DATA EVALUATION RECORD
MIDGE 60-DAY TOXICITY STUDY

1. **CHEMICAL:** Cypermethrin PC Code No.: 109702

2. **TEST MATERIAL:** [^{14}C]Cypermethrin Radiochemical purity: 99.8%

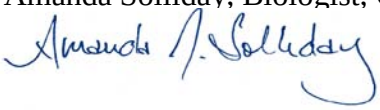
3. **CITATION:**

Authors:	Putt, A.E.
Title:	Cypermethrin – Life-Cycle Toxicity Test with Midge (*Chironomus tentans*) During a 60-Day Sediment Exposure.
Study Completion Date:	November 30, 2005
Laboratory:	Springborn Smithers Laboratories 720 Main Street Wareham, MA 02571-1037
Sponsor:	Pyrethroid Working Group Beveridge & Diamond 1350 I Street NW Washington, DC 20005
Laboratory Report ID:	13656.6112
MRID No.:	46725701
DP Barcode:	D325932

4. **REVIEWED BY:** Keith Sappington, Senior Advisor, OPP/EFED/ERBV

Signature:  **Date:** 2-22-11

REVIEWED BY: Amanda Solliday, Biologist, OPP/EFED/ERBV

Signature:  **Date:** 2-22-11

REVIEWED BY: Justin Housenger, Biologist, OPP/EFED/ERB5

Signature:  **Date:** 2-22-11

5. **STUDY PARAMETERS:**

Age of Test Organism:	First instar, <24 hours old
Definitive Test Duration:	60 days
Study Method:	Static renewal
Type of Concentrations:	Mean-measured

6. CONCLUSIONS:

The 60-day chronic toxicity of radiolabeled cypermethrin to midge larvae, *Chironomus tentans*, was studied under static renewal conditions in sediment-spiked exposures. Endpoints assessed included larval survival and growth (Day 20), percent emergence, development rate, time to death, number of eggs/female, and percent hatch of eggs laid.

Nominal sediment concentrations were 0 (negative and solvent controls), 3.1, 6.3, 13, 25, 50, and 100 µg a.i./kg dw. Undegraded cypermethrin (>97% of the recovered radioactivity) remained predominantly associated with the sediment matrix. Mean-measured sediment concentrations were <0.33 (LOQ, controls), 2.7, 4.7, 10, 20, 39, and 82 µg a.i./kg dw, respectively. Mean-measured pore water concentrations were <0.094 (LOQ, controls), 0.089, 0.11, 0.23, 0.51, 0.90, and 2.1 µg a.i./L, respectively. Pore water concentrations only increased slightly during the 60-day study, and radioactivity associated with the overlying water layer was <0.035 µg a.i./L (LOQ).

For percent emergence, there was a statistically-significant difference between the negative (70%) and solvent control (91%). However, both controls were within guideline standards for control performance (>50%), so this difference does not invalidate the test. In addition, no statistically-significant difference between controls was observed for mortality or dry weight and the percent emergence was lower for the solvent control (the opposite of what would be expected if the solvent was adversely impacting the test organisms). Therefore, the biological significance of the difference in percent emergence between the controls is questionable.

There was no significant effect on larval survival or percent emergence at any treatment level. Dry weight was adversely affected at the mean-measured sediment concentration of 82 µg a.i./kg dw sediment. Development rate was the most sensitive endpoint (statistically verified by the reviewer), with significant effects at the 20, 39 and 82 µg a.i./kg dw sediment concentration levels. The subsequent NOAEC and LOAEC were 10 and 20 µg a.i./kg dw sediment, respectively. The above effects were determined by comparing treatment to the negative control group.

Additionally, the study author reported significant adverse effects on the mean number of total eggs/female at the mean-measured 82 µg a.i./kg dw sediment concentration level relative to the pooled control. However, replicate data were not provided for the reviewer's verification of this endpoint. When compared to the negative control, the LOAEL appears to be in agreement with the study author's determination, based on the means and standard deviations reported for mean total of eggs/female (negative control=1088±178 mean total eggs/female, 82 µg a.i./kg dw sediment=759±148 mean total eggs/female). The study author also did not provide replicate data for mean days to death and percent hatch endpoints, but these endpoints did not appear to be significantly different from the negative control group at any treatment level.

The 20-day LC_{50} for larval survival was >82 µg a.i./kg dw sediment. The 20-day EC_{50} (with 95% C.I.) for midge growth (dry weight) was 74 (62-88) µg a.i./kg dw sediment.

This reviewer notes that HPLC/RAM analysis of cypermethrin concentrations in pore water (conducted only at the highest test concentration) indicate that the parent material declined to 30.4% and 4.5% of total radioactive residues measured at test initiation and termination (D60). In contrast, the recovery of parent compound from bulk sediment was 100% and 97.5% for the initial and terminal measurements, respectively. Given that recovery of parent chemical was high based on QA/QC sample spikes, the low concentrations of parent material in the pore water appear to reflect desportion of the degradation products from the sediment particles into the pore water phase. This presumption is consistent with the expected lower hydrophobicity of the degradation products compared to the parent compound. Since the measured pore water concentrations of cypermethrin do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured pore water concentrations.

Instead, this reviewer has estimated freely dissolved pore water endpoints based on measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (5.6%) and the mean K_{OC} (141,700 L/kg-OC; MRID 42129003) for cypermethrin (see Results Synopsis below). These estimated pore water endpoints, which are based on the freely dissolved test material (i.e., chemical that is not sorbed onto particulate organic carbon [POC] or dissolved organic carbon [DOC]), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that K_{OC} values for cypermethrin vary considerably depending on soil type (20,800 to 328,000). This range of K_{OC} likely reflects differences in organic carbon composition and other soil properties used to determine K_{OC}. Therefore, these estimated pore water endpoints are subject to the same uncertainty in determination and application of K_{OC} for cypermethrin.

This study was submitted to fulfill U.S. EPA data requirements for whole sediment chronic toxicity to freshwater invertebrates and is classified as SUPPLEMENTAL due to lack of reported raw data for selected reproductive endpoints (# eggs/female, % hatch, mean time to death). Although isolated incidents of low dissolved oxygen concentrations (< 2.5 mg/L recommended level) were observed on three replicates on two days during the course of the study, these levels were above DO threshold levels shown to cause adverse effects on the midge during long-term exposures (< 1.5 mg/L) and did not appear to adversely affect organisms in this test. This study provides useful information on the 60-day chronic toxicity of cypermethrin to sediment-dwelling midges (*Chironomus tentans*).

Results Synopsis:

Based on Mean Measured Sediment Concentrations	Based on Organic Carbon Normalized Sediment Concentrations	Based on Estimated Porewater Concentrations [1]
Midge Survival (Day 20) NOAEC: 82 µg a.i./kg dw LOAEC: >82 µg a.i./kg dw LC_{50}: >82 µg a.i./kg dw Probit Slope: N/A 95% C.I.: N/A	**Midge Survival (Day 20)** NOAEC: 1460 µg a.i./kg-OC LOAEC: >1460 µg a.i./kg-OC LC_{50}: >1460 µg a.i./kg-OC Probit Slope: N/A 95% C.I.: N/A	**Midge Survival (Day 20)** NOAEC: 0.010 µg a.i./L LOAEC: >0.010 µg a.i./L LC_{50}: >0.010 µg a.i./L Probit Slope: N/A 95% C.I.: N/A
Midge Growth (Day 20) NOAEC: 39 µg a.i./kg dw LOAEC: 82 µg a.i./kg dw EC_{50}: 72 µg a.i./kg dw 95% C.I.: 62-88µg a.i./kg dw Slope: 6.00±3.34	**Midge Growth (Day 20)** NOAEC: 696 µg a.i./kg-OC LOAEC: 1460 µg a.i./kg-OC EC_{50}: 1320 µg a.i./kg-OC 95% C.I.: 1110-1570 µg a.i./ kg-OC Slope: 6.00±3.34	**Midge Growth (Day 20)** NOAEC: 0.005 µg a.i./L LOAEC: 0.010 µg a.i./L EC_{50}: 0.009 µg a.i./L 95% C.I.: 0.008-0.011 µg a.i./L Slope: 6.00±3.34
Percent Emergence NOAEC: 82 µg a.i./kg dw LOAEC: >82 µg a.i./kg dw	**Percent Emergence** NOAEC: 1460 µg a.i./kg-OC LOAEC: >1460 µg a.i./kg-OC	**Percent Emergence** NOAEC: 0.010 µg a.i./L LOAEC: >0.010 µg a.i./L
Development Rate[2] **NOAEC: 10 µg a.i./kg dw** **LOAEC: 20 µg a.i./kg dw**	**Development Rate[2]** **NOAEC: 179 µg a.i./kg-OC** **LOAEC: 357 µg a.i./kg-OC**	**Development Rate[2]** **NOAEC: 0.0013 µg a.i./L** **LOAEC: 0.0025 µg a.i./L**
Time to Death[3] NOAEC: 82 µg a.i./kg dw LOAEC: >82 µg a.i./kg dw	**Time to Death[3]** NOAEC: 1460 µg a.i./kg-OC LOAEC: >1460 µg a.i./kg-OC	**Time to Death[3]** NOAEC: 0.010 µg a.i./L LOAEC: >0.010 µg a.i./L
Number of Eggs/Female[3] NOAEC: 39 µg a.i./kg dw LOAEC: 82 µg a.i./kg dw	**Number of Eggs/Female[3]** NOAEC: 696 µg a.i./kg-OC LOAEC: 1460 µg a.i./kg-OC	**Number of Eggs/Female[3]** NOAEC: 0.005 µg a.i./L LOAEC: 0.010 µg a.i./L
Percent Hatch[3] NOAEC: 82 µg a.i./kg dw LOAEC: >82 µg a.i./kg dw	**Percent Hatch[3]** NOAEC: 1460 µg a.i./kg-OC LOAEC: >1460 µg a.i./kg-OC	**Percent Hatch[3]** NOAEC: 0.010 µg a.i./L LOAEC: >0.010 µg a.i./L

[1] Freely dissolved pore water endpoints (ug a.i./L) estimated as:
Mean measured bulk sediment conc. (*ug a.i./kg-d.w.*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]
[2] The most sensitive endpoints are shown in bold (development rate).
[3] Endpoints are presented as reported by the study author. The reviewer was unable to re-calculate the statistics for time-to-death, number of eggs/female and percent hatch, as replicate data was not provided in the study report.

Endpoint(s) affected: growth, development rate, and number of eggs/female (unverified)
Most sensitive endpoint: development rate

7. ADEQUACY OF THE STUDY:

A. Classification: SUPPLEMENTAL

B. Rationale: Replicate data were not provided for days to death (individually and combined by sex), number of total eggs/female, and percent hatch.

C. Repairability:
Study may be upgradable with the submission of raw data as indicated above.

8. MAJOR GUIDELINE DEVIATIONS:

This study was compared to the draft OCSPP 850.1760 guideline (in prep.) and Test Method 100.5: Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants on *Chironomus dilutus* (formerly known as *C. tentans*) in Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (US EPA, 2000).

1. Replicate data were not provided for days to death (individually and combined by sex), number of total eggs/female, and percent hatch.

2. Some reproductive endpoints were not reported and/or analyzed: time to oviposition, sex ratio, number of egg cases oviposited, total number of eggs produced and adult mortality.

3. The dissolved oxygen (DO) levels were not provided in terms of percent saturation. During the study, levels ranged from 1.9-8.2 mg/L (based on daily measurements). It was reported that DO was maintained above 2.5 mg/L, with the exception of the measurement on Day 13 in the 25 and 50 µg a.i./kg nominal treatment levels and the measurement on Day 23 in the solvent control. It was not stated if aeration was employed to maintain acceptable levels. Raw data for dissolved oxygen measurements were not provided, only ranges for each test level. D.O. should be maintained above 2.5 mg/L in the test; however the Agency-wide guidelines indicate that exposure to brief periods of DO levels at 1.5 mg/L or higher did not appear to adversely affect midge health.

4. Pre-test mortality rates should have been provided. A group of organisms should not be used for a test if they appear unhealthy, discolored, behave abnormally, or greater than 20 percent mortality occurs during holding 48 hours before the start of a test.

9. SUBMISSION PURPOSE:

This study was submitted to provide information on the chronic toxicity of cypermethrin to sediment-dwelling chironomids.

10. MATERIALS AND METHODS:

A. Test Organisms

Agency Guideline Criteria	Reported Information
Species: *Hyalella azteca, Chironomus dilutus or Chironomus tentans*	*Chironomus tentans* (currently classified as *Chironomus dilutus*)
Life Stage: For *C. tentans*: larvae <24 hours old	Newly-hatched larvae, <24 hours 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.)	Egg masses were obtained from Aquatic BioSystems, Fort Collins, CO
All organisms from the same source?	Yes.

B. Source/Acclimation

Agency Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	Upon receipt, midge egg masses were placed in 270-mL crystallizing dishes containing approximately 200 mL of laboratory well water (same source as overlying water in definitive testing). Egg masses were observed daily until hatched. At test initiation, egg masses with hatched larvae still inside the egg case were transferred into clean laboratory water to facilitate the larvae to leave the egg mass. Midge larvae were <24 hours old at test initiation. No temperature was reported.
Feeding: Tetrafin® goldfish food, fed 1.5 mL daily to each test chamber starting Day -1 (1.0 mL contains 4.0 mg of dry solids)	Midge larvae were fed a diet consisting of a finely-ground flaked fish food suspension (4.0 mg/mL).

Agency Guideline Criteria	Reported Information
Pretest Mortality: The organisms should appear healthy, behave normally, feed well, and have low mortality in cultures, during holding (e.g., <20% for 48 h before the start of a test).	Pretest mortality was not reported.

C. Test System

Agency Guideline Criteria	Reported Information
Source of dilution water (overlying water) and sediment: Culture water, well water, surface water, site water, or reconstituted water. Overlying water should be from a source of water that has been demonstrated to support survival, growth, and reproduction of *C. tentans* in culture.	Overlying water was from the same source as the culture water (laboratory well water). Total hardness and total alkalinity ranged as calcium carbonate of 34-54 and 28-34 mg/L, respectively. Conductivity range of 130-180 Φmhos/cm and pH range of 7.1-7.9.
Formulated sediment is preferred, but uncontaminated natural sediment is acceptable. Sediment should have similar characteristics to those specified for *C. tentans* (EPA, 2000).	The sediment was collected from Glen Charlie Pond, Wareham, Massachusetts. Particle size distribution was characterized as 87% sand, 10% silt, and 3% clay. The average pH was 5.3 and total ammonia concentration was 4.5 mg/L as nitrogen. Average percent organic carbon was 5.6%.

Agency Guideline Criteria	Reported Information
Does water support test animals without observable signs of stress?	Yes
Quality Of Water Conductivity, hardness, alkalinity, and ammonia should be measured in all treatments at the beginning of the test, on Day 20, and at the end of the test. Dissolved oxygen (DO) and pH measurements should be taken at the beginning of a test and at least three times a week until the end of the test. Conductivity should be measured weekly.	No problems were reported. Periodic analysis of the dilution water for pesticides, PCBs and toxic metals indicated that none of the analytes were detected at toxic levels (actual results not provided). Pre-test particulate matter, total organic carbon (TOC), dissolved organic carbon (DOC), and residual chlorine levels were not provided for the dilution water. DOC levels measured in the pore water during the test ranged from 24.7-50.9 mg/L on Day 0 and 0.535-4.05 mg/L on Day 60.
Water Temperature 23±1°C. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Test water temperature was maintained at 22-25°C. Raw data were not provided (only temperature range data during the test based on daily measurements). At test initiation, Day 10 (initiation of the male auxiliary replicates), Day 20, and at test termination, temperature was measured in the overlying water of each replicate vessel of each test level used for biological monitoring. On remaining days, temperature was measured in one alternating replicate each day. Temperature was also continuously monitored in one replicate negative control vessel throughout the study.

Agency Guideline Criteria	Reported Information
pH For *C. tentans*, survival is best above pH 6.5. Poor control survival occurs at pH < 6.5 (OCSPP 850.1735).	pH ranged from 6.4 to 7.3. Raw data were not provided (only pH range data during the test based on measurements at days 0, 10, 20 and 60).
Dissolved Oxygen Tests should be managed toward a goal of DO >2.5 mg/L to insure satisfactory performance. If the DO level of the water falls below 2.5 mg/L for any one treatment, aeration is encouraged and should be done in all replicates for the duration of the test.	Dissolved oxygen (DO) ranged from 1.9 to 8.2 mg/L. It was reported that DO was maintained above 2.5 mg/L, with the exception of the measurement on Day 13 in the 25 and 50 µg a.i./kg nominal treatment levels (replicates L, 2.4 and 1.9 mg/L, respectively) and the measurement on Day 23 in the solvent control (replicate J, 2.0 mg/L). Raw data were not provided (only DO range data during the test based on daily measurements), and results were not provided in terms of percent saturation. At test initiation, Day 10 (initiation of the male auxiliary replicates), Day 20, dissolved oxygen concentrations were measured in the overlying water of each replicate vessel of each test level used for biological monitoring. On remaining days, DO was measured in one alternating replicate each day.

Agency Guideline Criteria	Reported Information
Total Hardness Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the test.	48-64 mg/L $CaCO_3$, measured on days 0, 10, 20 and 60. Total hardness of the overlying water was determined at test initiation, Day 10, Day 20, and test termination in a composite sample from each control and treatment level.
Conductivity Should be measured at the beginning, on Day 20, and at the end of a test.	170-240 Φmhos/cm Conductivity of the overlying water was determined at test initiation, Day 10, Day 20, and test termination in a composite sample from each control and treatment level.
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	pH: 5.3 Ammonia of pore water: 4.5 mg/L as nitrogen TOC: 5.6% Particle size distribution: 87% sand, 10% silt, and 3% clay Percent water content: not reported
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	A representative sample of the sediment source was analyzed for the presence of pesticides, PCBs and toxic metals; none of these compounds were detected at concentrations that would be considered to have an adverse impact on the results of the test (actual results not provided).

Agency Guideline Criteria	Reported Information
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	Radiolabeled test substance [Cyclopropane-1-^{14}C]cypermethrin Lot No.: CFQ13998 Batch No.: 1 CAS No.: 52315-07-8 Radiochemical purity: 99.8% Specific activity: 2.04 GBq/mmol Storage: In a freezer (-4°C) in the original container Description: Provided in toluene Water solubility: Not reported. The radiolabeled test substance was used for the definitive test and QC samples. Analytical methods were appropriate to accurately quantitate [^{14}C]cypermethrin in the different matrices. Non-radiolabeled test substance Cypermethrin technical Reference number: PL04-0113 CAS number: 52315-07-8 Purity: 95.6% Non-radiolabeled cypermethrin was stored at room temperature and used for the range-finding test.

Agency Guideline Criteria	Reported Information
Stock Solutions 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. Solvent is evaporated completely from the sediment prior to constructing test vessels.	The toluene was removed under nitrogen, and a primary stock solution was prepared in acetone (229 mg/L). From this, six individual dosing stock solutions were prepared in acetone for application of the test substance to the sediment. Negative and solvent controls were included in the test. All dosing stock solutions were clear and colorless with no visible un-dissolved test substance.
Test Concentrations For Spiked Sediment For LC_{50} calculation, test concentrations should bracket the predicted LC_{50}. Sediment may be mixed using rolling mill, feed mixer or hand mixer and equilibrium should be established.	Selection of nominal levels for the definitive study was based on toxicity information developed through preliminary testing. Test solutions were added to the sediment using the jar-rolling technique. A 9-mL aliquot of each dosing stock solution was applied to 0.0500 kg of coarse silica sand, and the solvent was allowed to evaporate off for 30 minutes. The dry treated sand was then added to 2.000 kg of wet sediment (1.3935 kg dw) in individual 1-gallon jars (1.4435 kg total dw). Each jar was then rolled for 4 hours at room temperature, and was stored at 4°C overnight. Treated sediments were equilibrated for a 27-day period in the refrigerator. Once a week during the equilibration period, the jars were mixed for an additional 2 hours at room temperature to ensure the sediment was homogeneous.

Agency Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water.	1. Glass beakers with 40-mesh Nitex® screen for drainage. 2. 300 mL. The approximate total volume was 275 mL with 100 mL (approximately 4.0-cm layer) of sediment and 175 mL of overlying water. On Day 17, emergence traps were placed over the test vessels to trap emergent flies for the remainder of the test. On Day 18 and thereafter, collected flies were paired in reproductive/oviposit chambers. Both the emergence traps and reproductive/oviposit chambers were constructed using 3.5-cm tall plexiglass tubes (i.d. of 6 cm) covered with mesh Nitex® screen. During the reproductive phase of the experiment, the chambers were placed on top of a 100 x 20-mm Petri dish containing 50 mL of laboratory well water. Egg masses were incubated in plastic cups containing 20 mL of laboratory well water.
Type of Dilution System Daily renewal or a flow-through system may be used.	Static renewal, with an intermittent delivery system in combination with calibrated water-distribution system.
Flow Rate 2 volume changes/day	The overlying water was either replaced by adding two volume additions per day or the water delivery system cycled approximately 21 times per day, providing approximately 6 overlying volume replacements per vessel per day (this is unclear in the study report, see Reviewer's Comments section)

Agency Guideline Criteria	Reported Information
Aeration If the DO level of the water falls below 2.5 mg/L for any one treatment, aeration is encouraged and should be done in all replicates for the duration of the test.	None reported.
Photoperiod 16 hours light, 8 hours dark at 100 to 1000 lux.	16 hours light, 8 hours dark. Light intensity ranged from 410 to 640 lux.
Solvents 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. Solvent is evaporated completely from the sediment prior to constructing test vessels.	Acetone, 9 mL per 1.4435 kg total dry mass of sediment spiked. The acetone was allowed to evaporate during the mixing procedure.

D. Test Design

Agency Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers. Overlying water is added to chambers in a manner that minimizes suspension of sediment.	Treated sediments were allocated to each test vessel 1 day prior to test initiation (Day -1). Overlying water was gently added to each vessel, which was then placed under the renewal system.

Agency Guideline Criteria	Reported Information
Renewal of Overlying Water: Renewal of overlying water is started on Day -1 before the addition of test organisms on Day 0. Renewal of overlying water is required during a test. Two volume additions of overlying water (continuous or intermittent) should be delivered to each test chamber daily. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%.	Renewal of the overlying water began on Day -1. The calibration of the overlying water renewal system was checked at test initiation and termination, and the system was visually inspected at least twice daily.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Midges were added impartially to each test vessel until each vessel contained 12 midges.
Range Finding Test It may be desirable to conduct a range-finding test in which the organisms are exposed to a control and three or more concentrations of the test material that differ by a factor of ten.	A 60-day range-finding test was conducted with unlabeled technical-grade cypermethrin (95.6% purity) at nominal sediment concentrations of 0 (negative and solvent controls), 0.010, 0.10, 1.0, 10, and 100 µg a.i./kg. Eight replicate vessels, each containing 12 midge larvae, were established for each level. Endpoints assessed included survival and ash-free dry weight of larvae on Day 20, percent emergence, time to emergence, time to death, reproduction (total number of eggs/female), and percent hatch. A statistically-significant reduction in larval dry weight was observed at the 100 µg a.i./kg treatment level compared to the solvent control (0.80 versus 1.20 mg, respectively). No other treatment-related differences in mortality, growth or reproduction were reported.

Agency Guideline Criteria	Reported Information
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance. The operation of the exposure system should be monitored daily.	Test vessels were observed daily for mortality and abnormal behavior. On Day 20, four (of the 12) replicate vessels were collected and survival and ash-free dry weights of the midge larvae were determined. Starting on Day 18 and daily thereafter, the number of male and female midges emerged were recorded. Midges that had completely emerged but had not escaped the surface tension of the water were observed an additional 24 hours before being recorded as an emerged or dead midge. Development rates were determined. Starting on Day 18 and daily thereafter, emerged male and female midge were collected and paired for reproduction. Reproductive/oviposit chambers were checked daily for dead adults and egg masses and dead flies were removed daily. The number of eggs produced in each primary egg mass was counted the day the egg mass was laid, using the ring method (details provided in Reviewer's Comment section). Egg masses were incubated at test temperature in 20 mL of laboratory well water. The number of unhatched eggs was counted following 4-6 days of incubation, and hatching success was determined. All levels were terminated on Day 60, when no further emergence occurred for ≥7 days in any of the treatments or controls.

Agency Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test 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), 3.1, 6.3, 13, 25, 50, and 100 µg a.i./kg dry weight Aqueous solubility was not reported, based on the 2005 USEPA RED (D293412), the solubility of cypermethrin is reported as 4 µg/L.
Number of Test Organisms 12 organisms per test chamber are recommended. The number of replicates tested depends in part on the significance level selected and the type of statistical analysis. For routine testing, a total of 16 replicates, each containing 12, <24-h-old larvae are tested for each treatment. For the total of 16 replicates the assignment of beakers is as follows: initially, 12 replicates are set up on Day -1 of which 4 replicates are used for 20-d growth and survival endpoints and 8 replicates for determination of emergence and reproduction. It is typical for males to begin emerging 4 to 7 d before females. Therefore, additional males, referred to as auxiliary males, need to be available during the prime female emergence period for each respective chamber/sediment. To provide these males, 4 additional replicates are stocked with 12, <24-h-old larvae 10 d following initiation of the test.	12 larvae/vessel, with 20 replicate vessels prepared per level and allotted for the following: 12 replicates – biological responses (4 for survival and growth on Day 20 and 8 for monitoring midge emergence) 4 replicates – additional vessels established on Day 9 for production of auxiliary males during the emergence/reproductive phase of the test 4 replicates – chemical analysis monitoring
Test organisms randomly or impartially assigned to test vessels?	Yes

Agency Guideline Criteria	Reported Information
Feeding *C. tentans* in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin7 suspension daily. A drop in DO levels below 2.5 mg/L may indicate over-feeding.	Finely ground flaked fish food suspension (4.0 mg/mL) introduced at a rate of 1.5 mL of suspension per test vessel, once daily. DO dropped below 2.5 mg/L on Day 13 in the 25 and 50 µg a.i./kg dw treatment levels and on Day 23 in the solvent control level. It was not reported if feeding was suspended during these times.
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	At test initiation, Day 10 (initiation of the male auxiliary replicates), Day 20, and at test termination, dissolved oxygen concentration, temperature, and pH were measured in the overlying water of each replicate vessel of each test level used for biological monitoring. On remaining days, DO and temperature were measured in one alternating replicate each day. Temperature was also continuously monitored in one replicate negative control vessel throughout the study. Test water temperature was maintained at 22-25°C. Raw data were not provided (only temperature range data during the test based on daily measurements). Total hardness, alkalinity, conductivity, and ammonia concentrations of the overlying water were determined at test initiation, Day 10, Day 20, and test termination in a composite sample from each control and treatment level.

Agency Guideline Criteria	Reported Information
Chemical Analysis Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Total radioactive residues were determined in duplicate sediment, overlying test water, and pore water samples on Days 0 and 60 (test initiation and termination) using LSC (aqueous) or LSC following combustion (sediment). Additional sediment and pore water samples from the highest treatment level (100 µg a.i./kg dw sediment) were analyzed for cypermethrin concentrations on Days 0 and 60 using HPLC/RAM.

11. REPORTED RESULTS:

A. General Results

Agency Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Control Criteria Average survival of *C. tentans* in the control sediment should be greater than or equal to 70% on Day 20. Average size of *C. tentans* in the control sediment at 20 d or 0.48 mg/surviving organism as ash-free dry weight.	21% mortality in the negative control and 6% mortality in the solvent control. 1.28 mg in the negative control and 1.47 mg in the solvent control
Percent Recovery of Chemical:	LSC – 93.0-118% of nominal (QC samples) HPLC/RAM – 100% of nominal (QC samples)
Data Endpoints - Larvae survival (Day 20 and test termination) -Pupae mortality -Adult mortality - Larval dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight; Day 20) - Percent emergence - Mean rate of emergence by gender - Days to death (M/F and combined sexes) - Sex ratio - Time to oviposition, first emergence - Total number of egg cases/treatment - Total number of eggs/female - Percent hatch of eggs laid	-Survival (Day 20) -Dry weight (Day 20; pooled surviving midges from each replicate and dried at 58-59ºC for 24 hours, then ashed at 550°C for 2 hours in a furnace) -Percent emergence (M/F and combined sexes) -Development rate, same as mean rate of emergence (M/F and combined sexes) -Days to death (M/F and combined sexes) -Total number of eggs/female -Percent hatch

Agency Guideline Criteria	Reported Information
Raw data included?	Replicate data were only provided for survival, dry weight, percent emergence, and development rate. **No raw reproductive data were provided.**

Reported Data on the Chronic Effects of Cypermethrin to *C. tentans*

Toxicant Concentration				Day 20		Day 60		Mean Days to Death				Mean Total Eggs/Female (SD)	Mean Percent Hatch (SD)
Nominal (µg a.i./kg)	Mean Measured (total [^{14}C]residues)												
	Sediment (µg a.i./kg)	Pore Water (µg a.i./L)	Overlying Water (µg a.i./L)	Percent Mortality (SD)	Dry Weight per Larvae (mg) (SD)	Percent Emergence (SD)	Develop-ment Rate (M/F Combined)	Unmated Males	Mates Males	Mated Females	Total		
Control	<LOQ	<LOQ	<LOQ	79 (21)	1.28 (0.21)	70 (13)	0.037 (0.0031)	3.3	6.5	6.3	5.8	1088 (178)	89 (11)
Solvent Control	<LOQ	<LOQ	<LOQ	94 (4)	1.47 (0.21)	91 (10)	0.038 (0.0025)	3.7	7.0	6.4	5.9	1041 (84)	89 (11)
3.1	2.7	0.089[a]	<LOQ	96 (5)	1.33 (0.25)	90 (10)	0.035 (0.0027)	3.6	6.6	6.4	6.0	886 [d] (166)	86 (12)
6.3	4.7	0.11	<LOQ	92 (17)	1.49 (0.27)	74 (28)	0.035 (0.0036)	3.5	6.9	6.6	5.7	963 (159)	94 (2)
13	10	0.23	<LOQ	85 (20)	1.47 (0.34)	84 (8)	0.035 (0.0018)	3.7	7.0	6.4	6.2	929 (170)	89 (8)
25	20	0.51	<LOQ	90 (21)	1.22 (0.11)	83 (14)	0.034 [b] (0.0020)	4.2	6.1	5.6	5.4	946 (127)	89 (5)
50	39	0.90	<LOQ	94 (8)	1.30 (0.39)	92 (15)	0.034 [b] (0.0029)	3.8	6.7	6.0	5.7	908 (130)	89 (5)
100	82	2.1	<LOQ	71 (14)	0.54 [b] (0.17)	64 (21) [c]	0.027[b] (0.0033)	3.5	7.3	6.2	6.0	759 [c] (148)	82 (15)

SD=standard deviation, calculated by study author. LOQ of 0.32-0.33 µg a.i./kg (sediment), <0.086-0.094µg a.i./L (pore water), <0.033-0.034 µg a.i./L (overlying water).
Note: Measured pore water conc. from the study are reported in this DER, but were not used to derive endpoints. See Verification of Statistical Results section for details.
[a] Since the Day 0 measured concentration was less than the LOQ, ½ the LOQ value (0.086 µg/L) and the Day 60 (0.091 µg/L) concentration were averaged.
[b] Statistically different compared to the negative control data, reviewer-calculated.
[c] Statistically different compared to the solvent control data, as reported by the study author.
[d] Statistically different compared to the pooled control data, as reported by the study author. However, the difference observed was not considered biologically relevant since no significant difference was observed at the next four higher treatment levels.

B. Reported Statistical Results

Method: Endpoints assessed included larval survival and growth (Day 20), percent emergence, development rate, time to death, number of eggs/female, and percent hatch of eggs laid. Analyses were performed using the mean replicate organism response and the mean-measured sediment concentrations via Toxstat v. 3.5. Survival data were arcsine transformed.

A t-test was conducted for each endpoint to compare the performance of the control and solvent control organisms. **Control responses indicated a significant difference between the negative and solvent control for percent emergence, and data obtained were therefore compared to the solvent control group. For all other endpoints, no significant differences were observed, and the data were pooled for subsequent comparisons.**

The Shapiro-Wilk's Test for normality was conducted on data obtained for survival and growth data from Day 20; the Chi-Square Test for normality was used for all other endpoints. Bartlett's Test was used to check on the assumption of homogeneity of variance for all endpoints. Survival and percent hatch data failed the qualifying test for normality and/or homogeneity and therefore data were analyzed using Wilcoxon's Rank Sum Test. Growth data passed both assumptions and data were analyzed using Williams' Test. Bonferroni's t-Test was used to analyze percent emergence, development rate, number of eggs per female, and days to death. The NOAEC and LOAEC were based on significance data.

The LC_{50} and EC_{50} values were calculated using the Inhibition Concentration Method (via Toxstat v. 3.5).

Based on Sediment Concentrations

Midge Survival (Day 20)
NOAEC: 82 µg/kg dw
LOAEC: >82 µg/kg dw
LC_{50}: >82 µg/kg dw 95% C.I.: N/A
Probit Slope: N/A

Midge Growth (Day 20)
NOAEC: 39 µg/kg dw
LOAEC: 82 µg/kg dw
EC_{50}: 72 µg/kg dw 95% C.I.: 62-79 µg/kg dw
Probit Slope: N/A

Percent Emergence
NOAEC: 39 µg/kg dw
LOAEC: 82 µg/kg dw

Development Rate
NOAEC: 10 µg/kg dw
LOAEC: 20 µg/kg dw

Time to Death
NOAEC: 82 µg/kg dw
LOAEC: >82 µg/kg dw

Number of Eggs/Female
NOAEC: 39 µg/kg dw
LOAEC: 82 µg/kg dw

Percent Hatch
NOAEC: 82 µg/kg dw
LOAEC: >82 µg/kg dw

Endpoint(s) affected: growth, percent emergence, development rate, and number of eggs/female
Most sensitive endpoint(s): development rate

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Survival, dry weight, emergence, and development data were statistically analyzed; replicate data were not provided for time to death, number of eggs/female, and percent hatch. For all endpoints, the solvent control data were compared to the negative control data using a two-sample t-test assuming either equal or unequal variances. **For comparison to treatments, only the negative control group was used, as per EFED guidance (Frankenberry *et al.*, 2008). The study reviewer's statistical analysis differed from the study author's, as the study author compared percent emergence in the treatments to the solvent control group because there was a statistically significant difference between the negative and solvent control observations for this parameter. For all parameters where data was available for statistical analysis (mortality, dry weight and development rate), the study reviewer made comparisons between the treatment responses and the negative control.**

All data were determined to be normally distributed and the variances were homogeneous, so the NOAEC and LOAEC were determined using ANOVA (survival and emergence), followed by William's test (development, dry weight). These analyses were conducted using Toxstat statistical software. For emergence, data for the "L" replicate of the 0.091 µg a.i./L treatment group were entered as "0"; the study author excluded this replicate (where no emergence was recorded) from the analysis. The LC_{50} is not definitive, as mortality did not exceed 50%, while the EC_{50} based on dry weight was determined using the Probit

method via Nuthatch statistical software. The reviewer reported results based on both estimated pore water and mean measured concentrations.

The mean rate of emergence for a given gender *g* for test vessel is calculated using the following equation:

j ($\overline{re_{gj}}$) represents the mean time span between test initiation (day 0 of the test) and emergence of adults of that gender within the test vessel. The mean rate of emergence ($\overline{re_{gj}}$) for a gender in replicate *j* is calculated using Equation 5.

Equation 5

$$\overline{re_{gj}} = \sum_{i=1}^{m} \frac{fe_{gji} r_{gi}}{nc_{gj}}$$

where:

g = gender of midge either male (m) or female (f);
j = index number of replicate in a test group from 1 to the total number of reproduction replicates in a test group;
i = index of inspection interval;
m = maximum number of inspection intervals;
fe_{gji} =number of midges of gender *g* in test vessel *j* that completely emerged during inspection interval *i*;
$r_{gi} = 1 \Big/ \left(day_i - \frac{l_i}{2} \right)$ = development rate of midges of gender *g* which emerged during inspection interval *i*;
day_i = inspection day (days since exposure *i.e.* day 0 of the test);
l_i = length of inspection interval *i* (days, usually 1 day); and
nc_{gj} = the total number of midges of gender g in test vessel *j* that underwent complete emergence by termination of the treatment group.

All of the above statistical analyses were performed in terms of the mean-measured sediment and pore water treatment concentrations. Sediment endpoints are also reported on an organic carbon-normalized basis, based on the following equation using an average TOC of 5.6%:

$$\text{mg/kg OC} = \frac{\text{mg/kg dry weight}}{\text{kg TOC/kg dry weight}}$$

Treatment-related effects were observed at the mean-measured 82 µg a.i./kg dw sediment concentration level for dry weight of the larvae (Day 20, 58% reduction). Reviewer-calculated % reduction is relative to the negative control. In addition, the development rate

(the most sensitive endpoint) was also affected by treatment at the 20 and 39 µg a.i./kg dw sediment concentration levels (8% reduction).

Reviewer's Summary of Endpoints:

Based on Mean Measured Sediment Concentrations	Based on Organic Carbon Normalized Sediment Concentrations	Based on Estimated Porewater Concentrations [1]
Midge Survival (Day 20) NOAEC: 82 µg a.i./kg dw LOAEC: >82 µg a.i./kg dw LC_{50}: >82 µg a.i./kg dw Probit Slope: N/A 95% C.I.: N/A	**Midge Survival (Day 20)** NOAEC: 1460 µg a.i./kg-OC LOAEC: >1460 µg a.i./kg-OC LC_{50}: >1460 µg a.i./kg-OC Probit Slope: N/A 95% C.I.: N/A	**Midge Survival (Day 20)** NOAEC: 0.010 µg a.i./L LOAEC: >0.010 µg a.i./L LC_{50}: >0.010 µg a.i./L Probit Slope: N/A 95% C.I.: N/A
Midge Growth (Day 20) NOAEC: 39 µg a.i./kg dw LOAEC: 82 µg a.i./kg dw EC_{50}: 72 µg a.i./kg dw 95% C.I.: 62-88 µg a.i./kg dw Slope: Slope: 6.00±3.34	**Midge Growth (Day 20)** NOAEC: 696 µg a.i./kg-OC LOAEC: 1460 µg a.i./kg-OC EC_{50}: 1320 µg a.i./kg-OC 95% C.I.: 1110-1570 µg a.i./kg-OC Slope: 6.00±3.34	**Midge Growth (Day 20)** NOAEC: 0.005 µg a.i./L LOAEC: 0.010 µg a.i./L EC_{50}: 0.009 µg a.i./L 95% C.I.: 0.008-0.011 µg a.i./L Slope: 6.00±3.34
Percent Emergence NOAEC: 82 µg a.i./kg dw LOAEC: >82 µg a.i./kg dw	**Percent Emergence** NOAEC: 1460 µg a.i./kg-OC LOAEC: >1460 µg a.i./kg-OC	**Percent Emergence** NOAEC: 0.010 µg a.i./L LOAEC: >0.010 µg a.i./L
Development Rate[2] **NOAEC: 10 µg a.i./kg dw** **LOAEC: 20 µg a.i./kg dw**	**Development Rate[2]** **NOAEC: 179 µg a.i./kg-OC** **LOAEC: 357 µg a.i./kg-OC**	**Development Rate[2]** **NOAEC: 0.0013 µg a.i./L** **LOAEC: 0.0025 µg a.i./L**
Time to Death[3] NOAEC: 82 µg a.i./kg dw LOAEC: >82 µg a.i./kg dw	**Time to Death[3]** NOAEC: 1460 µg a.i./kg-OC LOAEC: >1460 µg a.i./kg-OC	**Time to Death[3]** NOAEC: 0.010 µg a.i./L LOAEC: >0.010 µg a.i./L
Number of Eggs/Female[3] NOAEC: 39 µg a.i./kg dw LOAEC: 82 µg a.i./kg dw	**Number of Eggs/Female[3]** NOAEC: 696 µg a.i./kg-OC LOAEC: 1460 µg a.i./kg-OC	**Number of Eggs/Female[3]** NOAEC: 0.005 µg a.i./L LOAEC: 0.010 µg a.i./L
Percent Hatch[3] NOAEC: 82 µg a.i./kg dw LOAEC: >82 µg a.i./kg dw	**Percent Hatch[3]** NOAEC: 1460 µg a.i./kg-OC LOAEC: >1460 µg a.i./kg-OC	**Percent Hatch[3]** NOAEC: 0.010 µg a.i./L LOAEC: >0.010 µg a.i./L

[1] Freely dissolved pore water endpoints (ug a.i./L) estimated as:
Mean measured bulk sediment conc. (*ug a.i./kg-d.w.*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]
[2] The most sensitive endpoints are shown in bold (development rate).
[3] Endpoints are presented as reported by the study author. The reviewer was unable to re-calculate the statistics for time-to-death, number of eggs/female and percent hatch, as replicate data was not provided in the study report.

Endpoints affected (that reviewer could confirm statistically): growth and development rate
Most sensitive endpoint: development rate

13. REVIEWER'S COMMENTS:

For percent emergence, there was a statistically-significant difference between the negative (70%) and solvent control (91%). However, percent emergence in both controls were within guideline standards for control performance (>50%). Percent emergence consistently exceeded the negative control performance in the next 5 treatments, indicating no consistent expression of a "solvent effect" in the test, since all treatments were prepared with test material in the same amount of solvent. Furthermore, solvent was reportedly evaporated during the preparation of spiked sediment, thus substantially reducing the likelihood of solvent effects. Therefore, these findings indicate that the statistically-significant difference between the solvent and negative control for percent emergence is not likely related to solvent residue on spiked sediments, so this difference does not invalidate the test. In addition, no statistically-significant difference between controls was observed for mortality or dry weight so the biological significance of the difference in percent emergence between the controls is questionable.

The reviewer's conclusions were similar to the study author's for survival, dry weight, and development rate. Conclusions differed for emergence rate, where the study author compared treatment groups to the solvent control and the reviewer compared treatment groups for all endpoints to the negative control. **The reviewer was unable to verify the reproductive endpoints (number of total eggs/female and percent hatch) and mean days to death because the raw data was not reported.**

Cypermethrin residues remained primarily associated with the sediment matrix, as indicated by LSC analysis at 0 and 60 days of sediment, pore water, and overlying water. The mean percent recovery of total residues from the sediment ranged from 75-86% for all treatment levels. HPLC/RAM analysis of the highest treatment level indicated that >97.5% of the recovery radioactivity was parent cypermethrin. Total residues in pore water increased very slightly during the 60-day study, but remained ≤3.0 µg a.i./L (60-day value measured at the highest treatment level).

This reviewer notes that HPLC/RAM analysis of cypermethrin concentrations in pore water (conducted only at the highest test concentration) indicate that the parent material declined to 30.4% and 4.5% of total radioactive residues measured at test initiation and termination (D60). In contrast, the recovery of parent compound from bulk sediment was 100% and 97.5% for the initial and terminal measurements, respectively. Given that recovery of parent chemical was high based on QA/QC sample spikes, the low concentrations of parent material in the pore water appear to reflect desportion of the degradation products from the sediment particles into the pore water phase. This presumption is consistent with the expected lower hydrophobicity of the degradation products compared to the parent compound. Since the measured pore water concentrations of cypermethrin do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured pore water concentrations.

Instead, this reviewer has estimated freely dissolved pore water endpoints based on

measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (5.6%) and the mean K_{OC} (141,000 L/kg-OC; MRID 42129003) for cypermethrin (see Results Synopsis below). These estimated pore water endpoints, which are based on the freely dissolved test material (i.e., chemical that is not sorbed onto particulate organic carbon [POC] or dissolved organic carbon [DOC]), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that K_{OC} values for cypermethrin vary considerably depending on soil type (20,800 to 328,000). This range of K_{OC} likely reflects differences in organic carbon composition and other soil properties used to determine K_{OC}. Therefore, these estimated pore water endpoints are subject to the same uncertainty in determination and application of K_{OC} for cypermethrin.

It was explained in the study that females will generally lay a single primary egg mass, and that sometimes a second, generally smaller egg mass may be laid. As these second egg masses are prone to fungus and poor viability, they were not counted for egg numbers or used to determine hatch during the reproductive phase of the preliminary or definitive studies.

The number of eggs produced in each primary egg mass was counted the day the egg mass was laid, using the ring method. Five rings of eggs in each egg mass were selected at about equal distances along the length of the egg mass and the number of eggs in these five rings was then counted. The average number of eggs per ring was then multiplied times the number of rings in the egg mass to estimate the total number of eggs.

On Day 18, fungal growth was observed in all replicates of the nominal 100 µg a.i./kg dw level. On Day 19, excessive fungal growth was observed in two of the replicates, and was subsequently removed using a pipet.

The percent water content of the sediment should have been reported.

The survival at test termination should have been reported for all treatments.

An initial definitive exposure was initiated on February 4, 2005, but was terminated on February 28, 2005 due to low control survival of midge larvae on test day 20 (not further explained).

This study was conducted in compliance with all pertinent U.S. EPA GLP regulations with the exception of the routine water, food, and sediment contaminant screening analyses.

Experimental dates were April 8 – June 7, 2005.

14. <u>REFERENCES</u>:

Adams, W.J., *et al*. 1985. Aquatic safety assessment of chemicals sorbed to sediments. In: *Aquatic Toxicology and Hazard Assessment: Seventh Symposium* ASTM STP 854. R.D. Cardwell, R. Purdy and R.C. Bahner, Eds. American Society for Testing Materials. 1985. pp. 429-453.

APHA, AWWA, WPCF. 1995. *Standard Methods for the Examination of Water and Wastewater*. 19th Edition, Washington, DC.

ASTM. 2002. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Ditsworth, G.R., D.W. Schults, J.K.P. Jones. 1990. Preparation of Benthic Substrates for Sediment Toxicity Testing. *Environmental Toxicology and Chemistry*. Vol. 9, pp. 1523-1529.

Dunnett, C.W. 1995. A multiple comparison procedure for comparing several treatments with a control. *Journal of American Statistics Association.* 50: 1096-1121.

Dunnett, C.W. 1964, New tables for multiple comparisons with a control. *Biometrics* 20: 482-491.

Frankenberry M., Hammer C. and K. Sappington. Guidance for the Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicology Studies. Environmental Fate and Effects Division, Office of Pesticide Programs, US Environmental Protection Agency. Washington, DC.

Gulley, D.D., A.M. Boetler, and H.L. Bergman. 1996. Toxstat Release 3.5. University of Wyoming, Laramie, Wyoming.

Norberg-King, T.J. 1993. A Linear Interpolation method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. National Effluent toxicity Assessment Center, Environmental Research Laboratory – Duluth, U.S. Environmental Protection Agency, Duluth, Minnesota. Technical Report 03-93.

OECD. 2004. OECD Guideline 218. Sediment-Water Chironomid Toxicity Test Using Spiked Sediment. April 13, 2004. Paris, France.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry.* 2nd Edition. W.H. Freeman and Co. New York. 859 pp.

U.S. EPA 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act. Good Laboratory Practice Standards; Final Rule. Office of the Federal Register, National

Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

U.S. EPA 40 CFR, Part 158. U.S. Environmental Protection Agency. Data requirements for registration. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

U.S. EPA 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates, 2nd Edition. U.S. Environmental Protection Agency. Office of Research and Development. EPA/600/R-99/064.

Weber, C.I. *et al.* 1989. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. 2nd Edition. EPA/600/4-89-001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* 27: 103-117.

Williams, D.A. 1972. A comparison of several dose levels with a zero control. *Biometrics* 28: 519-531.

Zumwalt, D.C., *et al.* 1994. A water-renewal system that accurately delivers small volumes of water to exposure chambers. *Environmental Toxicology and Chemistry*. pgs. 1311-1314.

16. OUTPUT OF REVIEWER= STATISTICAL VERIFICATION:

Percent survival

	control	solvent control
	50	92
	83	92
	83	92
	100	100
mean	**79**	**94**
variance	**438**	**16**
t-test	**0.248011**	

Dry weight

	control	solvent control
	1.08	1.47
	1.17	1.47
	1.56	1.72
	1.32	1.21
mean	**1.2825**	**1.4675**
variance	**0.044025**	**0.043358**
t-test	**0.257278**	

Percent emergence

	control	solvent control
	83.3	100
	83.3	91.7
	75	100
	75	75
	50	75
	75	91.667
	58.333	91.667
	58.333	100
mean	**69.78325**	**90.62925**
variance	**157.2346**	**107.8972**
t-test	**0.002923**	

Dry weight

	control	solvent control
	0.0383	0.03691
	0.032	0.031
	0.038	0.037
	0.04	0.03
	0.03	0.04
	0.03	0.04
	0.041	0.041
	0.04	0.04
	0.038	0.041
	0.04	0.04
	0.037	0.038
	0.03	0.04
	0.039	0.043
	0.04	0.04
	0.044	0.039
	0.041	0.037
mean	**0.0365**	**0.04025**
variance	**2.03E-05**	**4.25E-06**
t-test	**0.201558**	

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survival
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                                ANOVA TABLE
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SOURCE	DF	SS	MS	F
Between	6	1909.429	318.238	1.206
Within (Error)	21	5539.250	263.774	
Total	27	7448.679		

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

survival
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	79.000	79.000		
2	2.7 (0.089)	96.000	96.000	-1.480	
3	4.7 (0.11)	91.750	91.750	-1.110	
4	10 (0.23)	85.250	85.250	-0.544	
5	20 (0.51)	89.500	89.500	-0.914	
6	39 (0.90)	93.750	93.750	-1.284	
7	82 (2.1)	71.000	71.000	0.697	

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

survival
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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	neg control	4			
2	2.7 (0.089)	4	28.251	35.8	-17.000
3	4.7 (0.11)	4	28.251	35.8	-12.750
4	10 (0.23)	4	28.251	35.8	-6.250
5	20 (0.51)	4	28.251	35.8	-10.500
6	39 (0.90)	4	28.251	35.8	-14.750
7	82 (2.1)	4	28.251	35.8	8.000

survival
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	79.000	79.000	89.208
2	2.7 (0.089)	4	96.000	96.000	89.208
3	4.7 (0.11)	4	91.750	91.750	89.208
4	10 (0.23)	4	85.250	85.250	89.208
5	20 (0.51)	4	89.500	89.500	89.208
6	39 (0.90)	4	93.750	93.750	89.208
7	82 (2.1)	4	71.000	71.000	71.000

survival
File: 5701s 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
neg control	89.208				
2.7 (0.089)	89.208	0.889		1.72	k= 1, v=21
4.7 (0.11)	89.208	0.889		1.80	k= 2, v=21
10 (0.23)	89.208	0.889		1.83	k= 3, v=21
20 (0.51)	89.208	0.889		1.84	k= 4, v=21
39 (0.90)	89.208	0.889		1.85	k= 5, v=21
82 (2.1)	71.000	0.697		1.85	k= 6, v=21

s = 16.241
Note: df used for table values are approximate when v > 20.

dry weight
File: 5701w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	2.478	0.413	5.986
Within (Error)	21	1.459	0.069	
Total	27	3.937		

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


dry weight
File: 5701w        Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2             Ho:Control<Treatment
-------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  SIG
----- --------------------    -----------    ------------------  ------  ---
  1            neg control       1.283              1.283
  2                2.7 (0.089)   1.333              1.333        -0.269
  3                4.7 (0.11)    1.490              1.490        -1.117
  4               10 (0.23)      1.470              1.470        -1.009
  5               20 (0.51)      1.223              1.223         0.323
  6               39 (0.90)      1.300              1.300        -0.094
  7               82 (2.1)       0.540              0.540         3.997  *
-------------------------------------------------------------------------
Dunnett table value =  2.46     (1 Tailed Value, P=0.05,  df=20,6)


dry weight
File: 5701w        Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 2 OF 2             Ho:Control<Treatment
-------------------------------------------------------------------------
                           NUM OF   Minimum Sig Diff  % of     DIFFERENCE
GROUP    IDENTIFICATION    REPS     (IN ORIG. UNITS)  CONTROL  FROM CONTROL
----- --------------------  -------  ----------------  -------  ------------
  1            neg control     4
  2                2.7 (0.089)4             0.457       35.6       -0.050
  3                4.7 (0.11) 4             0.457       35.6       -0.208
  4               10 (0.23)   4             0.457       35.6       -0.187
  5               20 (0.51)   4             0.457       35.6        0.060
  6               39 (0.90)   4             0.457       35.6       -0.017
  7               82 (2.1)    4             0.457       35.6        0.743
-------------------------------------------------------------------------


dry weight
File: 5701w        Transform: NO TRANSFORMATION

        WILLIAMS TEST  (Isotonic regression model)    TABLE 1 OF 2
-------------------------------------------------------------------------
GROUP                              ORIGINAL      TRANSFORMED    ISOTONIZED
          IDENTIFICATION       N      MEAN           MEAN           MEAN
------  --------------------  ---  -----------    -----------    -----------
```

1	neg control	4	1.283	1.283	1.394
2	2.7 (0.089)	4	1.333	1.333	1.394
3	4.7 (0.11)	4	1.490	1.490	1.394
4	10 (0.23)	4	1.470	1.470	1.394
5	20 (0.51)	4	1.223	1.223	1.261
6	39 (0.90)	4	1.300	1.300	1.261
7	82 (2.1)	4	0.540	0.540	0.540

dry weight
File: 5701w 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
neg control	1.394				
2.7 (0.089)	1.394	0.597		1.72	k= 1, v=21
4.7 (0.11)	1.394	0.597		1.80	k= 2, v=21
10 (0.23)	1.394	0.597		1.83	k= 3, v=21
20 (0.51)	1.261	0.114		1.84	k= 4, v=21
39 (0.90)	1.261	0.114		1.85	k= 5, v=21
82 (2.1)	0.540	3.983	*	1.85	k= 6, v=21

s = 0.264
Note: df used for table values are approximate when v > 20.

based on sediment
Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	39.	17.	92.	0.18	0.43
EC10	45.	23.	90.	0.14	0.50
EC25	57.	37.	88.	0.090	0.65
EC50	**74.**	**62.**	**88.**	**0.036**	**0.84**

Slope = 6.00 Std.Err. = 3.34

Goodness of fit: p = 0.60 based on DF= 4.0 21.

5701WS : dry weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change

0.00	4.00	1.28	1.36	-0.0778	100.	0.00
2.70	4.00	1.33	1.36	-0.0278	100.	1.63e-14
4.70	4.00	1.49	1.36	0.130	100.	3.39e-11
10.0	4.00	1.47	1.36	0.110	100.	9.05e-06
20.0	4.00	1.22	1.36	-0.137	100.	0.0321
39.0	4.00	1.30	1.30	0.00372	95.3	4.70
82.0	4.00	0.540	0.540	-0.000246	39.7	60.3

percent emerged
File: 5701e Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	4400.140	733.357	1.705
Within (Error)	49	21079.574	430.195	
Total	55	25479.714		

Critical F value = 2.34 (0.05,6,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

percent emerged
File: 5701e Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	69.783	69.783		
2	2.7 (0.089)	79.170	79.170	-0.905	
3	4.7 (0.11)	73.959	73.959	-0.403	
4	10 (0.23)	84.367	84.367	-1.406	
5	20 (0.51)	83.333	83.333	-1.307	
6	39 (0.90)	91.663	91.663	-2.110	
7	82 (2.1)	63.537	63.537	0.602	

Dunnett table value = 2.37 (1 Tailed Value, P=0.05, df=40,6)

percent emerged
File: 5701e Transform: NO TRANSFORMATION

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	neg control	8			
2	2.7 (0.089)	8	24.578	35.2	-9.387
3	4.7 (0.11)	8	24.578	35.2	-4.176
4	10 (0.23)	8	24.578	35.2	-14.584
5	20 (0.51)	8	24.578	35.2	-13.550
6	39 (0.90)	8	24.578	35.2	-21.879
7	82 (2.1)	8	24.578	35.2	6.246

percent emerged
File: 5701e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	8	69.783	69.783	80.379
2	2.7 (0.089)	8	79.170	79.170	80.379
3	4.7 (0.11)	8	73.959	73.959	80.379
4	10 (0.23)	8	84.367	84.367	80.379
5	20 (0.51)	8	83.333	83.333	80.379
6	39 (0.90)	8	91.663	91.663	80.379
7	82 (2.1)	8	63.537	63.537	63.537

percent emerged
File: 5701e 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
neg control	80.379				
2.7 (0.089)	80.379	1.022		1.68	k= 1, v=49
4.7 (0.11)	80.379	1.022		1.76	k= 2, v=49
10 (0.23)	80.379	1.022		1.79	k= 3, v=49
20 (0.51)	80.379	1.022		1.80	k= 4, v=49
39 (0.90)	80.379	1.022		1.80	k= 5, v=49
82 (2.1)	63.537	0.602		1.81	k= 6, v=49

s = 20.741
Note: df used for table values are approximate when v > 20.

development rate
File: 5701d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	8.301	1.383	6.881
Within (Error)	101	20.323	0.201	
Total	107	28.623		

Critical F value = 2.25 (0.05,6,60)
Since F > Critical F REJECT Ho:All groups equal

development rate
File: 5701d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	16	3.739	3.739	3.739
2	2.7 (0.089)	14	3.414	3.414	3.480
3	4.7 (0.11)	16	3.506	3.506	3.480
4	10 (0.23)	16	3.513	3.513	3.480
5	20 (0.51)	16	3.313	3.313	3.335
6	39 (0.90)	15	3.360	3.360	3.335
7	82 (2.1)	15	2.769	2.769	2.769

development rate
File: 5701d 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
neg control	3.739				
2.7 (0.089)	3.480	1.577		1.67	k= 1, v=101
4.7 (0.11)	3.480	1.633		1.75	k= 2, v=101
10 (0.23)	3.480	1.633		1.77	k= 3, v=101

```
              20 (0.51)   3.335     2.547      *       1.78       k= 4, v=101
              39 (0.90)   3.335     2.505      *       1.79       k= 5, v=101
              82 (2.1)    2.769     6.020      *       1.79       k= 6, v=101
-------------------------------------------------------------------------------
s =    0.449
Note: df used for table values are approximate when v > 20.
```