

**DATA EVALUATION RECORD  
WHOLE SEDIMENT CHRONIC TOXICITY, MARINE INVERTEBRATES  
NON-GUIDELINE**

1. **CHEMICAL:** Novaluron PC Code No.: 124002

2. **TEST MATERIAL:** Novaluron technical Purity: 100.0% (w:w)

3. **CITATION:**

Authors: Staggs, M.

Title: Novaluron – 28-Day Toxicity Test Exposing Estuarine Amphipods (*Leptocheirus plumulosus*) to a Test Substance Applied to Sediment under Static-Renewal Conditions Following EPA Test Methods

Study Completion Date: September 21, 2018

Laboratory: Smithers Viscient

Sponsor: ADAMA Makhteshim Ltd.

Laboratory Report ID: 14125.6112

MRID No.: 50691105

DP Barcode: 449149

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CDM/CSS-Dynamac JV

Signature: 

**Date:** 01/30/19

**APPROVED BY:** Adrian Graff, Environmental Scientist, CDM/CSS-Dynamac JV

Signature: 

**Date:** 2/11/19

5. **APPROVED BY:** Cassandra Kirk, PhD, Biologist, OPP/EFED/ERB - I

**Signature:**

**Date:** 9/18/19

6. **DISCLAIMER:** *This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

## 7. STUDY PARAMETERS:

<b>Scientific Name of Test Organism:</b>	<i>Leptocheirus plumulosus</i>
<b>Age/Size of Test Organism:</b>	Juvenile, 7 to 8 days old
<b>Definitive Test Duration:</b>	28 days
<b>Study Method:</b>	Intermittent flow-through
<b>Type of Concentrations:</b>	Time-weighted average (TWA) sediment (bulk and OC-normalized) and TWA pore water (TWA overlying water were <LOD)

## 8. CONCLUSIONS:

**Results Synopsis:** In a 28-day *Leptocheirus plumulosus* life cycle study, reproduction was the most sensitive endpoint, with treatment-related reductions in the number of offspring per surviving female at the two highest treatment levels. A treatment-related effect on survival was also observed at the highest treatment level. No further significant effects were noted.

In terms of TWA sediment concentrations:

NOAEC: 16 µg ai/kg

LOAEC: 46 µg ai/kg

In terms of TWA OC-normalized sediment concentrations:

NOAEC: 470 µg ai/kg-OC

LOAEC: 1400 µg ai/kg-OC

In terms of TWA pore water concentrations:

NOAEC: 0.051 µg ai/L

LOAEC: 0.16 µg ai/L

In terms of TWA overlying water concentrations:

NOAEC: N/A\*

LOAEC: N/A\*

\*Not applicable due to measured concentrations in four lowest levels being below LOQ.

Endpoint(s) Affected: Survival, reproduction

Most Sensitive Endpoint(s): Reproduction

## 9. ADEQUACY OF THE STUDY:

**A. Classification:** This study is **scientifically sound** and is classified as **acceptable**.

**B. Rationale:** This study was conducted following guidance outlined in the *Methods for*

*Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod Leptocheirus plumulosus* (U.S. EPA, 2001, EPA 600/R-01/020). This study does not fulfill any current U.S. EPA OCSPP data requirement.

**C. Repairability:** N/A

**10. MAJOR GUIDELINE DEVIATIONS:**

- Raw dry weight data (used to generate growth rates) were not provided.

Minor deviations from referenced guidance included:

- Instead of screening the neonate amphipods to obtain organisms between 0.425 and 0.60 mm, organisms of known age (7 to 8 days old) were used to initiate exposure (see Reviewer’s Comments).
- Replicate F in the TWA 16 µg ai/L treatment group was inadvertently initiated with 21 amphipods and not the guidance recommended number of organisms, 20. Despite this, survival was 100% for Replicate F.

**11. MATERIALS AND METHODS:**

**A. Test Organisms/Acclimation**

Guideline Criteria	Reported Information
<p><b><u>Species</u></b> <i>Leptocheirus plumulosus</i></p>	<p><i>Leptocheirus plumulosus</i></p>
<p><b><u>Source</u></b> Laboratory cultures or commercial or government sources.</p>	<p>Aquatic Research Organisms Hampton, New Hampshire</p>
<p><b><u>Acclimation Period/Culture Conditions</u></b> Periodic-renewal (two to three times weekly) culture system maintained at 20 to 25°C and under a 16-hour light/8-hour dark photoperiod. Amphipods are cultured in bins with a thin (<i>ca.</i> 1 to 1.5 cm) layer of clean, fine-grained sediment and at least 10 cm of clean, continuously-aerated dilution water (same as test water).</p>	<p>Purchased organisms (&lt;48 hours old) were acclimated to test conditions for <i>ca.</i> 6 days.  During acclimation, amphipods were maintained in 11-L plastic tubs containing <i>ca.</i> 9 L of 20‰ salinity seawater and marine sediment. Prior to testing, water quality parameters measured 24°C and 22‰ salinity.</p>

Guideline Criteria	Reported Information
<p><b><u>Life Stage</u></b> Neonates: age-selected (&lt;48 hours old) or size-selected (preferable): retained between 0.25-mm and 0.6-mm mesh screens.</p>	<p>Juvenile, 7 to 8 days old</p> <p>Dry weight averaged 0.053 mg/amphipod at study initiation (n=60).</p>
<p><b><u>Food</u></b> Finely-ground TetraMin® provided two or three times per week (in conjunction with media renewal). Other food sources (e.g., alga) are also acceptable.</p>	<p>Amphipods were fed periodically during acclimation by adding <i>ca.</i> 1 mL of 100 mg/mL flaked fish food suspension to each holding vessel.</p>
<p><b><u>Pretest Mortality</u></b> Do not use if organisms appear unhealthy, discolored, or otherwise stressed (e.g., &gt;20% mortality in 48-hr period preceding the test).</p>	<p>No mortality was observed in the test population upon receipt or during acclimation.</p>

### B. Test System

Guideline Criteria	Reported Information
<p><b><u>Test Materials</u></b></p>	<p>Identity: Novaluron technical IUPAC name: Not reported CAS name: Not reported CAS No.: 116714-46-6 Description: Light pink solid Lot/Batch No.: 96869065 Purity: 100.0%, w:w (expiration – 08/12/21) Storage: Dark, room temperature</p>
<p><b><u>Type of Test System</u></b> Static-renewal</p> <p>Siphon off and replace 400 mL of overlying water three times per week (Monday, Wednesday, and Friday).</p>	<p>Intermittent flow-through, with <i>ca.</i> 2 volume additions/chamber/day</p>

Guideline Criteria	Reported Information
<p><b><u>Source of dilution water (overlying water)</u></b>                      Clean seawater, natural or reconstituted water from the same source was used for culturing.</p>	<p>Natural seawater was collected at Cape Cod Canal, Bourne, Massachusetts (4 m offshore at a 0.5-m depth), diluted with laboratory well water to a salinity of 19 to 21‰ and filtered. Prior to use, the pH range of the seawater was 7.4 to 7.8.</p> <p>The TOC of the overlying water source (measured monthly) was 0.81 and 1.6 mg/L for May and June 2018, respectively.</p>
<p><b><u>Source of sediment</u></b>                      Uncontaminated natural sediment, which has been press-sieved (0.25-mm). Sediments should be stored at 4°C in the dark and used within 2 to 8 weeks of collection.</p>	<p>Natural marine sediment (Smithers Viscient Batch No. 121217) was collected from Sequim Bay, Sequim, Washington and wet-pressed sieved (0.25 mm).</p> <p>Particle size distribution – 17% sand, 58% silt, and 25% clay (USDA class: silt loam)                      Percent solids: 30.65%                      TOC – 3.4%                      pH – 7.5                      Moisture at 1/3 bar – 77.7%                      Ammonia concentration of pore water – 4.7 mg/L as N</p>
<p><b><u>Additional Sediment Analysis</u></b>                      Pore water ammonia should not exceed 60 mg/L at test initiation.                      Pore water salinity range of 1 to 35‰ during the study.</p>	<p>Salinity, ammonia (as N), and pH were determined in pore water from all levels at 0 and 28 Days.</p> <p>Salinity – 24 to 26‰ on Day 0 and 21‰ on Day 28                      Ammonia – 6.0 to 7.3 µg/L on Day 0 and 0.47 to 2.1 µg/L on Day 28                      pH – 7.2 to 7.4 on Day 0 and 6.7 on Day 28</p>

Guideline Criteria	Reported Information
<p><b><u>Sediment Spiking</u></b></p>	<p>A 22.2 µg ai/mL primary stock solution was prepared in acetone (purity of test material was 100%) and from this, five dosing stock solutions were prepared in acetone at 11.1, 3.73, 1.24, 0.418, and 0.140 µg ai/mL. All stocks were clear and colorless, with no visible undissolved test substance.</p> <p>A 10-mL volume of the appropriate stock was applied to 0.050 kg of fine silica sand, and the solvent was allowed to (completely) evaporate off for 45 minutes. The spiked sand was then mechanically mixed for 15 minutes with an additional 2.25 kg of wet sediment; the total dry weight of sand plus wet sediment was 0.7396 kg dw.</p> <p>Negative and solvent control groups were included in the test. The range of nominal concentrations chosen for the definitive test (1.9 to 150 µg ai/kg dw sediment) was based on the results of preliminary testing and was made in consultation with the Sponsor.</p>
<p><b><u>Sediment Conditioning</u></b></p>	<p>N/A – treated sediments were used immediately to establish test vessel systems</p>
<p><b><u>Sediment and Overlying Water Into Test Chambers</u></b>                      One day prior (Day -1) to start of test: each sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.</p>	<p>Test systems were established on Day -1. Overlying water was gently added to each vessel and the vessels were placed under the renewal system.</p>
<p><b><u>Introduction of Test Organisms</u></b></p>	<p>At test initiation (Day 0), 20 amphipods were impartially added (five at a time) to each replicate test vessel.</p>

Guideline Criteria	Reported Information
<p><b><u>Solvents</u></b></p>	<p>Acetone, 0.014 mL/g dw sediment (10 mL/0.7396 kg dw sediment)</p> <p>The acetone was allowed to completely evaporate during the dosing procedure.</p>
<p><b><u>Water Temperature</u></b> Overall: 25(±2)°C Daily limits: 25(±3)°C</p>	<p>Daily: 24 to 26°C Instantaneous: 24 to 27°C</p>
<p><b><u>pH</u></b> 7.0 to 9.0 pH units</p>	<p>7.2 to 8.3</p>
<p><b><u>Dissolved Oxygen</u></b> Overall: ≥4.4 mg/L (60% saturation) Daily limits: ≥3.6 mg/L (50% saturation)</p>	<p>4.1 to 6.8 mg/L (55 to 92% ASV)</p>
<p><b><u>Salinity</u></b> Overall: 5(±2)‰ (if estuarine sediment; pore water 1 to 10‰) or 20(±2)‰ (if marine sediment; pore water &gt;10 to 35‰) Daily limits: 5(±3)‰ or 20(±3)‰</p>	<p>19 to 20‰</p>
<p><b><u>Ammonia</u></b></p>	<p>0.77 to 1.4 µg/L (as N) on Day 0, decreasing to ≤0.10 to 0.18 µg/L (as N) on Day 28</p>
<p><b><u>Aeration</u></b> Overlying sea water should be continuously aerated except when test organisms are being added. Aeration rate should be recorded daily in all chambers.</p>	<p>Aeration with oil-free air was provided to each replicate with a constant flow of bubbles from a 1-mL glass pipette.</p>
<p><b><u>Test Vessels</u></b> 1-L glass chambers (10 cm id) containing 175 mL (<i>ca.</i> 2 cm) of sediment and <i>ca.</i> 725 mL of overlying water (total final volume of 900 mL).</p>	<p>1-L glass jars with 40-mesh nylon screens to retain organisms during water renewal</p> <p>Each vessel contained 120 mL (<i>ca.</i> 2-cm layer) of sediment and 600 mL of overlying water (<i>ca.</i> 10-cm layer). The total overlying water plus sediment volume was maintained at <i>ca.</i> 720 mL.</p>

Guideline Criteria	Reported Information
<p><b><u>Photoperiod</u></b> 16-hour light/8-hour dark photoperiod using wide-spectrum fluorescent light with an intensity of 500 to 1000 lux.</p>	16 hours light, 8 hours dark using fluorescent bulbs, with an intensity range of 500 to 580 lux
<p><b><u>Feeding</u></b> Three times per week after water renewal.</p> <p><b><u>Recommended rates</u></b> Days 0 to 13: 20 mg TetraMin® per chamber Days 14 to 28: 40 mg TetraMin® per chamber</p>	<p>Once daily with 1.0 mL of a flaked fish food suspension prepared in natural, filtered seawater and Neo-Novum shrimp diet suspension; diets were prepared at the appropriate concentrations for rates below:</p> <p>Days 0 to 6: 9.0 mg flaked fish food and 0.45 mg shrimp diet/replicate Days 7 to 13: 14 mg flaked fish food and 0.70 mg shrimp diet/replicate Days 14 to 20: 20 mg flaked fish food and 1.0 mg shrimp diet/replicate Days 21 to 27: 30 mg flaked fish food and 1.5 mg shrimp diet/replicate</p>

### C. Test Design

Guideline Criteria	Reported Information
<p><b><u>Duration</u></b> 28 days</p>	28 days
<p><b><u>Range-Finding Test</u></b></p>	<ul style="list-style-type: none"> <li>• 28-day exposure under test conditions at nominal concentrations of 0 (negative control), 0 (acetone solvent control), 0.63, 2.5, 10, 40, and 160 µg ai/kg</li> <li>• 20 amphipods per replicate, with four replicates per level (80 per level)</li> <li>• Day-28 survival averaged 88, 77, 90, 72, 75, 83, and 50%, respectively</li> <li>• Day-28 male growth rate averaged 0.076, 0.071, 0.083, 0.078, 0.076, 0.082, and 0.051 mg/amphipod/day, respectively</li> <li>• Day-28 female growth rate averaged 0.060,</li> </ul>



Guideline Criteria	Reported Information
	<p>0.056, 0.056, 0.052, 0.059, 0.065, and 0.042 mg/amphipod/day, respectively</p> <ul style="list-style-type: none"> <li>• Day-28 offspring per female averaged 27, 21, 30, 29, 18, 15, and 9, respectively</li> </ul>
<p><b><u>Nominal Sediment Concentrations</u></b> Control(s) and at least 5 test concentrations</p>	<p>0 (negative control), 0 (acetone solvent control), 1.9, 5.6, 17, 50, 150 and 2.6, 6.4, 16, 40, and 100 µg ai/kg dw sediment</p>
<p><b><u>Number of Test Organisms</u></b> 20 organisms per test chamber are recommended. Five replicates per treatment should be used.</p>	<p>20 amphipods per replicate vessel</p> <p>6 biological replicates per level</p> <p>An additional five (destructive) replicates per level were maintained for chemical analysis and pore water quality measurements.</p>
<p><b><u>Monitoring the test</u></b> Condition and activity of amphipods should be observed at each water renewal interval (i.e., three times per week).</p>	<p>Daily observations of organism mortality and behavior were made and the physical characteristics of the overlying water and sediment were recorded.</p>

Guideline Criteria	Reported Information
<p><b><u>Overlying Water Parameter Measurements</u></b>                      Daily temperature in water bath or test/dummy chamber – daily min/max is recommended. Temperature should also be measured in at least one replicate per treatment at study initiation, termination, and preceding water renewal intervals (three times weekly during the study).</p> <p>Salinity, DO, and pH should be measured in at least one replicate per treatment at study initiation, termination, and preceding water renewal intervals (three times weekly during the study).</p> <p>Total ammonia should be measured on Days 0 and 28 in one replicate per treatment.</p>	<p>Dissolved oxygen (DO), temperature, pH, and salinity were measured in the overlying water from each replicate vessel on Days 0 and 28, and in one alternating replicate from each level on Days 1 to 27. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature-controlled water bath used to house the test vessels.</p> <p>Ammonia (as nitrogen) was measured in one composite sample from all biological replicates per level on Days 0 and 28.</p>
<p><b><u>Pore Water Parameter Measurements</u></b>                      Total ammonia, salinity, temperature, and pH of pore water from surrogate containers on Days 0 and 28.</p>	<p>Pore water salinity, pH, and ammonia were determined from single (destructive) test vessels prepared at all control and treatment levels on Days 0 and 28.</p>

Guideline Criteria	Reported Information
<p><b><u>Chemical Analysis</u></b></p>	<p><u>In-life phase:</u> Concentrations of novaluron were determined in sediment, pore water, and overlying water from (supplemental destructive) test vessels collected on Days 0, 14, and 28.</p> <p>The overlying water was decanted and the sediment was centrifuged at <math>\geq 10,000</math> g for 30 minutes to isolate the sediment and pore water matrices. Sediment samples were then mixed well prior to analysis.</p> <p>Samples were analyzed using liquid chromatography with tandem mass spectrometry (LC/MS/MS) based on methodology validated at Smithers Viscient (see Reviewer's Comments).</p>

## 12. REPORTED RESULTS:

### A. General Results

Guideline Criteria	Reported Information
<p><b>Quality assurance and GLP compliance statements were included in the report?</b></p>	<p>Yes. This study was conducted in accordance with U.S. EPA GLP Standards as specified in 40 CFR, Part 160 with the following exceptions: routine water, sediment, and food contaminant screening analyses for potential contaminants. These analyses, however, were performed using standard validated methods.</p>
<p><b><u>Control Criteria</u></b> Minimum mean control survival of 80%</p>	<p><u>Control criteria met:</u> Negative control: 89% survival Solvent control: 94% survival</p>
<p><b>Were growth and reproduction measurable in all control replicates?</b></p>	<p>Yes</p>

Guideline Criteria	Reported Information
<p><b><u>Percent Recovery of Chemical</u></b></p>	<p><u>Based on QC samples analyzed concurrently with the sediment and overlying water samples</u></p> <p><u>Sediment</u> Spiked at 1.00, 15.0, and 150 µg ai/kg Recoveries of 72.8 to 105% of nominal (n=8); excludes one outlier of 66.8% LOD (MDL): 0.27 µg ai/kg</p> <p><u>Aqueous</u> Spiked at 0.0100, 0.500, and 5.00 µg ai/L Recoveries of 91.6 to 110% of nominal (n=8); excludes one outlier of &lt;LOD LOD (MDL): 0.0050 µg ai/L</p>
<p><b><u>Data Endpoints</u></b></p> <ul style="list-style-type: none"> <li>- Survival</li> <li>- Reproduction</li> <li>- Growth rate</li> </ul>	<ul style="list-style-type: none"> <li>- Survival</li> <li>- Reproduction</li> <li>- Growth rate (gender-specific)</li> </ul>
<p><b>Raw data included?</b></p>	<p>No, raw dry weight data were not reported</p>

Effects Data:

Survival averaged 89 and 94% for the negative and solvent control levels, respectively, and 94, 93, 88, 94, and 78% for the mean-measured 1.7, 5.2, 16, 46, and 140 µg ai/kg treatment levels, respectively. No statistically-significant differences were indicated at any level compared to the negative control. The NOAEC, LOAEC, and LC<sub>50</sub> were 140, >140, and >140 µg ai/kg, respectively, in terms of mean-measured sediment concentrations and 0.48, >0.48, and >0.48 µg ai/L, respectively, in terms of mean-measured pore water concentrations.

Toxicant Concentration			Percent Survival (± SD)	Male Growth Rate (± SD)	Female Growth Rate (± SD)	No. Young per Surviving Female (± SD)
Nominal Sediment (µg ai/kg)	Mean-Measured Sediment (µg ai/kg)	Mean-Measured Pore Water (µg ai/L)				
Negative control	<LOD <sup>(a)</sup>	<LOD <sup>(a)</sup>	89 ± 4.9	0.091 ± 0.012	0.064 ± 0.006	31 ± 6.8
Solvent control	<LOD <sup>(a)</sup>	<LOD <sup>(a)</sup>	94 ± 6.6	0.092 ± 0.011	0.066 ± 0.005	34 ± 3.7
1.9	1.7	0.0046	94 ± 5.8	0.094 ± 0.017	0.059 ± 0.005	35 ± 5.6
5.6	5.2	0.018	93 ± 5.2	0.086 ± 0.005	0.057 ± 0.003	27 ± 5.0
17	16	0.052	88 ± 7.6	0.094 ± 0.011	0.064 ± 0.008	33 ± 5.8
50	46	0.16	94 ± 5.8	0.091 ± 0.003	0.065 ± 0.009	22 ± 3.8*
100	140	0.48	78 ± 6.8	0.052 ± 0.011*	0.056 ± 0.012	14 ± 12*

<sup>(a)</sup> The LOD (=MDL) was 0.27 µg ai/kg for sediment samples and 0.0050 µg ai/mL for aqueous samples.

\* Statistically-significant difference compared to the negative control, based on Dunnett's Multiple Comparison Test (male growth rate) or the Mann-Whitney U Two-Sample Test (reproduction).

Male growth rates averaged 0.091 and 0.092 mg/amphipod/day for the negative and solvent control levels, respectively, and 0.094, 0.086, 0.094, 0.091, and 0.052 mg/amphipod/day for the mean-measured 1.7, 5.2, 16, 46, and 140  $\mu\text{g ai/kg dw}$  treatment levels, respectively. The difference was statistically-significant at the 140  $\mu\text{g ai/kg}$  level compared to the negative control ( $p < 0.05$ ). The NOAEC, LOAEC, and  $EC_{50}$  for male growth rate were 46, 140, and  $>140 \mu\text{g ai/kg}$ , respectively, based on mean-measured sediment concentrations and 0.16, 0.48, and  $>0.48 \mu\text{g ai/L}$ , respectively, based on mean-measured pore water concentrations.

Female growth rates averaged 0.064 and 0.066 mg/amphipod/day for the negative and solvent control levels, respectively, and 0.059, 0.057, 0.064, 0.065, and 0.056 mg/amphipod/day for the mean-measured 1.7, 5.2, 16, 46, and 140  $\mu\text{g ai/kg dw}$  treatment levels, respectively. No statistically-significant differences were indicated at any level compared to the negative control. The NOAEC, LOAEC, and  $EC_{50}$  were 140,  $>140$ , and  $>140 \mu\text{g ai/kg}$ , respectively, in terms of mean-measured sediment concentrations and 0.48,  $>0.48$ , and  $>0.48 \mu\text{g ai/L}$ , respectively, in terms of mean-measured pore water concentrations.

Reproduction was the most sensitive endpoint. The number of offspring per surviving female amphipod averaged 31 and 34 for the negative and solvent control levels, respectively, and 35, 27, 33, 22, and 14 for the mean-measured 1.7, 5.2, 16, 46, and 140  $\mu\text{g ai/kg}$  treatment levels, respectively. Differences were statistically-significant at the 46 and 140  $\mu\text{g ai/kg}$  levels compared to the negative control ( $p < 0.05$ ). The subsequent NOAEC and LOAEC for reproduction were 16 and 46  $\mu\text{g ai/kg}$ , respectively, based on mean-measured sediment concentrations and 0.052 and 0.16  $\mu\text{g ai/L}$ , respectively, based on mean-measured pore water concentrations. It was reported that although  $>50\%$  reproduction was observed at the highest treatment level tested, a robust  $EC_{50}$  value could not be calculated due to variability in the data set.

#### Analytical:

During testing, concentrations of novaluron were determined in sediment, overlying water, and pore water on Days 0, 14, and 28. Study results were reported in terms of mean-measured sediment and mean-measured pore water concentrations.

Sediment concentrations were relatively stable at all treatment levels from 0 to 14 Days, and then decreased at all levels from Days 14 to 28, resulting in coefficients of variation (CV) of 10 to 25% (see copy of Excel worksheet in Appendix I). Recoveries ranged from 97 to 120% of nominal levels on Day 0, from 92 to 100% of nominal levels on Day 14, and from 65 to 82% of nominal levels on Day 28. Mean-measured concentrations represented 91 to 95% of nominal values.

Pore water concentrations decreased following Day 0, and overall (0 through 28 Days) analytical variation was  $\geq 40\%$  for all levels (see Appendix I). At the nominal 1.9, 5.6, 17, 50, and 150  $\mu\text{g ai/kg dw}$  treatment levels, Day-0 concentrations measured 0.0088, 0.025, 0.078,

0.23, and 0.75 µg ai/L, respectively, Day-14 concentrations measured <0.0050 (<LOD), 0.020, 0.047, 0.16, and 0.33 µg ai/L, respectively, and Day-28-concentrations measured <0.0050 (<LOD), 0.0095, 0.032, 0.099, and 0.35 µg ai/L, respectively. Overall mean-measured pore water concentrations were 0.0046, 0.018, 0.052, 0.16, and 0.48 µg ai/L for the nominal 1.9, 5.6, 17, 50, and 150 µg ai/kg levels, respectively.

In overlying water samples, novaluron was detected only twice, at 0.011 µg ai/L on Day 0 and at 0.028 µg ai/L on Day 28 at the nominal 150 µg ai/kg treatment level. All other results were below the analytical method detection limit of 0.0050 µg ai/L.

## **B. Statistical Results (From Study Report)**

At study termination (Day 28), data obtained on amphipod survival, male and female growth rates, and reproduction (number of young per surviving female) were statistically analyzed. Growth rate was calculated using the following formula:  $\text{mg gain/amphipod/day} = (\text{mg/amphipod at termination} - \text{mg/amphipod at initiation})/28 \text{ days}$ . Statistical analyses were performed using CETIS™ Version 1.8 (2013) statistical software and reported in terms of mean-measured sediment and mean-measured pore water concentrations.

An Equal Variance Two-Sample t-Test or Fisher's Exact Test was used to compare the performance of the negative and solvent control responses for all endpoints. Negative control and solvent control data were statistically-similar for all endpoints.

Survival data were analyzed using Fisher's Exact Test with Bonferroni-Holm's Adjustment. Growth and reproduction data were tested for normality using the Shapiro-Wilks' test and for homogeneity of variance using Bartlett's test. All endpoints met both assumptions. Male and female growth rates were subsequently analyzed using Dunnett's Multiple Comparison test, and a Mann-Whitney U Two-Sample test was used to establish treatment effects for reproduction (as it resulted in the most conservative estimation of the NOAEC and LOAEC). The NOAEC and LOAEC values were assigned based on significance. All statistical analyses were conducted at the 95% level of certainty except in the case of the qualification tests (i.e., Shapiro-Wilks' Test), in which a 99% level of certainty was applied.

In this study, the LC/EC<sub>50</sub> values exceeded the highest concentration tested for all endpoints except reproduction. For reproduction, it was reported that a robust EC<sub>50</sub> value could not be calculated due to variability in the data set.

In terms of mean-measured sediment concentrations:

LC/EC<sub>50</sub>: >140 µg ai/kg                      95% C.I.: N/A  
 NOAEC: 16 µg ai/kg  
 LOAEC: 46 µg ai/kg

In terms of mean-measured pore water concentrations:

LC/EC<sub>50</sub>: >0.48 µg ai/L                      95% C.I.: N/A  
 NOAEC: 0.052 µg ai/L  
 LOAEC: 016 µg ai/L

Endpoint(s) Affected: male growth rate and reproduction

Most Sensitive Endpoint(s): reproduction

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Survival and reproduction data were analyzed using CETIS statistical software version 1.9.5.3 with database backend settings implemented by EFED on 7/25/17. Three test records were established for time-weighted average bulk sediment, OC-normalized sediment, and pore water, named “50691105 bulk”, “50691105 OC”, and “50691105 pore”, respectively. A test record was not established for TWA overlying water concentrations due to the four lowest measured concentrations being below the MDL. Hypothesis testing was conducted using TWA bulk sediment concentrations and findings were extrapolated to the other test records.

Negative and solvent control data were compared using an Equal Variance t Two-Sample test ( $\alpha = 0.05$ ) as homoscedasticity was achieved based on a Variance Ratio F test ( $\alpha = 0.01$ ). No statistically significant differences were noted. All further hypothesis testing was conducted comparing treatment data to negative control data only.

Reproduction data were checked for normality and homoscedasticity using a Shapiro-Wilk W Normality test ( $\alpha = 0.01$ ) and a Bartlett Equality of Variance test, respectively, resulting in a non-normal distribution. Consequentially, reproduction data were analyzed using a nonparametric Mann-Whitney U Two-Sample test ( $\alpha = 0.05$ ). Survival data were analyzed using a parametric Dunnett Multiple Comparison test ( $\alpha = 0.05$ ) as assumptions were met.

Endpoint	Methods	NOAEC (µg ai/kg)	LOAEC (µg ai/kg)
Survival	Dunnett	46	143
Reproduction	Mann-Whitney U	16	46

Endpoint(s) Affected: Survival, reproduction



Most Sensitive Endpoint(s): Reproduction

#### 14. **REVIEWER'S COMMENTS:**

The reviewer's and study author's conclusions were not in agreement for survival but were in complete agreement for reproduction. One explanation for the discrepancy over the survival endpoint is that the reviewer analyzed survival data using a Dunnett Multiple Comparison test while the study author used a Fisher's Exact Test with Bonferroni-Holm's adjustment. The reviewer's findings are presented in the Conclusions section of this DER.

Time-weighted average (TWA) concentrations were reviewer-calculated for all matrices using the following equation. TWA concentrations are considered more indicative of actual concentration levels and are reported in the Conclusions section of the DER (see associated Excel worksheet in Appendix I). Results were also provided in terms of OC-normalized sediment concentrations based on the sediment organic carbon content of 3.4%. OC-Normalized values were rounded to two significant figures.

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

$C_{TWA}$  is the time-weighted average concentration,

$C_j$  is the concentration measured at time interval  $j$  ( $j = 0, 1, 2, \dots, n$ )

$t_j$  is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval  $j$  (e.g.,  $t_0 = 0$  hours (test initiation),  $t_1 = 24$  hours,  $t_2 = 96$  hours).

The sediment and aqueous samples were analyzed for novaluron concentration using liquid chromatography with tandem mass spectrometry detection (LC/MS/MS) based on methodology validated at Smithers Viscient. The method validations established mean recoveries of  $103 \pm 5.53\%$  (RSD 5.38%) from artificial sediment and  $90.8 \pm 7.86\%$  (RSD 8.66%) from dilute, natural, filtered seawater.

For this study, amphipods of a known age (7 to 8 days old) were used to initiate exposure and this deviates from guidance which recommends the use of neonate organisms obtained from sieving. It was reported that the use of known-aged organisms has become a standard practice based on the recommendation of the OCSPP guideline 850.1740, which states, "for pesticide testing, starting with known-age 7- to 8-day old *L. plumulosus* is preferred to sieving". This practice is beneficial as it reduces the amount of handling and subsequent stress to the organisms and it likely reduces variability between individual organisms.

It was reported that fungal growth was observed in all replicates of the 150  $\mu\text{g ai/kg}$  treatment

level on Day 19. Otherwise, no uncharacteristic observations were noted throughout the exposure.

The experimental phase of the 28-day definitive test was conducted from May 24 to June 21, 2018.

**15. REFERENCES:**

No references were cited other than standard guidelines or statistical methodologies.

**APPENDIX I. COPY OF REVIEWER’S SUPPLEMENTAL CALCULATIONS:**

SEDIMENT (TOC = 3.4%)

Nominal Concentration (µg ai/kg dw)	Measured Concentration (µg ai/kg dw)			TWA (µg ai/kg dw)	Std. Dev.	CV (%)	TWA, OC Norm (µg ai/kg OC)	TWA, OC Norm (µg ai/kg OC) rounded
	Day 0	Day 14	Day 28					
Negative control	<0.27	<0.27	<0.27	<b>&lt;0.27</b>				
Solvent control	<0.27	<0.27	<0.27	<b>&lt;0.27</b>				
1.9	2.0	1.9	1.2	<b>1.8</b>	0.44	25	51	<b>51</b>
5.6	5.7	5.7	4.1	<b>5.3</b>	0.9	17	156	<b>160</b>
17	20	16	12	<b>16</b>	4.0	25	471	<b>470</b>
50	48	48	40	<b>46</b>	4.6	10	1353	<b>1400</b>
150	150	150	120	<b>143</b>	17	12	4191	<b>4200</b>

PORE WATER

Nominal Concentration (µg ai/kg dw)	Measured Concentration (µg ai/L)			TWA (µg ai/L)	Std. Dev.	CV (%)
	Day 0	Day 14	Day 28			
Negative control	<0.0050	<0.0050	<0.0050	<b>&lt;0.0050</b>		
Solvent control	<0.0050	<0.0050	<0.0050	<b>&lt;0.0050</b>		
1.9	0.0088	<b>0.0025</b>	<b>0.0025</b>	<b>0.0041</b>	0.004	89
5.6	0.025	0.020	0.0095	<b>0.019</b>	0.008	42
17	0.078	0.047	0.032	<b>0.051</b>	0.023	46
50	0.23	0.16	0.099	<b>0.16</b>	0.066	40
150	0.75	0.33	0.35	<b>0.44</b>	0.2369	54

<MDL, expressed as 1/2 \* 0.0050, or 0.0025

OVERLYING  
WATER

Nominal Concentration (µg ai/kg dw)	Measured Concentration (µg ai/L)			TWA (µg ai/L)	Std. Dev.	CV (%)
	Day 0	Day 14	Day 28			
Negative control	<0.0050	<0.0050	<0.0050	<b>&lt;0.0050</b>		
Solvent control	<0.0050	<0.0050	<0.0050	<b>&lt;0.0050</b>		
1.9	<0.0050	<0.0050	<0.0050	<b>&lt;0.0050</b>		
5.6	<0.0050	<0.0050	<0.0050	<b>&lt;0.0050</b>		
17	<0.0050	<0.0050	<0.0050	<b>&lt;0.0050</b>		
50	<0.0050	<0.0050	<0.0050	<b>&lt;0.0050</b>		
150	0.011	<b>0.0025</b>	0.028	<b>0.011</b>	0.013	118

<MDL, expressed as 1/2 \* 0.0050, or 0.0025

# CETIS Summary Report

Report Date: 08 Feb-19 18:39 (p 1 of 2)  
 Test Code/ID: 50691105 bulk / 01-7715-1882

## OPPTS 850.1780 Chronic Sediment Leptocheirus

Smithers Viscient

<b>Batch ID:</b> 11-4483-1304	<b>Test Type:</b> Chronic Sediment Leptocheirus	<b>Analyst:</b>
<b>Start Date:</b> 24 May-18	<b>Protocol:</b> OPPTS 850.1780 Chronic Sediment Leptoc	<b>Diluent:</b> Natural seawater & well water
<b>Ending Date:</b> 21 Jun-18	<b>Species:</b> Leptocheirus plumulosus	<b>Brine:</b>
<b>Test Length:</b> 28d 0h	<b>Taxon:</b> Malacostraca	<b>Source:</b> Aquatic Research Organisms <b>Age:</b> 7-8
<b>Sample ID:</b> 07-1700-6482	<b>Code:</b> 50691105 bulk	<b>Project:</b> Insecticide
<b>Sample Date:</b> 24 May-18	<b>Material:</b> Novaluron	<b>Source:</b> ADAMA Makhteshim, Ltd
<b>Receipt Date:</b> 21 Jun-18	<b>CAS (PC):</b>	<b>Station:</b>
<b>Sample Age:</b> n/a	<b>Client:</b> CDM Smith	

124002 50691105, TWA bulk sediment concentrations (ug ai/kg dw), stats performed by A. Graff

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
03-3643-0012	F0 Survival	Equal Variance t Two-Sample Test	0.1693	Solvent Blank passed f0 survival	1
01-2089-0347	Reproduction	Equal Variance t Two-Sample Test	0.3741	Solvent Blank passed reproduction	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	TU	PMSD	S
14-3845-6960	F0 Survival	Dunnett Multiple Comparison Test	46	143	81.1		9.24%	1
20-4850-2814	F0 Survival	Williams Multiple Comparison Test	46	143	81.1		7.21%	1
07-9765-1853	Reproduction	Jonckheere-Terpstra Step-Down Test	✓ 16	46	27.13		n/a	1
01-8758-0695	Reproduction	Mann-Whitney U Two-Sample Test	✓ 16	46	27.13		24.1%	1

### F0 Survival Summary

Conc-µg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	6	0.942	0.872	1.000	0.850	1.000	0.027	0.067	7.06%	0.00%
0	N	6	0.892	0.840	0.943	0.800	0.950	0.020	0.049	5.51%	5.31%
1.8		6	0.942	0.880	1.000	0.850	1.000	0.024	0.059	6.21%	0.00%
5.3		6	0.925	0.870	0.980	0.850	1.000	0.021	0.052	5.67%	1.77%
16		6	0.875	0.795	0.955	0.800	1.000	0.031	0.076	8.67%	7.08%
46		6	0.942	0.880	1.000	0.850	1.000	0.024	0.059	6.21%	0.00%
143		6	0.783	0.712	0.855	0.700	0.900	0.028	0.068	8.72%	16.81%

### Reproduction Summary

Conc-µg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	6	33.6	29.7	37.4	28.6	37.4	1.51	3.71	11.06%	0.00%
0	N	6	30.6	23.5	37.7	24.8	42	2.77	6.77	22.12%	8.74%
1.8		6	35.1	29.1	41	25.2	41.8	2.3	5.64	16.10%	-4.47%
5.3		6	27.1	21.9	32.3	20.8	35.6	2.03	4.97	18.34%	19.18%
16		6	32.5	26.4	38.6	28.3	44	2.37	5.81	17.87%	3.08%
46		6	21.9	17.9	25.9	17.1	27	1.55	3.79	17.29%	34.67%
143		6	14	1.31	26.8	5.5	38.3	4.96	12.1	86.44%	58.12%

# CETIS Summary Report

Report Date: 08 Feb-19 18:39 (p 2 of 2)  
Test Code/ID: 50691105 bulk / 01-7715-1882

OPPTS 850.1780 Chronic Sediment Leptocheirus

Smithers Viscient

## F0 Survival Detail

Conc- $\mu$ g ai/kg	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	S	0.900	1.000	1.000	1.000	0.850	0.900
0	N	0.900	0.900	0.900	0.950	0.900	0.800
1.8		1.000	0.850	1.000	0.950	0.950	0.900
5.3		0.950	0.950	0.850	0.900	1.000	0.900
16		0.800	0.900	0.900	0.800	0.850	1.000
46		1.000	0.850	0.900	0.950	0.950	1.000
143		0.750	0.800	0.700	0.900	0.800	0.750

## Reproduction Detail

Conc- $\mu$ g ai/kg	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	S	33.2	35.2	29.8	37.1	37.4	28.6
0	N	25.7	34.1	42	31.8	25.3	24.8
1.8		34.3	37.6	25.2	33.6	41.8	37.8
5.3		28.3	26.4	20.8	24	27.6	35.6
16		28.8	28.3	44	31.6	30.5	31.9
46		17.1	23.3	27	25	19.3	19.8
143		5.5	7	11.3	38.3	12	10.2

# CETIS Analytical Report

Report Date: 08 Feb-19 18:39 (p 1 of 6)  
 Test Code/ID: 50691105 bulk / 01-7715-1882

## OPPTS 850.1780 Chronic Sediment Leptocheirus

Smithers Viscient

<b>Analysis ID:</b> 14-3845-6960	<b>Endpoint:</b> F0 Survival	<b>CETIS Version:</b> CETISv1.9.5
<b>Analyzed:</b> 08 Feb-19 18:37	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Status Level:</b> 1
<b>Batch ID:</b> 11-4483-1304	<b>Test Type:</b> Chronic Sediment Leptocheirus	<b>Analyst:</b>
<b>Start Date:</b> 24 May-18	<b>Protocol:</b> OPPTS 850.1780 Chronic Sediment Leptoc	<b>Diluent:</b> Natural seawater & well water
<b>Ending Date:</b> 21 Jun-18	<b>Species:</b> Leptocheirus plumulosus	<b>Brine:</b>
<b>Test Length:</b> 28d 0h	<b>Taxon:</b> Malacostraca	<b>Source:</b> Aquatic Research Organism <b>Age:</b> 7-8

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	46	143	81.1		9.24%

### Dunnnett Multiple Comparison Test

Control	vs	Conc-µg ai/k	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.8	-1.42	2.34	0.082	10	CDF	0.9954	Non-Significant Effect
		5.3	-0.945	2.34	0.082	10	CDF	0.9809	Non-Significant Effect
		16	0.472	2.34	0.082	10	CDF	0.6586	Non-Significant Effect
		46	-1.42	2.34	0.082	10	CDF	0.9954	Non-Significant Effect
		143*	3.07	2.34	0.082	10	CDF	0.0095	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.108681	0.0217361	5	5.82	7.2E-04	Significant Effect
Error	0.112083	0.0037361	30			
Total	0.220764		35			

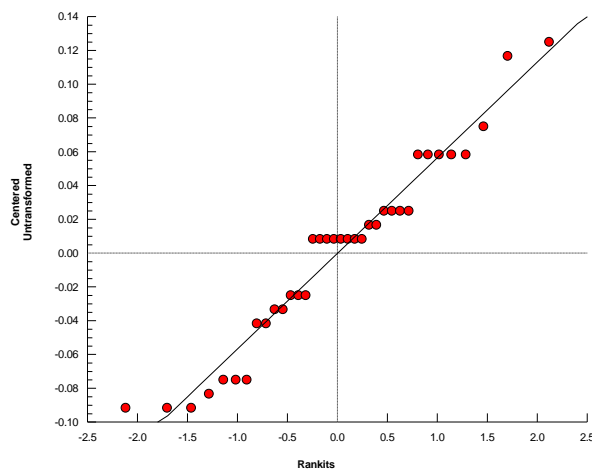
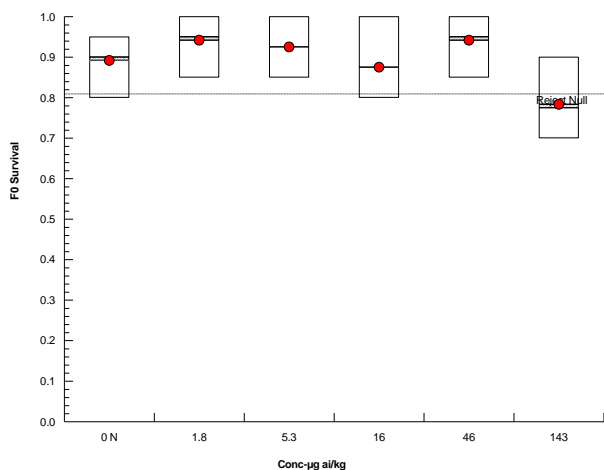
### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	1.24	15.1	0.9410	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.952	0.917	0.1191	Normal Distribution

### F0 Survival Summary

Conc-µg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	6	0.892	0.840	0.943	0.900	0.800	0.950	0.020	5.51%	0.00%
1.8		6	0.942	0.880	1.000	0.950	0.850	1.000	0.024	6.21%	-5.61%
5.3		6	0.925	0.870	0.980	0.925	0.850	1.000	0.021	5.67%	-3.74%
16		6	0.875	0.795	0.955	0.875	0.800	1.000	0.031	8.67%	1.87%
46		6	0.942	0.880	1.000	0.950	0.850	1.000	0.024	6.21%	-5.61%
143		6	0.783	0.712	0.855	0.775	0.700	0.900	0.028	8.72%	12.15%

### Graphics



# CETIS Analytical Report

Report Date: 08 Feb-19 18:39 (p 2 of 6)  
 Test Code/ID: 50691105 bulk / 01-7715-1882

## OPPTS 850.1780 Chronic Sediment Leptocheirus

Smithers Viscient

<b>Analysis ID:</b> 20-4850-2814	<b>Endpoint:</b> F0 Survival	<b>CETIS Version:</b> CETISv1.9.5
<b>Analyzed:</b> 08 Feb-19 18:37	<b>Analysis:</b> Parametric-Control vs Ord.Treatments	<b>Status Level:</b> 1
<b>Batch ID:</b> 11-4483-1304	<b>Test Type:</b> Chronic Sediment Leptocheirus	<b>Analyst:</b>
<b>Start Date:</b> 24 May-18	<b>Protocol:</b> OPPTS 850.1780 Chronic Sediment Leptoc	<b>Diluent:</b> Natural seawater & well water
<b>Ending Date:</b> 21 Jun-18	<b>Species:</b> Leptocheirus plumulosus	<b>Brine:</b>
<b>Test Length:</b> 28d 0h	<b>Taxon:</b> Malacostraca	<b>Source:</b> Aquatic Research Organism <b>Age:</b> 7-8

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	46	143	81.1		7.21%

### Williams Multiple Comparison Test

Control	vs	Conc-µg ai/k	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.8	-1.42	1.7	0.06	10	CDF	>0.05	Non-Significant Effect
		5.3	-0.945	1.78	0.063	10	CDF	>0.05	Non-Significant Effect
		16	0.472	1.8	0.064	10	CDF	>0.05	Non-Significant Effect
		46	-0.472	1.81	0.064	10	CDF	>0.05	Non-Significant Effect
		143*	3.07	1.82	0.064	10	CDF	<0.05	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.108681	0.0217361	5	5.82	7.2E-04	Significant Effect
Error	0.112083	0.0037361	30			
Total	0.220764		35			

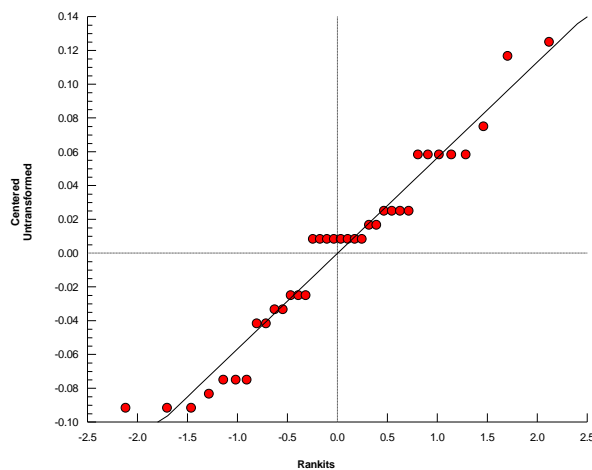
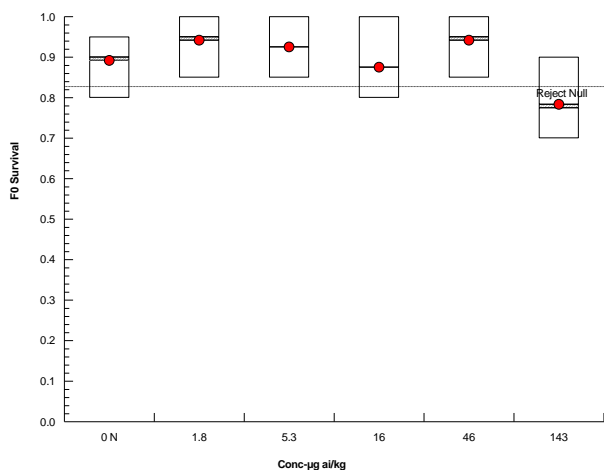
### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	1.24	15.1	0.9410	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.952	0.917	0.1191	Normal Distribution

### F0 Survival Summary

Conc-µg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	6	0.892	0.840	0.943	0.900	0.800	0.950	0.020	5.51%	0.00%
1.8		6	0.942	0.880	1.000	0.950	0.850	1.000	0.024	6.21%	-5.61%
5.3		6	0.925	0.870	0.980	0.925	0.850	1.000	0.021	5.67%	-3.74%
16		6	0.875	0.795	0.955	0.875	0.800	1.000	0.031	8.67%	1.87%
46		6	0.942	0.880	1.000	0.950	0.850	1.000	0.024	6.21%	-5.61%
143		6	0.783	0.712	0.855	0.775	0.700	0.900	0.028	8.72%	12.15%

### Graphics





# CETIS Analytical Report

Report Date: 08 Feb-19 18:39 (p 3 of 6)  
 Test Code/ID: 50691105 bulk / 01-7715-1882

## OPPTS 850.1780 Chronic Sediment Leptocheirus

Smithers Viscient

<b>Analysis ID:</b> 03-3643-0012	<b>Endpoint:</b> F0 Survival	<b>CETIS Version:</b> CETISv1.9.5
<b>Analyzed:</b> 08 Feb-19 18:37	<b>Analysis:</b> Parametric-Two Sample	<b>Status Level:</b> 1
<b>Batch ID:</b> 11-4483-1304	<b>Test Type:</b> Chronic Sediment Leptocheirus	<b>Analyst:</b>
<b>Start Date:</b> 24 May-18	<b>Protocol:</b> OPPTS 850.1780 Chronic Sediment Leptoc	<b>Diluent:</b> Natural seawater & well water
<b>Ending Date:</b> 21 Jun-18	<b>Species:</b> Leptocheirus plumulosus	<b>Brine:</b>
<b>Test Length:</b> 28d 0h	<b>Taxon:</b> Malacostraca	<b>Source:</b> Aquatic Research Organism <b>Age:</b> 7-8

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed f0 survival	8.43%

### Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	1.48	2.23	0.075	10	CDF	0.1693	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0075	0.0075	1	2.2	0.1693	Non-Significant Effect
Error	0.0341667	0.0034167	10			
Total	0.0416667		11			

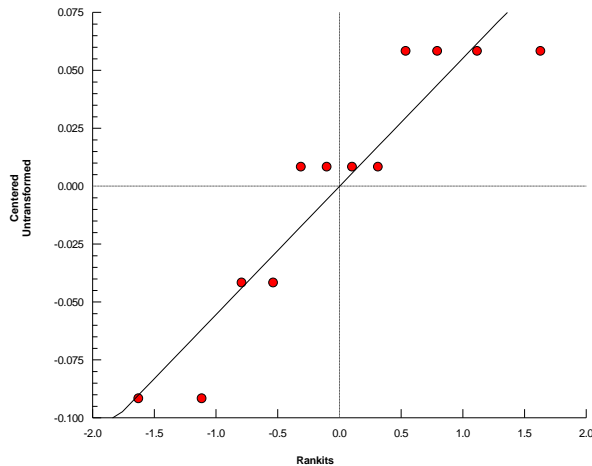
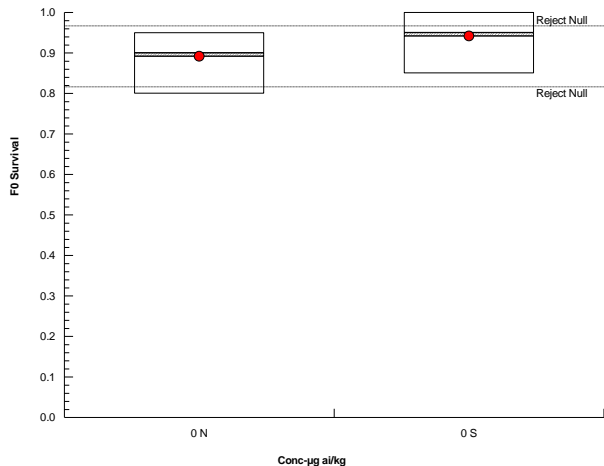
### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	1.83	14.9	0.5241	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.859	0.802	0.0479	Normal Distribution

### F0 Survival Summary

Conc-µg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	6	0.942	0.872	1.000	0.950	0.850	1.000	0.027	7.06%	0.00%
0	N	6	0.892	0.840	0.943	0.900	0.800	0.950	0.020	5.51%	5.31%

### Graphics



# CETIS Analytical Report

Report Date: 08 Feb-19 18:39 (p 4 of 6)  
 Test Code/ID: 50691105 bulk / 01-7715-1882

## OPPTS 850.1780 Chronic Sediment Leptocheirus

Smithers Viscient

<b>Analysis ID:</b> 01-8758-0695	<b>Endpoint:</b> Reproduction	<b>CETIS Version:</b> CETISv1.9.5
<b>Analyzed:</b> 08 Feb-19 18:37	<b>Analysis:</b> Nonparametric-Two Sample	<b>Status Level:</b> 1
<b>Batch ID:</b> 11-4483-1304	<b>Test Type:</b> Chronic Sediment Leptocheirus	<b>Analyst:</b>
<b>Start Date:</b> 24 May-18	<b>Protocol:</b> OPPTS 850.1780 Chronic Sediment Leptoc	<b>Diluent:</b> Natural seawater & well water
<b>Ending Date:</b> 21 Jun-18	<b>Species:</b> Leptocheirus plumulosus	<b>Brine:</b>
<b>Test Length:</b> 28d 0h	<b>Taxon:</b> Malacostraca	<b>Source:</b> Aquatic Research Organism <b>Age:</b> 7-8

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	16	46	27.13		24.09%

### Mann-Whitney U Two-Sample Test

Control	vs	Conc-µg ai/k	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.8	11	n/a	0	10	Exact	0.8799	Non-Significant Effect
		5.3	22	n/a	0	10	Exact	0.2944	Non-Significant Effect
		16	14	n/a	0	10	Exact	0.7576	Non-Significant Effect
		46*	32	n/a	0	10	Exact	0.0130	Significant Effect
		143*	31	n/a	0	10	Exact	0.0206	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1810.7	362.14	5	7.29	1.4E-04	Significant Effect
Error	1490.42	49.6808	30			
Total	3301.12		35			

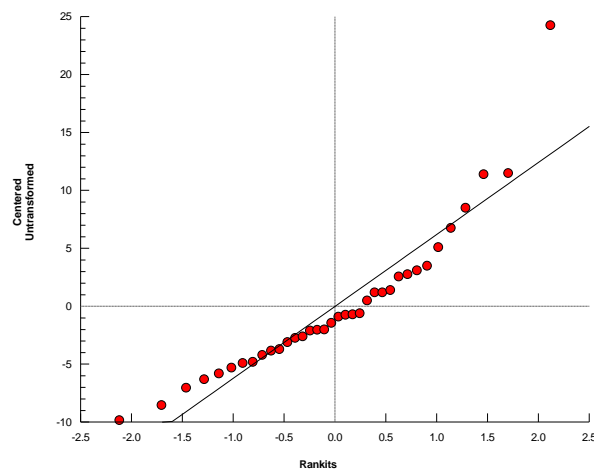
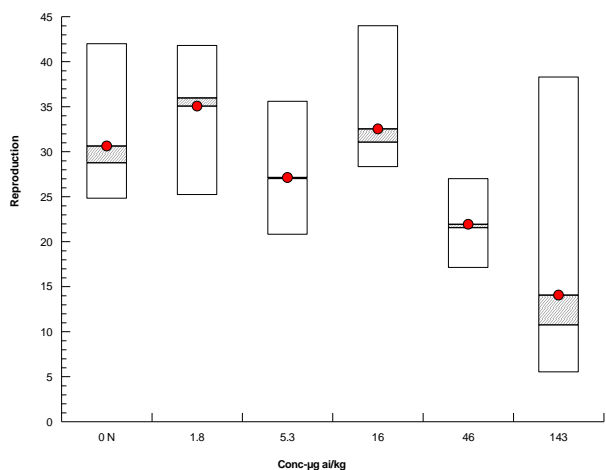
### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	8.17	15.1	0.1472	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.881	0.917	0.0010	Non-Normal Distribution

### Reproduction Summary

Conc-µg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	6	30.6	23.5	37.7	28.8	24.8	42	2.77	22.12%	0.00%
1.8		6	35	29.1	41	35.9	25.2	41.8	2.3	16.10%	-14.48%
5.3		6	27.1	21.9	32.3	27	20.8	35.6	2.03	18.34%	11.43%
16		6	32.5	26.4	38.6	31	28.3	44	2.37	17.87%	-6.21%
46		6	21.9	17.9	25.9	21.5	17.1	27	1.55	17.29%	28.42%
143		6	14	1.31	26.8	10.8	5.5	38.3	4.96	86.44%	54.11%

### Graphics



# CETIS Analytical Report

Report Date: 08 Feb-19 18:39 (p 5 of 6)  
 Test Code/ID: 50691105 bulk / 01-7715-1882

## OPPTS 850.1780 Chronic Sediment Leptocheirus

Smithers Viscient

<b>Analysis ID:</b> 07-9765-1853	<b>Endpoint:</b> Reproduction	<b>CETIS Version:</b> CETISv1.9.5
<b>Analyzed:</b> 08 Feb-19 18:37	<b>Analysis:</b> Nonparametric-Control vs Ord. Treatments	<b>Status Level:</b> 1
<b>Batch ID:</b> 11-4483-1304	<b>Test Type:</b> Chronic Sediment Leptocheirus	<b>Analyst:</b>
<b>Start Date:</b> 24 May-18	<b>Protocol:</b> OPPTS 850.1780 Chronic Sediment Leptoc	<b>Diluent:</b> Natural seawater & well water
<b>Ending Date:</b> 21 Jun-18	<b>Species:</b> Leptocheirus plumulosus	<b>Brine:</b>
<b>Test Length:</b> 28d 0h	<b>Taxon:</b> Malacostraca	<b>Source:</b> Aquatic Research Organism <b>Age:</b> 7-8

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C > T	16	46	27.13	

### Jonckheere-Terpstra Step-Down Test

Control	vs	Conc-µg ai/k	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control		1.8	11	n/a		Exact	0.8799	Non-Significant Effect
		5.3	63	n/a		Exact	0.5103	Non-Significant Effect
		16	-0.0257	1.64	1	Asymp	0.5103	Non-Significant Effect
		46*	2.21	1.64	1	Asymp	0.0136	Significant Effect
		143*	3.42	1.64	1	Asymp	3.1E-04	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1810.7	362.14	5	7.29	1.4E-04	Significant Effect
Error	1490.42	49.6808	30			
Total	3301.12		35			

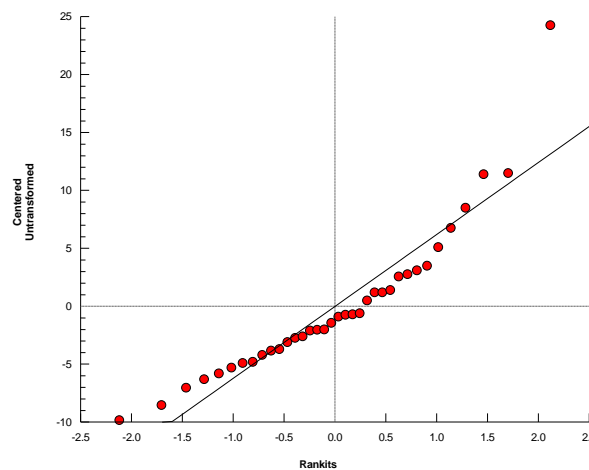
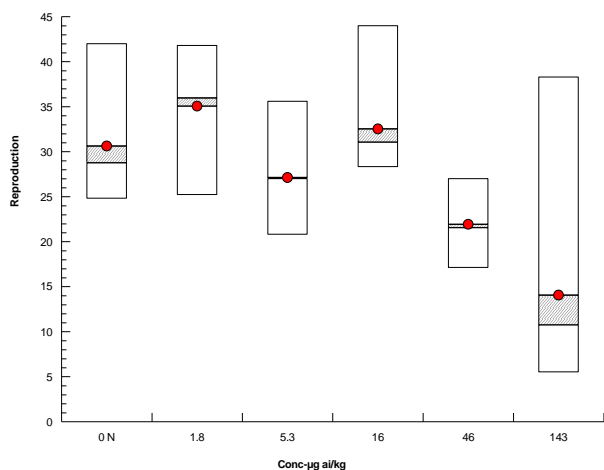
### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	8.17	15.1	0.1472	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.881	0.917	0.0010	Non-Normal Distribution

### Reproduction Summary

Conc-µg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	6	30.6	23.5	37.7	28.8	24.8	42	2.77	22.12%	0.00%
1.8		6	35	29.1	41	35.9	25.2	41.8	2.3	16.10%	-14.48%
5.3		6	27.1	21.9	32.3	27	20.8	35.6	2.03	18.34%	11.43%
16		6	32.5	26.4	38.6	31	28.3	44	2.37	17.87%	-6.21%
46		6	21.9	17.9	25.9	21.5	17.1	27	1.55	17.29%	28.42%
143		6	14	1.31	26.8	10.8	5.5	38.3	4.96	86.44%	54.11%

### Graphics



# CETIS Analytical Report

Report Date: 08 Feb-19 18:39 (p 6 of 6)  
 Test Code/ID: 50691105 bulk / 01-7715-1882

## OPPTS 850.1780 Chronic Sediment Leptocheirus

Smithers Viscient

<b>Analysis ID:</b> 01-2089-0347	<b>Endpoint:</b> Reproduction	<b>CETIS Version:</b> CETISv1.9.5
<b>Analyzed:</b> 08 Feb-19 18:37	<b>Analysis:</b> Parametric-Two Sample	<b>Status Level:</b> 1
<b>Batch ID:</b> 11-4483-1304	<b>Test Type:</b> Chronic Sediment Leptocheirus	<b>Analyst:</b>
<b>Start Date:</b> 24 May-18	<b>Protocol:</b> OPPTS 850.1780 Chronic Sediment Leptoc	<b>Diluent:</b> Natural seawater & well water
<b>Ending Date:</b> 21 Jun-18	<b>Species:</b> Leptocheirus plumulosus	<b>Brine:</b>
<b>Test Length:</b> 28d 0h	<b>Taxon:</b> Malacostraca	<b>Source:</b> Aquatic Research Organism <b>Age:</b> 7-8

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed reproduction	22.95%

### Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.93	2.23	7.03	10	CDF	0.3741	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	25.8133	25.8133	1	0.866	0.3741	Non-Significant Effect
Error	298.223	29.8223	10			
Total	324.037		11			

### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	3.33	14.9	0.2125	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.895	0.802	0.1370	Normal Distribution

### Reproduction Summary

Conc-µg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	6	33.6	29.7	37.4	34.2	28.6	37.4	1.51	11.06%	0.00%
0	N	6	30.6	23.5	37.7	28.8	24.8	42	2.77	22.12%	8.74%

### Graphics

