DATA EVALUATION RECORD
MIDGE 10-DAY TOXICITY STUDY
OPPTS 850.1735

1. **CHEMICAL:** Esfenvalerate PC Code No.: 109303

2. **TEST MATERIAL:** [Phenoxyphenyl ring-U-^{14}C]A.alpha fenvalerate Purity: 66.5% (radiochemical purity)

3. **CITATION:**

Author: Arthur E. Putt

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

Study Completion Date: June 30, 2005

Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, Massachusetts 02571-1075

Sponsor: Pyrethroid Working Group
Beveridge & Diamond
1350 I Street NW
Washington, DC 20005

Laboratory Report ID: 13656.6119

MRID No.: 465915-05

DP Barcode: D319265

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

Signature: **Date: Date:** 2/24/11

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

Signature: **Date:** 2/24/11

APPROVED BY: Keith Sappington, Senior Science Advisor**,** OPP/EFED/ERB5

Signature: **Date: Date:** 2/24/11

5. STUDY PARAMETERS:

Age of Test Organism:	2nd Instar, 8 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]esfenvalerate 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), 45, 90, 180, 350, 700, and 1400 µg/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 <0.87 (<LOQ; controls), and 70, 140, 250, 470, 970, and 1800 µg a.i./kg dry sediment, equivalent to mean recoveries of 160, 160, 140, 130, 140, and 130% of the nominal concentrations, respectively. Mean-measured (reviewer-calculated from Days 0 and 10) pore water concentrations were <0.22 (<LOQ; controls), and <0.22, <0.22, 0.19, 0.48, 0.89, and 1.47 µg a.i./L., respectively. Mean-measured (reviewer-calculated from Days 0 and 10) overlying water concentrations were <0.089 µg a.i./L (<LOQ) for the control and nominal 45 through 700 µg a.i./L treatment levels, and <0.28 µg a.i./L for the nominal 1400 µg a.i./L level. 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 11% in the negative and solvent control groups, respectively, and 11, 10, 9, 26, 51, and 78% for the 70, 140, 250, 470, 970, and 1800 µg a.i./kg dry sediment concentrations, respectively. Statistically-significant reductions ($p \leq 0.05$) in treatment survival on Day 10 compared to the negative control (9% mortality) were identified at the ≥ 470 µg a.i./kg dry sediment levels (the three highest levels tested). The Day-10 NOAEC, LOAEC, and LC_{50} (with 95% C.I.) for survival was 250, 470 and 1019 (867-1212) ug a.i/kg dry sediment., respectively, based on the mean-measured sediment treatment concentrations.

Dry weight per midge larvae averaged 2.26 and 2.35 mg in the negative and solvent control groups, respectively, and 2.19, 2.24, 1.47, 1.12, 0.59, and 0.28 mg the mean-measured 70, 140, 250, 470, 970, and 1800 µg a.i./kg dry sediment concentrations, respectively, and was significantly reduced ($p \leq 0.05$) compared to the negative control at the ≥ 250 1800 µg a.i./kg dry sediment concentrations. The Day-10 NOAEC, LOAEC and EC_{50} (with 95% C.I.) for dry weight was 140, 250, and 450 (380-540) µg a.i./kg dry sediment concentrations, respectively. 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 esfenvalerate concentrations in pore water (conducted only at the highest test concentration) indicate that the parent material declined somewhat over the course of this study (100 and 80% for initial and terminal measurements, respectively). In contrast, the recovery of parent compound from bulk sediment was generally high >95% for initial and terminal measurements. Given that recovery of parent chemical was high based on QA/QC sample spikes, the decline in concentrations of parent material by day 10 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.

This reviewer notes that measured concentrations of esfenvalerate in porewater likely reflect both "freely dissolved" chemical and chemical that is sorbed to dissolved organic carbon (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 esfenvalerate in porewater (by nearly two orders of magnitude) than would be expected based on estimated values of esfenvalerate calculated from bulk sediment concentrations, its mean K_{OC} (252,000), the fraction of total organic carbon in bulk sediment (5.5%). In addition, DOC reported in porewater ranged from 6.7 to 39 mg/L. For highly hydrophobic chemicals like esfenvalerate, it has been widely reported in the scientific literature that DOC in porewater can substantially reduce bioavailability and toxicity. Therefore, since the measured pore water concentrations of esfenvalerate do not accurately describe the exposure to the bioavailable 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} (252,000) L/kg-OC; MRID 45555102) for esfenvalerate (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 DOC), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that K_{OC} values for esfenvalerate vary considerably (85,700 to 596,200) 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 esfenvalerate.

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

Results Synopsis:

Based on ESTIMATED[1] Pore Water Concentrations

Survival

NOAEC: 0.018 µg a.i./L
LOAEC: 0.034 µg a.i./L
LC_{50}: 0.074 µg a.i./L 95% C.I.: 0.063-0.087
Slope: N/A

Dry Weight

NOAEC: 0.010 µg a.i./L
LOAEC: 0.018 µg a.i./L
EC_{50}: 0.033 µg a.i./L 95% C.I.: 0.027-0.039 µg a.i./L
Slope: 2.01 ± 0.165

[1] Freely dissolved pore water endpoints (ug/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 Mean-Measured Spiked Sediment Concentrations

Survival

NOAEC: 250 µg a.i./kg dry sediment
LOAEC: 470 µg a.i./kg dry sediment
LC_{50}: 1019 µg a.i./kg dry sediment 95% C.I.: 867-1212 µg a.i./kg dry sediment
Slope: N/A

Dry Weight

NOAEC: 140 µg a.i./kg dry sediment
LOAEC: 250 µg a.i./kg dry sediment
EC_{50}: 450 µg a.i./kg dry sediment 95% C.I.: 380-540 µg a.i./kg dry sediment
Slope: 2.01 ± 0.165

Endpoints affected: Survival and Ash-Free Dry Weight
Most sensitive endpoint: Ash-Free Dry Weight

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

Survival

LC_{50}: 18,527 µg a.i./kg TOC 95% C.I.: 15,764-22,036 µg a.i./kg TOC
Slope: N/A
NOAEC: 4,545 µg a.i./kg TOC
LOAEC: 8,545 µg a.i./kg TOC

Dry Weight

EC_{50}: 8,182 µg a.i./kg TOC 95% C.I.: 6,909-9,818 µg a.i./kg TOC

Slope: 2.01 ± 0.165
NOAEC: 2,545 µg a.i./kg TOC
LOAEC: 4,545 µg a.i./kg TOC

7. ADEQUACY OF THE STUDY:

A. Classification: ACCEPTABLE

B. Rationale:
Although dissolved oxygen levels dropped below the Agency's recommended 40% saturation level per OPPTS 850.1735, levels were at or above the more recent Agency-wide guidelines (EPA/600/R-99/064) recommended levels of 2.5 mg/L with one exception (Day 3, 90 ug a.i/kg sediment nominal). Given that the more recent Agency-wide guidelines are based on empirical data pertaining to DO tolerance of *C. tentans*, and that survival and growth was not significantly affected at this treatment level, this reviewer concludes that the low DO does not interfere with the study integrity.

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.

3. Dissolved oxygen (DO) concentrations were 2.5-7.3 mg/L throughout the exposure period with the following exceptions: on Day 3, the measured DO levels in replicate C of the nominal 45 and 90 ug a.i/kg sediment treatments were 2.5 and 2.0 mg/L,

respectively. As explained in the Reviewer's Comments section, 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.

9. SUBMISSION PURPOSE: This study was submitted to provide information on the toxicity of esfenvalerate 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.	2nd instar, 8 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 0.47 mm. Ash-free dry weight was confirmed at test initiation (sub-population of 20 midge larvae) to be 0.36 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 8 days prior to test initiation.

Guideline Criteria	Reported Information
Feeding 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 (e.g. <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 190-200 µmhos/cm, and a pH range of 7.4-7.7. 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 nitrogen.
Does water support test animals without observable signs of stress?	Yes

Guideline Criteria	Reported Information
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 0.75-1.4 mg/L based on measurements from the overlying water as a composite sample from each treatment and control group. Dissolved organic carbon concentrations were 6.7-39.0 mg/L based on measurements from the pore water as a composite sample from each treatment and control group on Day 0 and 10.
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 on all test days.
pH Not specified, but should be appropriate to the test species and should not deviate more than 0.4 pH units.	6.9-7.1 (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 was >2.5 mg/L (2.5-7.3 mg/L) throughout the exposure period with the following exceptions: on Day 3, the measured DO levels in replicate C of the nominal 45 and 90 ug a.i/kg sediment treatments were 2.5 and 2.0 mg/L, respectively. The actual percent DO saturation relative to the test temperature was not reported, but these values are 29 and 23% saturation respectively (reviewer calculated). With one exception, DO levels exceeded the Agency-wide guidelines of 2.5 mg/L
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 $CaCO_3$.

Guideline Criteria	Reported Information
Conductivity Not specified, but should be amenable to the test species.	230-260 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	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

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: Esfenvalerate Synonyms: GB800-001, Asana, DPX-GB800 (resolved *S,S* isomer) CAS Name: [S]-cyano(3-phenoxyphenyl)-methyl [S]-4-chloro-α-(1-methylethyl)benzeneaceate CAS no.: 66230-04-4 Batch no.: 1 (Lot no.) Purity: 99.7% 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: A.alpha Fenvalerate [phenoxyphenyl ring-U-^{14}C] Synonyms: [^{14}C]DPX-GB800, [^{14}C]esfenvalerate, DPX-GB800 (resolved *S,S* isomer) Batch no.: CF11429 (Lot no.) CAS No.: 66230-04-4 Specific activity: 49.3 µCi/mg (equiv. to 110,845 dpm/µg) Amount received: 89.92 mg (1.19 mCi,

Guideline Criteria	Reported Information
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	The initial primary [^{14}C]esfenvalerate stock was prepared by transferring 0.0001 g of the test material to a 10-mL amber vial and adding 2.00 mL of acetonitrile. Triplicate 20.0-µL aliquotes of the stock were then assayed via LSC. Based on this analysis and the specific activity of 49.93 µCi/mg (110,845 dpm/µg) provided by the supplier, the stock was determined to have a concentration of 0.0364 mg/L and a mean radiopurity of 53.7%. The [^{14}C]esfenvalerate was purified at Springborn Smithers by HPLC/RAM. Based on the purification and the supplier-reported specific activity of 110,845 dpm/µg, the purified stock solution was determined to have a concentration of 0.238 mg/mL (= 238 µg/mL). A mean radiopurity of 95.8% was determined by three repetitive injections of the stock solution. The stock was stored frozen until
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; Sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile	Nominal sediment treatment concentrations selected for the test were 45, 90, 180, 350, 700, and 1400 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) of sediment (equiv. 122 g wet weight per vessel or 71 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; 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.
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 435-660 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	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

Guideline Criteria	Reported Information
flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	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 approx. 2 volume additions per day per replicate test vessel (refer to Reviewer's Comments section).
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), 45, 90, 180, 350, 700, and 1400 µg/kg dry weight (ug a.i/kg sediment); nominal sediment treatment levels were determined based on the results of a range-finding study.

Guideline Criteria	Reported Information
	Aqueous solubility of the test material was not reported. According to Laskowski (2002) the solubility is low, 0.006 mg/L.
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.	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 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 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.

12. <u>REPORTED RESULTS</u>:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
<u>Control Mortality</u> Must be ≤ 30% in the sediment at end of the test.	Negative control: 9% Solvent control: 11%
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. <u>In overlying water</u>: N/A; see Reviewer's Comments section <u>In pore water:</u> <0.14% based on reviewer-determined mean-measured concentrations <u>In sediment</u>: 130-160% 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 26.1-1510 ug a.i/kg sediment, recoveries were 89.5-99.6% of nominal. In freshwater matrix spikes at 0.476-19.0 ug

Guideline Criteria	Reported Information
3) Limit of quantitation (LOQ)	a.i/L, recoveries were 93.8-106% of nominal, with a single outlier of 138% of nominal. 3) LOQ = 0.088-0.089 ug a.i/L. for overlying water samples; 0.22 ug a.i/L. for pore-water samples; and 0.81-0.87 ug a.i/kg sediment (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					
Nominal Sediment (ug a.i/kg sediment.)	Mean-Measured (Days 0 and 10)			Cumulative Number Dead (and %)	Mean Ash-Free Dry Weight per Midge, mg ± SD, (and % Inhibition)[4]
	Sediment (ug a.i./kg sediment.)[1]	Pore Water (ug a.i/L.)[2]	Overlying Water (ug a.i./L[3]		
Negative control	<0.87	<0.22	<0.089	7/80 (9)	2.26 ± 0.23
Solvent control	<0.87	<0.22	<0.089	9/80 (11)	2.35 ± 0.37
45	70	<0.22	<0.089	9/80 (11)	2.19 ± 0.16 (3)
90	140	<0.22	<0.089	8/80 (10)	2.24 ± 0.28 (1)
180	250	0.27[5]	<0.089	7/80 (9)	1.47 ± 0.28 (35)*
350	470	0.48	<0.089	21/80 (26)*	1.12 ± 0.25 (50)*
700	970	0.88	<0.089	41/80 (51)*	0.59 ± 0.23 (74)*
1400	1800	1.5	<0.28[6]	62/80 (78)*	0.28 ± 0.17 (88)*

[1] The LOQ for sediment samples was 0.81-0.87 ug a.i/kg sediment
[2] The LOQ for pore water samples was 0.22 ug 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 0.088-0.089 ug a.i/L
[4] Percent inhibition (reviewer-determined; note attached Excel e-file for calculations) 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 as the mean-measured concentrations for the lowest level tested since the Day 0 measured concentration for this treatment level was less than the LOQ (See Reviewer's Comments section).
[6] The Day 0 measured concentration was reported to be 0.28 ug a.i/L. while the Day 10 measured concentration was reported to be <0.88 (i.e., less than the LOQ).
N/A = Not applicable
* Statistically significant reduction ($p \leq 0.05$) compared to the negative control using William's Test.

B. Statistical Results

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 by the study author 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, William's Test was used to compare survival and growth treatment response data to the pooled control data. 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's Statistical Results (Survival)

LOAEC	470 ug a.i/kg sediment
NOAEC	250 ug a.i/kg sediment
LC_{50} (95% CI)	1100 ug a.i/kg sediment (820 – 1300)

Study Author's Statistical Results (Growth)

LOAEC	250 ug a.i/kg sediment
NOAEC	140 ug a.i/kg sediment
EC_{50} (95% CI)	450 ug a.i/kg sediment (350 – 570)

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): □fter 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. Since a *t*-Test indicated no statistically significant differences between the negative and solvent controls, Percent survival and growth treatment response data were statistically compared to the **negative control** to reflect current EFED guidance. The 10-day LC_{50} was determined by the study reviewer using the moving average method via the TOXANAL program. The study author used the ICp method to determine the LC_{50} and this difference in statistical method used likely reflects the difference in the values obtained from the study author and reviewer. The EC_{50} value for growth was determined using the Probit method via NUTHATCH statistical software. The above statistical analyses were performed in terms of the mean-measured sediment and pore water (reviewer-estimated) treatment concentrations (see Reviewer's Comments for further details).

Based on ESTIMATED[1] Pore Water Concentrations

Survival

NOAEC: 0.018 µg a.i./L
LOAEC: 0.034 µg a.i./L
LC_{50}: 0. 0.074 µg a.i./L 95% C.I.: 0.063-0.087
Slope: N/A

Dry Weight
NOAEC: 0.010 µg a.i./L
LOAEC: 0.018 µg a.i./L
EC_{50}: 0.033 µg a.i./L 95% C.I.: 0.027-0.039 µg a.i./L
Slope: 2.01 ± 0.165

[1] Freely dissolved pore water endpoints (ug/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 Mean-Measured Spiked Sediment Concentrations

Survival
NOAEC: 250 µg a.i./kg dry sediment
LOAEC: 470 µg a.i./kg dry sediment
LC_{50}: 1019 µg a.i./kg dry sediment 95% C.I.: 867-1212 µg a.i./kg dry sediment
Slope: N/A

Dry Weight
NOAEC: 140 µg a.i./kg dry sediment
LOAEC: 250 µg a.i./kg dry sediment
EC_{50}: 450 µg a.i./kg dry sediment 95% C.I.: 380-540 µg a.i./kg dry sediment
Slope: 2.01 ± 0.165

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

Survival
LC_{50}: 18,527 µg a.i./kg TOC 95% C.I.: 15,764-22,036 µg a.i./kg TOC
Slope: N/A
NOAEC: 4,545 µg a.i./kg TOC
LOAEC: 8,545 µg a.i./kg TOC

Dry Weight
EC_{50}: 8,182 µg a.i./kg TOC 95% C.I.: 6,910-9,820 µg a.i./kg TOC
Slope: 2.01 ± 0.165
NOAEC: 2,545 µg a.i./kg TOC
LOAEC: 4,545 µg a.i./kg TOC

14. REVIEWER'S COMMENTS:

This study was designed to fulfill proposed OPPTS Draft Guideline 850.1735 and provides information that may be useful for risk assessment purposes.

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} and EC_{50} values and their associated 95% confidence intervals (C.I.) for survival and growth, respectively. The reviewer-determined LC_{50} (with 95% C.I.) based on the sediment concentrations, 1019 (867-1212) µg a.i./kg dry sediment, was lower, and had a narrower confidence interval than that of the study author, 1100 (820-1300) µg a.i./kg dry sediment. The reviewer-determined EC_{50} (with 95% C.I.) based on the sediment concentrations, 450 (380-540) µg a.i./kg dry sediment, was also lower and had a narrower confidence interval than that of the study author, 450 (350-570) µg a.i./kg dry sediment. Consequently, the reviewer-determined LC_{50} and EC_{50} (with 95% C.I.s) is reported in the CONCLUSION section of this DER because they are more conservative and reliable. The observed differences were attributed to the different statistical methods and/or statistical software used (moving average for LC_{50} and Nuthatch for EC_{50} for study reviewer vs. ICp for LC/EC_{50} for study author.

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.

It should also be noted that DO concentrations were >2.5 mg/L (2.5-7.3 mg/L) throughout the exposure period with the following exceptions: on Day 3, the measured DO levels in replicate C of the nominal 45 and 90 µg/kg treatments were 2.5 and 2.0 mg/L, respectively. These DO values correspond to % saturation levels of 29% and 23%, respectively (reviewer calculated) which are below the Guideline recommended minimum of 40% saturation.. Although dissolved oxygen levels dropped below the Agency's recommended 40% saturation level per OPPTS 850.1735, levels were at or above the more recent Agency-wide guidelines (EPA/600/R-99/064) recommended levels of 2.5 mg/L with one exception (Day 3, 90 ug a.i./kg sediment nominal). Given that the more recent Agency-wide guidelines are based on empirical data pertaining to DO tolerance of *C. tentans,* and that survival and growth was not significantly affected at this treatment level, this reviewer concludes that the low DO does not interfere with the study integrity.

For the definitive test (MRID: 465915-05) a 190 µg/mL dosing stock solution was prepared by placing 20 mL of the 238 µg/mL primary stock solution (in toluene; prepared as described on pg. 11 of this DER) in a 25-mL volumetric flask and removing the toluene under a gentle stream of nitrogen. The material remaining was reconstituted with 25 mL of acetone. Five additional 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 (%)
238	20	25	190	100
190	4.98	10	94.6	100
190	2.49	10	47.3	100
190	1.28	10	24.3	100
190	0.64	10	12.2	100
190	0.32	10	6.08	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 (1.2168 kg dry weight based on a percent of solids of 58.34%) in individual 1-gallon jars. The total mass of sediment spiked on a dry weight basis for each treatment level and control was 1.21168 kg (0.0500 kg sand and 1.1668 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 nominal 1400 µg/kg dry weight treatment group were analyzed by HLPLC/RAM to determine the percent of [^{14}C]residue associated with the parent test material (measured concentrations, µg/kg as esfenvalerate equivalents). **Recoveries were 95.7% and 95.9% from the sediment samples on Day 0 and Day 10, respectively. Recoveries were 100% and 80.4% 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 esfenvalerate to midge larvae (10 days old). The nominal treatment levels tested were 0.0 (negative and solvent controls), and 0.15, 1.5, 15, 150, and 1500 µg a.i./kg dry sediment and were

prepared in the same manner as described for the definitive test, with the exception that the acetone was allowed to evaporate from the sand for 40 minutes. Three replicates per treatment and control group with 10 midge larvae per replicate were tested. By Day 10, 97 and 93, and 93, 83, 93, 83, and 0% survival was observed in the negative and solvent controls, and nominal 0.15, 1.5, 15, 150, and 1500 ug a.i/kg sediment treatment groups, respectively. Ash-free dry weight among surviving midge larvae averaged 0.93 and 0.98, and 1.13, 1.08, 0.88, and 0.76 mg per midge larvae in the negative and solvent controls, and nominal 0.15, 1.5, 15, and 150 ug a.i/kg sediment treatment groups, respectively. Dry weights were not recorded for the nominal 1500 ug a.i/kg sediment treatment level since 100% mortality had occurred by Day 10. The definitive nominal sediment test concentrations of 45, 90, 180, 350, 700, and 1400 µg a.i./kg dry sediment 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 12 times per day. However, this rate would be equivalent to 600 mL of water per vessel, or approximately 3.4 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 12.

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:

RESULTS BASED ON MEAN-MEASURED SEDIMENT CONCENTRATIONS

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

Housenger Esfenvalerate 10 day sediment tox

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1800	73	55	75.3425	0
970	73	34	46.5753	0
470	73	14	19.1781	0
250	73	0	0	0
140	73	1	1.3699	0
70	73	2	2.7397	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 1041.069

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.0753991	1018.794	866.9556	1212.089

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.5867928	8.15732	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 = 2.485316
95 PERCENT CONFIDENCE LIMITS = .5815043 AND 4.389128

INTERCEPT=-7.505615

LC50 = 1047.091
95 PERCENT CONFIDENCE LIMITS = 554.2197 AND 5509.861

LC25 = 560.5193
95 PERCENT CONFIDENCE LIMITS = 134.1834 AND 1105.417

LC10 = 319.3859
95 PERCENT CONFIDENCE LIMITS = 16.40789 AND 594.0684

LC05 = 228.1062

95 PERCENT CONFIDENCE LIMITS = 4.169387 AND 458.4435
****

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err. /Estimate	Lower Bound
EC5	2.8E+02	1.7E+02	4.5E+02	0.10	0.62
EC10	3.7E+02	2.5E+02	5.5E+02	0.086	0.67
EC25	6.1E+02	4.6E+02	7.9E+02	0.058	0.76
EC50	**1.0E+03**	**8.9E+02**	**1.2E+03**	0.034	0.86

Slope = 2.87 Std.Err. = 0.411

Goodness of fit: p = 0.94 based on DF= 4.0 57.

1505SSD : Percent Survival (mean-meas. sed. conc., ppb a.i.)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	16.0	0.900	0.904	-0.00435	100.	0.00
70.0	8.00	0.888	0.904	-0.0165	100.	0.0386
140.	8.00	0.900	0.899	0.00127	99.4	0.621
250.	8.00	0.913	0.870	0.0422	96.2	3.77
470.	8.00	0.737	0.759	-0.0216	83.9	16.1
970.	8.00	0.488	0.485	0.00291	53.6	46.4
1.80e+03	8.00	0.225	0.225	0.000408	24.8	75.2

Growth Statistics

Program: Nuthatch Date: 2/24/11

Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

Input file: ESFENGRW.TXT

Raw data:

Sediment toxicity - Esfenvalerate Dry Weight

In c:\nuthatch\ESFENGRW.TXT : `Neg. control`
Interpreted as Dose = 0

ESFENGRW.TXT : Sediment toxicity - Esfenvalerate Dry Weight

Williams Test

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

Dose	Isotone Means	T-bar	P-value	Significance
0	2.26	.		
70	2.22	0.3741	N.S.	
140	2.22	0.3741	N.S.	
250	1.47	6.798	<0.005	*
470	1.12	9.759	<0.005	*
970	0.595	14.25	<0.005	*
1800	0.279	16.95	<0.005	*

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

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err. /Estimate	Lower Bound
EC5	69.	41.	1.2E+02	0.11	0.60
EC10	1.1E+02	67.	1.6E+02	0.097	0.64
EC25	2.1E+02	1.5E+02	2.9E+02	0.072	0.72
EC50	4.5E+02	3.7E+02	5.6E+02	0.047	0.81

Slope = 2.02 Std.Err. = 0.181

Goodness of fit: p = 0.098 based on DF= 4.0 49.

ESFENGRW.TXT : Sediment toxicity - Esfenvalerate Dry Weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	8.00	2.26	2.33	-0.0727	100.	0.00
70.0	8.00	2.19	2.22	-0.0243	94.9	5.07
140.	8.00	2.24	1.98	0.263	84.9	15.1
250.	8.00	1.47	1.63	-0.166	69.9	30.1
470.	8.00	1.12	1.14	-0.0190	48.8	51.2

970.	8.00	0.595	0.591	0.00418	25.3	74.7
1.80e+03	8.00	0.279	0.266	0.0129	11.4	88.6

!!!Warning: EC5 not bracketed by doses evaluated.

Title: Mean Ash-Free Dry Weight (mg/larvae; m.m. p.w., ppb a.i
File: ESGRWSED.TXT Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	neg control	8	2.0200	2.7300	2.2613
2	70 ug/kg sed	8	1.9500	2.4200	2.1912
3	140 ug/kg sed	8	1.9000	2.7600	2.2438
4	250 ug/kg sed	8	0.9800	1.7800	1.4662
5	470 ug/kg sed	8	0.7200	1.5500	1.1200
6	970 ug/kg sed	8	0.2800	0.9200	0.5950
7	1800 ug/kg sed	8	0.0500	0.6400	0.2788

Title: Mean Ash-Free Dry Weight (mg/larvae; m.m. p.w., ppb a.i
File: ESGRWSED.TXT Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	neg control	0.0526	0.2292	0.0811	10.1382
2	70 ug/kg sed	0.0248	0.1574	0.0557	7.1844
3	140 ug/kg sed	0.0816	0.2856	0.1010	12.7289
4	250 ug/kg sed	0.0809	0.2845	0.1006	19.4033
5	470 ug/kg sed	0.0630	0.2509	0.0887	22.4055
6	970 ug/kg sed	0.0524	0.2290	0.0810	38.4828
7	1800 ug/kg sed	0.0277	0.1664	0.0588	59.7050

Title: Mean Ash-Free Dry Weight (mg/larvae; m.m. p.w., ppb a.i
File: ESGRWSED.TXT Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	32.3970	5.3995	98.6988
Within (Error)	49	2.6806	0.0547	

```
     Total              55             35.0777
  ---------------------------------------------------------------------------
                                                          (p-value = 0.0000)

     Critical F  = 3.1948  (alpha = 0.01, df = 6,49)
                 = 2.2904  (alpha = 0.05, df = 6,49)

     Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)
Title:  Mean Ash-Free Dry Weight (mg/larvae; m.m. p.w., ppb a.i
  File:      ESGRWSED.TXT             Transform:             NO TRANSFORMATION

         William's Test  - TABLE 1 OF 2        Ho: Control<Treatment
  ---------------------------------------------------------------------------
                                      ORIGINAL      TRANSFORMED    ISOTONIZED

  GROUP     IDENTIFICATION       N      MEAN           MEAN           MEAN
  ------  --------------------  ---  -----------    -----------    -----------
     1             neg control   8      2.2613         2.2613         2.2613
     2           70 ug/kg sed    8      2.1912         2.1912         2.2175
     3          140 ug/kg sed    8      2.2438         2.2438         2.2175
     4          250 ug/kg sed    8      1.4662         1.4662         1.4662
     5          470 ug/kg sed    8      1.1200         1.1200         1.1200
     6          970 ug/kg sed    8      0.5950         0.5950         0.5950
     7         1800 ug/kg sed    8      0.2788         0.2788         0.2788
  ---------------------------------------------------------------------------


  Title:  Mean Ash-Free Dry Weight (mg/larvae; m.m. p.w., ppb a.i
  File:      ESGRWSED.TXT             Transform:             NO TRANSFORMATION

         William's Test  - TABLE 2 OF 2        Ho: Control<Treatment
  ---------------------------------------------------------------------------
                       COMPARED     CALC.     SIG      TABLE      DEGREES OF
     IDENTIFICATION      MEANS     WILLIAMS   0.05   WILLIAMS    FREEDOM USED
  -------------------- ----------- ----------- -----  ----------- ------------
          neg control     2.2613
        70 ug/kg sed      2.2175    0.3741            1.6800       k= 1, v=40
       140 ug/kg sed      2.2175    0.3741            1.7600       k= 2, v=40
       250 ug/kg sed      1.4662    6.7979      *     1.7900       k= 3, v=40
       470 ug/kg sed      1.1200    9.7587      *     1.8000       k= 4, v=40
       970 ug/kg sed      0.5950   14.2478      *     1.8000       k= 5, v=40
      1800 ug/kg sed      0.2788   16.9520      *     1.8100       k= 6, v=40
  ---------------------------------------------------------------------------
  s =   0.2339

   WARNING: Procedure has used isotonized means which differ from original
            (transformed) means.
```