DATA EVALUATION RECORD
28-DAY WHOLE SEDIMENT *Leptocheirus plumulosus* TOXICITY TEST

1. CHEMICAL: Esfenvalerate PC Code: 109303

2. TEST MATERIAL: [^{14}C]Esfenvalerate Radiochemical Purity: 95.8%

3. CITATION:

Authors: Putt, A.E.
Title: Esfenvalerate – Toxicity to Estuarine Amphipods (*Leptocheirus plumulosus*) During a 28-Day Sediment Exposure.
Study Completion Date: August 2, 2005
Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, MA 02571-1037
Sponsor: Pyrethroid Working Group
Beveridge & Diamond
1350 I Street NW
Washington, DC 20005
Laboratory Report ID: 13656.6120
MRID No.: 466204-01

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

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

REVIEWED BY: Amanda Solliday, Biologist, OPP/EFED/ERB 5

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

REVIEWED BY: Keith Sappington, Senior Advisor, OPP/EFED/ERB 5

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

5. STUDY PARAMETERS

Scientific Name of Test Organism: *Leptocheirus plumulosus*
Age of Test Organism: Neonate

Definitive Test Duration: 28 days
Study Method: Static renewal
Type of Concentrations: Mean-measured sediment (total radioactive residues)

6. CONCLUSIONS:

The 28-day toxicity study of esfenvalerate to estuarine amphipods (*Leptocheirus plumulosus*) was conducted under a static renewal system in which the overlying water was renewed three times weekly. The endpoints assessed were survival and growth. The nominal spiked sediment test concentrations were 0 for the negative and solvent (acetone) controls, 1.9, 5.6, 17, 50, 150, and 450 ug a.i/kg sediment. Measured concentrations at Day-0 (excluding controls) were 3.0, 6.2, 16, 41, 130, and 410 ug a.i/kg, respectively and at test termination on Day-28 were measured at 1.6, 4.5, 13, 42, 120, and 390 ug a.i/kg, respectively. Liquid scintillation counting (LSC) analysis defined the mean measured test concentrations throughout the study as <0.82 (negative and solvent controls), 2.2, 5.1, 13, 40, 125, and 383 ug a.i./kg sediment.

Mean measured pore water analysis was not definitive as Day-0 measurements yielded the two lowest treatment levels below the assay limit of quantitation (LOQ) while the Day-28 measurements yielded the three lowest treatment levels below of the LOQ. This prohibited a statistical analysis of an EC_{50} for growth based on measured pore water concentrations from being calculated due to undefined concentrations at the lower treatment levels, in addition to issues associated with the accuracy of pore water measurements (discussed below). Endpoints were not calculated using overlying water concentrations, as the testing apparatus ensures volume replacement three times weekly, and it is the sediment, not the overlying water, that is spiked with esfenvalerate.

The study author pooled the negative and solvent control at test termination for statistical analysis as there was no statistical difference between negative and solvent control percent survival. The study reviewer, however, performed the statistical analysis based on differences from the negative control, as per EFED guidance (Frankenberry *et al.*, 2008). In ascending order of the treatment levels (including the negative and solvent controls), the percent survival after 28 days was determined to be 90, 94, 90, 82, 78, 89, 59, and 0%. The two highest treatment levels (125 and 383 ug a.i/kg) showed statistically significant differences ($p<0.05$) from the negative control. The 28-day NOAEC, LOAEC, and LC_{50} based on mean measured sediment concentrations were 40, 125, and 113 ug a.i./kg sediment, respectively. A 28-day EC_{50} for growth was determined to be >125 ug a.i/kg sediment based on a less than 50% reduction in growth at all treatment levels below this level tested. Furthermore, the highest treatment level was excluded from the statistical analysis for the growth endpoint due to complete mortality in these treatment levels. The OC-normalized NOAEC, LOAEC, and LC_{50} are 830, 2600, and 2350 ug a.i/kg TOC based on 4.8% organic carbon in the sediment.

At test termination, statistical analysis showed no significant difference between negative and solvent control growth. The study reviewer's statistical analysis based effects on survival and growth based on comparisons to the negative control. Due to complete mortality in the highest treatment level (383 ug a.i./kg sediment), growth data was not available for this treatment level.

This reviewer notes that HPLC analysis of esfenvalerate concentrations in porewater (conducted only at the highest test concentration) indicate that the parent material was only a small fraction of total radioactive residues measured over the course of this study (14 % to 0% for initial and terminal measurements, respectively). In contrast, the recovery of parent compound from bulk sediment was generally high (92.3% to 76% for initial and terminal measurements, respectively). Given that recovery of parent chemical was high based on QA/QC samples, the low concentrations of parent material in the porewater appear to reflect desportion of the degradation products from the sediment particles into the porewater phase. This presumption is consistent with the expected lower hydrophobicity of the degradation products compared to the parent compound. Given that the measured porewater concentrations of esfenvalerate do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured porewater concentrations.

Instead, this reviewer has estimated freely dissolved porewater endpoints based on measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment 4.8% and the mean Koc (251,700 mL/g-OC, MRID 4555102) for esfenvalerate. These estimated porewater 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 Koc values for esfenvalerate vary considerably (85,700 mL/g – 596,200 mL/g-OC) which likely reflect differences in organic carbon composition and other soil properties used to determine Koc. Therefore, these estimated porewater endpoints are subject to the same uncertainty in determination and application of Koc for esfenvalerate.

This study was submitted to fulfill U.S. EPA data requirements for whole sediment chronic toxicity to estuarine/marine invertebrates based on **"**Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod *Leptocheirus plumulosus.*" Office of Research and Development, U.S. EPA. Washington, DC EPA/600/R-01/020 (2001). Growth was adversely affected at all treatment levels (a NOAEC was not defined for this endpoint) and an EC_{50} value could not be determined by the reviewer since dry weight data (in treatments with no significant affects on survival) were unsuitable for statistical analysis with Nuthatch or the ICp method. Even though the study follows test methods outlined by the document cited above, reproduction is a required endpoint, and was not assessed in this study. This study is scientifically sound and still may be used in risk assessment for evaluation of effects of chronic exposure on growth and survival of *Leptocheirus.* **It is classified as SUPPLEMENTAL.**

Results Synopsis:

Based on mean-measured sediment concentrations (total radioactive residues):

Mortality:

LC_{50}: 113 µg ai/kg dry weight — 95% C.I.: 97-135 µg ai/kg dry weight
NOAEC: 40 µg ai/kg dry weight — Probit Slope: N/A
LOAEC: 125 ug a.i/kg dry weight

Growth (dry weight):

EC_{50}: >125 ug a.i/kg sediment — 95% C.I.: N/A
NOAEC: <2.2 µg ai/kg dry weight — Slope: N/A
LOAEC: 2.2 µg ai/kg dry weight

Based on OC-normalized sediment concentrations (mean measured)

Mortality:

LC_{50}: 2350 ug a.i/kg TOC — 95% C.I: 2020 – 2810 ug a.i/kg TOC
NOAEC: 830 ug a.i/kg TOC — Probit Slope: N/A
LOAEC: 2600 ug a.i./kg TOC

Growth (dry weight):

EC_{50}: >2604 ug a.i/kg TOC — 95% C.I: N/A
NOAEC: <46 ug a.i/kg TOC — Slope: NA
LOAEC: 46 ug a.i/kg TOC

Based on ESTIMATED[1] pore water concentrations:

Mortality:

LC_{50}: 0.009 ug a.i/L — 95% C.I: 0.008 ug a.i/L – 0.01 ug a.i/L
NOAEC: 0.003 ug a.i/L — Probit Slope: N/A
LOAEC: 0.01 ug a.i./L

Growth (dry weight):

EC_{50}: >0.010 ug a.i/L — 95% C.I: N/A
NOAEC: <0.0002 ug a.i/L — Slope: NA
LOAEC: 0.0002 ug a.i/L

[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*)]

Endpoints affected: survival and growth
Most sensitive endpoint(s): growth (based on the NOAEC value)

7. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: Even though the study follows test methods outlined by the document cited above, reproduction is a required endpoint and was not assessed in this study. Furthermore, growth was adversely affected at all treatment levels, and therefore a definitive NOAEC was not defined for this endpoint. This study is scientifically sound and still may be used in risk assessment for evaluation of effects of chronic exposure on growth and survival of *Leptocheirus.*

C. Repairability: This study is not repairable as a new study with reproduction as an endpoint will need to be conducted.

8. MAJOR GUIDELINE DEVIATIONS:

This study was compared to the draft OCSPP 850.1780 guideline (in prep.) and the Agency-wide guidance: "Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod *Leptocheirus plumulosus."* EPA 600/R-01/020 (USEPA 2001). The following deviations from the above cited guidance methods were observed:

1. Reproduction is a required endpoint for 28-day sediment toxicity studies and was not assessed in this study.

2. A definitive NOAEC for growth could not be defined for this study because adverse effects on growth were detected at all treatment levels compared to the negative control.

3. A physical description of the test substance was not provided. In addition, the aqueous solubility should have been reported

9. SUBMISSION PURPOSE: RED Follow-up

10. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: [^{14}C]Residues remained predominantly associated with the sediment during the study, with little variation in the day 0 and 28 total residue levels. Mean percent recoveries of total radioactive residues (based on LSC analyses) were 83-120% of nominal concentrations. On days 0 and 28 at the 450 µg ai/kg level (the only level analyzed by HPLC/RAM), 92.3 and 76.0% of the recovered radioactivity was parent material.

Less than an average of 9 µg/L was detected in the pore water during the study (based on LSC), and concentrations were consistent between 0 and 28 days. Mean recoveries were <0.22 µg/L (<LOQ) at the 1.9 and 5.6 µg/kg levels, and increased from 0.18 µg ai/L (reviewer-calculated using ½ the LOQ for the day-28 value) to 8.2 µg/L at the 450 µg/kg level. **Of the total radioactivity recovered from the 450 µg/kg level, only 14.7% was identified as [^{14}C]esfenvalerate on day 0, and none of the recovered radioactivity was identified as parent material on day 28 (based on HPLC/RAM analysis).**

Less than 1 µg/L was detected in the overlying water during the study (based on LSC), and samples were not further analyzed by HPLC/RAM.

Storage conditions of test chemical: In a freezer (< -4°C) in the original container

Physicochemical properties of Esfenvalerate.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapour pressure	Not reported	
UV adsorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species	*Leptocheirus plumulosus*
Source	Laboratory cultures
Culture Conditions	Adult amphipods were maintained in 11-L plastic bins containing a 2-cm layer of marine sediment and 7-8 L of 20‰ salinity seawater.
Age of Test Organisms	Neonates: size-selected (retained between 0.25 and 0.6-mm mesh screens)

Guideline Criteria	Reported Information
Food	During holding and acclimation, amphipods were fed daily a finely-ground suspension of Zeigler Prime flakes fish food. On days 0-13, 2 mL of a 10 mg/mL suspension was provided to each vessel. On days 14-28 4.0 mL of a 10 mg/mL suspension was provided to each vessel.
Health of parent culture stock	No mortality observed in the population 48 hours prior to test initiation.

B. Test System

Guideline Criteria	Reported Information
Type of Test System	Static-renewal
Test Water	Seawater was pumped from the Cape Cod Canal, Bourne, MA from about 4 m offshore at a depth of approx. 0.5 m. The seawater was filtered (not further specified) and adjusted to a salinity of 19-21‰ and a pH of 7.9-8.0 with laboratory well water.
Renewal of overlying water	3 times per week, 400 ml of the overlying was siphoned off and replaced with fresh overlying water. Care was taken to not disturb the sediment layer.
Test Sediment	Marine sediment was collected from Little Harbor Beach, Wareham, MA. The sediment was wet pressed through a 0.25-mm sieve to remove large particles.
Sediment Characterization	Particle size: 68% sand, 20% silt, and 12% clay pH: 6.6 Ammonia (as N) in pore water: 43.4 mg/L Percent organic carbon: 4.8%: Percent water content (1/3 bar): not reported Grain size: 32% silt/clay

Guideline Criteria	Reported Information
Test Material	[^{14}C]Esfenvalerate Description: not reported Lot no.: CF11429 CAS No.: 66230-04-4 Position of label: phenoxyphenyl ring –U-14C Radiochemical purity: 95.8% (purified) Specific activity: 49.93 µCi/mg (110,845 dpm/µg) Storage: freezer (< -4°C) Aqueous solubility: Not reported. According to Laskowski (2002), the solubility is low at 6 ug/L or 6 ppb.
Solvents	Acetone, 9 ml per 0.9184 kg sediment (dw basis). The acetone was allowed to evaporate during the mixing procedure. Both solvent control and negative control groups were included in the study.
Sediment Spiking	A jar-rolling technique was used to apply the test substance to the sediment. A 9-mL volume of each stock solution was applied to coarse silica sand and the solvent was allowed to evaporate off for 30 minutes. The sand was then added to 2.00 kg of wet sediment. Each jar was then rolled for 4 hours at room temperature at approx. 15 rpm. The jars were stored upright at 4°C overnight prior to conditioning.
Sediment Conditioning	The treated sediments were allowed to equilibrate for a 29-day period in the refrigerator. Once a week and prior to addition to the exposure vessels, the jars were mixed on the rolling mill for an additional 2 hours at room temperature to ensure the sediment was homogeneous.

Guideline Criteria	Reported Information
Sediment and Overlying Water Into Test Chambers	One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added. 1 L glass vessels containing 175 ml (approx. 2.0 cm layer) of sediment (equivalent to 190 g wet weight or 82 g dry weight per vessel) and 725 ml of overlying water. The total overlying water plus sediment volume was maintained at approx. 900 ml. Test vessels were covered with a plastic plate. Nine replicates were prepared for each test concentration and control. Five replicates were used to evaluate the biological response and the remaining four were used for chemical analysis and water quality measurements.
Aeration	Test chambers were aerated with oil-free air (rate not reported). It was not reported if aeration was stopped during introduction of the test organisms. (Rate reported in protocol as "constant trickle flow of bubbles," if levels fall below unacceptable levels).
Water Temperature	Overlying water: 24-26°C Pore water: not determined
pH	Overlying water: 6.9-8.1 Pore water: 6.0-7.0
Salinity	Overlying water: 20-21‰ Pore water: 20-21‰
Ammonia (as N)	Overlying water: 5.8-6.4 mg/L on day 0 and ≤0.10 mg/L on day 28 Pore water: 32-36 mg/L on day 0 and 1.8-3.3 mg/L on day 28
Dissolved Organic Carbon	Pore water: 25.9-50.7 mg/L on day 0 and 8.3-13.3 mg/L on day 28

Guideline Criteria	Reported Information
Dissolved Oxygen	5.2-7.1 mg/L (>60% saturation)
Photoperiod	16 hours light, 8 hours dark (600-850 lux)
Food	Finely ground flaked fish food suspension (10 mg/ml). Amphipods were fed three times per week, following renewal of the overlying water. Days 0-13: 2 ml of suspension Days 14-27: 4 ml of suspension

C. Test Design

Guideline Criteria	Reported Information
Duration	28 days
Nominal Concentrations	Negative control, solvent control, 1.8, 5.4, 16, 48, 144, and 431 µg ai/kg dw sediment Selection of nominal treatment levels for the definitive study was based on results from preliminary testing.
Mean-Measured Concentrations	<0.82 (controls), 2.2, 5.1, 13, 40, 125, and 383 µg total [^{14}C]esfenvalerate residues/kg dw sediment (based on LSC analysis)
Number of Test Organisms	100 amphipods per level, divided into 5 replicates each containing 20 amphipods
Test organisms randomly or impartially assigned to test vessels?	Yes, organisms were impartially assigned to test containers.

Guideline Criteria	Reported Information
Overlying Water Parameter Measurements	Dissolved oxygen, salinity, temperature, and pH were measured daily in each control and treatment level; measurements were taken from all replicate chambers on days 0 and 28, and from alternating chambers on days 1-27. Temperature was also continuously monitored in one representative test vessel (control, replicate H). Ammonia (as nitrogen) was measured on days 0 and 28 from a composite sample obtained for each control and treatment level.
Pore Water Parameter Measurements	Salinity, pH, ammonia, and dissolved organic carbon (DOC) were measured from a single replicate on days 0 and 28.
Chemical Analysis-Overlying Water	All control and treatment levels were analyzed on days 0 and 28 for total [^{14}C]residues using LSC.
Interstitial Water and Sediment Isolation Method	Centrifugation for 30 min at 10,000 *g*.
Chemical Analysis-Interstitial Water	All control and treatment levels were analyzed on days 0 and 28 for total [^{14}C]residues using LSC. In addition, samples from the 450 µg/kg level were analyzed for [^{14}C]esfenvalerate using HPLC/RAM.
Chemical Analysis-Bulk Sediment	All control and treatment levels were analyzed on days 0 and 28 for total [^{14}C]residues using LSC. In addition, samples from the 450 µg/kg level were analyzed for [^{14}C]esfenvalerate using HPLC/RAM.

11. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality	10% - negative control 6% - solvent control
Percent Recovery of Chemical:	Based on QC samples prepared and analyzed concurrently with sample analysis: LSC Sediment: 91.8-105% of nominal Overlying water: 92.1-101% of nominal on day 0 and 73.9-79.9% of nominal on day 28 HPLC/RAM Sediment: 94.7% associated with parent Pore water: 98-100% associated with parent
Data Endpoints	- Survival - Abnormal behavior - Dry weight
Observation Intervals	Daily for survival and abnormal behavior. Growth was determined from surviving organisms at day 28.
Raw data included?	Yes, mean replicate data provided

Effects Data (Reviewer-determined)

Toxicant Concentration [a]				Average Percent Survival, Day 28	Average Dry Weight/ Amphipod, mg
Nominal Sediment, µg ai/kg dw	Mean-measured Sediment, µg ai/kg dw	Mean-measured Pore Water, µg/L	Mean-measured, Overlying Water, µg/L		
Control	<0.82	<0.22	<0.088	90	1.29
Solvent Control	<0.82	<0.22	<0.088	94	1.02
1.9	2.2	<0.22	<0.088	90	0.86*
5.6	5.1	<0.22	<0.088	82	0.88*
17	13	0.18[b]	<0.088	78	0.86*
50	40	0.70	<0.088	89	1.06*
150	125	2.2	0.16	59*	0.78*
450	383	8.2	0.55	0*	--- [c]

[a]) All mean-measured values were based on LSC results of total radioactive residues.
[b] Reviewer-calculated using ½ the LOQ for the day 28 result.
[c] Excluded from statistical analysis due to complete mortality at this treatment level.
* Statistically different (≤0.05) compared to the negative control.

B. Statistical Results (From Study Report)

Endpoints analyzed were amphipod survival and growth (dry weight), both assessed on day 28 data. Analyses were performed with Toxstat Version 3.5 statistical software using the mean replicate organism response in each treatment group rather than individual response values. Survival data were arcsine square-root transformed prior to analysis.

For both endpoints, a t-Test was conducted to compare the performance of the negative and solvent control organisms. As no differences were observed, the data were pooled for subsequent comparisons. The data were then tested for normality using the Shapiro-Wilk's Test and for homogeneity of variance using Bartlett's Test. Growth data were normally distributed and met the assumption for homogeneity, and were analyzed using Williams'

Test to determine the NOAEC and LOAEC values. Survival data met the assumption of normality and failed the assumption of homogeneity, and were therefore analyzed using Wilcoxon's Rank Sum Test.

The Inhibition Concentration Method was used to calculate the 28-day LC/EC_{50} values with associated 95% confidence intervals.

Results were provided in terms of mean-measured sediment concentrations; these values were corrected for the radiopurity of the test material (95.8%) by the reviewer.

Study Author's Statistical Results

Endpoint	Methods	LC/EC_{50} (95% CI) (µg ai/kg dry weight)	NOAEC (µg ai/kg dry weight)	LOAEC (µg ai/kg dry weight)
Survival	ICp Wilcoxon's Rank Sum Test	172 (125-220)	40	125
Growth	ICp Williams' Test	192 (134-220)	40	125

12. VERIFICATION OF STATISTICAL RESULTS

Statistical Method(s): The Day 28 % survival data did not meet the assumptions of ANOVA (normality and homogeneity); therefore, the NOAEC and LOEAC values were determined using the non-parametric Wilcoxon's Rank Sum Test. The Day 28 mean dry weight per amphipod did meet the assumptions of ANOVA; the NOAEC and LOAEC values were determined using Dunnetts and Williams tests via Toxstat Statistical software. The negative and solvent control responses were compared for survival and growth endpoints using a Student's t-test; no significant differences were detected for either endpoint. The Day 28 LC_{50} (and 95% C.I.) value was determined using the moving average method via Toxanal Statistical software as a poor statistical fit was achieved using the Probit method. Differences in the study author's and reviewer's LC_{50} values (172 vs. 113 ug a.i./kg sediment, respectively) likely reflect differences in the statistical methods used (ICp vs. Moving Average). The mean dry weight data were unsuitable for statistical analysis with the Nuthatch and ICp programs. As such, the reviewer was unable to determine a definitive EC_{50} value and therefore reported it as >125 ug a.i/kg sediment due to less than a 50% reduction of growth effects at treatment concentrations lower than this level.. All toxicity values were determined using the mean-measured sediment

concentrations, which had been corrected for the radiopurity (95.8%) by the reviewer. The reviewer expressed the NOAEC and LOAEC based on the mean measured sediment and estimated pore water concentrations. The difference between the study author's NOAEC and LOAEC for growth (40 and 125 ug a.i./kg sediment, respectively) and those of the reviewer (<2.2 and 2.2 ug a.i./kg sediment, respectively) reflects differences in the controls used for comparison (pooled vs. negative control).

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 the concentration of esfenvalerate 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 esfenvalerate in porewater (by nearly two orders of magnitude) than would be expected based on estimated values using sediment esfenvalerate concentrations, its Koc, and sediment total organic carbon (TOC). For highly hydrophobic chemicals like esfenvalerate, 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.

Instead, this reviewer has estimated freely dissolved porewater endpoints based on measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment 4.8% and the mean Koc (251,700 mL/g-OC, MRID 4555102) for esfenvalerate. These estimated porewater 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 Koc values for esfenvalerate vary considerably (85,700 mL/g – 596,200 mL/g.-OC) which likely reflect differences in organic carbon composition and other soil properties used to determine Koc. Therefore, these estimated porewater endpoints are subject to the same uncertainty in determination and application of Koc for esfenvalerate.

Results Synopsis:

Based on mean-measured sediment concentrations (total radioactive residues):

Mortality:

LC_{50}: 113 µg ai/kg dry weight — 95% C.I.: 97-135 µg ai/kg dry weight
NOAEC: 40 µg ai/kg dry weight — Probit Slope: N/A
LOAEC: 125 ug a.i/kg dry weight

Growth (dry weight):

EC_{50}: >125 ug a.i/kg sediment — 95% C.I.: N/A
NOAEC: <2.2 µg ai/kg dry weight — Slope: N/A
LOAEC: 2.2 µg ai/kg dry weight

Based on OC-normalized sediment concentrations (mean measured)

Mortality:

LC_{50}: 2350 ug a.i/kg TOC — 95% C.I: 2020 – 2810 ug a.i/kg TOC
NOAEC: 830 ug a.i/kg TOC — Probit Slope: N/A
LOAEC: 2600 ug a.i./kg TOC

Growth (dry weight):

EC_{50}: >2604 ug a.i/kg TOC — 95% C.I: N/A
NOAEC: <46 ug a.i/kg TOC — Slope: NA
LOAEC: <46 ug a.i/kg TOC

Based on ESTIMATED[1] pore water concentrations:

Mortality:

LC_{50}: 0.009 ug a.i/L — 95% C.I: 0.008 ug a.i/L – 0.01 ug a.i/L
NOAEC: 0.003 ug a.i/L — Probit Slope: N/A
LOAEC: 0.01 ug a.i./L

Growth (dry weight):

EC_{50}: >0.010 ug a.i/L — 95% C.I: N/A
NOAEC: <0.0002 ug a.i/L — Slope: NA
LOAEC: 0.0002 ug a.i/L

[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*)]

Endpoints affected: survival and growth
Most sensitive endpoint(s): growth (based on the NOAEC value)

13. REVIEWER'S COMMENTS:

The review notes minor deviations from the recommended test methods that included the percent moisture content of the sediment not being specified and the pore water temperature measurements not being taken. These measurements are recommended at test initiation and termination. These deviations do not impact the acceptability of the study. The reviewer's analyses were conducted using only the negative control, whereas the study author's analyses were conducted using a pooled control. Additionally, the conclusions were based on the mean measured sediment concentrations. Therefore, the reviewer's results are reported in the Conclusions section of this DER and are all reviewer calculated. The study author was able to determine an EC_{50} value based on dry weight, while the reviewer was not. As the Probit method was unsuitable for the LC_{50} analysis because of poor goodness of fit, the reviewer used the moving average method. A 28-day EC_{50} for growth was determined to be >125 ug a.i/kg sediment based on a less than a 50% reduction in growth at all treatment levels below this level tested. Furthermore, the highest treatment level was excluded from the statistical analysis for the growth endpoint due to complete mortality in these treatment levels.

In this 28-day sediment toxicity study, 400 uL of fresh dilution water (not spiked with test material) replaced 400 uL of previously added overlying water three times per week. Care was taken when siphoning the water off as to not disturb the sediment layer beneath the overlying water. Following replacement of the overlying water, the food ration for that day was added to each vessel. The Day 0 measured overlying water concentrations were <0.088 (<LOQ), <0.088, <0.088, <0.088, <0.088, 0.13, and 0.42 ug a.i/L while the Day 28 measured concentrations were <0.085, <0.087, <0.087, <0.087, <0.087, 0.19, and 0.68 for the negative control and mean measured spiked sediment 2.2, 5.1, 13, 40, 125, and 383 ug a.i/kg dry sediment concentrations. The reviewer-determined mean measured overlying water concentrations were <0.087 (<LOQ), <0.87, <0.087, <0.087, <0.087, 0.16, and 55 ug a.i/L (average of the Day 0 and Day 28 measured concentrations). This particular type of test is designed to examine the effects of esfenvalerate to sediment dwelling organisms through pore water and sediment exposure, and the overlying water treatment concentrations are not the focus of this study.

Due to the significant reductions at all treatment levels regarding mean dry weigh per amphipod, a NOAEC was not determined for this, the most sensitive, endpoint.

For the definitive test (MRID 46620401), six individual dosing stock solutions were prepared in acetone for application to the test material to the sediment. These stock solutions were prepared using radiolabeled test material according to the following preparation scheme:

Conc. of Radiolabeled Stock Used (µg/mL)	Volume of Radiolabeled Stock Used (mL)	Diluted to Final Volume with Acetone (mL)	Dosing Stock Concentration (mg/mL)	Percent Radiolabeled (%)
236	4.8	25	46	100

46	3.3	10	15	100
46	1.1	10	5.1	100
46	0.38	10	1.7	100
46	0.124	10	0.57	100
46	0.042	10	0.19	100

All dosing stock 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 (0.8484 kg dry weight based on a percent of solids of 43.42%) in individual 1-gallon jars. The total mass of sediment spiked on a dry weight basis for each treatment level and control was 0.9148 kg (0.0500 kg sand and 0.8684 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 29 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.

A 28-day preliminary test was conducted with non-radiolabeled esfenvalerate (purity of 99.7%) at nominal treatment levels of 0 (negative and solvent controls), 0.15, 1.5, 15, 150, and 1500 µg ai/kg dw sediment. Three replicate vessels containing 20 amphipods each were exposed; otherwise, methods followed those described for the definitive study. Due to variable results, replicate A of the 0.15 µg ai/kg treatment and control levels was not used to calculate mean survival and mean dry weight. After 28 days of exposure, 73, 80, 42, 25, and 0% survival was observed among amphipods exposed to the 0.15, 1.5, 15, 150, and 1500 µg ai/kg treatment levels, respectively. In comparison, 88 and 98% survival was observed in the negative and solvent control groups, respectively. Dry weight among control amphipods averaged 1.74 and 2.09 mg for the negative and solvent control groups, respectively, compared to 0.73, 1.22, 1.36, and 0.74 mg for the 0.15, 1.5, 15, and 150 µg ai/kg treatment levels, respectively (100% mortality observed at the 1500 µg ai/kg level).

The [^{14}C]esfenvalerate had an initial radiopurity of 66.5%, and was purified to a radiopurity of 95.8% prior to study initiation.

This study was conducted in compliance with the U.S. EPA GLP regulations with the following exceptions: routine water, sediment and food contaminant screen analyses for pesticides, PCBs and toxic metals. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.

In-life dates were May 13 – June 10, 2005.

14. REFERENCES:

ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Bousfield, E.L. 1973. Shallow Water Gammeridean Amphipoda of New England. Ithaca, NY: Cornell University Press. Pp. 312.

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

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

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

Laskowski, D.A. 2002. Physical and Chemical Properties of Pyrethroids. Reviews Environmental Contaminant Toxicology 174: 49-170.

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

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

U.S. EPA. 2001. Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod Leptocheirus plumulosus. Office of Research and Development. Washington, DC. EPA/600/R-01/020.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act. Good Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, DC.

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

Weber, C.I., *et al.* 1989. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms.* 2nd edition. EPA/600/4-89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Wilcoxon, F. 1945. Individual comparisons by ranking methods. *Biom. Bull.* 1:80-83.

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

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

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

Mean % Survival, Day 28, ug ai/kg dry wt
File: 0401ps Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	90.0000	CALCULATED t VALUE =	-0.6911
GRP2 (BLANK CRTL) MEAN =	94.0000	DEGREES OF FREEDOM =	8
DIFFERENCE IN MEANS =	-4.0000		

TABLE t VALUE (0.05 (2), 8) = 2.306 **NO significant difference at alpha=0.05**
TABLE t VALUE (0.01 (2), 8) = 3.355 **NO significant difference at alpha=0.01**

Mean % Survival, Day 28, ug ai/kg dry wt
File: 0401PS Transform: NO TRANSFORMATION

WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	neg control	90.000				
2	2.2	90.000	27.50	16.00	5	
3	5.1	82.000	22.00	16.00	5	
4	13	78.000	21.50	16.00	5	
5	**40**	**89.000**	**26.50**	**16.00**	**5**	
6	125	59.000	16.00	16.00	5	*
7	383	0.000	15.00	16.00	5	*

Critical values use k = 6, are 1 tailed, and alpha = 0.05

Mean dry weight/amph. (mg), day 28, ug ai/kg dry wt
File: 0401dw Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.2920	CALCULATED t VALUE =	2.2541
GRP2 (BLANK CRTL) MEAN =	1.0220	DEGREES OF FREEDOM =	8
DIFFERENCE IN MEANS =	0.2700		

TABLE t VALUE (0.05 (2), 8) = 2.306 **NO significant difference at alpha=0.05**
TABLE t VALUE (0.01 (2), 8) = 3.355 **NO significant difference at alpha=0.01**

Mean dry weight/amph. (mg), day 28, ug ai/kg dry wt
File: 0401dw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.883	0.177	2.493
Within (Error)	24	1.707	0.071	
Total	29	2.590		

Critical F value = 2.62 (0.05,5,24)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Mean dry weight/amph. (mg), day 28, ug ai/kg dry wt
File: 0401dw Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	1.292	1.292		
2	2.2	0.864	0.864	2.540	*
3	5.1	0.876	0.876	2.469	*
4	13	0.864	0.864	2.540	*
5	40	1.060	1.060	1.377	
6	125	0.784	0.784	3.014	*

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=24,5)

Mean dry weight/amph. (mg), day 28, ug ai/kg dry wt
File: 0401dw Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	5			
2	2.2	5	0.398	30.8	0.428
3	5.1	5	0.398	30.8	0.416
4	13	5	0.398	30.8	0.428
5	40	5	0.398	30.8	0.232
6	125	5	0.398	30.8	0.508

Mean dry weight/amph. (mg), day 28, ug ai/kg dry wt
File: 0401dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	5	1.292	1.292	1.292
2	2.2	5	0.864	0.864	0.916
3	5.1	5	0.876	0.876	0.916
4	13	5	0.864	0.864	0.916
5	40	5	1.060	1.060	0.916
6	125	5	0.784	0.784	0.784

Mean dry weight/amph. (mg), day 28, ug ai/kg dry wt
File: 0401dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	1.292				
2.2	0.916	2.229	*	1.71	k= 1, v=24
5.1	0.916	2.229	*	1.79	k= 2, v=24
13	0.916	2.229	*	1.82	k= 3, v=24
40	0.916	2.229	*	1.83	k= 4, v=24
125	0.784	3.011	*	1.84	k= 5, v=24

s = 0.267
Note: df used for table values are approximate when v > 20.

Program: Nuthatch Date: 1/31/11

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	1.29	.		
2.2	0.916	2.229	0.018	*
5.1	0.916	2.229	0.02	*
13	0.916	2.229	0.021	*
40	0.916	2.229	0.022	*
125	0.784	3.011	<0.005	*

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

!!!Failure#1: near-singular matrix, model possibly unsuitable.
```

LC50-PERCENT SURVIVAL

```
THE NUMBER OF ORGANISMS USED IS TOO LARGE TO ALLOW CALCULATION
OF THE BINOMIAL PROBABILITY.  THE LC50 CALCULATIONS ARE UNAFFECTED.


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

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	1.538573E-02	113.3985	96.66426-135.0166

```
RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS    G              H            GOODNESS OF FIT PROBABILITY
 4             .9549075      19.49509      0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

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

SLOPE    =     1.525756
95 PERCENT CONFIDENCE LIMITS  = 3.479695E-02     AND    3.016716

LC50 =         111.6501
95 PERCENT CONFIDENCE LIMITS = 16.6587 AND  1.113517E+12

LC10 =         16.42423
95 PERCENT CONFIDENCE LIMITS = 3.634299E-26 AND  61.57737
```