

# **Exhibit 63**

**DATA EVALUATION RECORD**  
**LIFE-CYCLE SEDIMENT *Chironomus dilutus* TOXICITY TEST**  
**(FOLLOWING EPA TEST METHOD 100.5; 850.1760, in prep)**

1. **CHEMICAL:** Dacthal (DCPA) PC Code No.: 078701

2. **TEST MATERIAL:** Dacthal® Technical herbicide Purity: 99.3%

3. **CITATION:**

Authors: Picard, C.R.

Title: Dacthal Technical - Life-Cycle Toxicity Test Exposing Midges (*Chironomus dilutus*) to a Test Substance Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods.

Study Completion Date: March 10, 2016

Laboratory: Smithers Viscient  
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Laboratory Report ID: 11857.6110

MRID No.: 49865802

DP Barcode: 432681

4. **REVIEWED BY:** David A. McEwen, Staff Scientist, CDM Smith/CSS-Dynamac JV

Signature: 

**Date:** 12/01/2016

**APPROVED BY:** Moncie V. Wright, Environmental Scientist, CDM Smith/CSS-Dynamac JV

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**Date:** 01/06/2017

5. **APPROVED BY:** Christina M. Wendel, Biologist, OPP/EFED/ERB2

Signature:

**Date:** 12/01/2021

Susan Thomas, Biologist, OPP/EFED/ERB4

Signature:

**Date:** 06/02/2021

Michael Wagman, Senior Scientist, OPP/EFED/ERB2

Signature:

**Date:** 11/30/2021

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.

## 7. STUDY PARAMETERS:

<b>Scientific Name of Test Organism:</b>	<i>Chironomus dilutus</i>
<b>Age of Test Organism:</b>	First instar larvae, 3 days old
<b>Definitive Test Duration:</b>	60 days
<b>Study Method:</b>	Intermittent flow-through
<b>Type of Concentrations:</b>	Time-weighted average (TWA) sediment (bulk and OC-normalized), TWA pore water and TWA overlying water concentrations

## 8. EXECUTIVE SUMMARY:

The 60-day chronic toxicity of Chlorthal-dimethyl (Technical DCPA) to *Chironomus dilutus* was studied under static renewal conditions at nominal concentrations of 0 (negative and solvent controls), 2.6, 6.4, 16, 40 and 100 mg a.i./kg-sediment. Given the length of time between measurements of DCPA in various sample matrices (sediment, and/or water), on test days 0, 17 and 60, respectively, the reviewer calculated the time-weighted (TWA) concentrations and these are as follows for each representative sample matrix: <0.21 (<LOD, negative and solvent controls), 1.7, 4.4, 11, 29, and 84 mg a.i./kg in bulk sediment (OC-normalized treatment concentrations equivalent to 81, 209, 537, 1373 and 3981 mg a.i./kg OC, based on 2.1% OC), <0.0029 (<LOD, negative and solvent controls), 0.020, 0.061, 0.13, 0.22 and 0.24 mg a.i./L in pore water and <0.0029 (<LOD, negative and solvent controls), 0.0020, 0.0036, 0.0073, 0.015 and 0.024 mg a.i./L in overlying water. All pore water samples were generated by centrifuging the sediment samples for approximately 30 minutes.

**Results Synopsis:** It was determined that the solvent control positively impacted numerous parameters, as noted by the significant differences (and worse performance for negative controls) that were observed between the solvent and negative control for the following parameters: no. eggs/emerged female, no. eggs/primary egg case, % emerged, and egg cases/emerged female. Even for those endpoints where the solvent control was not statistically significantly different from negative controls (other than the time-based endpoints), the solvent control had substantial differences (solvent had mean 24%↑ in biomass, 26%↑ mortality/↓survival at D17). Therefore, it was determined that the results reported for the negative control were likely the effect of the solvent and not the test substance. There were no treatment-related effects at the highest test level based on the solvent control. Clearly for some endpoints they are impacted by the solvent, but it cannot be ruled out entirely for the other endpoints either, therefore this is an uncertainty of this study, and is considered a study deficiency.

**Based on Comparison to Negative Control (all concentrations are based on time-weighted averages)**

Endpoint	Bulk Sediment (mg a.i./kg)	OC-Normalized Sediment (mg a.i./g OC)	Pore Water (mg a.i./L)	Overlying Water (mg a.i./L)
Day 17 Survival	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
Day 17 AFDW	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
Time to death	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
No. eggs/emerged female*	NOAEC: 1.7 LOAEC: 4.4	NOAEC: 0.081 LOAEC: 0.21	NOAEC: 0.020 LOAEC: 0.061	NOAEC: 0.0020 LOAEC: 0.0036
No. eggs/primary egg case*	NOAEC: <1.7 LOAEC: 1.7	NOAEC: <0.081 LOAEC: 0.081	NOAEC: <0.020 LOAEC: 0.020	NOAEC: <0.0020 LOAEC: 0.0020
% emergence*	NOAEC: 1.7 LOAEC: 4.4	NOAEC: 0.081 LOAEC: 0.21	NOAEC: 0.020 LOAEC: 0.061	NOAEC: 0.0020 LOAEC: 0.0036
Primary Egg cases/emerged female*	NOAEC: 1.7 LOAEC: 4.4	NOAEC: 0.081 LOAEC: 0.21	NOAEC: 0.020 LOAEC: 0.061	NOAEC: 0.0020 LOAEC: 0.0036
Time to oviposition	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024

\* Statistically significant effects for no. of eggs/emerged female, no. of eggs/primary egg case, % emergence, and primary egg cases/emerged female.

**Based on Comparison to Solvent Control (all concentrations are based on time-weighted averages)**

Endpoint	Bulk Sediment (mg a.i./kg)	OC-Normalized Sediment (mg a.i./g OC)	Pore Water (mg a.i./L)	Overlying Water (mg a.i./L)
Day 17 Survival	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
Day 17 AFDW	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
Time to death	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
No. eggs/emerged female	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
No. eggs/primary egg case	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
% emergence	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
Primary Egg cases/emerged female	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024
Time to oviposition	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024

Endpoint(s) Affected: None (based on comparison to solvent control)

## 9. ADEQUACY OF THE STUDY:

A. Classification: This study **is scientifically sound** and is classified as **supplemental and may be used for risk characterization only**.

B. Rationale: This study protocol followed methods described in the “Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates”, 2<sup>nd</sup> Edition, Test Method 100.5 (U.S. EPA 2000). The solvent that was used in this test, acetone, impacted numerous parameters, as noted by the significant differences that were observed between the solvent and negative control (no. eggs/emerged female, no. eggs/primary egg case, % emerged, and egg cases/emerged female). It was determined that the reported results for the negative control were in fact likely the effect of the solvent and not the test substance. However, both Test Method 100.5 and OCSPP 850.1000 guideline recommend that if solvents are used, they not adversely affect test organisms. Clearly for some endpoints they are impacted by the solvent, but it cannot be ruled out entirely for the other endpoints either, therefore this is an uncertainty of this study, and is considered a study deficiency. The test used first instar larvae, (3 days old); however, the 100.5 test method recommends starting with younger (<24-hr) larvae and states that starting with substantially older organisms may compromise the emergence and reproductive endpoints.

C. Reparability: N/A

## 10. GUIDELINE DEVIATIONS:

This study falls under the U.S. EPA guideline 850.1760 that is currently in prep. The study methods were assessed according to the EPA Test method 100.5, and the following deficiencies were observed:

- The solvent that was used in this test, acetone, impacted numerous parameters, and it was determined that the reported results for the negative control were likely the effect of the solvent and not the test substance.
- The test used first instar larvae, 3 days old; however, the 100.5 test method recommends starting with younger (<24-hr) larvae and states that starting with substantially older organisms may compromise the emergence and reproductive endpoints. This is considered a minor deviation.
- The study report did not clearly lay out what the difference was between the total # of surviving ‘midge’ and total # of surviving ‘larvae’ in the columns in the day 17 table on page 124. The study author used the total # of surviving ‘midge’ column and the reviewer used the total # of surviving ‘larvae’ to estimate the % survival calculations and statistical analysis. The negative control % survival was 90% and met the control criteria. However, the solvent control % survival was barely below the control criteria of 70% (67%).

**11. MATERIALS AND METHODS:**

**A. Test Organisms/Acclimation**

Guideline Criteria	Reported Information
<p><b><u>Species</u></b>  <i>Chironomus dilutus</i> (formerly <i>C. tentans</i>)</p>	<p>Midges  <i>Chironomus dilutus</i></p>
<p><b><u>Life Stage</u></b>                      &lt;24-hr old larvae</p>	<p>First instar larvae, 3 days old at test initiation</p>
<p><b><u>Source</u></b>                      Collect a min. of 6 to 8 egg cases.</p>	<p>Newly-oviposited egg masses were obtained from the laboratory's culture facility.</p>
<p><b>All organisms from the same source?</b></p>	<p>Yes</p>
<p><b><u>Culture Conditions</u></b>                      Transfer all egg cases to a crystallizing dish containing control water. Discard larvae that have already left the cases.</p> <p>Test organisms must be cultured and tested at 23°C, ideally in the same water used for testing.</p>	<p>Egg masses were isolated from the main culture and held in laboratory well water at a temperature of <i>ca.</i> 23°C and observed daily until hatch.</p> <p>Hatched larvae were transferred to a shallow glass bowl containing <i>ca.</i> 1 L of laboratory well water (culture water) and reared under static conditions with gentle oil-free aeration for 3 days. During rearing, the temperature was maintained at <i>ca.</i> 23°C and the dissolved oxygen ranged from 7.2 to 7.6 mg/L. The larvae reared in the culture bowls for 3 days after hatching were used to provide first instar larvae for use during the exposure period.</p>
<p><b><u>Feeding</u></b></p>	<p>Hatched larvae were provided with 2.5 mL of <i>Ankistrodesmus falcatus</i> (<math>4 \times 10^7</math> cells/mL) at the start of the 3-day rearing period to serve as both a substrate and food.</p>

**B. Test System**

Guideline Criteria	Reported Information
<p><b><u>Dilution water (overlying water)</u></b>                      Culture water, well water, surface water, site water, or reconstituted water</p>	<p>Laboratory well water characterized as having a total hardness and total alkalinity of 52 and 20 mg/L as CaCO<sub>3</sub>, respectively, a pH of 6.6, and a conductivity of 320 μS/cm.                      Representative samples of the overlying water were analyzed periodically for pesticides, PCBs, and toxic metals by GeoLabs, Inc., Braintree, MA. None of these compounds were detected in any of the samples analyzed in at concentrations that are considered toxic in agreement with ASTM (2007) standard practice. The results of these analyses were not provided in the study report for reference. Samples of overlying water were analyzed monthly for total organic carbon (TOC) concentration, which ranged from 0.26 to 0.89 mg/L for Sept. to Nov. 2015, respectively.</p>
<p><b>Does water support test animals without observable signs of stress?</b></p>	<p>Yes</p>
<p><b><u>Water Temperature</u></b>                      23 ± 1°C</p>	<p>Overall range (Daily meas. Overlying water): 21 to 24°C                      Continuous monitoring in auxiliary vessel: 21 to 25°C (see Reviewer’s Comments)</p>
<p><b><u>pH</u></b></p>	<p>Overall range (overlying water): 6.4 to 7.2                      Overall range (pore water): 5.9 to 6.8                      Overall (in the sediment): 7.1</p>
<p><b><u>Dissolved Oxygen</u></b>                      &gt;2.5 mg/L</p>	<p>Overall range (overlying water): 2.5 to 7.6 mg/L</p>
<p><b><u>Ammonia</u></b>                      Should not vary more than 50%.</p>	<p>Overall range (overlying water): ≤0.10 to 0.87 mg/L as N                      Overall range (pore water): 1.1 to 2.5 mg/L as N</p>
<p><b><u>Hardness</u></b>                      Should not vary more than 50%.</p>	<p>Overall range (overlying water): 52 to 80 mg/L as CaCO<sub>3</sub></p>
<p><b><u>Alkalinity</u></b>                      Should not vary more than 50%.</p>	<p>Overall range (overlying water): 14 to 26 mg/L as CaCO<sub>3</sub></p>

Guideline Criteria	Reported Information
<b><u>Conductivity</u></b>	Overall range (overlying water): 380 to 510 $\mu\text{S}/\text{cm}$
<b><u>Test Sediment</u></b> Natural or formulated sediment	<p>Artificial sediment (Batch No. 072915) was prepared according to OECD Guideline No. 218 (2004) by mixing the following components (on a dry weight basis): 2.4 kg sphagnum peat, 9.6 kg kaolin clay and 36 kg fine sand (5.0, 20, and 75%, respectively). While blending the sediment components using a large-scale laboratory mixer, a total of 9.6 L of laboratory well water was also added.</p> <p>Prior to use, the peat was pre-soaked in dilution water for 1 week. During this time, the peat was amended with 160 g of powdered <math>\text{CaCO}_3</math> to increase the pH from 3.3 to 6.0.</p> <p>Representative samples of the sediment were analyzed periodically for pesticides, PCBs, and toxic metals by GeoLabs, Inc., Braintree, MA. None of these compounds were detected at concentrations that are considered toxic in any of the samples analyzed in agreement with ASTM (2007) standard practice. The results of these analyses were not provided in the study report for reference.</p>
<b><u>Sediment Characterization</u></b> pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content should be reported	<p>Characterized by Agvise Laboratories, Northwood, North Dakota</p> <p>Particle distribution – 83% sand, 4% silt, 13% clay</p> <p>Percent organic carbon – 2.1%</p> <p>Percent solids – 67.26%</p> <p>Percent moisture at 1/3 bar – not reported</p> <p>pH – 7.1</p> <p>Ammonia concentration of pore water on Day 0 in the control group – 2.5 mg/L (as N)</p>
<b><u>Test Material</u></b>	<p>Identity: Dacthal<sup>®</sup> technical herbicide (DCPA a.i.)</p> <p>IUPAC name: Tetrachloroterephthalic acid</p> <p>CAS name: 2,3,5,6-tetrachloro-1,4-benzenedicarboxylic acid</p> <p>CAS No.: 1861-32-1</p>



Guideline Criteria	Reported Information
	<p>Description: Not reported  Batch No.: 120904-1  Purity: 99.3%  Storage: Room temperature, in a dark ventilated cabinet  Aqueous solubility: Not reported</p> <p>A 25 mg/mL primary stock solution was prepared by bringing 0.6298 g of test substance to 25 mL with acetone. Five individual dosing stock solutions were then prepared (at 0.600 to 23.0 mg/mL) by diluting the appropriate volume of primary stock into 10 mL acetone. All dosing stock solutions were clear and colorless with no visible undissolved test substance.</p>
<b><u>Solvents</u></b>	<p>Acetone, 10 mL/2.068 kg dw</p> <p>The acetone was allowed to evaporate during the mixing procedure.</p> <p>Both solvent control and negative control groups were included in the study.</p>
<b><u>Sediment Spiking</u></b>	<p>A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand and the solvent was allowed to evaporate off for 50 minutes. The dry sand was then added to 3.0 kg of wet sediment (total of 2.068 kg dw based on percent solids of 67.26% and including the 0.05 kg fine silica sand) in individual glass jars (e.g., 4-L). Each jar was then rolled at room temperature for 4 hours at <i>ca.</i> 15 rpm.</p> <p>The range of nominal concentrations (nominally 2.6 to 100 mg/kg dw) was based upon the results of a preliminary testing and in consultation with the Sponsor.</p>
<b><u>Sediment Conditioning</u></b>	<p>Prior to the definitive study, a 28-day test was conducted in order to establish the time required to reach equilibration in spiked sediment (Smithers Viscient Study No. 11857.6112;</p>

Guideline Criteria	Reported Information
	<p>Appendix 2; pg. 93-95). The sediment was treated at a single nominal level of 10 mg/kg dw as previously-described by dosing sand, allowing the solvent to evaporate for 45 minutes, and incorporating the treated sand into formulated sediment. The treated sediment was rolled for 4 hours on a rolling mill at 15 rpm and then stored upright overnight at 2 to 8°C. At 1, 7, 14, 21 and 28 days, the treated sediment was rolled for an additional 2 hours, sampled in triplicate and samples centrifuged at 10,000 g for 15 to 30 minutes to generate pore water samples. The results demonstrated that pore water concentrations on Days 14 and 21 were statistically similar and suggestive of pore water equilibrium. Based on these results, and consultation with the study sponsor, an approximate 14-day equilibration time of spiked sediments was used in the sediment toxicity testing with dacthal.</p> <p>The jars containing treated sediment were stored upright in a dark refrigerator at 2 to 8°C for a 14-day equilibration period (see Reviewer’s Comments).</p> <p>Once a week during the 14-day equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill at room temperature for 2 hours to ensure the sediment was homogeneous.</p>
<p><b><u>Test Vessels</u></b>                      300 mL high-form lipless glass beakers containing 100 mL of sediment and 175 ml of overlying water</p>	<p>300-mL glass vessels, with two slots cut on the top edge covered with 40-mesh Nitex® screen for drainage.</p> <p>Each vessel contained 100 mL (ca. 4.0-cm layer) of sediment (equivalent to 172 g wet weight or 116 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at approx. 275 mL.</p> <p>On Day 14, emergence traps were placed over the test vessels to trap emergent flies for the remainder of the test. The emergence traps were 3.5-cm tall Plexiglass® tubes (6-cm id)</p>

Guideline Criteria	Reported Information
	covered with wide-mesh Nitex® screen.
<b><u>Reproductive/oviposit chambers</u></b>	Plexiglass® tubes (3.5-cm length, 6-cm id) covered on the top with wide-mesh Nitex® screen and placed within a 100 x 20-mm Petri dish. With midge addition, <i>ca.</i> 50 mL of laboratory well water was added to the Petri dish.
<b><u>Type of Dilution System</u></b> Continuous or intermittent	Intermittent flow-through
<b><u>Flow Rate</u></b> 2 volume additions/day	Days 0 to 5: 2 volume additions/day Days 6 to 60: 4 volume additions/day
<b><u>Aeration</u></b> None, unless DO in overlying water drops <2.5 mg/L	None reported
<b><u>Photoperiod</u></b> 16-hours light, 8-hours dark using wide-spectrum fluorescent lights; intensity of 100 to 1000 lux	16-hour light/8-hour dark photoperiod using fluorescent bulbs at an intensity range of 290 to 460 lux.
<b><u>Feeding</u></b>	Midge larvae were fed a finely-ground flaked aqueous fish food suspension (4.0 mg/mL). During exposure, the food was introduced at a rate of 1.5 mL of flaked fish food suspension per test vessel per day. Representative samples of the food source were analyzed periodically for pesticides, PCBs, and toxic metals by GeoLabs, Inc., Braintree, MA. None of these compounds have been detected at concentrations that are considered toxic in any of the samples analyzed in agreement with ASTM (2007) standard practice.

**C. Test Design**

<b>Guideline Criteria</b>	<b>Reported Information</b>
<p><b><u>Duration</u></b> About 50 to 65 days; each treatment is ended separately when no additional emergence occurs for 7 consecutive days. When no emergence is recorded from a treatment, termination of that level is based on the control sediment using the 7-day criterion.</p>	60 days
<p><b><u>Sediment 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 using a turbulence reducer, and each vessel was placed under the renewal system.</p> <p>Additional test systems were established on Day 9 for auxiliary male production.</p>
<p><b><u>Renewal of Overlying Water</u></b> Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.</p>	<p>The overlying water was renewed by adding two volume additions of water per test vessel per day via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected twice daily for proper functioning. The water system cycled approx. seven times per day and increased to 14 times per day on test day 5 (two and four volume additions per vessel per day, respectively).</p>
<p><b><u>Placing Organisms in Test Chambers</u></b> Should be handled as little as possible and introduced into overlying water below the air-water interface.</p>	<p>At test initiation (Day 0), first instar midge larvae (3-days old) were impartially added to replicates A-L; until each contained 12 larvae. On Day 10 of the test, first instar midge larvae (up to 3-days old) were impartially added to the four auxiliary male replicates M-P until each contained 12 larvae.</p>
<p><b><u>Monitoring the test</u></b> All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.</p>	<p>Test systems were observed daily for dead organisms (larvae or pupae) on the sediment surface, abnormal behavior, and characteristics of test solutions.</p> <p>Also daily beginning on Day 17, the number of emerged midges was recorded (male and female), and once established, reproductive/oviposit (R/O) chambers were</p>

Guideline Criteria	Reported Information
	checked daily for dead adults and egg masses. Survival of individual midges was recorded daily until death.
<p><b><u>Range Finding Test</u></b> A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment.</p>	<p><b><u>Preliminary toxicity assessment</u></b></p> <ul style="list-style-type: none"> <li>• 42-day exposure at nominal levels of 0 (negative control), 0 (acetone solvent control), 0.39, 1.6, 6.3, 25, and 100 mg/kg (dw) sediment</li> <li>• Dosed sediments were equilibrated for 14 days</li> <li>• Twelve, 3-day old (first instar) larvae per replicate, with eight replicates per level initiated on Day 0</li> <li>• Compared to the negative control, there were statistically-significant reductions observed at the 25 and 100 mg/kg levels in Day-20 AFDW and at the 0.39, 6.3 and 100 mg/kg levels in Day 42 percent emergence; however, no clear dose-response was observed, and the reductions were not considered to be treatment-related.</li> <li>• Based on these results, and in consultation with the Sponsor, nominal sediment concentrations were selected for the definitive test.</li> </ul>
<p><b><u>Nominal Concentrations of Definitive Test</u></b></p>	0 (negative and solvent controls), 2.6, 6.4, 16, 40, and 100 mg ai/kg dw sediment
<p><b><u>Number of Test Organisms</u></b> 12 organisms per test chamber are recommended; 16 replicates per treatment should be used (twelve at Day -1 and four for auxiliary males on Day 10)</p>	<p>12 organisms per test chamber; 144 individuals per treatment level or control</p> <p>12 biological replicates per level initiated on Day -1</p> <p>4 biological replicates per level initiated on Day 10 for production of auxiliary males</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	Yes

Guideline Criteria	Reported Information
<p><b><u>Water Parameter Measurements</u></b>            Conductivity, hardness, alkalinity, and ammonia should be measured in all treatments at the beginning, on Day 20, and at the end of the test. Conductivity should also be determined weekly.</p> <p>DO and pH should be measured at the beginning of the test and at least three times per week thereafter.</p> <p>Temperature should be measured daily in one test chamber from each treatment. The temperature of the water bath should be continuously monitored.</p>	<p><u>Overlying water:</u>            For all levels, total hardness, alkalinity, conductivity, and ammonia (as N) concentrations were measured in a composite sample of overlying water from all available biological replicates on Days 0, 10, 17, and 60.</p> <p>DO, temperature, and pH were measured in the overlying water of each replicate vessel used for biological monitoring on Days 0, 10, 17 and 60. On the remaining test days, DO and temperature were measured daily in one alternating replicate from each level. The temperature was continuously monitored in an auxiliary vessel in the water bath.</p> <p><u>Pore water:</u>            The pH and ammonia (as N) were measured in isolated pore water from the negative control level and replicates Q, R and T (chemical analysis replicates) on Days 0, 17 and 60.</p>
<p><b><u>Chemical Analysis</u></b></p>	<p><u>Pre-test analyses:</u>            Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for dacthal.</p> <p><u>In-life analyses:</u>            Concentrations of dacthal were determined in sediment, pore water and overlying water samples from surrogate test vessels collected on Days 0, 17 and 60.</p> <p>The overlying water was decanted, and the sediment was centrifuged at <i>ca.</i> 10,000 <i>g</i> for 30 minutes to isolate the sediment and pore water matrices. Sediment samples were then mixed well prior to analysis.</p> <p>Aqueous samples were analyzed using high performance liquid chromatography with ultraviolet detection (HPLC/UV) and sediment samples were analyzed using gas chromatography with micro-electron capture detection (GC/<math>\mu</math>ECD) based on methodology validated at Smithers Viscient (see Reviewer's</p>

Guideline Criteria	Reported Information
	Comments).

**12. REPORTED RESULTS:**

**A. General Results**

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR, Part 160 with the following exceptions: routine water, sediment and food contaminant screening analyses for pesticides, PCBs, and toxic metals. It was reported, however, that these analyses were conducted following standard validated methods.
<p><b><u>Control Criteria</u></b>  <b>Was control survival at least 70% and dry weight at least 0.6 mg/organism (0.48 mg/organisms AFDW) on Day 20?</b></p> <p><b>Was control emergence <math>\geq 50\%</math>?</b></p> <p><b>Was the mean number of control eggs/egg case <math>\geq 800</math> and was the percent hatch <math>\geq 80\%</math>?</b></p>	<p><u>All control criteria met:</u>                      Negative control: 90% and 1.67 mg AFDW/ larva                      Solvent control: 67% and 2.07 mg AFDW/ larva</p> <p>Negative control: 85%                      Solvent control: 62%</p> <p>Negative control: 1358 eggs/mass and 89%                      Solvent control: 1078 eggs/mass and 88%</p>
<b><u>Percent Recovery of Chemical</u></b>	<p><u>Procedural recoveries (from QC samples) conducted concurrently with sample analysis:</u>  <u>Sediment:</u>                      Spiked at 1.00, 16.0, and 100 mg/kg                      Recovery range of 88.5 to 109% (n=9)                      LOQ<sub>sediment</sub> = 0.21 mg a.i./kg  <u>Aqueous:</u>                      Spiked at 0.005, 0.500, and 10.0 mg/L                      Recovery range of 99.6 to 115% (n=9)                      LOQ<sub>water</sub> = 0.0025 to 0.0029 mg a.i./L</p>

Guideline Criteria	Reported Information
<b><u>Data Endpoints</u></b>	<p><u>Day 17:</u></p> <ul style="list-style-type: none"> <li>- Survival</li> <li>- Ash-free dry weight (AFDW)</li> </ul> <p><u>Day 60:</u></p> <ul style="list-style-type: none"> <li>- Percent emergence</li> <li>- Male and female emergence rates</li> <li>- Male and female days to death (for mated midges)</li> <li>- Eggs masses per mated female</li> <li>- Eggs per egg mass</li> <li>- Eggs per mated female</li> <li>- Percent hatch</li> <li>- Days to oviposition</li> </ul>
<b>Raw data included?</b>	Yes

Effects Data (study author results):Day 17 Endpoints:

Survival on Day 17 averaged 90 and 92% in the negative and solvent controls, respectively, and was 85, 75, 73, 94, and 83% in the mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, respectively. No statistically-significant differences were reported at any treatment level compared to the negative control. In terms of mean-measured sediment concentrations, the reported NOAEC and LOAEC for Day 17 survival were 86 and >86 mg a.i./kg, respectively. In terms of mean-measured pore water concentrations, the reported NOAEC and LOAEC for Day 17 survival were 0.25 and >0.25 mg a.i./L, respectively.

Ash-free dry weight (AFDW) on Day 17 averaged 1.67 and 2.07 mg per larvae for the negative and solvent controls, respectively, and was 1.64, 2.60, 2.32, 2.19, and 2.05 mg per larvae in the mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, respectively. No statistically-significant differences were reported at any treatment level compared to the negative control. The reported NOAEC and LOAEC for Day 17 AFDW were 86 and >86 mg a.i./kg, respectively, using mean-measured sediment concentrations. In terms of mean-measured pore water concentrations, the reported NOAEC and LOAEC were 0.25 and >0.25 mg a.i./L, respectively.



Test Concentration		Day 17	
Mean-measured (and Nominal) Sediment, mg a.i./kg	Mean-measured Pore Water, mg a.i./L	Survival (% ± SD) <sup>1</sup>	AFDW (mg/larva ± SD)
Negative control		90 ± 8 (90)	1.67 ± 0.17
Solvent control		92 ± 12 (67)	2.07 ± 0.53
1.8 (2.6)	0.021	85 ± 4 (75)	1.64 ± 0.35
4.8 (6.4)	0.060	75 ± 7 (65)	2.60 ± 0.33
12 (16)	0.12	73 ± 18 (69)	2.32 ± 0.26
30 (40)	0.21	94 ± 8 (90)	2.19 ± 0.35
86 (100)	0.25	83 ± 15 (73)	2.05 ± 0.77

LOQ = 0.21 mg a.i./kg dw for sediment samples and 0.0025-0.0029 mg a.i./L for aqueous samples.

<sup>1</sup> Reviewer-calculated numbers differed from the study author in some cases and are presented in (parentheses). The study author used the total # of surviving 'midge' column and the reviewer used the total # of surviving 'larvae' to estimate the % survival calculations and statistical analysis (Day 17 table, pg 124 of study report).

#### Day 60 Endpoints:

Percent emergence averaged 85 and 62% in the negative and solvent controls, respectively, and was 88, 60, 68, 79, and 69% in the mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, respectively. A statistically-significant difference was reported at the 12 mg ai/kg level ( $p < 0.05$ , Steel's Many-One Rank Sum Test) compared to the negative control. However, this reduction was not considered to be treatment related due to the lack of a clear dose response. In terms of mean-measured sediment concentrations, the reported NOAEC and LOAEC for percent emergence were 86 and >86 mg a.i./kg, respectively. In terms of mean-measured pore water concentrations, the reported NOAEC and LOAEC were 0.25 and >0.25 mg a.i./L, respectively.

Test Concentration		Day 60				
Mean-measured (and Nominal) Sediment, mg a.i./kg	Mean-measured Pore Water, mg a.i./L	Percent Emergence $\pm$ SD	Male Emergence Rate $\pm$ SD	Female Emergence Rate $\pm$ SD	Male Days to Death $\pm$ SD	Female Days to Death $\pm$ SD
Negative control		85 $\pm$ 9	0.0459 $\pm$ 0.0055	0.0404 $\pm$ 0.0024	4.1 $\pm$ 0.63	4.2 $\pm$ 0.53
Solvent control		62 $\pm$ 10	0.0515 $\pm$ 0.0009	0.0460 $\pm$ 0.0015	4.8 $\pm$ 2.4	3.9 $\pm$ 0.45
1.8 (2.6)	0.021	88 $\pm$ 9	0.0470 $\pm$ 0.0033	0.0432 $\pm$ 0.0032	4.2 $\pm$ 0.68	4.1 $\pm$ 0.57
4.8 (6.4)	0.060	60 $\pm$ 38	0.0499 $\pm$ 0.0024	0.0438 $\pm$ 0.0035	4.5 $\pm$ 1.5	3.7 $\pm$ 0.73
12 (16)	0.12	68 $\pm$ 11 <sup>a</sup>	0.0489 $\pm$ 0.0035	0.0448 $\pm$ 0.0031	3.7 $\pm$ 1.9	4.0 $\pm$ 0.46
30 (40)	0.21	79 $\pm$ 16	0.0484 $\pm$ 0.0028	0.0435 $\pm$ 0.0028	4.1 $\pm$ 0.74	3.5 $\pm$ 0.63 <sup>b</sup>
86 (100)	0.25	69 $\pm$ 25	0.0460 $\pm$ 0.0028	0.0418 $\pm$ 0.0026	3.7 $\pm$ 1.1	3.8 $\pm$ 0.46

a Statistically-significant reduction compared to the negative control ( $p < 0.05$ ; Steel's Many-One Rank Sum Test). However, this reduction was not considered to be treatment related due to the lack of a clear dose response.

b Statistically-significant reduction compared to the negative control ( $p < 0.05$ ; Bonferroni's Adjusted t-Test). However, this reduction was not considered to be treatment related due to the lack of a clear dose response.

LOQ = 0.21 mg a.i./kg dw for sediment samples and 0.0025-0.0029 mg a.i./L for aqueous samples.

Male emergence rates averaged 0.0459 and 0.0515 for the negative and solvent controls, respectively, and were 0.0470, 0.0499, 0.0489, 0.0484, and 0.0460 in the mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, respectively. No statistically-significant differences were reported at any treatment level compared to the negative control. The reported NOAEC and LOAEC for male emergence rate were 86 and >86 mg a.i./kg, respectively. In terms of mean-measured pore water concentrations, the reported NOAEC and LOAEC were 0.25 and >0.25 mg a.i./L, respectively.

Female emergence rates averaged 0.0404 and 0.0460 for the negative and solvent controls, respectively, and were 0.0432, 0.0438, 0.0448, 0.0435, and 0.0418 for the mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, respectively. No statistically-significant differences were reported at any treatment level compared to the negative control. The reported NOAEC and LOAEC for female emergence rate were 86 and >86 mg ai/kg, respectively. In terms of mean-measured pore water concentrations, the reported NOAEC and LOAEC were 0.25 and >0.25 mg a.i./L, respectively.

For the negative control, solvent control, and mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, male time to death averaged 4.1, 4.8, 4.2, 4.5, 3.7, 4.1, and 3.7 days, respectively, and female time to death averaged 4.2, 3.9, 4.1, 3.7, 4.0, 3.5, and 3.8 days, respectively. For male days to death, no statistically-significant differences were reported compared to the negative control at any treatment level. For female days to death, a statistically-significant difference was reported at the 30 mg a.i./kg level ( $p < 0.05$ , Bonferroni's Adjusted t-Test) compared to the negative control. However, this reduction was not considered to be treatment related due to the lack of a clear dose response. The reported NOAEC and LOAEC for both endpoints were 86 and >86 mg a.i./kg, respectively. In terms of mean-measured pore water concentrations, the reported NOAEC and LOAEC were 0.25 and >0.25 mg a.i./L, respectively.

The mean number of eggs per egg mass averaged 1358 and 1078 for the negative and solvent controls, respectively, and was 1135, 1162, 1023, 1241, and 1011 in the mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, respectively. A treatment-related decrease ( $p < 0.05$ , Bonferroni's Adjusted t-Test) in the number of eggs per egg mass was reported at 86 mg a.i./kg compared to the negative control. The significant decrease noted at 12 mg a.i./kg was not considered to be related to treatment due to the lack of a clear dose response. The mean number of egg masses per mated female averaged 0.96 and 0.79 for the negative and solvent controls, respectively, and was 0.95, 0.65, 0.74, 0.98, and 0.72 in the mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, respectively. A treatment-related decrease ( $p < 0.05$ , Wilcoxon's Test with Bonferroni's Adjustment) in the mean number of egg masses per mated female was reported at 86 mg a.i./kg compared to the negative control. The significant decrease noted at 12 mg a.i./kg was not considered to be related to treatment due to the lack of a clear dose response. The mean number of eggs per mated female averaged 1316 and 841 for the negative and solvent controls, respectively, and was 1083, 752, 764, 1207, and 715 in the mean-

measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, respectively. A treatment-related decrease ( $p < 0.05$ , Bonferroni's Adjusted t-Test) in the mean number of eggs per mated female was reported at 86 mg a.i./kg compared to the negative control. The significant decreases noted at 4.8 and 12 mg a.i./kg were not considered to be related to treatment due to the lack of a clear dose response. In terms of mean-measured sediment concentrations, the reported NOAEC and LOAEC for these parameters were 30 and 86 mg a.i./kg, respectively. In terms of mean-measured pore water concentrations, the reported NOAEC and LOAEC were 0.21 and 0.25 mg a.i./L, respectively.

No treatment-related effects were reported on mean percent hatch or mean days to oviposition. For the negative control, solvent control, and mean-measured 1.8, 4.8, 12, 30, and 86 mg a.i./kg treatment levels, the mean percent hatch averaged 89, 88, 93, 74, 76, 97, and 86%, respectively and mean days to oviposition averaged 1.3, 1.3, 1.2, 1.3, 1.4, 1.2, and 1.1, respectively. The reported NOAEC and LOAEC for these reproductive parameters were 86 and >86 mg a.i./kg, respectively. In terms of mean-measured pore water concentrations, the reported NOAEC and LOAEC were 0.25 and >0.25 mg a.i./L, respectively.

Test Concentration		Day 60				
Mean-measured (and Nominal) Sediment, mg a.i./kg	Mean-measured Pore Water, mg a.i./L	Mean Eggs per Egg Mass $\pm$ SD	Mean Percent Hatch $\pm$ SD	Mean Egg Masses per Mated Female $\pm$ SD	Mean No. of Eggs per Mated Female $\pm$ SD	Mean Days to Oviposition $\pm$ SD
Negative control		1358 $\pm$ 252	89 $\pm$ 13	0.96 $\pm$ 0.072	1316 $\pm$ 310	1.3 $\pm$ 0.36
Solvent control		1078 $\pm$ 229	88 $\pm$ 12	0.79 $\pm$ 0.16	841 $\pm$ 222	1.3 $\pm$ 0.35
1.8 (2.6)	0.021	1135 $\pm$ 113	93 $\pm$ 8	0.95 $\pm$ 0.091	1083 $\pm$ 168	1.2 $\pm$ 0.22
4.8 (6.4)	0.060	1162 $\pm$ 81	74 $\pm$ 15	0.65 $\pm$ 0.46	752 $\pm$ 526 <sup>a</sup>	1.3 $\pm$ 0.17
12 (16)	0.12	1023 $\pm$ 157 <sup>a</sup>	76 $\pm$ 18	0.74 $\pm$ 0.15 <sup>b</sup>	764 $\pm$ 219 <sup>a</sup>	1.4 $\pm$ 0.44
30 (40)	0.21	1241 $\pm$ 342	97 $\pm$ 2	0.98 $\pm$ 0.071	1207 $\pm$ 340	1.2 $\pm$ 0.33
86 (100)	0.25	1011 $\pm$ 197 <sup>c</sup>	86 $\pm$ 9	0.72 $\pm$ 0.16 <sup>d</sup>	715 $\pm$ 136 <sup>c</sup>	1.1 $\pm$ 0.19

a Statistically-significant reduction compared to the negative control ( $p < 0.05$ ; Bonferroni's Adjusted t-Test). However, this reduction was not considered to be treatment related due to the lack of a clear dose response.

b Statistically-significant reduction compared to the negative control ( $p < 0.05$ ; Wilcoxon's Test with Bonferroni's Adjustment). However, this reduction was not considered to be treatment related due to the lack of a clear dose response.

c Statistically-significant reduction compared to the negative control ( $p < 0.05$ ; Bonferroni's Adjusted t-Test).

d Statistically-significant reduction compared to the negative control ( $p < 0.05$ ; Wilcoxon's Test with Bonferroni's Adjustment).

LOQ = 0.21 mg a.i./kg dw for sediment samples and 0.0025-0.0029 mg a.i./L for aqueous samples.

Analytical Data:

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for dacthal. Recoveries in the stock solutions ranged from 98 to 110% of nominal concentrations. Analysis of the spiked sediment samples after mixing and prior to allocation into the test vessels resulted in recoveries ranging from 100 to 120% of nominal concentrations. During testing, concentrations of dacthal were determined in sediment, overlying water and pore water on Days 0, 17 and 60.

In sediment, concentrations of dacthal were slightly declining during the 60-day period (with reviewer-calculated coefficients of variation of 18 to 32%; see Appendix 1). For the nominal 2.6, 6.4, 16, 40, and 100 mg a.i./kg treatment levels, sediment concentrations averaged 2.3, 6.3, 15, 37, and 98 mg a.i./kg, respectively, on Day 0; 1.8, 4.5, 12, 30, and 90 mg a.i./kg, respectively, on Day 17; and 1.3, 3.5, 8.8, 24, and 69 mg a.i./kg, respectively, on Day 60. Overall mean-measured sediment concentrations were 1.8, 4.8, 12, 30, and 86 mg a.i./kg, representing 69 to 86% of nominal sediment levels.

In pore water, concentrations of dacthal were relatively consistent during the 60-day exposure period (with reviewer-calculated coefficients of variation of 6 to 21%; see Appendix 1). For the nominal 2.6, 6.4, 16, 40, and 100 mg a.i./kg treatment levels, pore water concentrations averaged 0.023, 0.058, 0.11, 0.17, and 0.25 mg a.i./L, respectively, on Day 0; 0.020, 0.064, 0.15, 0.26, and 0.21 mg a.i./L, respectively, on Day 17; and 0.019, 0.057, 0.12, 0.19, and 0.28 mg a.i./L, respectively, on Day 60. Overall mean-measured pore water concentrations were 0.021, 0.060, 0.12, 0.21, and 0.25 mg a.i./L.

In overlying water, maximum concentrations of dacthal were observed on Day 0 and concentrations decreased during the 60-day exposure period (with reviewer-calculated coefficients of variation of 103 to 177%; see Appendix 1). For the nominal 2.6, 6.4, 16, 40, and 100 mg a.i./kg sediment treatment levels, overlying water concentrations averaged 0.0059, 0.013, 0.023, 0.038, and 0.058 mg a.i./L, on Day 0; <LOQ (<0.0025), <LOQ (<0.0025), 0.0058, 0.017, and 0.025 mg a.i./L, respectively, on Day 17; and <LOQ (<0.0029), 0.0031, 0.0032, 0.0044, and 0.0093 mg a.i./L, respectively, on Day 60.

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

Endpoints that were statistically-analyzed included larval survival and growth (ash-free dry weight; AFDW) on Day 17; and percent emergence, emergence rate (gender-specific), time to death (gender-specific), eggs masses per mated female, eggs per egg mass, eggs per mated female, percent hatch, and days to oviposition on Day 60. Analyses were performed with CETIS<sup>TM</sup> (Version 1.8, 2013) statistical software. Percent survival, percent emergence and percent hatch data were transformed (*e.g.*, arcsine square-root percentage) prior to analysis. Results were provided in terms of mean-measured sediment and mean-measured pore water concentrations.

An Equal Variance Two-Sample t-Test, Unequal Variance Two-Sample t-Test, or Wilcoxon’s Rank Sum Two-Sample Test was used to compare the performance of the negative control and solvent control data. The negative control and solvent control data were similar for all endpoints. Treatment-level data were compared to the negative control data for all endpoints.

For all endpoints, the data were tested for normality using the Shapiro-Wilks’ Test and for homogeneity of variance using Bartlett’s Test. Data pertaining to egg masses per female and days to oviposition did not meet the assumption for normality and data pertaining to egg masses per female and percent emergence did not meet the assumption of homogeneity of variance; these endpoints were subsequently analyzed using Wilcoxon’s Test with Bonferroni’s Adjustment or Steel’s Many-One Rank Sum Test. All remaining endpoints met both assumptions and were assessed using Dunnett’s Multiple Comparison Test or Bonferroni’s Adjustment t-Test. 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’, Bartlett’s), in which a 99% level of certainty was applied.

Endpoint	Methods	Mean-measured Sediment, mg a.i./kg	Mean-measured Pore Water, mg a.i./L
Day 17 survival	Dunnett’s Multiple Comparison Test	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25
Day 17 AFDW	Dunnett’s Multiple Comparison Test	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25
Percent emergence	Steel’s Many-One Rank Sum Test	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25
Male emergence rate	Bonferroni’s Adjustment t-test	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25
Female emergence rate	Bonferroni’s Adjustment t-test	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25
Male days to death	Bonferroni’s Adjustment t-test	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25
Female days to death	Bonferroni’s Adjustment t-test	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25
Eggs/egg mass	Bonferroni’s Adjustment t-test	NOAEC: 30 LOAEC: 86	NOAEC: 0.21 LOAEC: 0.25
Percent hatch	Bonferroni’s Adjustment t-test	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25

Endpoint	Methods	Mean-measured Sediment, mg a.i./kg	Mean-measured Pore Water, mg a.i./L
Egg masses/mated female	Wilcoxon's Test with Bonferroni's Adjustment	NOAEC: 30 LOAEC: 86	NOAEC: 0.21 LOAEC: 0.25
Eggs per mated female	Bonferroni's Adjustment t-test	NOAEC: 30 LOAEC: 86	NOAEC: 0.21 LOAEC: 0.25
Days to oviposition	Wilcoxon's Test with Bonferroni's Adjustment	NOAEC: 86 LOAEC: >86	NOAEC: 0.25 LOAEC: >0.25

Endpoint(s) Affected: eggs per egg mass, egg masses per mated female, and eggs per mated female

### 13. VERIFICATION OF STATISTICAL RESULTS:

#### Statistical Method:

The reviewer analyzed the data using CETIS™ statistical software version 1.8.7.12 with database backend settings implemented by EFED on 10/20/2015. The negative and solvent control data were compared using the Equal Variance Two-Sample t-test. Significant differences between negative and solvent controls were detected for the following endpoints: no. eggs/emerged female, no. eggs/primary egg case, % emerged, and egg cases/emerged female. Given these differences, all subsequent analyses were conducted by separately comparing treatment data to the negative and solvent controls for comparison. CETIS summary and analytical output files for each analysis are attached to this DER, outputs note Day 20; however, study measurements were taken on Day 17, this is an artifact of the CETIS template.

Data were then tested for normality using Shapiro-Wilk's test ( $\alpha = 0.01$ ) and for homogeneity of variance using Bartlett's test ( $\alpha = 0.01$ ). Data that were normal and homogeneous were analyzed via Dunnett's test, and data that were not normal and/or not homogenous were analyzed via the Mann-Whitney U Two-Sample Test, unless otherwise noted.

Results were expressed as time-weighted average bulk and OC-normalized sediment, overlying, and pore water concentrations.



**Based on Comparison to Negative Control**

Endpoint*	Bulk Sediment (mg a.i./kg)	OC-Normalized Sediment (mg a.i./g OC)	Pore Water (mg a.i./L)	Overlying Water (mg a.i./L)	Statistical Test Used
Day 17 Survival	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Dunnett
Day 17 AFDW	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Dunnett
Time to death	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Jonckheere- Terpstra
No. eggs/emerged female*	NOAEC: 1.7 LOAEC: 4.4	NOAEC: 0.081 LOAEC: 0.21	NOAEC: 0.020 LOAEC: 0.061	NOAEC: 0.0020 LOAEC: 0.0036	Williams
No. eggs/primary egg case*	NOAEC: <1.7 LOAEC: 1.7	NOAEC: <0.081 LOAEC: 0.081	NOAEC: <0.020 LOAEC: 0.020	NOAEC: <0.0020 LOAEC: 0.0020	Mann-Whitney
% emergence*	NOAEC: 1.7 LOAEC: 4.4	NOAEC: 0.081 LOAEC: 0.21	NOAEC: 0.020 LOAEC: 0.061	NOAEC: 0.0020 LOAEC: 0.0036	Mann-Whitney
Primary Egg cases/emerged female*	NOAEC: 1.7 LOAEC: 4.4	NOAEC: 0.081 LOAEC: 0.21	NOAEC: 0.020 LOAEC: 0.061	NOAEC: 0.0020 LOAEC: 0.0036	Mann-Whitney
Time to oviposition	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Mann-Whitney

\* Statistically significant effects for no. of eggs/emerged (mated) female, no. of eggs/primary egg case, % emergence, and primary egg cases/emerged female.

**Based on Comparison to Solvent Control**

Endpoint	Bulk Sediment (mg a.i./kg)	OC-Normalized Sediment (mg a.i./g OC)	Pore Water (mg a.i./L)	Overlying Water (mg a.i./L)	Statistical Test Used
Day 17 Survival	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Dunnett
Day 17 AFDW	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Dunnett
Time to death	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Jonckheere- Terpstra
No. eggs/emerged female	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Mann-Whitney
No. eggs/primary egg case	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Mann-Whitney
% emergence	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Mann-Whitney
Primary Egg cases/emerged female	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Mann-Whitney
Time to oviposition	NOAEC: 84 LOAEC: >84	NOAEC: 4.0 LOAEC: >4.0	NOAEC: 0.24 LOAEC: >0.24	NOAEC: 0.024 LOAEC: >0.024	Mann-Whitney

**14. REVIEWER'S COMMENTS:**

The reviewer's and the study author's results were not in agreement. It was determined that the solvent control positively impacted numerous parameters, as noted by the significant differences (and worse performance for negative controls) that were observed between the solvent and negative control for the following parameters: no. eggs/emerged female, no. eggs/primary egg

case, % emerged, and egg cases/emerged female. Even for those endpoints where the solvent control was not statistically significantly different from negative controls (other than the time-based endpoints), the solvent control had substantial differences (solvent had mean 24%↑ in biomass, 26%↑ mortality/↓survival at D17). Therefore, it was determined that the results reported for the negative control were likely the effect of the solvent and not the test substance. There were no treatment-related effects at the highest test level based on the solvent control. Clearly for some endpoints they are impacted by the solvent, but it cannot be ruled out entirely for the other endpoints either, therefore this is an uncertainty of this study, and is considered a study deficiency. The reviewer determined that the results reported for the negative control were in fact the effect of the solvent and not the test substance, and as a result there were no effects at the highest test level based on the solvent control. The reviewer used the TWA concentrations, while the study authors used the mean-measured concentrations. The reviewer calculated TWA concentrations given the length of time between measurements of DCPA in the various sample matrices (sediment, and/or water), on test days 0, 17 and 60, respectively.

Although when the results were initially compared to the negative control, the reviewer had determined that there were statistically significant differences for the following endpoints: emergence, no. of eggs/primary egg case, no. of eggs/emerged (mated) female, and no. egg cases/emerged (mated) female. The study authors determined that the observed effects (eggs per egg mass, egg masses per mated female, and eggs per mated female) were not considered to be toxicant related due to the lack of a clear dose response, and that none of the concentrations tested resulted in ≥50% reductions. The reviewer's results indicated statistically significant effects at lower treatment levels for no. of eggs/primary egg case and no. of eggs/emerged (mated) female. For % emergence and no. egg cases/emerged (mated) female, the reviewer determined biologically significant reductions at lower test concentrations than those indicated by the statistical analyses of either the reviewer or the study author (the study author's results indicated no effect on emergence). However, due to the influence of the solvent for numerous endpoints, the reviewer determined that the results reported for the negative control were in fact the effect of the solvent and not the test substance, and as a result there were no effects at the highest test level based on the solvent control.

The study report did not clearly lay out what the difference was between the total # of surviving 'midge' and total # of surviving 'larvae' in the columns in the day 17 table on page 124. The study author used the total # of surviving 'midge' column and the reviewer used the total # of surviving 'larvae' to estimate the % survival calculations and statistical analysis. The negative control % survival was 90% and met the control criteria. However, the solvent control % survival was barely below the control criteria of 70% (67%).

Time-weighted average (TWA) concentrations were reviewer-calculated for all matrices (refer to copy of Excel worksheet in Appendix I) and are reported in the Conclusions sections of the DER. TWA concentrations were calculated by the reviewer using the following equation:

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

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).

As per U.S. EPA request, the NOAEC and LOAEC values were also reported in terms of OC-normalized sediment concentrations in the Conclusion section of the DER. The TOC of the sediment was 2.1%, yielding OC-normalized sediment concentrations of 0.081, 0.21, 0.54, 1.4, and 4.0 mg a.i./g OC (81, 209, 537, 1373, 3981 mg a.i./kg OC). The TWA pore water and overlying water concentrations were 0.020, 0.061, 0.13, 0.22, and 0.24 mg a.i./L, and 0.0020, 0.0036, 0.0073, 0.015, and 0.024 mg a.i./L, respectively.

On test days 23, 26, 30, 38 and 50, the temperature in the test vessels fell to 21°C, below the protocol acceptable range at 23±1°C. The temperature of the water bath (continuous measurement) also exceeded the protocol range of either low (21°C) or high (25°C) on days 16, 24, 27, 30, 38, 39, 48, 49, 58 and 59. It was reported that since the temperatures observed were within the acceptable tolerance of the species and since changes would have occurred at a gradual rate, these deviations did not have a negative impact on the results or interpretation of the study.

Aqueous samples were analyzed for dacthal technical concentration using high performance liquid chromatography with ultraviolet detection (HPLC/UV) and sediment samples were analyzed using gas chromatography with micro-electron capture detection (GC/μECD) based on methodology validated at Smithers Viscient. The method validations were conducted prior to the initiation of the definitive study and established average recoveries of 102±3.90% from artificial sediment and 97.9±4.86% from filtered seawater. It was reported that conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation studies with the following exception related to analysis of the aqueous samples only: the calibration curve from the validated method was prepared at concentrations from 0.00200 to 0.0500 mg/L. Three additional standards at concentrations of 0.100, 0.250 and 0.500 mg/L were added to the calibration curve to better enable the quantitation of the aqueous samples.

The pH and ammonia (as N) were measured in isolated pore water from the negative control level on Days 0, 17 and 60. Ammonia levels measured 2.5, 1.2 and 1.1 mg/L on Days 0, 17, and 60, respectively. The pH ranged from 5.9 to 6.8.

The experimental phase of the definitive test was performed from September 11 to November 10, 2015.

This study **is scientifically sound** and is classified as **supplemental and may be used for risk characterization only**.

DP Barcode: 432681

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**15. REFERENCES:**

None, other than standard guidelines and methodologies.

**APPENDIX I. Copy of Excel Worksheet with TWA Concentrations**

SEDIMENT								
Nominal Concentration (mg ai/kg)	Measured Concentration (mg ai/kg)			TWA (mg ai/kg)	Std. Dev.	CV (%)	TWA, OC Normalized (mg ai/g OC)	TWA, OC Normalized (mg ai/kg OC)
	Day 0	Day 17	Day 60					
Negative Control	<0.21	<0.21	<0.21	<b>&lt;0.21</b>				
Solvent Control	<0.21	<0.21	<0.21	<b>&lt;0.21</b>				
2.6	2.3	1.8	1.3	<b>1.7</b>	0.50	30	<b>0.081</b>	<b>81</b>
6.4	6.3	4.5	3.5	<b>4.4</b>	1.42	32	<b>0.21</b>	<b>209</b>
16	15	12	8.8	<b>11</b>	3.10	27	<b>0.54</b>	<b>537</b>
40	37	30	24	<b>29</b>	6.51	23	<b>1.4</b>	<b>1373</b>
100	98	90	69	<b>84</b>	14.98	18	<b>4.0</b>	<b>3981</b>

PORE WATER						
Nominal Concentration (mg ai/kg)	Measured Concentration (mg ai/L)			Time-Weighted Average (mg ai/L)	Std. Dev.	CV (%)
	Day 0	Day 17	Day 60			
Negative Control	<0.0026	<0.0025	<0.0029	<b>&lt;0.0029</b>		
Solvent Control	<0.0026	<0.0025	<0.0029	<b>&lt;0.0029</b>		
2.6	0.023	0.020	0.019	<b>0.020</b>	0.0021	10
6.4	0.058	0.064	0.057	<b>0.061</b>	0.0038	6
16	0.11	0.15	0.12	<b>0.13</b>	0.0208	16
40	0.17	0.26	0.19	<b>0.22</b>	0.0473	21
100	0.25	0.21	0.28	<b>0.24</b>	0.0351	15

OVERLYING WATER						
Nominal Concentration (mg ai/kg)	Measured Concentration (mg ai/L)			Time-Weighted Average (mg ai/L)	Std. Dev.	CV (%)
	Day 0	Day 17	Day 60			
Negative Control	<0.0026	<0.0025	<0.0029	<b>&lt;0.0029</b>		
Solvent Control	<0.0026	<0.0025	<0.0029	<b>&lt;0.0029</b>		
2.6	0.0059	0.00125	0.00145	<b>0.0020</b>	0.0026	133
6.4	0.013	0.00125	0.0031	<b>0.0036</b>	0.0063	177
16	0.023	0.0058	0.0032	<b>0.0073</b>	0.0108	147
40	0.038	0.017	0.0044	<b>0.015</b>	0.0170	110
100	0.058	0.025	0.0093	<b>0.024</b>	0.0249	103

Values for the nominal 2.6 and 6.4 mg ai/kg groups on Day 17 and the 2.6 mg ai/kg group on Day 60 were below the LOQ. When necessary, values of 1/2 of the LOQ were used for TWA calculations.