

US EPA ARCHIVE DOCUMENT

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

- 1. **CHEMICAL:** Cypermethrin PC Code: 109702
- 2. **TEST MATERIAL:** [¹⁴C]Cypermethrin Radiochemical Purity: 99.8%
- 3. **CITATION:**

Authors: Putt, A.E.

Title: Cypermethrin – 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: 13656.6111

MRID No.: 465915-03

- 4. **APPROVED BY:** Justin Housenger, Biologist OPP/EFED/ERB5

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

Amanda Solliday, Biologist OPP/EFED/ERB5

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

Keith Sappington, Senior Advisor OPP/EFED/ERB5

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 and pore water (total radioactive residues)

6. CONCLUSIONS:

The 28-day toxicity of cypermethrin 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 test concentrations were 0 for the negative and solvent (acetone) controls, 1.9, 5.6, 17, 50, 150, and 450 µg a.i/kg dw sediment. Measured concentrations at Day-0 (excluding controls) were 2.0, 6.2, 21, 54, 190, and 560 ug a.i/kg sediment, and on day 28, the concentrations were measured at 0.89, 2.7, 8.9, 24, 82, and 260 ug a.i/kg, respectively. The mean measured concentrations that were therefore defined as the test concentration throughout the course of the study were 1.4, 4.5, 15, 39, 130, and 410 ug a.i/kg sediment, respectively.

The study author pooled the negative and solvent control for statistical analysis for the survival endpoint and after determining a statistical difference between the negative and solvent control for the growth endpoint, used the solvent control for statistical analysis. For this evaluation, the reviewer will use the negative control only for both survival and growth statistical analysis, 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 84, 76, 94, 86, 83, 55, 15, and 1%. The three highest treatment levels (39, 130, and 410 ug a.i/kg sediment) showed statistically significant differences ($p < 0.05$) from the negative control. The 28-day NOAEC, LOAEC, and LC₅₀ based on mean measured sediment concentrations were 15, 39 and 61 ug/kg, respectively. A 28-day EC₅₀ for growth was determined to be 99 ug a.i/kg sediment (77 – 125) using the ICp program. Note that the highest treatment level was excluded from the statistical analysis for the growth endpoint due to a lack of sufficient sample size to provide a representative growth response due to almost complete mortality (99%) in this treatment level. The OC-normalized NOAEC, LOAEC, and LC₅₀ for mortality were 366, 951, and 1488 ug/kg TOC based on 4.1 % organic carbon in the test sediment.

The reviewer noted a statistically significant reduction in growth (dry weight) in the solvent control compared to the negative control. Growth in the treatments was also significantly reduced relative to the negative control. Although ordinarily these results would be suggestive of a solvent effect in a water column test system, such an effect appears highly unlikely in this test because solvent was evaporated on silica sand prior to mixing with sediment. Further, all treatments including the controls contained the same amount of silica sand, thus indicating that

this is not an effect of the addition of sand. Other water and sediment quality characteristics are similar among the solvent and negative controls. Lastly, this reviewer notes that no statistically significant differences between negative and solvent controls were reported for three other 28-d tests with *Leptocheirus* conducted by the same laboratory using the same sediment and over the same time period. Therefore, in considering these lines of evidence, this reviewer concludes the statistical difference between the solvent and negative controls is not likely a result of solvent addition. These differences may reflect natural biological variability in growth and/or other unexplained influences. As a result, they cast some uncertainty on interpretation of the test results, though not to a level that would invalidate the test results in this reviewer's professional opinion. According to EFED policy, all comparisons were made to the negative control.

This reviewer notes that HPLC analysis of cypermethrin 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 (3.3% for both the initial and terminal measurements). In contrast, the recovery of parent compound from bulk sediment was generally high (92% for both the initial and terminal measurements). 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 desorption 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 cypermethrin 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 mean measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (4.1%) and the mean Koc (141,700 L/kg-OC, MRID 42129002) for cypermethrin. 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 cypermethrin vary considerably (20,800 – 328,500 L/kg) 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 cypermethrin.

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 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 uncertain based on the inability of a definitive NOAEC to be determined for growth. 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.

Results Synopsis:

Based on mean-measured sediment concentrations:

Mortality:

LC ₅₀ : 61 µg a.i/kg sediment	95% C.I.: 49 – 78 ug a.i/kg sediment
NOAEC: 15 µg a.i/kg sediment	Probit Slope: N/A
LOAEC: 39 µg a.i/kg sediment	

Growth (dry weight):

EC ₅₀ : 99 µg a.i/kg sediment	95% C.I.: 77 – 125 ug a.i/kg sediment
NOAEC: <1.4 µg a.i/kg sediment	Slope: N/A
LOAEC: 1.4 µg a.i/kg sediment	

Based on ESTIMATED¹ Pore Water Concentrations:

Mortality:

LC ₅₀ : 0.011 µg a.i/L	95% C.I.: 0.008 – 0.013 ug a.i/L
NOAEC: 0.003 µg a.i/L	Probit Slope: N/A
LOAEC 0.007 µg a.i /L	

Growth (dry weight):

EC ₅₀ : 0.017 µg a.i/L	95% C.I.: 0.013 – 0.022 ug a.i/L
NOAEC: <0.0002 µg a.i/L	Slope: N/A
LOAEC: 0.0002 µg a.i/L	

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

Mortality:

LC ₅₀ : 1488 ug a.i/kg TOC	95% C.I.: 1195 – 1902 ug a.i/kg TOC
NOAEC: 366 ug a.i/kg TOC	Probit Slope: N/A
LOAEC: 951 ug a.i/kg TOC	

Growth (dry weight):

EC ₅₀ : 2415 ug a.i/kg TOC	95% C.I.: 1885 – 3049 ug a.i/kg TOC
NOAEC: <34 ug a.i/kg TOC	Slope: N/A
LOAEC: 34 ug 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 (dry weight); based on the 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. 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. 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. Neonate amphipods were acclimated and tested under differing temperatures. The acclimation temperature for 48 hours prior to test initiation was 18°C, and the testing temperature was 25 ± 2°C.
2. A physical description of the test substance was not provided. In addition, the aqueous solubility should have been reported.
3. Reproduction is a required endpoint for 28-day sediment toxicity studies and was not assessed in this study.
4. A NOAEC could not be determined in this study because significant adverse effects in dry weight were detected at all treated levels, when compared to the negative control group.

9. SUBMISSION PURPOSE: RED follow-up

10. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: [¹⁴C]Residues were predominantly associated with the sediment, but declined approximately 60% between test initiation and termination. Mean percent recoveries of total radioactive residues (reviewer-calculated from LSC results) were 95-127% of nominal concentrations on day 0, declining to 47-58% of nominal on day 28. On days 0 and 28 at the 450 µg ai/kg level (the only level analyzed by HPLC/RAM), 92% of the recovered radioactivity was parent material.

Less than an average of 20 µg/L was detected from the pore water during the study (based on LSC), and concentrations were generally consistent between 0 and 28 days. Mean recoveries increased from 0.076 µg/L at the 1.9 µg/kg level to 17 µg/L at the 450 µg/kg level. Of the total radioactivity recovered from the 450 µg/kg level, only 3.3% was identified as [¹⁴C]cypermethrin (based on HPLC/RAM analysis on days 0 and 28).

Less than 2 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 Cypermethrin.

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, Kow, vapor pressure of test compound)

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
<u>Species</u>	<i>Leptocheirus plumulosus</i>
<u>Source</u>	Laboratory cultures

Guideline Criteria	Reported Information
<u>Culture Conditions</u>	Adult amphipods were maintained at 18°C in 11-L plastic bins containing a 2-cm layer of marine sediment and 7-8 L of 20 ppt salinity seawater.
<u>Age of Test Organisms</u>	Neonates: size-selected (retained between 0.25 and 0.6-mm mesh screens)
<u>Food</u>	During holding and acclimation, amphipods were fed daily a finely-ground suspension of Zeigler Prime flakes fish food (i.e., 100 mg/ml).
<u>Health of parent culture stock</u>	No mortality observed in the population 48 hours prior to test initiation.

B. Test System

Guideline Criteria	Reported Information
<u>Type of Test System</u>	Static-renewal
<u>Test Water</u>	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 $20 \pm 1\%$ and a pH of 7.7-7.9 with laboratory well water.
<u>Renewal of overlying water</u>	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.
<u>Test Sediment</u>	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.

Guideline Criteria	Reported Information
<u>Sediment Characterization</u>	Particle size: 68% sand, 19% silt, and 13% clay pH: 6.9 Ammonia (as N) in pore water: 7.5 mg/L TOC: 4.1% Percent water content (1/3 bar): 39% Grain size: 32% silt/clay
<u>Test Material</u>	[¹⁴C]Cypermethrin Description: not reported Lot no.: CFQ13998 CAS No.: not reported Position of label: 1-cyclopropane Radiochemical purity: 99.8% Specific activity: 2.04 Gbq/mmol (292,035 dpm/μg) Storage: in the freezer (<-4°C) Aqueous solubility: Not reported. According to Laskowski, 2002, the solubility is low at 4 ug/L or 4 ppb.
<u>Solvents</u>	Acetone, 9 ml per 0.8644 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.
<u>Sediment Spiking</u>	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.

Guideline Criteria	Reported Information
<u>Sediment Conditioning</u>	The treated sediments were allowed to equilibrate for 28-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.
<u>Sediment and Overlying Water Into Test Chambers</u>	<p>One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added.</p> <p>1 L glass vessels containing 175 ml (approx. 2.3 cm layer) of sediment (equivalent to 185 g wet weight or 80 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.</p> <p>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.</p>
<u>Aeration</u>	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.
<u>Water Temperature</u>	Overlying water: 23-27°C Pore water: 18-19°C
<u>pH</u>	Overlying water: 7.0-8.4 Pore water: 6.7-7.2
<u>Salinity</u>	Overlying water: 19-21‰ Pore water: 20-23‰

Guideline Criteria	Reported Information
<u>Ammonia (as N)</u>	Overlying water: 1.6-1.9 mg/L on day 0 and ≤ 0.83 mg/L on day 28 Pore water: 7.5-11mg/L on day 0 and 0.89-6.8 mg/L on day 28
<u>Dissolved Organic Carbon</u>	Pore water: 19.2-41.8 mg/L on day 0 and 7.6-16.5 mg/L on day 28
<u>Dissolved Oxygen</u>	5.1-7.0 mg/L (>60% saturation)
<u>Photoperiod</u>	16 hours light, 8 hours dark (500-1000 lux)
<u>Food</u>	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
<u>Duration</u>	28 days
<u>Nominal Concentrations</u>	Negative control, solvent control, 1.9, 5.6, 17, 50, 150, and 450 $\mu\text{g ai/kg dw}$ sediment Selection of nominal treatment levels for the definitive study was based on results from preliminary testing.
<u>Mean-Measured Concentrations</u>	<0.33 (controls), 1.4, 4.5, 15, 39, 130, and 410 $\mu\text{g total } [^{14}\text{C}]\text{cypermethrin residues/kg dw}$ sediment (based on LSC analysis)

Guideline Criteria	Reported Information
<u>Number of Test Organisms</u>	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.
<u>Overlying Water Parameter Measurements</u>	<p>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.</p> <p>Temperature was also continuously monitored in one representative test vessel (solvent control, replicate H).</p> <p>Ammonia (as nitrogen) was measured on days 0 and 28 from a composite sample obtained for each control and treatment level.</p>
<u>Pore Water Parameter Measurements</u>	Salinity, pH, temperature, ammonia, and dissolved organic carbon (DOC) were measured from a single replicate on days 0 and 28.
<u>Chemical Analysis-Overlying Water</u>	All control and treatment levels were analyzed on days 0 and 28 for total [¹⁴ C]residues using LSC.
<u>Interstitial Water and Sediment Isolation Method</u>	Centrifugation for 30 min at 10,000 g.
<u>Chemical Analysis-Interstitial Water</u>	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]cypermethrin using HPLC/RAM.

Guideline Criteria	Reported Information
<u>Chemical Analysis-Bulk Sediment</u>	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]cypermethrin 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
<u>Control Mortality</u>	16% - negative control 24% - solvent control
Percent Recovery of Chemical:	Based on QC samples prepared and analyzed concurrently with sample analysis: <u>LSC</u> Sediment: 86.9-101% of nominal Overlying water: 96.0-103% of nominal <u>HPLC/RAM</u> Sediment: 100% associated with parent Pore water: 100% associated with parent
<u>Data Endpoints</u>	- Survival - Abnormal behavior - Dry weight
<u>Observation Intervals</u>	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, $\mu\text{g}/\text{kg dw}$	Mean-measured Sediment, $\mu\text{g}/\text{kg dw}$	Mean-measured Pore Water, $\mu\text{g}/\text{L}$	Mean-measured, Overlying Water, $\mu\text{g}/\text{L}$		
Control	<0.33	<0.083	<0.034	84	2.15
Solvent Control	<0.33	<0.083	<0.034	76	1.57**
1.9	1.4	0.076 ^(b)	<0.034	94	1.78*
5.6	4.5	0.16 ^(b)	<0.034	86	1.76*
17	15	0.57	0.056	83	1.67*
50	39	2.0	0.21	55*	1.74*
150	130	6.1	0.64	15*	0.95*
450	410	17	1.8	1*	0.46* ^(c)

^(a) All mean-measured values were based on LSC results of total radioactive residues.

^(b) Reviewer-calculated using $\frac{1}{2}$ the LOQ for the day 28 result.

^(c) Excluded from statistical analysis of growth due to a lack of a sufficient sample size in order to provide a representative growth response.

* Statistically different (≤ 0.05) compared to the negative control

**The reviewer's analysis detected a significant difference ($p < 0.01$) between the solvent and negative control groups.

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. No differences were observed for survival data which were therefore

pooled for subsequent comparisons. Growth data, however, were statistically different, and therefore responses from the solvent control were used in subsequent comparisons. The data were then tested for normality using the Shapiro-Wilk's Test and for homogeneity of variance using Bartlett's Test. Both sets of data were normally distributed and met the assumption for homogeneity, and were analyzed using Williams' Test to determine the NOAEC and LOAEC values.

The Inhibition Concentration Method was used to calculate the 28-day LC/EC₅₀ 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 Williams' Test	67 (44-84)	15	39
Growth	ICp Williams' Test	>130	39	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 satisfied these assumptions, 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 between the control groups for survival; however, for dry weight, the solvent control group was significantly lower than the negative control group. The negative control group was used for comparison to the treatment groups. These analyses were conducted using Toxstat statistical software. The 28-day LC₅₀ value was determined using the Moving Average Method, as a poor statistical fit was achieved using the Probit method. Differences in the study author's and reviewer's LC₅₀ values (67 and 61 ug a.i/kg sediment, respectively) likely reflect the differences in the statistical methods used (ICp vs. Moving Average). The EC₅₀ was determined with ICp program with the data from the highest treatment level being excluded due to a lack of a sufficient sample size in order to provide a representative growth response (99 % mortality at this

treatment level). 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 (39 and 130 ug a.i./kg sediment, respectively) and those of the reviewer (<1.4 and 1.4 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 HPLC analysis of cypermethrin 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 (3.3% for both the initial and terminal measurements). In contrast, the recovery of parent compound from bulk sediment was generally high (92% for both the initial and terminal measurements). 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 desorption 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 cypermethrin 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 mean measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (4.1%) and the mean Koc (141,700 L/kg-OC, MRID 42129002) for cypermethrin. 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 cypermethrin vary considerably (20,800 – 328,500 L/kg) 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 cypermethrin.

Results Synopsis:

Based on mean-measured sediment concentrations:

Mortality:

DP Barcode: D319265

MRID No.: 465915-03

LC₅₀: 61 µg a.i/kg sediment
NOAEC: 15 µg a.i/kg sediment
LOAEC: 39 µg a.i/kg sediment

95% C.I.: 49 – 78 ug a.i/kg sediment
Probit Slope: N/A

Growth (dry weight):

EC₅₀: 99 ug a.i/kg sediment
NOAEC: <1.4 µg a.i/kg sediment
LOAEC: 1.4 µg a.i/kg sediment

95% C.I.: 77 – 125 ug a.i/kg sediment
Slope: N/A

Based on ESTIMATED¹ Pore Water Concentrations:

Mortality:

LC₅₀: 0.011 µg a.i/L
NOAEC: 0.003 µg a.i/L
LOAEC 0.007 µg a.i/L

95% C.I.: 0.008 – 0.013 ug a.i/L
Probit Slope: N/A

Growth (dry weight):

EC₅₀: 0.017 µg a.i/L
NOAEC: <0.0002 µg a.i/L
LOAEC: 0.0002 µg a.i/L

95% C.I.: 0.013 – 0.022 ug a.i/L
Slope: N/A

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

Mortality:

LC₅₀: 1488 ug a.i/kg TOC
NOAEC: 366 ug a.i/kg TOC
LOAEC: 951 ug a.i/kg TOC

95% C.I.: 1195 – 1902 ug a.i/kg TOC
Probit Slope: N/A

Growth (dry weight):

EC₅₀: 2415 ug a.i/kg TOC
NOAEC: <34 ug a.i/kg TOC
LOAEC: 34 ug a.i/kg TOC

95% C.I.: 1885 – 3049 ug a.i/kg TOC
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 (dry weight); based on the 28-day NOAEC

13. REVIEWER'S COMMENTS:

The reviewer's conclusions differed from the study author's. Both the reviewer and the study author detected a significant difference between the control groups for the dry weight endpoint, where the solvent control dry weights were significantly lower (27%) than the negative control

dry weights. The study author subsequently compared treated groups to the solvent control, while the reviewer compared treatment responses to the negative control, per EFED guidance (Frankenberry et al 2008). Additional discussion of this issue is provided in the Conclusions section. The reviewer's analysis detected significant reductions in dry weight for all treatment groups, compared to the negative control group. As a result, a NOAEC could not be determined for this study. The reviewer's analysis of the survival endpoint revealed similar results for the NOAEC and a slightly lower LC₅₀ with a narrower 95% confidence interval. The reviewer's toxicity values are reported in the Conclusions section.

As the Probit method was unsuitable for LC₅₀ analysis because of poor goodness of fit, the reviewer used the moving average method. The EC₅₀ was determined with ICp program with the data from the highest treatment level being excluded due to a lack of a sufficient sample size in order to provide a representative growth response (99 % mortality at this treatment level). The reviewer expressed the NOAEC and LOAEC based on the mean measured sediment and estimated pore water concentrations.

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 not to 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 <0.033 (<LOQ), <0.033, <0.033, 0.074, 0.24, 0.71, and 1.9 ug a.i/L while the Day 28 measured concentrations were <0.034, <0.034, <0.034, 0.038, 0.18, 0.57, and 1.7 ug a.i/L for the negative control and mean measured spiked sediment 1.4, 4.5, 15, 39, 130, and 410 ug a.i/kg dry sediment concentrations. The reviewer-determined mean measured overlying water concentrations were <0.033, <0.033, <0.033, 0.056, 0.21, 0.64, and 1.8 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 cypermethrin 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 46591503), six individual 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	5.02	25	46	100
46	3.31	10	15	100
46	1.10	10	5.1	100
46	0.375	10	1.7	100
46	0.124	10	0.57	100
46	0.042	10	0.19	100

All dosing stock solutions were clear and colorless with no visible undissolved test substance.

An appropriate amount (9 mL) of each individual dosing stock solution (above) was added to 0.0500 kg of coarse 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 (0.8644 kg dry weight based on a percent of solids of 43.22%) in individual 1-gallon jars. The total mass of sediment spiked on a dry weight basis for each treatment level and control was 0.9144 kg (0.0500 kg sand and 0.8644 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 cypermethrin (purity of 95.6%) 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, 88, 95, 72, 82, and 2% 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, 95 and 100% survival was observed in the negative and solvent control groups, respectively. Dry weight among control amphipods averaged 2.37 and 2.09 mg for the negative and solvent control groups, respectively, compared to 2.15, 2.38, 1.20, 1.30, and 0.58 mg for the 0.070, 0.70, 7.0, 70, and 700 µg ai/kg treatment levels, respectively.

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 January 27 – February 24, 2005.

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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:

survival (sediment)

File: 1503s Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

 -
 GRP1 (SOLVENT CRTL) MEAN = 84.0000 CALCULATED t VALUE = 1.5396
 GRP2 (BLANK CRTL) MEAN = 76.0000 DEGREES OF FREEDOM = 8
 DIFFERENCE IN MEANS = 8.0000

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: 1503s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	42337.143	7056.190	89.399
Within (Error)	28	2210.000	78.929	
Total	34	44547.143		

Critical F value = 2.45 (0.05,6,28)
 Since F > Critical F REJECT Ho:All groups equal

survival (sediment)

File: 1503s 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	84.000	84.000		
2	1.4	94.000	94.000	-1.780	
3	4.5	86.000	86.000	-0.356	
4	15	83.000	83.000	0.178	
5	39	55.000	55.000	5.161	*
6	130	15.000	15.000	12.280	*
7	410	1.000	1.000	14.772	*

 Dunnett table value = 2.43 (1 Tailed Value, P=0.05, df=24,6)

survival (sediment)
 File: 1503s 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	1.4	5	13.654	16.3	-10.000
3	4.5	5	13.654	16.3	-2.000
4	15	5	13.654	16.3	1.000
5	39	5	13.654	16.3	29.000
6	130	5	13.654	16.3	69.000
7	410	5	13.654	16.3	83.000

survival (sediment)
 File: 1503s 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	84.000	84.000	89.000
2	1.4	5	94.000	94.000	89.000
3	4.5	5	86.000	86.000	86.000
4	15	5	83.000	83.000	83.000
5	39	5	55.000	55.000	55.000
6	130	5	15.000	15.000	15.000
7	410	5	1.000	1.000	1.000

survival (sediment)
 File: 1503s 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	89.000				
1.4	89.000	0.890		1.70	k= 1, v=28
4.5	86.000	0.356		1.78	k= 2, v=28
15	83.000	0.178		1.81	k= 3, v=28
39	55.000	5.161	*	1.82	k= 4, v=28

130	15.000	12.280	*	1.83	k= 5, v=28
410	1.000	14.772	*	1.83	k= 6, v=28

s = 8.884

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	12.	7.1	20.	0.11	0.59
EC10	17.	11.	26.	0.098	0.63
EC25	29.	20.	41.	0.075	0.70
EC50	53.	42.	69.	0.053	0.78

Slope = 2.53 Std.Err. = 0.277

Goodness of fit: p = 0.87 based on DF= 4.0 28.

1503S : survival (sediment)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	5.00	84.0	88.4	-4.40	100.	0.00
1.40	5.00	94.0	88.4	5.60	100.	0.00305
4.50	5.00	86.0	88.1	-2.11	99.7	0.323
15.0	5.00	83.0	81.2	1.75	91.9	8.09
39.0	5.00	55.0	56.2	-1.22	63.6	36.4
130.	5.00	15.0	14.5	0.482	16.4	83.6
410.	5.00	1.00	1.11	-0.106	1.25	98.7

dry weight sediment

File: 1503d Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	2.1440	CALCULATED t VALUE =	4.5738
GRP2 (BLANK CRTL) MEAN =	1.5760	DEGREES OF FREEDOM =	8
DIFFERENCE IN MEANS =	0.5680		

TABLE t VALUE (0.05 (2), 8) = 2.306** SIGNIFICANT DIFFERENCE at alpha=0.05
 TABLE t VALUE (0.01 (2), 8) = 3.355** SIGNIFICANT DIFFERENCE at alpha=0.01

dry weight sediment

DP Barcode: D319265

MRID No.: 465915-03

File: 1503d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3.331	0.666	9.652
Within (Error)	23	1.581	0.069	
Total	28	4.912		

Critical F value = 2.64 (0.05,5,23)
 Since F > Critical F REJECT Ho:All groups equal

dry weight sediment
 File: 1503d Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	2.144	2.144		
2	1.4	1.784	1.784	2.167	
3	4.5	1.758	1.758	2.323	
4	15	1.666	1.666	2.877	*
5	39	1.738	1.738	2.444	
6	130	0.945	0.945	6.804	*

Bonferroni T table value = 2.50 (1 Tailed Value, P=0.05, df=23,5)

dry weight sediment
 File: 1503d Transform: NO TRANSFORMATION

BONFERRONI T-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	1.4	5	0.415	19.4	0.360
3	4.5	5	0.415	19.4	0.386
4	15	5	0.415	19.4	0.478
5	39	5	0.415	19.4	0.406
6	130	4	0.441	20.5	1.199

dry weight sediment

File: 1503d 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	2.144	2.144	2.144
2	1.4	5	1.784	1.784	1.784
3	4.5	5	1.758	1.758	1.758
4	15	5	1.666	1.666	1.702
5	39	5	1.738	1.738	1.702
6	130	4	0.945	0.945	0.945

dry weight sediment

File: 1503d 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	2.144				
1.4	1.784	2.171	*	1.72	k= 1, v=23
4.5	1.758	2.328	*	1.80	k= 2, v=23
15	1.702	2.666	*	1.83	k= 3, v=23
39	1.702	2.666	*	1.84	k= 4, v=23
130	0.945	6.817	*	1.85	k= 5, v=23

s = 0.262

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	32.	9.7	1.0E+02	0.25	0.30
EC10	44.	17.	1.1E+02	0.20	0.39
EC25	74.	44.	1.3E+02	0.11	0.59
EC50	1.3E+02	1.0E+02	1.8E+02	0.062	0.74

Slope = 2.63 Std.Err. = 1.08

Goodness of fit: p = 0.078 based on DF= 3.0 23.

1503D : dry weight sediment

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs.	Pred.	Obs.	Pred.	%Change
------	--------	------	-------	------	-------	---------

		Mean	Mean	-Pred.	%Control	
0.00	5.00	2.14	1.85	0.295	100.	0.00
1.40	5.00	1.78	1.85	-0.0651	100.	9.66e-06
4.50	5.00	1.76	1.85	-0.0910	100.	0.00538
15.0	5.00	1.67	1.84	-0.172	99.4	0.623
39.0	5.00	1.74	1.70	0.0361	92.0	7.96
130.	4.00	0.945	0.949	-0.00428	51.3	48.7

!!!Warning: EC50 not bracketed by doses evaluated.

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

Cypermethrin 28-day sediment tox

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
410	84	83	98.80949	0
130	84	69.00001	82.1429	0
39	84	29	34.5238	0
15	84	1.000001	1.1905	0
4.5	100	14	14	0
1.4	100	6	6	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 56.71837

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	2.048059E-02	61.41688	49.13084	78.36615

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.6015801	15.01268	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 = 1.465711
95 PERCENT CONFIDENCE LIMITS = .3288822 AND 2.60254**

INTERCEPT=-2.428131

**LC50 = 45.35479
95 PERCENT CONFIDENCE LIMITS = 9.513966 AND 446.4905**

**LC25 = 15.71944
95 PERCENT CONFIDENCE LIMITS = .3914179 AND 53.14685**

**LC10 = 6.05669
95 PERCENT CONFIDENCE LIMITS = 8.224561E-03 AND 21.07576**

**LC05 = 3.422704
95 PERCENT CONFIDENCE LIMITS = 7.181392E-04 AND 13.75445**
