TEXT SEARCHABLE DOCUMENT 2011

DATA EVALUATION RECORD
MIDGE 10-DAY TOXICITY STUDY

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

2. **TEST MATERIAL:** [Cyclopropane-1-^{14}C]cypermethrin — Purity: 99.8% (radiochemical purity)

3. **CITATION:**

Author: Arthur E. Putt

Title: Cypermethrin - Toxicity to Midge *(Chironomus tentans)* During a 10-Day Sediment Exposure

Study Completion Date: June 29, 2005

Laboratory: Springborn Smithers Laboratories
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Laboratory Report ID: 13656.6110

MRID No.: 465915-04

DP Barcode: D319265

4. **REVIEWED BY:** Amanda Solliday, Biologist, OPP/EFED/ERB5

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REVIEWED BY: Keith Sappington, Senior Advisor, OPP/EFED/ERB5

Signature: **Date:** 2-24-11

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5. STUDY PARAMETERS:

Age of Test Organism:	2nd-3rd Instar, 11 days old
Definitive Test Duration:	10 days
Study Method:	Intermittent flow-through
Type of Concentrations:	Mean-measured

6. CONCLUSIONS:

The 10-day acute toxicity of [^{14}C]cypermethrin to midge larvae, *Chironomus tentans*, was studied under an intermittent flow-through system in sediment-spiked exposures. Endpoints assessed included survival and growth (ash-free dry weight).

The nominal spiked sediment test concentrations were 0 (negative and solvent controls), 22, 44, 88, 180, 350, and 700 µg a.i./kg dry sediment. The majority of radioactive residues remained predominately associated with the sediment during the 10-day study. Mean-measured sediment concentrations (Days 0 and 10) were <0.31 (<LOQ; controls), and 22, 43, 81, 170, 340, and 650 µg a.i./kg dry sediment, equivalent to mean recoveries of 100, 98, 92, 97, 96, and 93% of the nominal concentrations, respectively. All subsequent sediment concentrations in this review are mean-measured, unless otherwise noted. Mean-measured (reviewer-calculated from Days 0 and 10) pore water concentrations were <0.083 (<LOQ; controls), and 0.096, 0.20, 0.43, 0.77, 1.70, and 2.70 µg a.i./L. Mean-measured (reviewer-calculated from Days 0 and 10) overlying water concentrations were <0.034 (<LOQ, controls), and <0.034, <0.034, 0.036, 0.063, 0.14, and 0.19 µg a.i./L, respectively. The low overlying water concentrations likely result (at least in part) from the flow-through system employed, which ensured at least two volume replacements per vessel per day.

There is some uncertainty regarding the statistically significant difference between survival and dry weight in the negative and solvent control. Mortality was significantly reduced in the solvent control (8%) relative to the negative control (1%). The mortality in all other treatments except for the second lowest treatment level (44 µg a.i./kg dry sediment) differ statistically from the negative control. The measured dry weights for the negative control and solvent control differed statistically, but the three lowest treatment levels (22, 44, 88 µg a.i./kg dry sediment) did not significantly differ from the negative control dry weights. Because solvent was evaporated on silica sand prior to mixing with sediment and all treatments contained the same amount of initially-added solvent as the solvent control, the statistically significant reduction in survival in the solvent control appears to be a spurious result and is not likely related to the effect of the solvent. Considering all available information, the biological significance of the statistically higher mortality and lower dry weight seen in the solvent control is questionable and is not considered sufficiently robust as to interfere with the integrity of this test. Therefore, the reviewer relies on comparisons to the negative control for derivation of endpoints, in accordance with EFED guidance (Frankenberry *et al.*, 2008).

After 10 days, 1 and 8% mortality occurred in the negative and solvent control groups, respectively, and 15, 14, 16, 39, 74 and 99% in the 22, 43, 81, 170, 340, and 650 µg a.i./kg dry sediment treatments, respectively. When compared to the negative control, statistically significant reductions ($p \leq 0.05$) in treatment survival on Day 10 were identified at the 22, 81, 170, 340, and 650 µg a.i./kg dry sediment treatments (all tested concentrations except for the second lowest level tested, 43 µg a.i./kg dry sediment). The Day-10 NOAEC, LOAEC, and LC_{50} (with 95% C.I.) for survival were <22, 22 and 164 (72-496) µg a.i./kg dry sediment, based on the mean-measured bulk sediment concentrations compared to the negative control. Organic carbon-normalized endpoints based on sediment concentrations are given in the results synopsis below.

Dry weight per midge larvae averaged 2.04 and 1.79 mg in the negative and solvent control groups, respectively, and 2.00, 1.91, 1.98, 1.31, 0.21, and 0.82 mg for the 22, 43, 81, 170, 340, and 650 µg a.i./kg dry sediment treatments, respectively. Reviewer-determined percent inhibitions in dry weight compared to the negative control were 2, 6, 3, 36, 90, and 60% for the 22, 43, 81, 170, 340, and 650 µg a.i./kg dry sediment treatments, respectively. When compared to the negative control, dry weight was significantly reduced ($p \leq 0.05$) at the ≥170 µg a.i./kg dry sediment treatments (three highest treatment levels tested). When compared to the negative control, the 10-day NOAEC, LOAEC and EC_{50} (with 95% C.I.) for dry weight were 81, 170, and 200 (180-230) µg a.i./kg dry sediment, respectively. Organic carbon-normalized endpoints based on sediment concentrations are given in the results synopsis below.

This reviewer notes that HPLC/RAM analysis of cypermethrin concentrations <u>in pore water</u> (conducted only at the highest test concentration) indicate that the parent material declined to a negligible fraction of total radioactive residues measured over the course of this study (0% at the 10-d measurement). In contrast, the recovery of parent compound from bulk sediment was 100% for the initial and terminal measurements. 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 <u>will not</u> be expressed in terms of <u>measured</u> pore water concentrations.

Instead, this reviewer has <u>estimated</u> freely dissolved pore water endpoints based on measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (5.5%) 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 328,500). 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.

No abnormal behavior or additional sub-lethal effects were reported for surviving midges in the controls or treatment groups during the exposure period.

This study was designed to fulfill the OPPTS Draft Guideline 850.1735. This study is classified as **acceptable** and provides information on the 10-day toxicity of cypermethrin to sediment-dwelling midges (*Chironomus tentans*).

Results Synopsis:

Treatment comparison to negative control

Based on ESTIMATED[1] Pore Water Concentrations	
Survival	
LC_{50}: 0.021 µg a.i./L	95% C.I.: 0.009- 0.060 µg a.i./L
Probit Slope: NA	
NOAEC: <0.003 µg a.i./L	
LOAEC: 0.003 µg a.i./L	
Dry Weight	
EC_{50}: 0.026 µg a.i./L	95% C.I.: 0.023-0.030 µg a.i./L
Slope: 5.49	
NOAEC: 0.010 µg a.i./L	
LOAEC: 0.022 µg a.i./L	
Based on Bulk Sediment Concentrations (mean-measured)	
Survival	
LC_{50}: 164 µg a.i./kg dry sediment	95% C.I.: 72-469 µg a.i./kg dry sediment
Probit Slope: 1.96	
NOAEC: <22 µg a.i./kg dry sediment	
LOAEC: 22 µg a.i./kg dry sediment	
Dry Weight	
EC_{50}: 200 µg a.i./kg dry sediment	95% C.I.: 180-230 µg a.i./kg dry sediment
Slope: 5.49	
NOAEC: 81 µg a.i./kg dry sediment	

LOAEC: 170 µg a.i./kg dry sediment	
Based on OC-normalized Sediment Concentrations (mean-measured)	
Survival	
LC_{50}: 2980 µg a.i./kg TOC	95% C.I.: 1310-8530 µg a.i./kg TOC
Probit Slope: 1.96	
NOAEC: <400 µg a.i./kg TOC	
LOAEC: 400 µg a.i./kg TOC	
Dry Weight	
EC_{50}: 3640 µg a.i./kg TOC	95% C.I.: 3270-4180 µg a.i./kg TOC
Slope: 5.49	
NOAEC: 1470 µg a.i./kg TOC	
LOAEC: 3090 µg a.i./kg TOC	

[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*)]

Endpoints affected: Survival and Dry Weight

Most sensitive endpoint: Survival

7. ADEQUACY OF THE STUDY:

A. Classification: ACCEPTABLE

B. Rationale: Study was conducted according to EPA/OPPTS Guidelines (850.1735) with only minor guideline deviations.

C. Repairability: N/A

8. 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 (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, DC EPA/600/R-99/064. March 2000.

1) Physical descriptions and water solubilities of the test substances (radio-labeled and

unlabeled) were not reported.

2) Dissolved oxygen (DO) concentrations ranged 3.0-8.0 mg/L throughout the exposure period, based on daily measurements. Only the overall range of DO concentrations was reported. On test Day 5, to prevent DO levels from decreasing below 2.5 mg/L, the rate of the flow-through system was increased from 7 to 10 cycles per day (7 cycles=2 volume replacements, 10 cycles=2.8 volume replacements). As explained in the Reviewer's Comments section, the episodic nature of these DO excursions combined with the information on DO tolerance of *C. tentans* suggest that they are not sufficient to invalidate the study results.

3) The overlying water and sediment were analyzed for the presence of pesticides, PCBs and toxic metals. The study author noted that "none of these compounds were found at concentrations that would be considered to have an adverse impact on the results of the test." However, the results of this screen were not reported.

9. SUBMISSION PURPOSE: This study was submitted to provide information on the toxicity of cypermethrin to sediment-dwelling chironomids (larvae).

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Acute whole sediment toxicity tests are outlined in 850.1735 specifically for the amphipod *Hyalella azteca* and the midge *Chironomus tentans*.	*Chironomus tentans*
Life Stage Second to third instar larvae (about 10 days old larvae with at least 50% at third instar)	2nd-3rd instar, 11 days old. Age was confirmed by measuring the head capsule widths of 20 midge larvae from a sub-sample of the test population used to initiate the test. Sizes ranged from 0.25 to 0.45 mm. Ash-free dry weight was confirmed at test initiation (sub-population of 20 midge larvae) to be 0.29 mg dry weight per midge larvae.
Supplier Brood stock can be obtained from	In-house laboratory cultures.

Guideline Criteria	Reported Information
laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	
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 hours. Avoid unnecessary stress, crowding and rapid temperature and water quality changes.	Reared under test conditions for 11 days prior to test initiation.
Feeding During acclimation, feeding should begin on day 0 and continue through day 9 unless food is not being eaten.	During acclimation, midges were fed a finely-ground flaked fish food suspension (4.0 mg/mL) daily based on the number and size of the larvae in each rearing vessel.
Pretest Mortality A group of organisms should not be used if they appear unhealthy, discolored and there should be <20% mortality 48 h before the beginning of a test.	No mortalities 48 hours prior to test initiation. No other abnormalities were reported.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (Overlying water) and sediment Soft reconstituted water or water from a natural source, not de-chlorinated tap water. 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 (laboratory well water). The water was characterized as having total hardness and total alkalinity ranges as calcium carbonate of 46-50 and 32-34 mg/L, respectively, a specific conductivity range of 180-190 µmhos/cm, and a pH range of 7.4-7.5. Natural sediment was collected from Glen Charlie Pond, Wareham, MA (sub-batch 13656.6106 from the Pyrethroid Working Group-Freshwater Sediment Batch), wet pressed (2.0 mm sieve) to remove large particles, and was characterized by Agvise Laboratories (Northwood, ND). Analysis of the sediment pore water determined an ammonia concentration of 8.1 mg/L as nitrogen.
Does water support test animals without observable signs of stress?	Yes
Quality Of Water 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. Ammonia concentrations as nitrogen were <0.10-0.89 mg/L based on measurements from the overlying water as a composite sample from each treatment and control group. Dissolved organic carbon concentrations were 7.4-13.7 mg/L based on measurements from the pore water as a composite sample from each treatment and control group.
Water Temperature 23°C ± 1°C. Daily mean test temperature Must not deviate more than ±1°C and instantaneous temperature must be within ±3 °C. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning,	Daily measurement range of 23-24°C. Continuous monitoring range of 23-25°C in replicate J of the negative control group.

Guideline Criteria	Reported Information
middle and end of the test in all test chambers.	
pH Not specified, but should be appropriate to the test species and should not deviate more than 0.4 pH units.	6.5-6.8 (overlying water)
Dissolved Oxygen Should be measured at the beginning and end of short term tests. DO should be >40 percent and <100 percent saturation.	DO ranged from 3.0-8.0 mg/L. The actual percent DO saturation relative to the test temperature was not reported.
Total Hardness Prefer 40 - 200 mg/L as $CaCO_3$.	40-72 mg/L as $CaCO_3$ Total alkalinity was 26-52 mg/L as $CaCO_3$.
Conductivity Not specified for chironomids, but should be amenable to the test species.	240-260 µmhos/cm
Sediment Characterization 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: 4.9 Avg. TOC: 5.5% Total volatile sulfides: Not reported Particle size distribution: 83% sand, 12% silt, and 5.5% clay Water holding capacity: Not reported Moisture content @ 1/3 bar: 31%
Additional Sediment Analysis 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.	The sediment was screened for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc. (Braintree, MA) and none of these compounds were reported to be at concentrations that would be considered to have an adverse impact on the test results. The actual results of the screening were not reported.

Guideline Criteria	Reported Information
Laboratory Spiked Sediment 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.	Nonradiolabeled test material: Cypermethrin tech. Synonyms: None reported CAS no.: Not listed Batch no.: PL04-0113 Purity: 95.6% Physical description: Not reported Water solubility: Not reported Storage condition: Room temp., dark ventilated cabinet This test material was used to spike the sediments used for the range-finding test only. The test concentrations were adjusted for the purity of the test material. Radiolabeled test material: [Cyclopropane-1-^{14}C]cypermethrin Synonyms: None reported Batch no.: 1; Lot No. CFQ13998 Specific activity: 2.04 Gbq/mmole (equiv. to 292,035 dpm/µg) Amount received: 1 mCi (37.0 MBq) Radiochemical Purity: 99.8% Physical description: Not reported Water solubility: Not reported Storage condition: Freezer (<-4°C) This test material was used to spike the sediments used for the definitive test and QC samples.
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.	The primary [^{14}C]cypermethrin stock was prepared by removing the toluene from the test material under a gentle stream of nitrogen and then transferring the entire amount of test substance to a 50-mL volumetric flask and bringing to volume with acetone. Triplicate 25.0-µL aliquotes of the stock were then assayed via LSC.

Guideline Criteria	Reported Information
	Based on this analysis and the specific activity of 292,035 dpm/µg provided by the supplier, the stock was determined to have a concentration of 229 mg/L (229 µg/mL). The stock was stored frozen (-80°C) until use. The mean radiopurity of this stock solution was 99.5% by HPLC/RAM based on the results from three repetitive injections. Six individual dosing stock solutions were prepared in acetone for application of the test material to the sediment. See Reviewer's Comments section of this DER for further details on the exact dosing preparation scheme. Negative and solvent controls were also tested.
Test Concentrations For Spiked Sediment For LC_{50} calculation, test concentrations should bracket the predicted LC_{50}; 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.	Nominal sediment treatment concentrations selected for the test were 22, 44, 88, 180, 350, and 700 µg/kg dry sediment (ug a.i/kg sediment).
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100ml of sediment and 175 ml of overlying water.	1. Glass vessels (test chambers) 2. 300 mL; containing a 100-mL layer (~4.0-cm depth) of sediment (equiv. 151 g wet weight per vessel or 91 g dry weight) and 175 mL of overlying water. Total volume was maintained at 275 mL. The test vessels were all positioned in a water bath to maintain temperature.
Covers Static: Test vessels should be covered	Flow-through: Test chambers had two mesh-covered slots on the top edge of the

Guideline Criteria	Reported Information
with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	vessel to allow for drainage from the vessels during the cycling.
Type of Dilution System Must provide reproducible supply of toxicant.	N/A. Sediment was spiked with test material, not the overlying water.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	An intermittent delivery system in combination with a calibrated water-distribution system was used to renew the overlying water during the exposure period. Through Day 4, the water delivery system cycled approx. seven times per day (50 mL of water per cycle), providing two volume additions (i.e. 350 mL) per vessel per day. On Day 5, the intermittent-flow rate was increased to ten cycles per day to maintain acceptable DO levels (increasing the turnover rate to 2.8 volume additions/vessel/day). The renewal rate was visually checked at least two times per day.
Aeration 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.	Not reported
Photoperiod 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 was 360-670 lux.

Guideline Criteria	Reported Information
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.	A solvent control was prepared in the same manner as the treated sediment by adding 9 mL of acetone, containing no test material, to 0.05 kg of course silica sand. The solvent was allowed to evaporate off. The dried sand was then added to 2.0 kg of wet sediment and processed in the same manner as the treated sediments.

D. Test Design

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	The bulk quantity of spiked treatment sediments were subdivided and allocated to the replicate test vessels one day prior to test initiation. The overlying water was gently added to each vessel and the vessels were then placed in the water bath under the renewal system.
Renewal of Overlying Water: Renewal is required and flow rates should not differ by more than 10% in any two test chambers and should begin on day -1.	The overlying dilution water (not spiked) was renewed with 2 to 2.8 volume additions (increasing to 2.8 volume additions on day 5) per day per replicate test vessel.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	On Day 0, ten midge larvae were impartially and gently added to each of eight replicate test vessels/level.
Range Finding Test	The nominal treatment levels tested were 0.0 (negative and solvent controls), and 0.070, 0.70, 7.0, 70, and 700 µg a.i./kg dry sediment and were prepared in the same manner as described for the definitive test. See Reviewer's Comments section for details and Results.

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.	All replicate test vessels were observed daily for abnormal behavior, number of mortalities and signs of toxicity.
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.	0 (negative and solvent controls), 22, 44, 88, 180, 350, and 700 µg a.i./kg dry weight (ug a.i/kg sediment). Nominal sediment treatment levels were determined based on the results of a range-finding study. Aqueous solubility of the test material was not reported. Based on the 2005 USEPA RED (D293412), the solubility
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	10 midge larvae/replicate, with 10 replicates per level. Eight replicates (A-H) were prepared for biological response and water quality measurements, and two additional replicates (I and J) were prepared for chemical analysis of the test material in the overlying water, pore-water, and sediment.
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding 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.	Fed 1.5 mL of a 4-mg/mL suspension of a finely-ground flaked fish food once daily during the definitive test.

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.	pH was measured in all biological replicates at test initiation and termination. DO was measured in all biological replicates at test initiation and termination and daily in overlying water in one alternating replicate test vessel of each treatment level and control. Temperature was measured in all biological replicates at test initiation and termination and daily in overlying water in one alternating replicate test vessel of each treatment level and control. Temperature was also measured and recorded continuously in one replicate of the negative control. Hardness, alkalinity, conductivity, ammonia as nitrogen, and DOC were measured at study initiation and termination in a composite sample from the controls and each treatment group.
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.	The six treatment levels and both solvent and negative control sediments were sampled and analyzed for total [^{14}C]residue concentrations prior to the allocation of the sediments into the replicate exposure vessels and following the 12-day mixing and equilibration period. During the definitive exposure period, sediment, pore water, and overlying water samples were removed from replicates I and J on test Days 0 and 10, respectively, and analyzed for total [^{14}C]residue concentrations using liquid scintillation counting (LSC). Overlying water samples were removed from the test vessels by pipetting into a graduated cylinder. The pore water was then removed by removing the entire sediment sample and centrifuging for 30 minutes at

Guideline Criteria	Reported Information
	10,000 rpm. The resulting pore water was pipetted from the centrifuge tube. In addition, six QC samples (three aqueous and three sediment) were prepared and analyzed with each analytical sampling of the test vessels.

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Control Mortality Mean control mortality must be ≤ 20% at end of the test.	Negative control: 1% Solvent control: 8%
Percent Recovery of Chemical: 1) % of nominal; 2) Procedural recovery;	1) All recoveries are based on the reported mean-measured treatment concentrations and were determined by LSC analysis. In sediment: 92-100% of nominal sediment concentrations. 2) Based on QC samples fortified and analyzed concurrently with the sediment and overlying water test samples (on Days 0 and 10). In sediment matrix spikes at 18.2-730 µg a.i./kg dry sediment, recoveries were 76.5-102% of nominal. In freshwater matrix spikes at 0.916-22.9 µg a.i./L, recoveries were 93.6-101% of nominal. 3) LOQ = 0.034 µg a.i./L for overlying

Guideline Criteria	Reported Information
3) Limit of quantitation (LOQ)	water samples; 0.082-0.083 µg a.i./L for pore-water samples; and 0.31 µg a.i./kg dry weight for sediment samples.
Data Endpoints - Survival of Larvae - Ash-free dry weight should be determined by pooling all living organisms from a replicate and drying to a constant weight (e.g. 60°C for 24 h)	- Survival of larvae - Ash-free dry weight.
Raw data included?	Yes, mean replicate data provided

Effects Data (reviewer-determined)

Toxicant Concentration

Nominal Sediment (ug a.i./kg sediment)	Mean-Measured (Days 0 and 10)				
	Sediment (µg a.i./kg dry sediment)[1]	Pore Water (µg a.i./L)[2]	Overlying Water (µg a.i./L)[3]		
Negative control	<0.31	<0.083	<0.034	1/80 (1)	2.04
Solvent control	<0.31	<0.083	<0.034	6/80 (8)*	1.79*
22	22	0.096[5]	<0.034	12/80 (15)*	2.00 (2%)
44	43	0.20	<0.034	11/80 (14)	1.91 (6%)
88	81	0.43	0.036[5]	13/80 (16)*	1.98 (3%)
180	170	0.77	0.063[6]	31/80 (39)*	1.31 (36%)**
350	340	1.7	0.14[6]	59/80 (74)*	0.21 (90%)**
700	650	2.7	0.19[6]	79/80 (99)*	0.82 (60%)[7]

[1] The LOQ for sediment samples was 0.31 µg a.i./kg dry sediment.
[2] The LOQ for pore water samples was 0.082 (Day 0) - 0.083 (Day 10) µg a.i./L. Note: Measured concentrations from the study are reported in this DER, but were not used to derive endpoints. See Verification of Statistical Results section for further details.
[3] The LOQ for overlying water samples was 0.034 µg a.i./L.
[4] Percent inhibition (reviewer-calculated) is relative to the negative control.
[5] Since the Day 0 measured concentration was less than the LOQ, the LOQ value and the Day 10 concentration were averaged.
[6] Reviewer-determined as the average of the Day 0 and Day 10 measured concentrations. Note: The overlying water was renewed at least two times per day to maintain water quality. Concentrations would presumably be higher under static conditions.
[7] Excluded from statistical analysis due to significant effect on survival.
* Statistically significant reduction ($p \leq 0.05$) compared to the negative control using Steel's Many-One Rank Test.
*Statistically significant compared to the negative control using a t-test ($p \leq 0.05$, reviewer-calculated).
** Statistically significant reduction ($p \leq 0.05$) compared to the negative control using Bonferroni's Test (reviewer-calculated).

B. Statistical Results (From Study Report)

Statistical Method(s): Endpoints assessed included percent midge larvae survival and ash-free dry weight (growth) per larvae. Analyses were performed using the mean replicate organism response and the mean-measured sediment treatment concentrations via Toxstat v. 3.5.

Survival and growth treatment response data were compared to the solvent control data since a *t*-Test indicated statistically significant differences between the negative and solvent controls. All data were assessed for normality using the Chi-Square test for normality and for homogeneity of variance using Bartlett's Test. Survival and growth data meet the assumptions of ANOVA. Therefore, Dunnett's Test and Bonferroni's Test were used to compare survival and growth treatment response data to the solvent control data, respectively. The 10-day LC_{50} and EC_{50} values and associated 95% confidence intervals (95% C.I.) based on midge survival and growth, respectively, were determined visually using the Inhibition Concentration Method (Norberg-King, 1993) via Toxstat. Note the study author excluded the highest treatment level tested from the statistical analysis of the growth data due to the statistically significant reduction in survival by Day 10 at this treatment level.

Study Author's Statistical Results (Survival)

LOAEC	170 ug a.i/kg sediment
NOAEC	81 ug a.i/kg sediment
LC_{50} (95% CI)	290 ug a.i/kg sediment (240 – 330)

Study Authors' Statistical Results (Growth)

LOAEC	170 ug a.i/kg sediment
NOAEC	81 ug a.i/kg sediment
EC_{50} (95%CI)	220 ug a.i/kg sediment

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Methods:

Percent survival and growth treatment response data were statistically compared to both the negative and solvent control. T-tests indicated statistically significant differences in the dry weights of the solvent and negative control ($p \leq 0.05$), but not mortality ($p=0.98$). For mortality data, the data passed Shapiro-Wilk's tests for normality, but not an F-test for homogeneity of variance. Therefore, Steel's Many-One Rank test (non-parametric) was used to calculate the NOAEC and LOAEC values with Toxstat v. 3.5 statistical software. The study author and study reviewers' obtained different NOAEC and LOAEC values for survival due to the study author comparing statistical difference in the analysis against the pooled control while the study reviewer compared to the negative control. The 10-day LC_{50} value (164 ug a.i./kg sediment) was calculated using the probit method with Toxanal v. 1.04 software. Although the probit method indicated significant lack of fit ($p<0.001$), it was selected because: 1) the 95% confidence interval (72-469 ug a.i./sediment) better reflects uncertainty in the LC_{50} caused by the shallow slope compared to the moving average method (144-196 ug a.i./kg sediment); 2) the estimated probit slope (1.96) is consistent w/ the shallow slope of observed data; and 3) the LC_{50} values determined by the probit and moving average methods were nearly identical (164 vs. 167 ug ai/kg sediment, respectively). . The difference in statistical methods (ICp for the study author vs. probitfor the study reviewer) likely reflects the difference in LC_{50} values obtained.

After confirming normality and homogeneity of variances, NOAEC and LOAEC values based on mean dry weight per larvae (growth) data were determined parametrically using ANOVA and Bonferroni t-test (due to unequal reps) via Toxstat v. 3.5 statistical software. The 10-day EC_{50} values for dry weight data were calculated using the probit method via Nuthatch statistical software. Like the study author, the reviewer also excluded the highest treatment level tested from the statistical analysis of the dry weight data due to the statistically significant reduction in survival by Day 10 at this treatment level. The study author, however used the ICp method, while the study reviewer used Nuthatch, which explains the difference in EC_{50} values obtained by the two statistical analyses. Statistical outputs are presented in Appendix I.

The above statistical analyses were performed in terms of the mean-measured sediment and estimated pore water treatment concentrations. Sediment endpoints are also reported on an OC-normalized basis, based on the following equation:

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

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 a negligible fraction of total radioactive residues measured over the course of this study (0% at the 10-d measurement). In contrast, the recovery of parent compound from bulk

sediment was 100% for the initial and terminal measurements. 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 (5.5%) 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.

Results Synopsis (reviewer-calculated):

Treatment comparison to negative control

Based on ESTIMATED[1] Pore Water Concentrations

Survival

LC_{50}: 0.021 µg a.i./L 95% C.I.: 0.009- 0.060 µg a.i./L
Probit Slope: NA
NOAEC: <0.003 µg a.i./L
LOAEC: 0.003 µg a.i./L

Dry Weight

EC_{50}: 0.026 µg a.i./L 95% C.I.: 0.023-0.030 µg a.i./L
Slope: 5.49
NOAEC: 0.011 µg a.i./L
LOAEC: 0.022 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*)]

Based on Bulk Sediment Concentrations (mean-measured)

Survival

LC_{50}: 164 µg a.i./kg dry sediment 95% C.I.: 72-469 µg a.i./kg dry sediment
Probit Slope: 1.96
NOAEC: <22 µg a.i./kg dry sediment
LOAEC: 22 µg a.i./kg dry sediment

Dry Weight

EC_{50}: 200 µg a.i./kg dry sediment 95% C.I.: 180-230 µg a.i./kg dry sediment
Slope: 5.49
NOAEC: 81 µg a.i./kg dry sediment
LOAEC: 170 µg a.i./kg dry sediment

Based on OC-normalized Sediment Concentrations (mean-measured)

Survival

LC_{50}: 2980 µg a.i./kg TOC 95% C.I.: 1310-8530 µg a.i./kg TOC
Probit Slope: 1.96
NOAEC: <400 µg a.i./kg TOC
LOAEC: 400 µg a.i./kg TOC

Dry Weight

EC_{50}: 3640 µg a.i./kg TOC 95% C.I.: 3270-4180 µg a.i./kg TOC
Slope: 5.49
NOAEC: 1470 µg a.i./kg TOC
LOAEC: 3090 µg a.i./kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's conclusions differ from those of the study author for both survival and ash-free dry weight. The reviewer-determined LC_{50} (with 95% C.I.) based on the sediment concentrations, 164 (72-496) µg a.i./kg dry sediment, was lower than that of the study author, 290 (240-330) µg a.i./kg dry sediment. The reviewer-determined EC_{50} (with 95% C.I.) based on the sediment concentrations, 200 (180-230) µg a.i./kg dry sediment, was also lower than that of the study author, 220 (200-250) µg a.i./kg dry sediment. This is presumably due to a difference in statistical methods as the study author used the ICp method while the study reviewer used Nuthatch. All toxicity values reported in the Conclusions section of this DER are reviewer-determined. The reviewer-determined NOAEC for survival is <22 µg a.i./kg dry sediment, also lower than the NOAEC reported by the study author, 81 µg a.i./kg dry sediment. The reviewer-determined NOAEC for dry weight is the same as that reported in the study. The reviewer used the negative control for statistical comparisons, while the study author compared treatments to the solvent control,

and this can account for majority of the differences in the endpoints. The reviewer also normalized the endpoints for bulk sediment on an organic carbon basis, reported in the Conclusions and Verification of Statistical Results sections of this DER.

There are statistically significant differences between the negative and solvent control for both mortality and dry weight in this study ($0.01 < p < 0.05$). Mortality was 1% (1/80) in the negative control and 8% (6/80) in the solvent control. Mean dry weight was 2.04 mg/midge for the negative control and 1.79 mg/midge for the solvent control. The mortality in the second lowest treatment level (44 µg a.i./kg dry sediment) did not differ statistically from the negative control. The measured dry weights for the negative control and solvent control also differed statistically, but the three lowest treatment levels (22, 44, 88 µg a.i./kg dry sediment) did not significantly differ from the negative control dry weights. Because solvent was evaporated on silica sand prior to mixing with sediment and all treatments contained the same amount of initially-added solvent as the solvent control, the statistically significant reduction in survival in the solvent control appears to be a spurious result and is not likely related to the effect of the solvent. Considering all available information, the biological significance of the statistically higher mortality and lower dry weight seen in the solvent control is questionable and is not considered sufficiently robust as to interfere with the integrity of this test. Therefore, the reviewer relies on comparisons to the negative control for derivation of endpoints, in accordance with EFED guidance (Frankenberry *et al.*, 2008). Statistical comparisons to the solvent control are presented in Appendix II for comparative purposes only.

In addition, the reviewer determined LC_{50}/EC_{50}, NOAEC and LOAEC values for survival and dry weight based on the reviewer-determined estimated pore water concentrations. These toxicity values are reported in the Conclusions and Verification of Statistical Results sections of this DER.

In this 10-day flow-through test, fresh dilution water (not spiked with test material) was slowly added to the each test vessel via an automated intermittent renewal system at a rate of approximately 2 to 2.8 volume additions per day. The aged overlying water from each replicate test vessel was displaced and/or diluted by the incoming fresh dilution water and allowed to leave the test vessels via two overflow slots located at the top edge of the vessel. This renewal system was used to maintain adequate water quality throughout the exposure period. However, the renewal system also allowed any test material that moved from the sediment and pore-water to the overlying water to escape the exposure system. The Day-0 overlying water concentrations were <0.034 (<LOQ), <0.034, <0.034, 0.089, 0.19, and 0.24 µg a.i./L while the Day-10 measured concentrations were <0.034(<LOQ), <0.034, 0.037, 0.037, 0.082, and 0.14 µg a.i./L for the 22, 43, 81, 170, 340, and 650 µg a.i./kg dry sediment treatment concentrations, respectively. The reviewer-determined mean-measured overlying water concentrations were <0.034 (negative and solvent controls), <0.034, <0.034, 0.036,

0.063, 0.14, and 0.19 µg a.i./L, respectively, (average of the Day 0 and Day 10 measured concentrations). The reviewer was unable to determine toxicity values in terms of the overlying water concentrations since the measured test material concentrations on Day 0 for the three lowest treatment levels tested were less than the LOQ (<0.034 µg a.i./L) while the Day 10 measured concentrations were <LOQ in the two lowest treatment levels tested (note further details below). This particular type of test is designed to examine the effects of cypermethrin to sediment dwelling organisms through pore water and sediment exposure, and the overlying water treatment concentrations are not the focus of this study.

Dissolved oxygen (DO) concentrations ranged 3.0-8.0 mg/L throughout the exposure period, based on daily measurements. Only the overall range of DO concentrations was reported. On test Day 5, to prevent DO levels from decreasing below 2.5 mg/L, the rate of the flow-through system was increased from 7 to 10 cycles per day (7 cycles=2 volume replacements, 10 cycles=2.8 volume replacements). The actual percent DO saturation relative to the test temperature was not reported and was estimated by the reviewer. According to OPPTS Guideline 850.1735, the DO should be between 40% and 100% saturation. During this test, 40% saturation would be approximately 3.3 – 3.4 mg/L assuming 100% oxygen saturation at 23-25°C is 8.2-8.6 mg/L at standard pressure, according to the US Geological Survey Techniques for Water Resources Investigations Book 9 (Radtke *et al*, 1998). Although OPPTS Guideline 850.1735 (1996 Public Draft), indicate that the DO in overlying water should be between 40% and 100% saturation (which is 3.4 mg/L and above at 23.5 C; Radtke *et al*, 1998), more recent sediment toxicity testing protocols (USEPA 2000) and expected revision to the 1996 draft 850.1735 recommend that DO be maintained > 2.5 mg/L based on measured DO tolerances of *C. tentans*. Furthermore, USEPA (2000) indicates that 10-d exposures to concentrations as low as 1.5 mg/L did not adversely affect *C. tentans* survival or development. Although the potential interaction of these low DO events and toxicant exposure on midge growth cannot be discounted with absolute certainty, it appears as if the low oxygen levels did not impact the test organisms in the controls given the tolerance of *C. tentans* to DO as low as 2.5 mg/L.

For the definitive test, six individual dosing stock solutions were prepared in acetone for application to the test material to the sediment. These stock solutions were prepared using radiolabeled test material according to the following preparation scheme:

Conc. of Radiolabeled Stock Used (µg/mL)	**Volume of Radiolabeled Stock Used (mL)**	**Diluted to Final Volume with Acetone (mL)**	**Dosing Stock Concentration (mg/mL)**	**Percent Radiolabeled (%)**
229	10.63	25	97.4	100

97.4	5.02	10	48.7	100
97.4	2.58	10	25.0	100
97.4	1.26	10	12.2	100
97.4	0.631	10	6.12	100
97.4	0.315	10	3.06	100

All dosing stocks were clear and colorless with no visible undissolved test material.

An appropriate amount (9 mL) of each individual dosing stock solution (above) was added to 0.0500 kg of course silica sand and placed in glass petri dishes. The solvent was allowed to evaporate for 30 minutes. The dry sand, containing the test material, was then added to the 2.0000 kg of wet sediment (1.2027 kg dry weight based on a percent of solids of 60.11%) in individual 1-gallon jars. The total mass of sediment spiked on a dry weight basis for each treatment level and control was 1.2522 kg (0.0500 kg sand and 1.2022 kg dry weight sediment). The jars were sealed and rolled horizontally on a rolling mill for 4 hours at room temperature at approx. 15 rpm. Following the 4 hours of rolling, the jars were stored upright at 4°C overnight. The treated sediments were then allowed to equilibrate for 31 days in the refrigerator prior to allocation into the replicate test vessels. During the equilibration period the treated sediments were rolled on the mill for an additional 2 hours once per week.

On Day 0 and Day 10 sediment samples from the nominal 700 µg a.i./kg dry weight were analyzed by HLPLC/RAM to determine the percent of [^{14}C]residue associated with the parent test material (measured concentrations, µg/kg as cypermethrin equivalents). Recoveries were 100% from the sediment samples on Day 0 and Day 10. On Day 0, insufficient radioactivity was observed at the cypermethrin retention time to determine the radioactive distribution (i.e., percent associated with cypermethrin) in a pore water sample collected form the nominal 180 µg a.i./kg dry weight treatment level and analyzed by HLPLC/RAM. **On Day 10, none (0%) of the radioactivity was associated with cypermethrin in a pore water sample collected from the nominal 350 µg a.i./kg dry sediment treatment level and analyzed by HPLC/RAM. All of the extracted radioactivity from this sample was observed in one peak.**

The study author noted that prior to the initiation of the definitive test, a preliminary 10-day exposure was conducted to determine the relative toxicity of non-radiolabeled cypermethrin to midge larvae (9 days old). The nominal treatment levels tested were 0.0 (negative and solvent controls), and 0.070, 0.70, 7.0, 70, and 700 µg a.i./kg sediment (ug a.i/kg sediment) and were prepared in the same manner as described for the definitive test. Three replicates

per treatment and control group with 10 midge larvae per replicate were tested. By Day 10, 97 and 90, 97, 97, 90, and 0% survival was observed in the controls, and nominal 0.070, 0.70, 7.0, 70, and 700 µg a.i./kg sediment treatment groups, respectively. Ash-free dry weight among surviving midge larvae averaged 1.34 and 1.29, and 1.31, 1.31, 1.18, 1.17, and 1.04 mg per midge larvae in the negative and solvent controls, and nominal 0.070, 0.70, 7.0, 70, and 700 µg a.i./kg sediment treatment groups, respectively. The definitive nominal sediment test concentrations of 22, 44, 88, 180, 350, and 700 µg a.i./kg sediment were selected based on the preliminary results.

The study author mentions two protocol deviations, and neither of these deviations impact the results of the study:

1. *The study protocol states that the water temperature of the test solutions will be maintained at 23±1°C. During this study, on test day 10, the minimum/maximum reading ranged from 24 to 25 °C.* The OPPTS 850.1735 guidelines state that mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.
2. *The study protocol states that the data will be tested for normality using Shapiro-Wilk's test. During this test, due to the number of data points being greater than 50, Chi-Square test was used.* The reviewer agrees with the study author's determinations of normality for the mortality and growth data.

This study was conducted in compliance with all pertinent US EPA GLP regulations. Signed quality assurance, GLP and no data confidentiality statements were provided.

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

Comparisons to negative control

```
Title:  mortality cypermethrin 10-day sediment tox C. tentans
  File:      46591504.TXT            Transform:            NO TRANSFORMATION

                        Chi-Square Test for Normality
  ---------------------------------------------------------------------------
                       Actual and Expected Frequencies

  INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
               -----     -------------     -----------    -----------    -----
  EXPECTED    3.7520       13.5520           21.3920        13.5520     3.7520
  OBSERVED     4            13                23             13          3
  ---------------------------------------------------------------------------

       Chi-Square =   0.3330             (p-value = 0.9876)

       Critical Chi-Square   = 13.277  (alpha = 0.01 , df = 4)
                             =  9.488  (alpha = 0.05 , df = 4)
  ---------------------------------------------------------------------------

  Data PASS normality test (alpha = 0.01). Continue analysis.


  Title:  mortality cypermethrin 10-day sediment tox C. tentans
  File:      46591504.TXT            Transform:            NO TRANSFORMATION

                   Shapiro - Wilk's Test for Normality
  ---------------------------------------------------------------------------

             ******** Shapiro - Wilk's Test is aborted ********

      This test can not be performed because total number of replicates
      is greater than 50.

      Total number of replicates =   56

  ---------------------------------------------------------------------------
  Title:  mortality cypermethrin 10-day sediment tox C. tentans
  File:      46591504.TXT            Transform:            NO TRANSFORMATION

                 Hartley's Test for Homogeneity of Variance
  ---------------------------------------------------------------------------
```

```
Calculated H statistic (max Var/min Var) = 21.5714

                        Table H statistic =   20.00  (alpha = 0.01)
                                              11.80  (alpha = 0.05)
             Used  df =   7
             Based on R (# groups) =   7
-----------------------------------------------------------------------------

Data FAIL homogeneity test (alpha = 0.01). Try another transformation.


Title:  mortality cypermethrin 10-day sediment tox C. tentans
File:      46591504.TXT            Transform:            NO TRANSFORMATION

               Bartlett's Test for Homogeneity of Variance
-----------------------------------------------------------------------------

Calculated B1 statistic = 24.6657              (p-value = 0.0004)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.
-----------------------------------------------------------------------------

Critical B = 16.8119  (alpha = 0.01, df =  6)
           = 12.5916  (alpha = 0.05, df =  6)

Title:  mortality cypermethrin 10-day sediment tox C. tentans
File:      46591504.TXT            Transform:            NO TRANSFORMATION

           Summary Statistics on Data             TABLE 1 of 2
-----------------------------------------------------------------------------

        GRP  IDENTIFICATION    N      MIN        MAX        MEAN
        --- ---------------- ---- ---------- ---------- ----------
         1  negative contro    8     9.0000    10.0000     9.8750
         2           22 ppb    8     7.0000    10.0000     8.5000
         3           44 ppb    8     7.0000    10.0000     8.6250
         4           88 ppb    8     7.0000     9.0000     8.3750
         5          180 ppb    8     4.0000     9.0000     6.1250
         6          350 ppb    8     0.0000     4.0000     2.6250
         7          700 ppb    8     0.0000     1.0000     0.1250
-----------------------------------------------------------------------------


Title:  mortality cypermethrin 10-day sediment tox C. tentans
File:      46591504.TXT            Transform:            NO TRANSFORMATION

           Summary Statistics on Data             TABLE 2 of 2
-----------------------------------------------------------------------------
   GRP  IDENTIFICATION      VARIANCE       SD         SEM         C.V. %
   --- ---------------- -------------- ---------- ----------  ----------
    1  negative contro        0.1250     0.3536     0.1250      3.5803
    2           22 ppb        0.8571     0.9258     0.3273     10.8920
    3           44 ppb        1.4107     1.1877     0.4199     13.7708
    4           88 ppb        0.5536     0.7440     0.2631      8.8839
    5          180 ppb        2.6964     1.6421     0.5806     26.8095
    6          350 ppb        2.2679     1.5059     0.5324     57.3692
```

7	700 ppb	0.1250	0.3536	0.1250	282.8427

Title: mortality cypermethrin 10-day sediment tox C. tentans
File: 46591504.TXT Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	negative contro	9.8750				
2	22 ppb	8.5000	42.00	46.00	8.00	*
3	44 ppb	8.6250	46.50	46.00	8.00	
4	88 ppb	8.3750	38.00	46.00	8.00	*
5	180 ppb	6.1250	36.50	46.00	8.00	*
6	350 ppb	2.6250	36.00	46.00	8.00	*
7	700 ppb	0.1250	36.00	46.00	8.00	*

Critical values are 1 tailed (k = 6)

Title: mortality cypermethrin 10-day sediment tox C. tentans
File: 46591504.TXT Transform: NO TRANSFORMATION

Kruskal - Wallis' ANOVA by Ranks - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	negative contro	9.8750	9.8750	401.0000
2	22 ppb	8.5000	8.5000	289.0000
3	44 ppb	8.6250	8.6250	304.0000
4	88 ppb	8.3750	8.3750	278.0000
5	180 ppb	6.1250	6.1250	186.5000
6	350 ppb	2.6250	2.6250	96.5000
7	700 ppb	0.1250	0.1250	41.0000

H Value = 46.0878 Critical H = 12.5916 (alpha = 0.05 , df = 6)
(p-value 0.0000)
Since Calc H > Crit H REJECT Ho:All groups are equal at alpha = 0.05

Title: mortality cypermethrin 10-day sediment tox C. tentans
File: 46591504.TXT Transform: NO TRANSFORMATION

Dunn's Multiple Comparison - Kruskal - Wallis - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 7	0 6	0 5	0 4	0 2	0 3	0 1
7	700 ppb	0.1250	0.1250	\						
6	350 ppb	2.6250	2.6250	.	\					
5	180 ppb	6.1250	6.1250	.	.	\				

```
  4            88 ppb      8.3750      8.3750  * . . \
  2            22 ppb      8.5000      8.5000  * . . . \
  3            44 ppb      8.6250      8.6250  * * . . . \
  1   negative contro      9.8750      9.8750  * * * . . . \
-----------------------------------------------------------------------

* = significant difference (alpha = 0.05)      . = no significant difference
Table q value = 3.0380 (0.05, 7)          SE =   8.0642
```

Percent survival (ppb a.i.); Estimates of LC%:

Bulk Sediment:

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Solliday cypermethrin 10day sediment chironomus
****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
650	79	78	98.7342	0
340	79	58	73.4177	0
170	79	30	37.9747	0
81	79	12	15.1899	0
43	79	10	12.6582	0
22	79	11	13.9241	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 214.1013

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	1.940274E-02	166.9954	143.98	196.077

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.4537505	9.333191	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.957117
95 PERCENT CONFIDENCE LIMITS = .6387835 AND 3.275451

INTERCEPT=-4.334408

LC50 = 163.9419
95 PERCENT CONFIDENCE LIMITS = 72.25875 AND 469.1022

LC25 = 74.13963
95 PERCENT CONFIDENCE LIMITS = 12.67939 AND 146.292

```
     LC10 =      36.29521
95 PERCENT CONFIDENCE LIMITS = 1.705532 AND 79.56277

     LC05 =      23.67114
95 PERCENT CONFIDENCE LIMITS = .4844497 AND 58.56903
*********************************************************************
```

Dry Weight (nominal conc., ppb a.i.); NOAEC and LOAEC:

```
Title:  dry weight cypermethrin 10-day sediment tox C. tentans
  File:      DW4659~1.TXT            Transform:            NO TRANSFORMATION

                        Chi-Square Test for Normality
  ---------------------------------------------------------------------------
                       Actual and Expected Frequencies

  INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
               -----     -------------     -----------    -----------    -----
  EXPECTED    3.1490       11.3740           17.9540        11.3740     3.1490
  OBSERVED     4            12                16             13          2
  ---------------------------------------------------------------------------

       Chi-Square =   1.1288            (p-value = 0.8897)

       Critical Chi-Square   = 13.277  (alpha = 0.01 , df = 4)
                             =  9.488  (alpha = 0.05 , df = 4)
  ---------------------------------------------------------------------------

  Data PASS normality test (alpha = 0.01). Continue analysis.

  Title:  dry weight cypermethrin 10-day sediment tox C. tentans
  File:      DW4659~1.TXT            Transform:            NO TRANSFORMATION

                   Shapiro - Wilk's Test for Normality
  ---------------------------------------------------------------------------
       D =   2.4239
       W =   0.9747

       Critical W  = 0.9280 (alpha = 0.01 , N = 47)
                W  = 0.9460 (alpha = 0.05 , N = 47)

  ---------------------------------------------------------------------------

  Data PASS normality test (alpha = 0.01). Continue analysis.


  Title:  dry weight cypermethrin 10-day sediment tox C. tentans
  File:      DW4659~1.TXT            Transform:            NO TRANSFORMATION

                 Hartley's Test for Homogeneity of Variance
  ---------------------------------------------------------------------------

  Calculated H statistic (max Var/min Var) = 10.0132

                        Table H statistic =   18.40  (alpha = 0.01)
```

```
                                                  10.80  (alpha = 0.05)

              Used Closest df =   7
                 (Actual value: df (avg # reps - 1) =   6.83)
              Based on R (# groups) =   6

 ---------------------------------------------------------------------------

 Data PASS homogeneity test (alpha = 0.01). Continue analysis.

 ---------------------------------------------------------------------------
 NOTE: This test requires equal replicate sizes. If they are unequal
       but do not differ greatly, Hartley's test may still be used
       as an approximate test. Average df should be used as above.


 Title:  dry weight cypermethrin 10-day sediment tox C. tentans
 File:      DW4659~1.TXT            Transform:            NO TRANSFORMATION

                Bartlett's Test for Homogeneity of Variance
 ---------------------------------------------------------------------------

 Calculated B1 statistic = 14.7058              (p-value = 0.0117)

 Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

 ---------------------------------------------------------------------------

 Critical B = 15.0863  (alpha = 0.01, df =  5)
            = 11.0705  (alpha = 0.05, df =  5)

 ---------------------------------------------------------------------------

                     Using Average Degrees of Freedom
                (Based on average replicate size of    7.83)

 Calculated B2 statistic = 12.5397              (p-value = 0.0281)

 Data PASS B2 homogeneity test at 0.01 level. Continue analysis.

Title:  dry weight cypermethrin 10-day sediment tox C. tentans
 File:      DW4659~1.TXT            Transform:            NO TRANSFORMATION

            Summary Statistics on Data              TABLE 1 of 2
 ---------------------------------------------------------------------------
         GRP  IDENTIFICATION    N      MIN        MAX        MEAN
         --- ---------------- ---- ---------- ---------- ----------
          1  negative contro    8     1.7400     2.2500     2.0400
          2           22 ppb    8     1.8900     2.2000     2.0050
          3           44 ppb    8     1.3200     2.4100     1.9088
          4           88 ppb    8     1.7900     2.1300     1.9813
          5          180 ppb    8     0.8100     1.6100     1.3138
          6          350 ppb    7     0.0030     0.6900     0.2154
 ---------------------------------------------------------------------------
```

Title: dry weight cypermethrin 10-day sediment tox C. tentans
File: DW4659~1.TXT Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	negative contro	0.0234	0.1529	0.0541	7.4940
2	22 ppb	0.0161	0.1271	0.0449	6.3369
3	44 ppb	0.1317	0.3629	0.1283	19.0146
4	88 ppb	0.0132	0.1147	0.0406	5.7891
5	180 ppb	0.0970	0.3114	0.1101	23.7066
6	350 ppb	0.0757	0.2751	0.1040	127.7113

Title: dry weight cypermethrin 10-day sediment tox C. tentans
File: DW4659~1.TXT Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	18.8593	3.7719	63.7998
Within (Error)	41	2.4239	0.0591	
Total	46	21.2832		

(p-value = 0.0000)

Critical F = 3.5007 (alpha = 0.01, df = 5,41)
= 2.4434 (alpha = 0.05, df = 5,41)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: dry weight cypermethrin 10-day sediment tox C. tentans
File: DW4659~1.TXT 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 0.05
1	negative contro	2.0400	2.0400		
2	22 ppb	2.0050	2.0050	0.2879	
3	44 ppb	1.9088	1.9088	1.0796	
4	88 ppb	1.9813	1.9813	0.4832	
5	180 ppb	1.3138	1.3138	5.9738	*
6	350 ppb	0.2154	0.2154	14.4991	*

Bonferroni t critical value = 2.4208 (1 Tailed, alpha = 0.05, df = 5,41)

Title: dry weight cypermethrin 10-day sediment tox C. tentans
File: DW4659~1.TXT Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	negative contro	8			
2	22 ppb	8	0.2943	14.4	0.0350
3	44 ppb	8	0.2943	14.4	0.1312
4	88 ppb	8	0.2943	14.4	0.0587
5	180 ppb	8	0.2943	14.4	0.7262
6	350 ppb	7	0.3046	14.9	1.8246

Dry Weight (ppb a.i.); Estimates of EC%:

Bulk Sediment:
In c:\NUTHATCH\DWNEGSED.TXT : `control`
Interpreted as Dose = 0

DWNEGSED.TXT : dry weight cypermethrin 10-day sediment tox C. tentans

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	2.04	.		
22	2	0.2879	N.S.	
43	1.95	0.7814	N.S.	
81	1.95	0.7814	N.S.	
170	1.31	5.974	<0.005	*
340	0.215	14.5	<0.005	*

"*"=Significant; "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err. /Estimate	Lower Bound
EC5	1.0E+02	79.	1.3E+02	0.055	0.77
EC10	1.2E+02	95.	1.5E+02	0.048	0.80
EC25	1.5E+02	1.3E+02	1.8E+02	0.035	0.85
EC50	2.0E+02	1.8E+02	2.3E+02	0.024	0.89

Slope = 5.49 Std.Err. = 0.686

Goodness of fit: p = 0.92 based on DF= 3.0 41.

DWNEGSED.TXT : dry weight cypermethrin 10-day sediment tox C. tentans

---

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	8.00	2.04	1.99	0.0495	100.	0.00
22.0	8.00	2.00	1.99	0.0145	100.	6.02e-06
43.0	8.00	1.91	1.99	-0.0815	100.	0.0110
81.0	8.00	1.98	1.96	0.0196	98.6	1.45
170.	8.00	1.31	1.32	-0.00250	66.1	33.9
340.	7.00	0.215	0.215	0.000459	10.8	89.2