DATA EVALUATION RECORD
MIDGE 10-DAY TOXICITY STUDY
Non Guideline

1. **CHEMICAL:** Cyfluthrin PC Code No.: 128831

2. **TEST MATERIAL:** [Phenoxy-UL-^{14}C]cyfluthrin Purity: 99% (radiochemical purity)

3. **CITATION:**

Author: Arthur E. Putt

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

Study Completion Date: June 29, 2005

Laboratory: Springborn Smithers Laboratories
790 Main Street
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Sponsor: Pyrethroid Working Group
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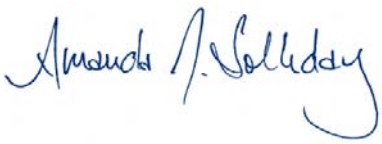
Laboratory Report ID: 13656.6115

MRID No.: 465915-07

DP Barcode: D319265

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

Signature:  **Date:** 02/24/11

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

Signature:  **Date:** 02/24/11

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

Signature:  **Date:** 02-24-11

5. STUDY PARAMETERS:

Age of Test Organism:	2^{nd}-3^{rd} Instar, 10 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]cyfluthrin 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), 31, 63, 125, 250, 500, and 1000 ppb (µg/kg dry sediment). The majority of radioactive residues remained predominately associated with the sediment during the 10-day study. Mean-measured sediment concentrations (reviewer-calculated from Days 0 and 10) were <0.15 (<LOQ; controls), and 29, 63, 120, 240, 460, and 870 µg a.i./kg d.w., equivalent to mean recoveries of 93, 100, 96, 94, 92, and 87% of the nominal concentrations, respectively. Mean-measured (reviewer-calculated from Days 0 and 10) pore water concentrations were <0.039 (<LOQ; controls), and 0.043, 0.059, 0.20, 0.42, 0.55, and 0.85 µg a.i./L, respectively, based on total radioactive residues. Mean-measured (reviewer-calculated from Days 0 and 10) overlying water concentrations were <0.016 (<LOQ; controls), and <0.016, <0.016 <0.021, <0.026, 0.037, and 0.17 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.

After 10 days, mortality averaged 9 and 10% in the negative and solvent control groups, respectively, and 14, 18, 23, 50, 74, and 94% in the mean-measured sediment concentrations of 29, 63, 120, 240, 460, and 870 µg a.i./kg d.w., respectively. Statistically-significant reductions ($p \leq 0.05$) in treatment survival on Day 10 compared to the negative control (91% survival) were identified at the ≥120 µg a.i./kg d.w. levels (the four highest levels tested). The Day-10 NOAEC, LOAEC, and LC_{50} (with 95% C.I.) for survival was 63, 120, and 290 (240-350) µg a.i./kg d.w., respectively, based on the mean-measured sediment treatment concentrations. Mean dry weight per midge was 1.61 and 1.79, and 1.70, 1.63, 1.64, 1.54, 1.19, and 0.69 mg for the negative and solvent controls, and the mean-measured 29, 63, 120, 240, 460, and 870 µg a.i./kg d.w. concentrations, respectively. Reviewer-determined percent inhibition in dry weight compared to the negative control (1.61 mg) was -6, -1, -2, 4, 26, and 57 % for the mean-measured 29, 63, 120, 240, 460, and 870 µg a.i./kg d.w. concentrations, respectively, and was significantly reduced ($p \leq 0.05$) compared to the negative control at the mean-measured 460 and 870 µg a.i./kg d.w.

treatment levels, respectively. The Day-10 NOAEC, LOAEC and EC_{50} (with 95% C.I.) for dry weight was 240, 460, and 740 (670-830) µg a.i./kg d.w., respectively, based on the mean-measured sediment treatment concentrations. No sub-lethal effects or abnormal behavior was reported for surviving midges in the controls or treatment groups during the exposure period.

This reviewer notes that HPLC/RAM analysis of cyfluthrin concentrations in pore water (conducted only at the highest test concentration) indicate that the parent material represented about half (50.6%) of total radioactive residues measured at test termination. In contrast, the recovery of parent compound from bulk sediment was generally high >96% for 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 cyfluthrin 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} (124,000 L/kg-OC; MRID 00131495, 00137544, 45022103) for cyfluthrin (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 cyfluthrin vary considerably (74,500 to 180,300) which 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 cyfluthrin.

This study was designed to fulfill proposed OPPTS Draft Guideline 850.1735, and does not fulfill any current U.S. EPA FIFRA guideline. This study is classified as ACCEPTABLE, and provides information on the 10-day toxicity of cyfluthrin to sediment-dwelling midges (*Chironomus tentans*).

Results Synopsis:

Based on ESTIMATED[1] Pore Water Concentrations	
Survival	
NOAEC: 0.009 µg a.i./L	
LOAEC: 0.018 µg a.i./L	
LC_{50} 0.039 µg a.i./L	95% C.I.: 0.034-0.045 µg a.i./L

Probit Slope: NA

Growth (Ash-Free Dry Weight)
NOAEC: 0.035 µg a.i./L
LOAEC: 0.067 µg a.i./L
EC_{50}: 0.109 µg a.i./L 95% C.I.: 0.098-0.122 µg a.i./L
Slope: Not reported

Based on Mean-Measured Spiked Sediment Concentrations

Survival
NOAEC: 63 µg a.i./kg d.w.
LOAEC: 120 µg a.i./kg d.w.
LC_{50}: 265 µg a.i./kg d.w. 95% C.I.: 229 - 308 µg a.i./kg d.w.
Probit Slope: NA

Growth (Ash-Free Dry Weight)
NOAEC: 240 µg a.i./kg d.w.
LOAEC: 460 µg a.i./kg d.w.
EC_{50}: 740 µg a.i./kg d.w. 95% C.I.: 670-830 µg a.i./kg d.w.
Slope: Not reported

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

Survival
LC_{50}: 4,818 µg a.i./kg TOC 95% C.I.: 4,164-5,600 µg a.i./kg TOC
Probit Slope: 2.92 ± 0.350
NOAEC: 1,150 µg a.i./kg TOC
LOAEC: 2,180 µg a.i./kg TOC

Growth (Ash-Free Dry Weight)
EC_{50}: 13,500 µg a.i./kg TOC
95% C.I.: 12,200-15,100 µg a.i./kg TOC
Slope: Not reported
NOAEC: 4,360 µg a.i./kg TOC
LOAEC: 8,360 µg a.i./kg TOC

[1] Freely dissolved pore water endpoints (ug/L) estimated as: Mean measured bulk sediment conc. (*ug/kg-d.w.*) / [Fraction TOC (*kg OC/kg-d.w.*) * K_{OC} (*L/kg-OC*)]

Endpoints affected: Survival and Ash-Free 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. Natural sediments were not analyzed for total volatile sulfides, BOD, COD, Eh, total inorganic carbon, total volatile solids, acid volatile sulfides; these analyses are suggested in the guidance documents.

2. Physical descriptions and water solubilities of the test substances (radio-labeled and unlabeled) were not reported.

9. SUBMISSION PURPOSE: This study was submitted to provide information on the toxicity of cyfluthrin to sediment-dwelling chironomids (larvae) for the purpose of pesticide registration (new use).

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Chironomus tentans *Other species which can be used are Hyalella azteca, Chironomus riparius, Daphnia sp., Ceriodaphnia sp.* (Specific criteria for these species are not listed in this report)	*Chironomus tentans*
Life Stage Second to third instar larvae (about 10 d old larvae with at least 50% at third instar.	2^{nd}-3^{rd} instar, 10 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 0.32 to 0.48 mm. Ash-free dry weight was confirmed at test initiation (sub-population of 20 midge larvae) to be 0.34 mg dry weight per midge larvae.
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.)	In-house laboratory cultures.
All organisms from the same source?	Yes.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Brood stock must be acclimated to culture water gradually from transport water to 100% culture water; water temperature exchange rate not to exceed 2°C within 24 hr; Avoid unnecessary stress, crowding and rapid temperature and water quality changes.	Reared under test conditions for 10 days prior to test initiation.
Feeding Feeding should begin on day 0 and continue through day 9 unless food is not being consumed.	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 (eg <20% mortality 48 h before the beginning of a test).	No mortalities 48 hours prior to test initiation.

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 50-56 and 34-35 mg/L, respectively, a specific conductivity range of 180 to 200 µmhos/cm, and a pH range of 7.5-7.7. 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 3.9 mg/L as

Guideline Criteria	Reported Information
	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 0.48-1.0 mg/L based on measurements from the overlying water as a composite sample from each treatment and control group. Dissolved organic carbon concentrations were 11-27 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 ±. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	Test water temperature was 22-24°C all test days.
pH Not specified, but should be appropriate to the test species and should not deviate more than 0.4 pH units.	6.7-7.1 (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 4.0-7.6 mg/L. The actual percent DO saturation relative to the test temperature was not reported, but is calculated by this reviewer to be > 46% saturation.
Total Hardness Prefer 40 - 200 mg/L as $CaCO_3$.	44-56 mg/L as $CaCO_3$ Total alkalinity was 26-36 mg/L as $CaCO_3$.
Conductivity Not specified, but should be amenable to the test species.	230-280 µmhos/cm

Guideline Criteria	Reported Information
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.
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: Cyfluthrin tech. Synonyms: Baythroid tech. CAS no.: 68359-37-5 Batch no.: SA07163S29 Purity: 93.3% 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: [Phenoxy-UL-^{14}C]cyfluthrin Synonyms: None reported Batch no.: 2003BRP-176-179 (Ref. no.) Specific activity: 121.9 mCi/mmole (equiv. to 623,113 dpm/µg)

Guideline Criteria	Reported Information
	Amount received: 1552.5 µCi in hexane (57.4 MBq) Radiochemical Purity: 99% Physical description: Not reported Water solubility: Not reported Storage condition: Room temp. This test material was used to spike the sediments used for the definitive test.
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]cyfluthrin stock was prepared by removing the benzene 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. Based on this analysis and the specific activity of 121.9 mCi/mmole provided by the supplier, the stock was determined to have a concentration of 123 mg/L. The stock was stored frozen until use. The mean radiopurity of this stock solution was 98.4% 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.

Guideline Criteria	Reported Information
Test Concentrations For Spiked Sediment For LC_{50} calculation, test concentrations should bracket the predicted LC50; Sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	Nominal sediment treatment concentrations selected for the test were 31, 63, 125, 250, 500, and 1000 µg a.i./kg dry sediment (ppb).
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) 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 with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	Flow-through: Test chambers had two mesh-covered slots on the top edge of the vessel to allow for drainage from the vessels during the cycling.
Type of Dilution System Must provide reproducible supply of toxi-cant.	N/A. Sediment was spiked with test material; overlying was not spiked.
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. The water delivery system cycled approx. seven (refer to Reviewer's Comments section) times per day (50 mL of water per cycle), providing two volume additions (i.e. 350 mL) per vessel per day. The renewal rate was visually checked at least two times per day.

Guideline Criteria	Reported Information
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 600-900 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.	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.

Guideline Criteria	Reported Information
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 approximately two volume additions 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. **Range Finding Test**	On Day 0, ten midge larvae were impartially and gently added to each of eight replicate test vessels/level. See Reviewer's Comments section for details and Results.
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), 31, 63, 125, 250, 500, and 1000 µg/kg dry weight (ppb); nominal sediment treatment levels were determined based on the results of a range-finding study. Aqueous solubility of the test material was not reported. According to Laskowski (2002) the solubility is low, 2.3 ppb at 20^{o}C.
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

Guideline Criteria	Reported Information
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 finely-ground flake food once daily during the definitive test.
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 10-day mixing and equilibration period. During the definitive exposure period sediment, pore water, and overlying water samples were removed from replicates I and J and analyzed by liquid scintillation counting (LSC) for total [^{14}C]residue concentration on test Days 0 and 10, respectively. Overlying

Guideline Criteria	Reported Information
	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 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 Must be ≤ 30% in the sediment at end of the test.	Negative control: 9% Solvent control: 10%
Percent Recovery of Chemical: 1) % of nominal;	1) All recoveries are based on the reported mean-measured treatment concentrations and were determined by LSC analysis. In overlying water: N/A; see Reviewer Comments section In pore water: N/A; see Reviewer Comments section In sediment: 87-100% of nominal sediment concentrations.
2) Procedural recovery;	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 2.02-1010 ppb, recoveries were 84.7-

Guideline Criteria	Reported Information
3) Limit of quantitation (LOQ)	103% of nominal, with a single outlier of 17.3% on Day 0. In freshwater matrix spikes at 0.204-20.4 ppb, recoveries were 91.4-105% of nominal. 3) LOQ = 0.016 ppb a.i. for overlying water samples; 0.039 ppb a.i. for pore-water samples; and 0.15 ppb a.i. (dry weight) for sediment samples.
Data Endpoints - Survival of Larvae - Ash-free dry weight (AFDW) should be determined by pooling all living organisms from a replicate and drying to a constant weight (e.g. 60°C for 24 h) **Raw data included?**	- Survival of larvae - Ash-free dry weight. Yes, mean replicate data provided.

Effects Data (Reviewer-determined)

Toxicant Concentration					
	Mean-Measured (Days 0 and 10)				
Nominal Sediment (ppb a.i.)	Sediment (ppb a.i.)[1]	Pore Water (ppb a.i.)[2]	Overlying Water (ppb a.i.)[3]	Cumulative Number Dead (and %)	Mean Ash-Free Dry Weight per Midge, mg ± SD, (and % Inhibition)[4]
Negative control	<0.15	<0.039	<0.016	7/80 (9)	1.61 ± 0.08
Solvent control	<0.15	<0.039	<0.016	8/80 (10)	1.79 ± 0.26
31	29	0.066[5]	<0.016	11/80 (14)	1.70 ± 0.18 (-6)
63	63	0.098[5]	<0.016	11/60[7] (18)	1.63 ± 0.36[7,8] (-1)
125	120	0.20	<0.021	18/80 (23)*	1.64 ± 0.44 (-2)
250	240	0.42	<0.026	40/80 (50)*	1.54 ± 0.35 (4)
500	460	0.55	0.037[6]	59/80 (74)*	1.19 ± 0.13 (26)*
1000	870	0.86	0.17[6]	75/80 (94)*	0.69 ± 0.14 (57)*

[1] The LOQ for sediment samples was 0.15 ppb a.i.

[2] The LOQ for pore water samples was 0.039 ppb a.i., Note: Measured concentrations in pore water are reported in this DER but were not used to calculate endpoints. See Verification of Statistical Results section for further details.

[3] The LOQ for overlying water samples was 0.016 ppb a.i..

[4] Percent inhibition (reviewer-determined) is relative to the negative control; a negative percent inhibition represents an increase in dry weight relative to the negative control.

[5] Results from the Day 10 analysis were reported by the study author as the mean-measured concentrations for the two lowest levels tested since the Day 0 measured concentrations for these two treatment levels were less than the LOQ. Reviewer calculated mean measured concentrations in porewater are 0.043 and 0.059 ppb for the 29 and 63 ppb sediment dry weight treatments, respectively (See Reviewer's Comments section).

[6] Reviewer-determined as the average of the Day 0 and Day 10 measured concentrations.

[7] Test organisms were inadvertently not added to two of the eight replicate test vessels at test initiation.

[8] Weighing tin for one replicate was inadvertently not recovered from the drying oven for the determination of the actual ash-free dry weight.

N/A = Not applicable

* Statistically significant reduction ($p \leq 0.05$) compared to the negative control using William's Test.

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 pooled control data since a *t*-Test indicated no 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, the study authors used the William's Test to compare survival and growth treatment response data to the pooled 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 using the Inhibition Concentration Method (Norberg-King, 1993) via Toxstat.

Study Author Statistical Results – Midge Survival

LOAEC	120 ug ai/kg sediment
NOAEC	63 ug a.i/kg sediment
LC_{50} (95% CI)	280 ug a.i/kg sediment (230 – 340)

Study Author Statistical Results – Midge Growth

LOAEC	460 ug a.i/kg sediment
NOAEC	240 ug a.i/kg sediment
EC_{50} (95% CI)	740 ug a.i/kg sediment (670 – 830)

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): After confirming normality and homogeneity of variances, NOAEC and LOAEC values based on percent survival and mean ash-free dry weight per larvae (growth) data were determined parametrically using ANOVA and William's multiple comparison Test via Toxstat statistical software. Percent survival and growth treatment response data were statistically compared to the negative control since, according to EFED guidance, a *t*-Test indicated no statistically significant differences between the negative and solvent controls. The 10-day LC_{50} for survival was determined using TOXANAL and the moving average method as the probit method was unsuitable for use due to a poor goodness of fit. The EC50 value for growth was determined using the NUTHATCH statistical software. The above statistical analyses were performed in terms of the mean-measured sediment and reviewer-determined estimated pore water treatment concentrations (see Reviewer's Comments for further details).

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 cyfluthrin concentrations in pore water (conducted only at the highest test concentration) indicate that the parent material represented about half (50.6%) of total radioactive residues measured at test termination. In contrast, the recovery of parent compound from bulk sediment was generally high >96% for 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 cyfluthrin 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} (124,000 L/kg-OC; MRID 00131495, 00137544, 45022103) for cyfluthrin (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 cyfluthrin vary considerably (74,000 to 180,000) which 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 cyfluthrin.

Results Synopsis

Based on ESTIMATED[1] Pore Water Concentrations

Survival
NOAEC: 0.009 µg a.i./L
LOAEC: 0.018 µg a.i./L
LC_{50} 0.039 µg a.i./L 95% C.I.: 0.034-0.045 µg a.i./L
Slope: N/A

Growth (Ash-Free Dry Weight)
NOAEC: 0.035 µg a.i./L
LOAEC: 0.067 µg a.i./L

EC_{50}: 0.109 µg a.i./L 95% C.I.: 0.098-0.122 µg a.i./L
Probit Slope: Not reported
[1] Freely dissolved pore water endpoints (ug/L) estimated as:
Mean measured bulk sediment conc. (*ug/kg-dw*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]

Based on Mean-Measured Spiked Sediment Concentrations

Survival
NOAEC: 63 µg a.i./kg d.w.
LOAEC: 120 µg a.i./kg d.w.
LC_{50}: 265 µg a.i./kg d.w. 95% C.I.: 229 - 308 µg a.i./kg d.w.
Slope: N/A

Growth (Ash-Free Dry Weight)
NOAEC: 240 µg a.i./kg d.w.
LOAEC: 460 µg a.i./kg d.w.
EC_{50}: 740 µg a.i./kg d.w. 95% C.I.: 670-830 µg a.i./kg d.w.
Probit Slope: Not reported

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

Survival
LC_{50}: 4,818 µg a.i./kg TOC 95% C.I.: 4,164-5,600 µg a.i./kg TOC
Slope: N/A
NOAEC: 1,150 µg a.i./kg TOC
LOAEC: 2,180 µg a.i./kg TOC

Growth (Ash-Free Dry Weight)
EC_{50}: 13,500 µg a.i./kg TOC
95% C.I.: 12,200-15,100 µg a.i./kg TOC
Slope: Not reported
NOAEC: 4,360 µg a.i./kg TOC
LOAEC: 8,360 µg a.i./kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study author's based on the mean-measured sediment concentrations with the exception of the LC_{50} values and its associated 95% confidence intervals (C.I.) for survival. The reviewer-determined LC_{50} (with 95% C.I.) based on the sediment concentrations, 265 (229-308) µg a.i./kg d.w., was similar to that determined by the study author, 280 (230-340) µg a.i./kg d.w. Consequently, the reviewer-determined LC_{50} (with 95% C.I.) is reported in the CONCLUSION section of this DER. The study author used the ICp method while the study reviewer used the moving average method as the Probit method was unsuitable for use due to a poor goodness of fit. The EC_{50} was determined by the study author using the ICp method while the study

reviewer used NUTHATCH. Both methods yielded the same EC_{50} for growth. These toxicity values are reported in the CONCLUSION and VERIFICATION OF STATISTICAL RESULTS sections of this DER. Note, the measured pore water concentrations on Day 0 for the nominal 31 and 63 µg a.i./kg d.w. treatment levels were reported to be <0.039 µg a.i./L (<LOQ) and were estimated by the reviewer to be 1/2 the reported LOQ (0.020 µg a.i./L) for calculation of the mean-measured pore water concentrations (see associated Excel e-file for actual calculations). Thus, the reviewer-calculated porewater concentrations associated with the 31 and 63 µg a.i./kg d.w. treatments are 0.043 and 0.059 µg a.i./L, respectively.

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 two 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 an overflow hole located at the top edge of vessel. This renewal system was presumably 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 <0.016 (<LOQ), <0.016, <0.016, <0.016, 0.033, and 0.058 µg a.i./L, while the Day 10 measured concentrations were <0.016 (<LOQ), <0.016, 0.021, 0.026, 0.041, and 0.29 µg a.i./L for the nominal 31, 63, 125, 250, 500, and 1000 µg a.i./kg d.w. spiked sediment treatment concentrations, respectively. The reviewer-determined mean-measured overlying water concentrations were <0.016 (<LOQ), <0.016, <0.021, <0.026, 0.037, and 0.17 µg a.i./L, respectively (average of the Day 0 and Day 10 measured concentrations).

This reviewer notes that the concentration of cyfluthrin measured in porewater likely reflects both "freely dissolved" chemical (i.e., chemical that is not sorbed onto particulate organic carbon (POC) or dissolved organic carbon (DOC) in addition to dissolved chemical that is sorbed to DOC. This finding is indicated by the fact that the extraction and analytical methods used in this study do not distinguish among the two phases of chemical (freely dissolved and DOC-sorbed). It is also indicated by the much higher measured concentrations of cyfluthrin in porewater than would be expected based on estimated values using sediment cyfluthrin concentrations, its Koc, and sediment total organic carbon (TOC). For highly hydrophobic chemicals like cyfluthrin, DOC in porewater can substantially reduce its bioavailability and toxicity. It is further noted that the porewater estimated environmental concentrations (EECs) generated using the Agency's PRZM/EXAMS model are based on freely dissolved chemical. Therefore, some downward adjustment of these porewater toxicity values using appropriate methods (e.g., Koc and DOC concentration in porewater) will likely be needed when comparing these values to freely dissolved EECs generated using PRZM/EXAMS.

For the definitive test (MRID: 465915-07), five additional 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 (%)
105	4.98	10	52.3	100
105	2.49	10	26.1	100
105	1.25	10	13.1	100
105	0.627	10	6.6	100
105	0.309	10	3.24	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 (0.8906 kg dry weight based on a percent of solids of 44.53%) in individual 1-gallon jars. The total mass of sediment spiked on a dry weight basis for each treatment level and control was 0.9406 kg (0.0500 kg sand and 0.8906 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 and pore water samples from the nomianl 1000 µg/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 cyfluthrin equivalents). **Recoveries were 98.5% and 96.6% from the sediment samples on Day 0 and Day 10, respectively. Recoveries were 100% and 50.6% from the pore water samples on Day 0 and Day 10, respectively.**

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 nonradiolabeled cyfluthrin 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 d.w. 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 93, 97, 97, 87,

and 10% survival was observed in the controls, and nominal 0.070, 0.70, 7.0, 70, and 700 µg a.i./kg d.w. treatment groups, respectively. Ash-free dry weight among surviving midge larvae averaged 1.27 and 1.28, and 1.29, 1.27, 1.28, 1.27, and 1.28 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 d.w. treatment groups, respectively. The definitive nominal sediment test concentrations of 31, 63, 125, 250, 500, and 1000 µg a.i./kg d.w. were selected based on the preliminary results.

A minor discrepancy was observed regarding the renewal rate of the overlying water. It was reported that the intermittent delivery system provided 50 mL of water per cycle to each replicate vessel, and that the delivery system cycled approximately 14 times per day. However, this rate would be equivalent to 700 mL of water per vessel, or 4 vessel turnovers per day. It was also reported that the water delivery system cycled such that approximately 350 mL of water was provided per vessel per day, or approximately 2 vessel turnovers per day. This rate would be equivalent to 7 cycles per day, not 14.

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

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2	29	8	0.7000	1.0000	0.8625
3	63	6	0.6000	1.0000	0.8167
4	120	8	0.4000	1.0000	0.7750
5	240	8	0.3000	0.7000	0.5000
6	460	8	0.0000	0.4000	0.2625
7	870	8	0.0000	0.3000	0.0625

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2	29	0.0084	0.0916	0.0324	10.6217
3	63	0.0297	0.1722	0.0703	21.0906
4	120	0.0307	0.1753	0.0620	22.6135
5	240	0.0171	0.1309	0.0463	26.1861
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1	[illegible]	0.9125	0.9125	
2	29	0.8625	0.8625	0.7721
3	63	0.8167	0.8167	1.3701
4	**120**	**0.7750**	**0.7750**	**2.1234**
5	240	0.5000	0.5000	6.3701 *
6	460	0.2625	0.2625	10.0378 *
7	870	0.0625	0.0625	13.1263 *

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3	63	6	0.1736	19.0	0.0958
4	120	8	0.1608	17.6	0.1375
5	240	8	0.1608	17.6	0.4125
6	460	8	0.1608	17.6	0.6500
7	870	8	0.1608	17.6	0.8500

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2	29	8	0.8625	0.8625	0.8625
3	63	6	0.8167	0.8167	0.8167
4	120	8	0.7750	0.7750	0.7750
5	240	8	0.5000	0.5000	0.5000
6	460	8	0.2625	0.2625	0.2625
7	870	8	0.0625	0.0625	0.0625

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870	0.0625	13.1263	*	1.8100	[illegible]= 6, [illegible]=40

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2	0.043	8	0.1523	0.2810	0.2273
3	0.059	5	0.1335	0.3118	0.2040
4	0.20	8	0.0864	0.4065	0.2033
5	0.42	8	0.0569	0.3263	0.1786
6	0.55	7	0.0253	0.1553	0.0720
7	0.85	3	-0.2147	-0.0706	-0.1643

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3	0.059	0.0088	0.0936	0.0419	45.8928
4	0.20	0.0111	0.1053	0.0372	51.8034
5	0.42	0.0094	0.0969	0.0342	54.2326
6	0.55	0.0020	0.0446	0.0169	61.9638
7	0.85	0.0066	0.0812	0.0469	-49.4429

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2	0.043	0.2273	1.6963	-0.5908	
3	0.059	0.2040	1.6300	0.0325	
4	0.20	0.2033	1.6413	0.0540	
5	**0.42**	**0.1786**	**1.5425**	**0.7177**	
6	0.55	0.0720	1.1857	3.4584	*
7	0.85	-0.1643	0.6933	*7.3273*	*

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2	0.043	8	0.3096	19.3	-0.0900	
3	0.059	5	0.3479	21.7	-0.0238	
4	0.20	8	0.3096	19.3	-0.0350	
5	0.42	8	0.3096	19.3	0.0637	
6	0.55	7	0.3193	19.9	0.4205	
7	0.85	3	0.4042	25.2	0.9129	

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2	0.043	8	1.6963	0.2273	0.2163
3	0.059	5	1.6300	0.2040	0.2040
4	0.20	8	1.6413	0.2033	0.2033
5	0.42	8	1.5425	0.1786	0.1786
6	0.55	7	1.1857	0.0720	0.0720
7	0.85	3	0.6933	-0.1643	-0.1643

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[illegible]: [illegible]~2.[illegible] [illegible]: [illegible] 10([illegible])

[illegible]'[illegible] - [illegible] 2 [illegible] 2 [illegible]: [illegible]

[illegible]	[illegible]	[illegible]	[illegible] 0.05	[illegible]	[illegible]
[illegible] 0.2053					
0.043	0.2163	-0.2954		2.6000	[illegible]= 1, [illegible]=40
0.059	0.2040	0.0325		2.6600	[illegible]= 2, [illegible]=40
0.20	0.2033	0.0540		2.6800	[illegible]= 3, [illegible]=40
0.42	**0.1786**	**0.7177**		**2.6900**	**k= 4, v=40**
0.55	0.0720	3.4584	*	2.7000	[illegible]= 5, [illegible]=40
0.85	-0.1643	7.3273	*	2.7000	[illegible]= 6, [illegible]=40

[illegible] = 0.0745

[illegible]: [illegible], [illegible], [illegible]'[illegible].

[illegible] 10 [illegible]

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[illegible].	[illegible]	[illegible]	[illegible]	[illegible].([illegible])
870	73	68	93.15069	0
460	73	52.00001	71.2329	0
240	73	34	46.5753	0
120	73	11	15.0685	0
63	54.75	5.750001	10.5023	0
29	73	4.000001	5.4795	0

[illegible], [illegible] 95 [illegible]. [illegible].

[illegible] 50 [illegible] 262.0784

[illegible]

[illegible]	[illegible]	[illegible] 50	95 [illegible]	
4	2.878648[illegible]-02	264.9074	228.8742	307.5636

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
4	.1372806	2.480374	4.177076[illegible]-02

[illegible] 0.05, [illegible].

[illegible] = 2.213006

95 [illegible] = 1.393056 [illegible] 3.032956

[illegible]=-5.307031

[illegible]50 = 250.0977

95 [illegible] = 175.556 [illegible] 365.6053

□□25 = 123.9711
95 □□□□□□□□ □□□□□□□□□□ □□□□□□ = 71.46323 □□□ 176.5094

□□10 = 65.91556
95 □□□□□□□□ □□□□□□□□□□ □□□□□□ = 28.19076 □□□ 103.4599

□□05 = 45.16709
95 □□□□□□□□ □□□□□□□□□□ □□□□□□ = 15.81944 □□□ 76.75556
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