TEXT SEARCHABLE DOCUMENT 2011

DP Barcode: D319265 MRID No.: 465915-01

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

1. CHEMICAL: Bifenthrin PC Code: 128825

2. TEST MATERIAL: [^{14}C]Bifenthrin Radiochemical Purity: 96.4%

3. CITATION:

Authors: Putt, A.E.
Title: Bifenthrin – Toxicity to Estuarine Amphipods (*Leptocheirus plumulosus*) During a 28-Day Sediment Exposure.
Study Completion Date: June 29, 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: 13656.6107
MRID No.: 465915-01

4. REVIEWED BY: Justin Housenger, Biologist, OPP/EFED/ERB 5

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

2086346

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 and pore water (total radioactive residues)

6. CONCLUSIONS:

The 28-day toxicity study of [^{14}C] bifenthrin to the estuarine amphipod *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. Reproduction is a required endpoint, yet was not assessed.

The nominal spiked sediment test concentrations were 0 for the solvent and negative control (acetone, 9 ml per 0.8330 kg sediment (dry weight basis), 5.6, 17, 50, 150, 450, and 1350 µg a.i/kg sediment. Measured concentrations in the sediment at Day-0 (excluding controls) were 5.5, 20, 51, 140, 460, and 1500 µg a.i/kg sediment, respectively and at test termination n Day-28 were measured at 5.4, 20, 49, 120, 420, and 1500 µg a.i/kg sediment, respectively. Mean percent recoveries based on the nominal values were 97, 120, 100, 89, 98, and 110%, respectively.

Mean measured pore water analysis was not definitive as Day-0 measurements yielded the lowest treatment level below the assay limit of quantitation (LOQ) while the Day-28 measurements yielded the two lowest treatment levels below LOQ. Overlying water mean measured concentration analysis is determined to be trivial relative to porewater and bulk sediment exposures as the testing apparatus ensures volume replacement three times weekly, and it is the sediment, not the overlying water, that is spiked with bifenthrin. 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 per EFED guidance (Frankenberry *et al.*, 2008). In ascending order of the treatment levels (including the negative and solvent controls), the percent survival was 94, 95, 98, 98, 96, 75, 0 and 0%. The two highest treatment levels (440 and 1500 µg a.i/kg sediment) showed statistically significant ($p<0.05$) differences from the negative control. The 28-day NOAEC, LOAEC for survival based on mean measured sediment concentrations were 50 and 130 µg a.i/kg sediment, respectively. The 28-day LC_{50} for survival was 168 µg a.i/kg sediment (95% CI of 146 and 195 µg a.i/kg sediment). A 28-day EC_{50} for growth was determined to be >130 ug a.i/kg sediment based on less than a 50% reduction in growth at all treatment levels below this level tested. Furthermore, the two highest treatment levels were excluded from the statistical analysis for the growth endpoint due to complete mortality in these treatment levels.

This reviewer notes that HPLC/RAM analysis of bifenthrin concentrations <u>in pore water</u> (conducted only at the highest test concentration) indicate that the parent material was only a

fraction of total radioactive residues measured over the course of this study 30.6-69% for initial and terminal measurements. In contrast, the recovery of parent compound from bulk sediment was generally high >99% 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 bifenthrin 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 (4.1%) and the mean K_{OC} (236,800 L/kg-OC; MRID 00141203) for bifenthrin (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 bifenthrin vary considerably (131,000 to 302,000) 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 bifenthrin.

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). This study is was able to achieve a NOAEC for both the survival and growth endpoints; however reproduction is a required endpoint based on the above study guidelines and was not assessed in this study. The most sensitive endpoint in this study was growth based on a lower EC_{50}, although the NOAEC for growth and mortality were identical. This study still provides useful information that can be used in risk assessments (e.g., chronic effects on amphipod survival and growth). It is classified as SUPPLEMENTAL.

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

Mortality:

LC_{50}: 168 µg a.i./kg sediment
NOAEC: 50 µg a.i/kg sediment
LOAEC 130 µg a.i./kg sediment

95% C.I.: 146-195 µg a.i./kg sediment
Probit Slope: N/A

Growth:

EC_{50}: > 130 µg a.i./kg sediment
NOAEC: 50 µg a.i./kg sediment
LOAEC: 130 µg a.i./kg sediment

95% C.I.: N/A
Slope: N/A

Based on ESTIMATED[1] pore water concentrations (total radioactive residues):

Mortality:

LC_{50}: 0.017 µg a.i./L
NOAEC: 0.005 µg a.i./L
LOAEC: 0.013 µg a.i./L

95% C.I.: 0.015 – 0.020 µg a.i./L
Probit Slope: N/A

Growth:

EC_{50}: >0.013 µg a.i./L
NOAEC: 0.005 µg a.i./L
LOAEC: 0.013 µg a.i./L

95% C.I.: N/A
Slope: N/A

Based on OC-normalized sediment concentrations (mean measured):

Mortality:

LC_{50} mortality: 4100 µg a.i/kg TOC
NOAEC (mortality): 1220 µg a.i/kg TOC
LOAEC (mortality): 3170 µg a.i/kg TOC

95% C.I.: 3560- 4760 µg a.i./kg
Probit Slope: N/A

Growth:

EC_{50} growth: >3170 µg a.i/kg TOC
NOAEC (growth): 1220 µg a.i/kg TOC
LOAEC (growth): 3170 µg a.i/kg TOC

95% C.I.: N/A
Slope: N/A

[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 and survival (based on the same 28-day NOAEC)

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. However, this study was able to achieve a NOAEC for both the survival and growth endpoints and is considered scientifically sound for those endpoints. The NOAEC for growth and mortality were identical. 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. Reparability: This study is not repairable as a new study will need to be conducted with reproduction as an endpoint.

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 according to the ORD testing guidelines but was not measured in this study.

2. Neonate amphipods were acclimated and tested under differing temperatures. The acclimation temperature for 48 hours prior to test initiation was 20°C, and the testing temperature was 25 ± 2°C.

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]Bifenthrin remained predominantly associated with the sediment and was stable in sediment under the conditions of the study. Mean percent recoveries of total radioactive residues (based on LSC analyses) were 89-120% of nominal concentrations. On days 0 and 28 at the 1350 µg ai/kg level (the only level analyzed by HPLC/RAM), 99.7% of the recovered radioactivity was parent material.

Less than an average of 15µg/L was detected in the pore water during the study (based on LSC), with mean recoveries of <0.20 µg/L at the 5.6 µg/kg level increasing to 14 µg/L at the 1350 µg/kg level. Of the total radioactivity recovered from the 1350 µg/kg level, <70% of the recovered was identified as [^{14}C]bifenthrin (based on HPLC/RAM analysis), with specific recoveries of 30.6 and 69.0% as parent compound on days 0 and 28, respectively. 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 Bifenthrin.

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 at 20°C 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)
Food	During holding and acclimation, amphipods were fed daily a finely-ground suspension of Zeigler Prime flakes fish food (i.e., 100 mg/ml).
Health of parent culture stock	No mortality observed in the population 48 hours prior to test initiation.

B. Test System

Guideline Criteria	Reported Information

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 ppt and a pH of 7.7-7.9 with laboratory well water.
Renewal of overlying water	3 times per week (Monday, Wednesday, and Friday), 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, 19% silt, and 13% clay pH: 6.9 Ammonia (as N) in pore water: 21.5 mg/L TOC: 4.1% Percent water content (1/3 bar): 39% Grain size: 32% silt/clay
Test Material	[^{14}C]Bifenthrin Description: not reported Lot no.: 021H9236 CAS No.: not reported Position of label: phenyl ring Radiochemical purity: 96.4% Specific activity: 24.4 mCi/mmole (128,208 dpm/µg) Storage: in the freezer (<-4°C) Aqueous solubility: Not reported. According to Laskowski (2002) the solubility is low, 0.0000140 mg/L or 0.0140 ppb.

Guideline Criteria	Reported Information
Solvents	Acetone, 9 ml per 0.8330 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. An appropriate 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 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 217 g wet weight or 84 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.
Water Temperature	Overlying water: 23-27°C Pore water: not determined
pH	Overlying water: 7.3-8.1 Pore water: 6.6-7.1
Salinity	Overlying water: 19-21‰ Pore water: 20-24‰
Ammonia (as N)	Overlying water: 2.8-3.1 mg/L on day 0 and ≤0.17 mg/L on day 28 Pore water: 15.2-18.2 mg/L on day 0 and 1.6-3.4 mg/L on day 28
Dissolved Organic Carbon	Pore water: 11.5-60.7 mg/L
Dissolved Oxygen	5.0-6.2 mg/L (>60% saturation)

Guideline Criteria	Reported Information
Photoperiod	16 hours light, 8 hours dark (620-950 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 **Mean-Measured Concentrations**	Negative control, solvent control, 5.6, 17, 50, 150, 450, and 1350 µg ai/kg dw sediment Selection of nominal treatment levels for the definitive study was based on results from preliminary testing. <0.73 (controls), 5.4, 20, 50, 130, 440, and 1500 µg total [^{14}C]bifenthrin 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 (solvent 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 1350 µg/kg level were analyzed for [^{14}C]bifenthrin 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 1350 µg/kg level were analyzed for [^{14}C]bifenthrin 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	6% - negative control 5% - solvent control
Percent Recovery of Chemical:	Based on QC samples prepared and analyzed concurrently with sample analysis: LSC Sediment: 97.2-102% of nominal Overlying water: 95.3-124% of nominal HPLC/RAM Sediment: 98.8% associated with parent Pore water: 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/kg dw	Mean-measured Sediment, µg/kg dw	Mean-measured Pore Water, µg/L	Mean-measured, Overlying Water, µg/L		
Control	<0.73	<0.20	<0.077	94	2.61
Solvent Control	<0.73	<0.20	<0.077	95	2.64
5.6	5.4	<0.20	<0.077	98	1.86*
17	20	0.20 [b]	<0.077	98	2.76
50	50	0.50	<0.077	96	2.35
150	130	1.4	0.066 [b]	75*	1.41*
450	440	4.8	0.24	0*	--- [c]
1350	1500	14	0.66	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 of growth 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. Although both sets of data were normally

distributed, survival data did not meet the assumption for homogeneity. Therefore, survival data were analyzed using Wilcoxon's Rank Sum Test, and growth data were analyzed using Bonferroni's Test to determine the NOAEC and LOAEC values.

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.

Study Author's Statistical Results

Endpoint	Methods	LC/EC_{50} (95% CI) (µg/kg)	NOAEC (µg/kg)	LOAEC (µg/kg)
Survival	ICp Wilcoxon's Rank Sum Test	240 (220-260)	50	130
Growth	ICp Bonferroni's Test	150 (130-180)	50	130

12. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Data for survival and dry weight were tested using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett tests for homogeneity of variances. Data for survival did not satisfy these assumptions, so the NOAEC and LOAEC were determined using the non-parametric Steele's many-one rank test. Data for dry weight satisfied the assumptions of ANOVA, so the NOAEC and LOAEC were determined using this test, followed by William's multiple comparison test. For both endpoints, the solvent control data were compared to the negative control data using a Student's t-test. No significant differences were detected and the negative control group was used for comparison to the treatment groups. These analyses were conducted using Toxstat statistical software. The LC_{50} was calculated using the Moving Average method, as poor statistical fit was achieved using the Probit method. Differences in the study author's and reviewer's LC_{50} values (240 and 168 ug a.i./kg sediment, respectively) likely reflect differences in the statistical methods used (ICp vs. Moving Average). The EC_{50} was not bracketed by the doses that were evaluated and therefore a suitable EC_{50} for growth could not be determined with the Nuthatch and ICp programs. The reviewer expressed the NOAEC and LOAEC based on the mean measured sediment and estimated pore water

concentrations.

This reviewer notes that HPLC/RAM analysis of bifenthrin concentrations in pore water (conducted only at the highest test concentration) indicate that the parent material was only a fraction of total radioactive residues measured over the course of this study 30.6-69% for initial and terminal measurements. In contrast, the recovery of parent compound from bulk sediment was generally high >99% 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 bifenthrin 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 (4.1%) and the mean K_{OC} (236,800 L/kg-OC; MRID 00141203) for bifenthrin (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 bifenthrin vary considerably (131,000 to 302,000) 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 bifenthrin.

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

Mortality:

LC_{50}: 168 µg a.i./kg sediment
NOAEC: 50 µg a.i/kg sediment
LOAEC 130 µg a.i./kg sediment

95% C.I.: 146-195 µg a.i./kg sediment
Probit Slope: N/A

Growth:

EC_{50}: >130 µg a.i./kg sediment
NOAEC: 50 µg a.i./kg sediment
LOAEC: 130 µg a.i./kg sediment

95% C.I.: N/A
Slope: N/A

Based on ESTIMATED[1] pore water concentrations (total radioactive residues):

Mortality:

LC_{50}: 0.017 µg a.i./L
NOAEC: 0.005 µg a.i./L

95% C.I.: 0.015 – 0.020 µg a.i./L
Probit Slope: N/A

LOAEC: 0.013 µg a.i./L

Growth:
EC_{50}: >0.013 µg a.i./L 95% C.I.: N/A
NOAEC: 0.005 µg a.i./L Slope: N/A
LOAEC: 0.013 µg a.i./L

Based on OC-normalized sediment concentrations (mean measured):
Mortality:
LC_{50} mortality: 4100 µg a.i/kg TOC 95% C.I.: 3560- 4760 µg a.i./kg
NOAEC (mortality): 1220 µg a.i/kg TOC Probit Slope: N/A
LOAEC (mortality): 3170 µg a.i/kg TOC

Growth:
EC_{50} growth: >3170 µg a.i/kg TOC 95% C.I.: N/A
NOAEC (growth): 1220 µg a.i/kg TOC Slope: N/A
LOAEC (growth): 3170 µg a.i/kg TOC

[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 and survival (based on the same 28-day NOAEC)

13. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the NOAEC and LOAEC values were identical to the study author's; however, the reviewer was unable to use the Probit method to verify the study author's LC_{50} estimates. New estimates of the LC_{50} using the negative control are provided in the results synopsis and conclusion sections. As the probit method was unsuitable for 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 >130 ug a.i/kg sediment based on less than a 50% reduction in growth at all treatment levels below this level tested. Furthermore, the two highest treatment levels were excluded from the statistical analysis for the growth endpoint due to complete mortality in these treatment levels. The NOAEC and LOAEC for this endpoint were also 50 and 130 µg a.i/kg dw sediment. Additionally, the reviewer expressed the NOAEC and LOAEC for survival and growth endpoints based on mean measured sediment concentrations as well as estimated pore water concentrations. For the reasons outlined previously in this DER, the study reviewer is expressing these endpoints using estimated pore water concentrations rather than mean measured pore water concentrations. All toxicity values reported in the CONCLUSIONS and RESULTS SYNOPSIS section of this DER were reviewer-determined.

In this 28 day test, 400 uL fresh dilution water (not spiked with test material) replaced 400 uL of existing overlying water b siphoning three times weekly. Care was taken not to disturb the sediment layer underneath. The Day 0 overlying water concentrations were <0.076 (<LOQ), <0.076, <0.076, <0.076, 0.093, 0.33, and 0.96 while the Day 28 measured concentrations were <0.077, <0.076, <0.077, <0.077, <0.077, 0.14, and 0.35 for the negative control and the mean-measured spiked sediment 5.4, 20, 50, 130, 440, and 1500 ug/kg dry sediment concentrations. The reviewer-determined mean-measured overlying water concentrations were <0.076 (<LOQ), <0.076, <0.076, <0.076, <0.085, 0.24, and 1.31 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 bifenthrin to sediment dwelling organisms through pore water and sediment exposure, and the overlying water treatment concentrations are not the focus of this study.

For the definitive test (MRID 46591501), six individuals dosing stock solutions were prepared in acetone for the application of 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 (%)
408	7.65	25	125	100
125	3.33	10	42	100
125	1.11	10	14	100
125	0.37	10	4.6	100
125	0.13	10	1.6	100
125	0.415	10	0.52	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 (0.7830 kg dry weight based on a percent of solids of 39.15%) in individual 1-gallon jars. The total mass of sediment spiked on a dry weight basis for each treatment level and control was 0.8330 kg (0.0500 kg sand and 0.7830 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.

On Day 0 and Day 28, sediment and pore water samples from the nominal 1350 µg a.i./kg dry sediment level were analyzed by HLPLC/RAM to determine the percent of [^{14}C]residues associated with the parent test material (measured concentrations dry sediment as bifenthrin equivalents). Recoveries were 99.7% from the sediment, µg/kg samples on Day 0 and Day 28,. **Recoveries were 30.6% and 69.0% from the pore water samples on Day 0 and Day 28, respectively, which indicates that a substantive portion of the LSC measured residues in pore water was not parent compound (bifenthrin).**

A 28-day preliminary test was conducted with non-radiolabeled bifenthrin (purity of 93.0%) at nominal treatment levels of 0 (negative and solvent controls), 0.070, 0.70, 7.0, 70, and 700 µg ai/kg dw sediment. Three replicate vessels containing 20 amphipods each were exposed; otherwise, methods followed those described for the definitive study. After 28 days of exposure, 85, 80, 100, 85, and 0% survival was observed among amphipods exposed to the 0.070, 0.70, 7.0, 70, and 700 µg ai/kg treatment levels, respectively. In comparison, 97 and 93% survival was observed in the negative and solvent control groups, respectively. Dry weight among control amphipods averaged 2.08 and 2.37 mg for the negative and solvent control groups, respectively, compared to 1.83, 2.18, 2.31, and 1.52 mg for the 0.070, 0.70, 7.0, and 70 µg a.i/kg treatment levels, respectively (100% mortality observed at the 700 µg a.i/kg level).

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 February 3 to March 3, 2005.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

```
survival (sediment)
File: 1501s        Transform: NO TRANSFORM

       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
-----------------------------------------------------------------------------
-
 GRP1 (SOLVENT CRTL) MEAN =    94.0000     CALCULATED t VALUE =    -0.3430
 GRP2 (BLANK CRTL) MEAN   =    95.0000     DEGREES OF FREEDOM =     8
 DIFFERENCE IN MEANS      =    -1.0000
-----------------------------------------------------------------------------
-
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


survival (sediment)
File: 1501s        Transform: NO TRANSFORM

          STEELS MANY-ONE RANK TEST       -       Ho:Control<Treatment
-----------------------------------------------------------------------------
                                TRANSFORMED    RANK     CRIT.
GROUP    IDENTIFICATION           MEAN         SUM     VALUE     df    SIG
----- --------------------     -----------   -------   ------   -----  ---
  1            neg control      94.000
  2                    5.4      98.000        34.00     16.00    5.00
  3                     20      98.000        34.50     16.00    5.00
  4                     50      96.000        31.50     16.00    5.00
  5                    130      75.000        16.00     16.00    5.00   *
  6                    440       0.000        15.00     16.00    5.00   *
  7                   1500       0.000        15.00     16.00    5.00   *
-----------------------------------------------------------------------------
  Critical values use k = 6, are 1 tailed, and alpha = 0.05


Estimates of EC%
-----------------------------------------------------------------------
Parameter   Estimate     95% Bounds        Std.Err.  Lower Bound
                       Lower     Upper                /Estimate
EC5          1.2E+02  2.2E-308     +INF   2.9E+04  1.9E-310
EC10         1.2E+02  2.2E-308     +INF   1.7E+04  1.8E-310
EC25         1.3E+02  2.2E-308     +INF   2.9E+03  1.7E-310
EC50         1.4E+02  2.2E-308     +INF   2.5E+04  1.6E-310

         Slope =     18.3  Std.Err. = 1.09e+07


Goodness of fit: p =      0.80  based on DF=       4.0       28.
-----------------------------------------------------------------------
1501S : survival (sediment)
-----------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
-----------------------------------------------------------------------
```

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	5.00	94.0	96.5	-2.50	100.	0.00
5.40	5.00	98.0	96.5	1.50	100.	2.95e-14
20.0	5.00	98.0	96.5	1.50	100.	2.95e-14
50.0	5.00	96.0	96.5	-0.500	100.	2.95e-14
130.	5.00	75.0	75.0	-1.56e-13	77.7	22.3
440.	5.00	0.00	2.33e-17	-2.33e-17	2.42e-17	100.
1.50e+03	5.00	0.00	5.89e-76	-5.89e-76	6.10e-76	100.

dry weight (sediment)
File: 1501d Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN = 2.6060 CALCULATED t VALUE = -0.2367
GRP2 (BLANK CRTL) MEAN = 2.6420 DEGREES OF FREEDOM = 8
DIFFERENCE IN MEANS = -0.0360

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

dry weight (sediment)
File: 1501d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	6.212	1.553	21.569
Within (Error)	20	1.448	0.072	
Total	24	7.660		

Critical F value = 2.87 (0.05,4,20)
Since F > Critical F REJECT Ho:All groups equal

dry weight (sediment)
File: 1501d 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	2.606	2.606		
2	5.4	1.862	1.862	4.384	*
3	20	2.760	2.760	-0.907	
4	50	2.350	2.350	1.508	
5	130	1.408	1.408	7.059	*

Dunnett table value = 2.30 (1 Tailed Value, P=0.05, df=20,4)

dry weight (sediment)
File: 1501d 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	5.4	5	0.390	15.0	0.744
3	20	5	0.390	15.0	-0.154
4	50	5	0.390	15.0	0.256
5	130	5	0.390	15.0	1.198

dry weight (sediment)
File: 1501d 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	2.606	2.606	2.606
2	5.4	5	1.862	1.862	2.324
3	20	5	2.760	2.760	2.324
4	50	5	2.350	2.350	2.324
5	130	5	1.408	1.408	1.408

dry weight (sediment)
File: 1501d 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	2.606				
5.4	2.324	1.657		1.72	k= 1, v=20
20	2.324	1.657		1.81	k= 2, v=20

```
                 50      2.324     1.657              1.83       k= 3, v=20
                130      1.408     7.040      *       1.85       k= 4, v=20
-----------------------------------------------------------------------------
s =    0.269
Note: df used for table values are approximate when v > 20.


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

     kgs  bifenthrin  28-D SEDIMENT
     ***************************************************************************
     CONC.     NUMBER        NUMBER        PERCENT       BINOMIAL
               EXPOSED       DEAD          DEAD          PROB.(PERCENT)
      1500      94            94            100           0
      440       94            94            100           0
      130       94            19            20.2128       0
      50        100           4             4             0
      20        100           2             2             0
      5.4       100           2             2             0

     THE BINOMIAL TEST SHOWS THAT 130 AND 440 CAN BE
     USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
     CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
     ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.


     AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 187.5119


     RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
        SPAN     G            LC50          95 PERCENT CONFIDENCE LIMITS
      4        9.400663E-03    168.4454      146.2311      194.9723


     RESULTS CALCULATED USING THE PROBIT METHOD
     ITERATIONS             G              H            GOODNESS OF FIT
PROBABILITY
         6                  25.04049      534.2074                    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    =               2.773531
     95 PERCENT CONFIDENCE LIMITS =-11.10535    AND    16.65241

     INTERCEPT=-6.151197

     LC50 =     165.1282
     95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

              LC25 =          94.32563
     95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
```

```
          LC10 =          56.98216
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

          LC05 =          42.14547
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
*************************************************************************
```