > 20.