Joint Exhibit 82

EPA OALJ Docket No. FIFRA-HQ-2022-0002

DP Barcode: D420871 MRID No.: 49307512

DATA EVALUATION RECORD
MYSID LIFE CYCLE TEST
OCSPP 850.1350

1. CHEMICAL: DCPA PC Code No.: 078701

2. TEST MATERIAL: Dacthal Technical Purity: 98.3%

3. CITATION

Authors:	Claude MB, Martin KH, Gallagher SP, and HO Krueger.
Title:	Dacthal: A flow-through life-cycle toxicity test with the saltwater mysid (*Americamysis bahia*).
Study Completion Date:	January 15, 2014
Laboratory:	Wildlife International (Evans Analytical Group), Easton, Maryland, USA
Sponsor:	Amvac Chemical Corporation, Los Angeles, California, USA
Laboratory Report ID:	246A-116A
MRID No.:	49307512
DP Barcode:	420871

4. REVIEWED BY: Moncie V. Wright, Staff Scientist, CDM Smith/CSS-Dynamac JV

Signature: Moncie V Wright **Date:** 10-11-2016

APPROVED BY: John Marton, Ph.D., Staff Scientist, CDM Smith/CSS-Dynamac JV

Signature: John Marton **Date:** 11/4/2016

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

Signature: **Date:** 12/01/2021

Michael Wagman, Senior Scientist, OPP/EFED/ERB2

Signature: **Date:** 11/30/2021

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

6. STUDY PARAMETERS

Scientific Name of Test Organism:	*Americamysis bahia*
Age of Test Organism:	Juveniles, <24-h post release from brood sac
Definitive Test Duration:	28 days (1st generation) and 96 hours (2nd generation)
Study Method:	Flow-through
Type of Concentrations:	Mean-measured

7. CONCLUSIONS:

Results Synopsis
NOAEC: <10 µg a.i./L
LOAEC: 10 µg a.i./L

Endpoint(s) Affected: All reproductive and growth endpoints (*e.g.,* F_0 Male dry weight and length; Offspring/female; Time to first brood; F_0 Female dry weight and length)
Most sensitive endpoint: F_0 Male dry weight and length

8. ADEQUACY OF THE STUDY

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

B. Rationale: A definitive NOAEC could not be established in the study as dose-responsive effects on male weight and length were observed at all doses. Additionally, although no significant differences were observed between the solvent and negative controls, there was a potential slight negative interaction with the solvent and the test substance for the F0 male dry weight endpoint (13%↓ ; p = 0.07) as well as a statistically significant difference between controls in the number of offspring per surviving female endpoint (-159%↑ ; p= 0.04), resulting in uncertainties as to whether the solvent had an impact on the effects. Only a tabulation of the total live young produced was provided, a count of the total number young produced per day comparing the number of dead versus live young produced was not provided, although one could be estimated based on the information provided by the reviewer (Appendix II). Given the drastic difference in young produced and live young available for the G2 phase of the experiment this information is especially important. The study authors provided no rationale; however it can be postulated that the difference was attributed to potential cannibalism (either from G1 mysids or G2 cohorts), which is a possibility; however, given the great number difference, that may not be the only reason.

C. Repairability: A NOAEC could not be determined in the study, as dose-responsive effects on male weight and length were observed at all doses. Additional data that includes lower concentrations is needed to reach a definitive NOAEC value. It is also recommended that any new test consider using a different solvent, as there may be an interaction between the test substance and the solvent.

9. GUIDELINE DEVIATIONS: This study was conducted according to a protocol based on procedures outlined in the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines, OCSPP (formerly OPPTS) Number 850.1350: *Mysid Chronic Toxicity Test* and ASTM Standard E 1191-03a: Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids. The reviewer assessed the study methods and results according to the updated U.S. EPA OCSPP (form. OPPTS) Guideline 850.1350: *Mysid Chronic Toxicity Test*. The following deviations and/or deficiencies from OCSPP 850.1350 were noted.

1) The study authors did not report if they randomly assigned treatments to test chamber locations.
2) An exploratory range-finding test was completed, and the concentrations were selected in consultation with the Sponsor and were based on exploratory range-finding toxicity data. However the results were not presented nor discussed within the study report.
3) The total organic carbon (TOC) content of the well water used to dilute the sea water was not reported. However, the lack of this information is considered to be a minor deficiency as the Kow and solubility of DCPA, (4.3 and 0.5 mg/L, respectively), in water would likely not result in an underestimation of toxicity.
4) A definitive NOAEC was not established in the study. This is considered a major deficiency of the study for assessing chronic risk to estuarine/marine invertebrates.
5) All live young produced were tabulated for each replicate and treatment level, and live young were kept for observation. However, the total of dead young vs. live young that were initially observed, was never quantified by the study author and so it is not clear how many were actually lost to cannibalism by the G1 parents or G2 cohorts or if they may have actually been lost due to treatment. Regardless, only a few LIVE G2 mysids were collected or available for the 96 hour test for further observations (Appendix 17 vs Appendix 20 of the study report, respectively), and no ***live*** G2 mysids were collected for the highest test concentration, although two young were produced (indicating they died before the G2 phase began) (G2 175 µg a.i./L level; the 157 µg a.i./L G1 level, respectively). However based on the information that was provided in the Appendices from the study report the total number of dead F1 young in the negative and solvent controls and treatment groups was estimated by the reviewer (Appendix II).
6) There was a statistically significant difference between the number of offspring produced in the solvent control compared to the negative control (159%↑, p=0.04). According to OCSPP 850.1000 guideline, solvents controls should not confound test results or affect test organisms at the concentration used. Whether the improved performance observed in the solvent control for the number of offspring endpoint is a true difference caused by the solvent or more reflective of the high variability of this endpoint is not known, but this response would be considered unusual.

These deficiencies ***did*** have an impact on the acceptability and scientific integrity of this study. The lack of a definitive NOAEC prevents the ability to use this study to quantitatively evaluate chronic risk to estuarine/marine invertebrates.

10. MATERIALS AND METHODS:

A. Biological System

Guideline Criteria	Reported Information
Species: An estuarine shrimp species, preferably *Americamysis bahia.*	Test species is the saltwater mysid (*Americamysis bahia*).
Duration of the Test: A mysid test must not be terminated before 7 days past the median time of 1st brood release in the control treatment.	1st generation mysids (G1, F_0): 28 days [which was at least 7 days past the median time of the first brood release for the G1 negative and solvent controls (Day 17)]. 2nd generation mysids (G2, F_1): 96 hours following the release of the mysids from the brood pouch
Source (or supplier)	In-house cultures maintained by the testing laboratory (Wildlife International).
Parental Acclimation 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	1.) Adult mysids were held in the laboratory ≥ 14 days before juveniles (<24 hours old) were collected for testing. The culture was maintained in a re-circulating (flow-through) saltwater system using water from the same source as used during the test. During the 2-week period immediately preceding the test, water temperatures in the cultures were 25.5 to 26.0ºC, pH ranged from 8.2 to 8.6, salinity ranged from 20 to 21 ppt (‰), and DO concentrations were ≥6.7 mg/L (≥91% of saturation). 2.) The pre-test health of the mysids was not reported.
Parental Acclimation Period At least 14 days	At least 14 days.
Chamber Location: Treatments should be randomly assigned to test chamber locations.	Organisms were impartially distributed to the test compartments. The delivery system and test chambers were placed in a temperature-controlled environmental chamber. Prior to pairing, mysids in each treatment and control group were held in one test compartment placed in each of four replicate test chambers. After mysids attained sexual maturity and were paired on

Guideline Criteria	Reported Information
	Day 14, reproductive pairs were placed in reproductive compartments, one pair per compartment, with up to five compartments in each replicate test chamber. The G2 mysids were initiated in a separate test system using the same test apparatus as described above and with the same test chambers and compartments as used for the Gl mysids after sexual maturity. Treatment vessel assignments with respect to location to each other were not reported.
Brood Stock: Test started with mysids: 1) from only one brood stock or 2) from brood stock which has not obtained sexual maturity or had been maintained for > 14 days in a laboratory with same food, water, temperature, and salinity used in the test.	The test was started with mysids that had been maintained for >14 days with similar food, water, temperature, and salinity used in the tests.
Distribution: **No. of mysids before pairing**: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/treatment level. **No. of mysids after pairing**: ≥ 20 randomly selected pairs/treatment (excess males should be held in separate compartment in same treatment to replace paired males).	***Before pairing***: 60/level; 15 mysids per compartment, one compartment per replicate test chamber (4 replicates) for a total of 60 mysids per control and treatment group. ***After pairing***: when possible, 20 pairs per level; one pair was placed in each compartment, with up to 5 compartments in each replicate chamber, resulting in 20 randomly selected pairs per control and treatment group (an additional compartment was maintained, if necessary, to house any remaining males).

Guideline Criteria	Reported Information
Pairing: 1) Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation). 2) Should be paired on the same day	After mysids (parental; G1 generation) attained sexual maturity they were paired on Day 14. At pairing, the sex and maturity of each G1 mysid was determined by microscopic examination, when possible five male/female pairs were assigned to reproductive compartments in each replicate test chamber, with one pair per compartment. Any immature G1 mysids or extra G1 females were discarded; sexually immature G1 males were maintained until G1 test termination.
Feeding: 1) Mysids should be fed live brine shrimp nauplii at least once daily. 2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	Mysids in cultures were fed live brine shrimp nauplii (*Artemia* sp.) daily supplied by INVE Aquaculture, Salt Lake City, Utah. The brine shrimp periodically were enriched with a nutrient enrichment (A1 DHA Selco, INVE Thailand, Ltd., Thailand). During the test mysids were fed live brine shrimp nauplii (*Artemia* sp.) up to four times daily, with one feeding per day consisting of brine shrimp enriched with a nutrient formula. Food was periodically supplemented with the saltwater algae *Skeletonema costatum* (in-house Wildlife International cultures). Excess food and waste were siphoned out daily during observations.
Counts: Live adult mysids should be counted 1) at initiation, 2) at pairing, 3) and daily after pairing. 4) Live young must be counted and removed daily. 5) Missing or impinged animals should be recorded.	G1 mysids (1st generation, F_0) were counted daily throughout the test. **Live** young (G2 mysids) produced by G1 mysids were counted, recorded, and removed daily after pairing. They were pooled and placed in a test compartment with up to 10 mysids per compartment. This G2 test chamber contained the same nominal test substance as the G1 chamber from where they originated. There was no minimum amount of G2 mysids to be initiated in the G2 phase each day. When available observations on mortality,

Guideline Criteria	Reported Information
	abnormal development, and behavior were recorded for G2 mysids for approximately 96 hours.
Controls: Negative control and carrier control (when applicable) are required.	Negative (dilution water only) and solvent (HPLC DMF, 0.1 mL/L) control.

Comments: The criteria for death included lack of movement, absence of respiratory movements, and lack of reaction to gentle prodding. At paring on Day 14, microscopic examination was used, and any immature G1 mysids or extra G1 females were discarded. Following pairing, excess mature male organisms were maintained in a separate compartment within the replicate. If a G1 male died in a reproductive compartment, it was replaced with another male, if available, from the pool of males maintained in the same replicate. All ***live*** young produced were tabulated for each replicate and treatment level, and live young were kept for observation. However, the total of dead young vs. live young that were initially observed, was not quantified by the study author and so it is not clear how many were lost to cannibalism by the G1 parents or G2 cohorts or if they may have actually been lost due to treatment. Regardless, only a few Live G2 mysids were collected or available for the 96 hour test for further observations (Appendix 17 vs Appendix 20 in the study report, respectively[1]), no ***live*** G2 mysids were collected for the highest test concentration, although two young were produced (indicating they died before the G2 phase began) (G2 175 µg a.i./L level; the 157 µg a.i./L G1 level, respectively). However based on the information that was provided in the Appendices from the study report the total number of dead F1 young in the negative and solvent controls and treatment groups was estimated by the reviewer (Appendix II).

The in-life phase of the definitive test was conducted from August 28, 2013 to September 29, 2013.

B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) May be natural (sterilized and filtered) or a commercial mixture; 2) Water must be free of pollutants. 3) During the test, difference between highest and lowest measured salinities must be less	1.) Natural seawater collected at Indian River Inlet, Delaware. The water was ozonated, filtered to remove particles >25-micron, diluted to a salinity of 20 ppt (‰) with onsite well water, then aerated with spray nozzles. Prior to use, the water was filtered to 0.45-

1 According to Appendix 17, 103 young were produced in the negative control. However, according to Appendix 20, only 18 F1 organisms from this dose level were exposed in the 96-hour G2 survival tests. This difference is consistent for all other the dose levels as well, without explanation.

Guideline Criteria	Reported Information
than 10‰ (parts per thousand). Should be measured daily. 4) Salinity should be between 15 and 30 l. 5) pH should be measured at the beginning, end of test and weekly. 6) DO must be measured @ each conc. @ least once a wk. 7) See details in ASTM E-1191.	micron to remove fine particles and subsequently passed through a UV sterilizer. The total organic carbon (TOC) content of the well water used to dilute the sea water was not reported. 2.) Pesticides, organics, and metals were not detected in the dilution water, except for barium, calcium, chloride, magnesium, potassium, sodium, and sulfate (Appendix 4 in study report; pg. 66-67). 3.) The difference between the highest and lowest values was <10 ppt (‰). 4.) Salinity ranged from 19 to 21 ppt in the G1 28-day exposure and from 20 to 21 ppt (‰) in the G2 96-hour exposure. *G1 (1st generation, F_0) exposure*: Salinity was measured daily in one replicate of the negative control, with measurements rotating among replicates at each measurement interval. *G2 (2nd generation, F_1) exposure*: Salinity was measured in each negative control replicate as it was initiated with mysids. Thereafter, it was measured daily in one replicate test chamber of the negative control during the test period and at test termination. 5.) *G1 (1st generation, F_0) exposure*: The pH was measured in one replicate test chamber of each control and treatment group at test initiation and termination, and weekly during the test with measurements rotating among replicates at each measurement interval. *G2 (2nd generation, F_1) exposure*: The pH was measured in each control and treatment group as it was initiated with mysids. Thereafter, it was measured in one replicate test chamber of each control and treatment group weekly and at test termination, with measurements rotating among replicates. 6.) *G1 (1st generation, F_0) exposure*: Prior to pairing, the DO was measured in one replicate test chamber from each control and

Guideline Criteria	Reported Information
	treatment group at test initiation and weekly during the test period, with measurements typically rotating among the replicates in each group at each measurement interval. After pairing, DO was measured daily until test termination one replicate test chamber of each control and treatment group, with measurements rotating among the replicates in each group. *G2 (2nd generation, F1) exposure*: DO was measured in each control and treatment group replicate as it was initiated with mysids. Thereafter, it was measured daily in one replicate chamber of each control and treatment group and at test termination, with measurements rotating among replicates. The DO in the G1 test ranged from 5.5 to 7.4 mg/L, and in the G2 test ranged from 5.7 to 7.4 mg/L. A DO concentration of 4.4 mg/L represents 60% saturation at 25ºC in saltwater with a salinity of 20‰.
Test Temperature: 1) Measured daily in one chamber and at least 3 times in all chambers. 2) Mean measured temperature for each chamber at test termination should be within 1EC of selected test temperature. 3) Each individual measured temperature must be within 3°C of the mean of the time-weighted averages. 4) For mysid shrimp, 27°C is recommended. 5) Whenever temp. is measured concurrently in more than one test chamber the highest & lowest temp. must not differ by more than 2°C.	1.) *G1 (1st generation, F0) exposure*: The temperature was measured in each test chamber at test initiation and termination, and weekly during the test. *G2 (2nd generation, F1) exposure*: The temperature was measured in a test chamber at test initiation and termination. Temperature was monitored continuously in one negative control test chamber in both the G1 and G2 exposures. 2.) The mean-measured temperatures for each chamber at test termination were within 1ºC of the selected temperature of 25ºC. 4.) The test temperatures in the G1 test ranged from 24.7 to 26.2ºC, and in the G2 test ranged from 24.1 to 25.8ºC.
Photoperiod: Recommend 16L/8D. 14L/10D also acceptable.	Ambient laboratory light consisted of fluorescent light bulbs that emit wavelengths similar to natural sunlight and provided a

Guideline Criteria	Reported Information
	photoperiod of 14L:10D, with a 120-minute transition period of low light intensity provided when lights went on and off. Light intensity was measured over the surface of one representative test chamber at test initiation in both G1 and G2 exposures, and measured 257 and 115 lux, respectively.
Dosing Apparatus: 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations 3) A dilution factor not greater than 0.5 and controls should be used.	Continuous-flow diluter equipped with syringe pumps (Harvard Apparatus, South Natick, Massachusetts) that delivered stock solutions or solvent to mixing chambers where the stock or solvent were mixed with dilution water prior to delivery to the test chambers.
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	Mixing chambers were used. Rotameters controlled the flow of dilution water into each mixing chamber. After mixing, the flow from each mixing chamber was split to deliver the test water to the four replicate chambers. Flow splitting accuracy varied by no more than ±10% of the mean flow rate for the four replicates.
Test Vessels: 1) Material: all glass, No. 316 stainless steel, or perflorocarbon plastic **Test Chambers:** 1) Most common: 300x450x150 mm deep with solution depth of 100 mm. 2) Should be covered. **Test Compartments (within chambers):** 1) Size: 250 ml beaker with side cutouts covered with nylon mesh or stainless steel screen. or 2) 90 or 140 mm inside dia. glass Petri dish bottoms with collars made of 200 - 250 um mesh screen.	***Prior to pairing***: Test compartments: 2-L glass containers measuring 12-cm in diameter and 19-cm in height, with two nylon mesh covered holes on opposite sides of the container (6.7-cm). Test vessels/chambers: 9-L glass aquaria, containing ~2.5-L of test solution (depth 6.9-cm). ***After pairing on Day 14***: Test vessels/chambers: 19-L glass aquaria, containing ~14.5-L of test solution (G1 exposure: depth 17.7-cm; G2 exposure: depth 18.5-cm). Reproductive compartments: 10-cm diameter glass petri dishes with sides constructed of nylon mesh screen (G1 exposure: depth 17.4-

Guideline Criteria	Reported Information
	cm; G2 exposure: depth 17.5-cm).
Flow Rate: 1) Flow rates should provide 5 to 10 volume additions per 24 hr. 2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. 3) Meter systems calibrated before study and checked twice daily during test period.	1.) Prior to pairing, the appropriate amount of the five test substance stock solutions were injected into the diluter mixing chambers assigned to treatment groups at a rate of 12.5 µL/min where they were mixed with dilution water delivered at a rate of 125 mL/min to achieve the desired test concentrations. Following pairing, the stock solutions were injected into the diluter mixing chambers assigned to treatment groups at a rate of 25.0 µL/min where they were mixed where they were mixed with dilution water delivered at a rate of 250 mL/min to achieve the desired test concentrations. The flow of dilution water into each mixing chamber was controlled using rotameters and adjusted to provide each adult and juvenile test chamber with at least 6 and 18 volume additions of test water per day, respectively. 2.) DO was maintained at ≥75% of saturation (5.5 mg/L) in both the G1 and G2 exposures. 3.) The rotameters used to control the flow of the dilution water to the mixing chambers were calibrated prior to the test and verified or recalibrated weekly during the test. Delivery of test solutions to the test chambers was initiated 6-days prior to the introduction of the mysids to the water. The operation of the exposure system was checked visually at least once on the first and last days of the test, and at least twice per day during the test.
Aeration: 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks may be aerated.	1.) The dilution water was aerated prior to use in the test. 2.) The test tanks were not aerated.

Comments: Results of periodic analysis of the dilution water for pesticides, organics, and metals were provided from water collected on December 26, 2012 (non-GLP), ca. 6 months prior to the definitive experiment. These results indicated that none of these compounds were detected at

concentrations that are considered toxic in any of the water samples analyzed. A dissolved oxygen concentration of 4.4 mg/L represents 60% saturation at 25ºC in saltwater with a salinity of 20‰. The DO concentrations remained ≥75% saturation (5.5mg/L) at 25ºC in saltwater with a salinity of 20‰ for both G1 and G2 exposures.

Delivery of the test solutions into the test chambers was initiated 6 days prior to test initiation in order to achieve equilibrium of the test substance. Delivery system and test chambers were placed in a temperature-controlled environmental chamber to maintain the target temperature throughout the test period.

C. Chemical System:

Guideline Criteria	Reported Information
Concentrations: 1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. 2) Toxicant conc. must be measured in one tank at each treatment level every week. 3) One concentration must adversely affect a life stage and one concentration must not affect any life stage. 4) The measured conc. of the test material of any treatment should be at least 50% of the time-weighted average measured conc. for >10% of the duration of the test. 5) The measured conc. for any treatment level should not be more than 30% higher than the time-weighted average measured conc. for more than 5% of the duration of the test.	1.) There were 5 nominal test concentrations (13, 25, 50, 100, 200 µg a.i./L), and a negative and solvent control. 2.) *G1 (1st generation, F_0) exposure*: Analytical verification was performed on samples collected on days -1, 0, 7, 14, 15, 16, 21, and 28. Samples were collected mid-depth and processed immediately for analysis. Mean-measured G1: <7.5 (<LOD, negative and solvent controls), 10, 21, 39, 76, and 157 µg a.i./L) *G2 (2nd generation, F_1) exposure*: Analytical verification was performed on samples collected on days -1, 0, 6, 13, and 15. Mean-measured G2: <7.5 (<LOD, negative and solvent controls), 12, 24, 45, 84, and 175 µg a.i./L). Samples were collected mid-depth and processed immediately for analysis. Refer to copy of Excel worksheet in Appendix III. Measured concentrations of Dacthal (DCPA) in G1 the samples ranged from approximately 61.2 to 101% of nominal. Measured concentrations of Dacthal (DCPA) in G2 the samples ranged from approximately 80.2 to 97.5% of nominal.
Solvents: 1) Should not exceed 0.1 ml/L in a flow-through system. 2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.	HPLC-grade dimethylformamide (DMF) was used at a concentration of 0.1 mL/L (0.01%).

Comments: Nominal concentrations were selected in consultation with the Sponsor. No preliminary range-finding data were reported.

The test solutions in the mixing chambers and test chambers appeared clear and colorless during the test, with no evidence of precipitation observed in any control or treatment solution.

The analysis of Dacthal (DCPA) in saltwater was based on methodology developed by Wildlife International. The analytical method consisted of diluting the samples with saltwater, as necessary, and analyzed using HPLC with ultraviolet (UV) absorbance detection at 220 nm. The limit of quantitation (LOQ) for the analysis of Dacthal in saltwater was 7.50 µg a.i./L.

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with the Good Laboratory Practice Standards as published by the U.S. EPA (40 CFR Parts 160 and 792), as well as the Japan and OECD Principles of GLP.
Controls: 1) Survival of the first-generation controls (between pairing and test termination) must not be less than 70%. 2) At least 75% of the paired 1st generation females in the controls produced young or 3) The average number of young produced by the 1st generation females in the control(s) was at least 3.	1.) Survival after pairing: Negative control: 81%; Solvent control: 86% 2.) 90 and 94% of surviving females in the negative and solvent control, respectively, produced young. 3) The average number of young produced by the 1st generation females was 5.2 in the negative control and 13.5 in the solvent control.
Data Endpoints must include: 1) Survival of first-generation mysids Female Male 2) Number of live young produced per female 3) Dry weight of each first-generation mysid alive at the end of the test Female Male 4) Length of each first-generation mysid alive at the end of the study Female Male	1.) Survival of 1st generation (G1, F_0) mysids. 2.) Number of live young produced per female. 3.) Dry weight of each 1st generation (G1, F_0) mysid alive at the end of the test (male and female). 4.) Length of each 1st generation (G1, F_0) mysid alive at the end of the test (male and female). 5.) Abnormal development was recorded (G1, F_0 and G2, F_1). 6.) Aberrant behavior was recorded if it occurred (G1, F_0 and G2, F_1).

Guideline Criteria	Reported Information
5) Incidence of pathological or histological effects; 6) Observations of other effects or clinical signs.	7.) Survival of 2nd generation (G2, F_1) mysids
Raw data included? (Y/N) At a minimum, individual data should be included for: 1) Surviving 1st generation % and & mysids. 2) Number of live young produced per female. 3) Individual length measurements of % and & mysids. 4) Individual dry weight measurements for % and & mysids at the end of the test.	Raw data including individual data for each replicate and test compartment was provided, with one exception. The data for the number of *live young produced per living female*, was not provided, though the supporting data needed to calculate the endpoint were provided.

Comments: For each female the number of reproductive days was defined as the number of days that the female was alive from the day of first brood release of any female in the test to the end of the test. Therefore, if a female dies the number of reproductive days ends on the last day that she was alive. The percent surviving females producing young and number of your per surviving female were calculated based on the total number of surviving females present at test termination. Females that died during the test prior to test termination and the young that they produced were excluded from the calculation of percent females producing the mean number of young per female. The day of first brood release (by any female) was reported as Day 17. This parameter was not assessed as a toxicological endpoint by the study author; however, the reviewer analyzed this parameter as part of the verification of statistical analysis. All ***live*** young produced were included for the reproductive endpoints.

Effects Data:

1st generation (G1, F_0)

Toxicant Conc. (µg ai/L)		Percent Survival (Mean)*	
Nominal	Mean-measured	Pre-Pairing (Days 0-14)	Post-Pairing (Days 14-28)[1]
Negative control	<LOQ[(a)]	100	81 (85)
Solvent control	<LOQ[(a)]	95	86 (85)
13	10	95	87 (87)
25	21	98	88 (95)
50	39	100	84 (80)
100	76	95	86 (86)
200	157	97	86 (88)

[a] LOQ = 7.50 µg a.i./L
* Study author found that there were no statistically significant decreases in survival in comparison to the pooled, negative or solvent control using Fisher's Exact test ($p > 0.05$).
[1] Reviewer-calculated numbers differed slightly in some cases and are presented in parentheses.

There were no treatment-related effects on survival, either before pairing or afterward. Before pairing, survival was 100 and 95%, respectively, in the negative and solvent control. Survival ranged from 95 to 100% in the treatment groups. After pairing, survival was 81 and 86%, respectively, in the negative and solvent control. Survival ranged from 84 to 88% in the treatment groups. The reported NOAEC for survival both pre- and post-pairing was 157 µg a.i./L.

Toxicant Conc. (µg ai/L)		Reproduction (mean or mean ± SD)		
Nominal	Mean-measured	Number of Young Per Reproductive Day ± SD	Percent of Females Producing Young[1]	Number of Offspring per Surviving Female ± SD[1]
Negative control[2]	<LOQ[(a)]	0.429 ± 0.204	90	5.2 ± 2.5
Solvent control[2]	<LOQ[(a)]	1.08 ± 0.461	94	13.5 ± 6.3
13	10	0.590 ± 0.084	100	7.1 ± 1.0
25	21	0.325 ± 0.136	65	3.9 ± 1.6
50	39	0.351 ± 0.028	75	3.5 ± 1.5
100	76	0.219 ± 0.072*	79	3.7 ± 2.1
200	157	0.008 ± 0.017*	7**	0.1 ± 0.2*

[a] LOQ = 7.50 µg ai/L
* Study author determined there was a statistically significant decrease in reproduction and mean number of young per surviving female in comparison to the negative control using Dunnett's test ($p < 0.05$).
** Study author determined there was a statistically significant decrease in percent of surviving females producing young in comparison to the pooled control using Fisher's Exact test ($p < 0.05$).
[1] Calculated by the study author based on the total number of surviving females present at test termination. Females that died prior to test termination and the young that they produced were excluded from the calculation of the mean percent of females producing young and the mean number of young per female.
[2] The study author determined that there was a statistically significant difference between the negative and solvent control groups ($p < 0.05$). The mean reproduction values in the solvent control group for this study were higher than normal when compared to historical control data. Therefore, comparisons were made to the negative control for the reproductive endpoint.

The percent of surviving females that produced young averaged 90 and 94% in the negative and solvent controls, respectively, as compared to 100, 65, 75, 79, and 7% in the mean-measured 10, 21, 39, 76, and 157 µg a.i./L treatment groups, respectively. The treatment groups were compared to the negative control. The number of young produced per surviving female averaged 5.2 and 13.5 in the negative and solvent controls, respectively, as compared to 7.1, 3.9, 3.5, 3.7, and 0.1 in the mean-measured 10, 21, 39, 76, and 157 µg a.i./L treatment groups, respectively. The NOAEC for both endpoints was reported to be 76 µg a.i./L.

The number of young produced per reproductive day averaged 0.429 and 1.08 in the negative and solvent controls, respectively, as compared to 0.590, 0.325, 0.351, 0.219, and 0.008 in the mean-measured 10, 21, 39, 76, and 157 µg a.i./L treatment groups, respectively. The treatment groups were compared to the negative control. The NOAEC for this endpoint was reported to be 39 µg a.i./L.

Toxicant Conc. (µg a.i./L)		Growth (mean ± SD)			
Nominal	Mean-measured	Total Body Length (mm)		Dry Body Weight (mg)	
		Male	Female	Male	Female
Negative control	<LOQ[(a)]	7.23 ± 0.170	7.47 ± 0.141	1.10 ± 0.131	1.33 ± 0.048
Solvent control	<LOQ[(a)]	7.19 ± 0.195	7.39 ± 0.187	0.96 ± 0.093	1.20 ± 0.177
13	10	6.87 ± 0.262	7.27 ± 0.141	0.90 ± 0.096	1.17 ± 0.066
25	21	6.95 ± 0.038	7.37 ± 0.130	0.91 ± 0.015	1.28 ± 0.012
50	39	6.83 ± 0.059	7.15 ± 0.176	0.87 ± 0.066	1.17 ± 0.106
100	76	6.70 ± 0.202	7.14 ± 0.215	0.77 ± 0.102	1.08 ± 0.178
200	157	6.20 ± 0.079	6.42 ±0.207	0.58 ± 0.059	0.77 ± 0.045

a LOQ = 7.50 µg a.i./L

Total male body length averaged 7.23 and 7.19 mm in the negative and solvent controls, respectively. In the treatment groups, total male body length averaged 6.87, 6.95, 6.83, 6.70, and 6.20 mm in the mean-measured 10, 21, 39, 76, and 157 µg a.i./L treatment groups, respectively. The treatment groups were compared to the pooled controls. The NOAEC for male length was reported to be 21 µg a.i./L.

Male dry weight averaged 1.10 and 0.96 mg in the negative and solvent controls, respectively. In the treatment groups, male dry weight averaged 0.90, 0.91, 0.87, 0.77, and 0.58 mg in the mean-measured 10, 21, 39, 76, and 157 µg a.i./L treatment groups, respectively. The treatment groups were compared to the pooled controls. The NOAEC for male weight was reported to be 21 µg a.i./L.

Total female body length averaged 7.47 and 7.39 mm in the negative and solvent controls, respectively. In the treatment groups, total female body length averaged 7.27, 7.37, 7.15, 7.14, and 6.42 mm in the mean-measured 10, 21, 39, 76, and 157 µg a.i./L treatment groups, respectively. The treatment groups were compared to the pooled controls. The NOAEC for female length was reported to be 21 µg a.i./L.

Female dry weight averaged 1.33 and 1.20 mg in the negative and solvent controls, respectively. In the treatment groups, female dry weight averaged 1.17, 1.28, 1.17, 1.08, and 0.77 mg in the mean-measured 10, 21, 39, 76, and 157 µg a.i./L treatment groups, respectively. The treatment groups were compared to the pooled controls. The NOAEC for female weight was reported to be 39 µg a.i./L.

2nd generation (G2, 2nd generation, F_1)

Toxicant Conc. (µg a.i./L)		Percent Survival (Mean)
Nominal	Mean-measured	After 96 hours
Negative control	<LOQ[a]	61
Solvent control	<LOQ[a]	92
13	12	83
25	23	25
50	45	68
100	84	100
200	175	--[b]

a LOQ = 7.50 µg a.i./L
b The required number of live young were not produced.

Survival averaged 61 and 92% in the negative and solvent control, respectively. In the treatment groups, survival ranged from 25 to 100%. The controls were pooled for analysis and used for comparison. Although a statistically significant reduction was determined for the 23 µg a.i./L treatment group, this was based on a sample size of 4 mysids, and the two higher treatment groups lacked statistically significant decreases. The reported NOAEC for G2 survival was 84 µg a.i./L.

Toxicity Observations:

1st generation (G1, F_0):

Surviving mysids in the controls and 10, 21, 39, and 76 µg a.i./L treatment groups mostly appeared normal from test initiation to pairing on Day 14. Some organisms appeared smaller than the controls, but these observations were infrequent. Mysids in the 157 µg a.i./L treatment group exhibited signs of a smaller size as compared to the controls.

Surviving mysids in the treatment groups mostly appeared normal from Day 14 to Day 28. Some organisms exhibited lethargy or got stuck above the waterline, but these observations were infrequent.

2nd generation (G2, F_1):

Generally, the young produced by the first generation (G1) were dead, lethargic, or appeared normal, and did so consistently throughout the controls and treatment groups. After collection, surviving mysids in the controls and treatment groups appeared normal with a few cases of

lethargy and erratic swimming during the 96-hour evaluation period.

All live young produced were included for the reproductive endpoints; however, only live G2 mysids that were produced were used to initiate the G2 phase of the study. Only a few live G2 mysids were collected or available for the 96 hour test for further observations (Appendix 17 vs Appendix 20 of the study report, respectively), and no ***live*** G2 mysids were collected for the highest test concentration, although two young were produced (indicating they died before the G2 phase began; G2 175 µg a.i./L level; the 157 µg a.i./L G1 level, respectively). However, based on the information that was provided in the Appendices from the study report the total number of dead F1 young in the negative and solvent controls and treatment groups was estimated by the reviewer (Appendix II).

Statistical Results:

The study authors analyzed G1 (F_0) and G2 (F_1) survival, G1 (F_0) reproduction endpoints, and G1 (F_0) growth endpoints (total body length and dry weight). First, the negative and solvent control were compared using an appropriate statistical test. No differences were detected for survival or growth, so the control data were pooled for comparison among the treatment groups. There were significant differences between the controls for the reproductive endpoints. The mean reproductive values in the solvent control group for this study were higher than normal when compared to historical control data (Appendix 6 of the study report). Therefore, the study authors compared the treatment group data to the negative control.

Survival and percent of surviving females producing young were considered to be discrete variable data and were analyzed using the Chi-square and Fisher's Exact tests. The number of young produced per reproductive day, the number of young produced per surviving female, and growth data were classified as continuous variable data. All continuous data were evaluated for normality and homogeneity of variance using Shapiro-Wilk's and Levene's test, respectively ($p = 0.01$). All data were normal with homogeneity of variance. The growth endpoints were analyzed using Jonckheere-Terpstra's trend test, and the reproduction data were analyzed using Dunnett's test ($p \leq 0.05$).

The mean-measured test concentrations were used for the analysis. All statistical tests were conducted using SAS software.

Endpoint	Method	NOEC	LOEC	MATC
G1 (F_0) Survival	Chi-square and Fisher's Exact tests	157	>157	--
G1 (F_0) percent of surviving females producing young	Chi-square and Fisher's Exact test	76	157	--

Endpoint	Method	NOEC	LOEC	MATC
G1 (F_0) Number of young produced per reproductive day	Dunnett's test	39	76	--
G1 (F_0) Number of young produced per surviving female	Dunnett's test	76	157	--
G1 (F_0) Dry weight, female	Jonckheere-Terpstra's trend test	39	76	--
G1 (F_0) Dry weight, male	Jonckheere-Terpstra's trend test	39	76	--
G1 (F_0) Total length, female	Jonckheere-Terpstra's trend test	76	157	--
G1 (F_0) Total length, male	Jonckheere-Terpstra's trend test	21	39	29
G2 (F_1) Survival	Chi-square and Fisher's Exact tests	84	>84	--

Endpoint(s) Affected: All reproductive and growth endpoints
Most Sensitive Endpoint(s): G1 male total length

12. REVIEWER'S STATISTICAL RESULTS:

The reviewer analyzed F_0 survival (pre- and post-pairing), F_0 time to first brood, F_0 reproduction (offspring per female), F_0 growth (sex-specific dry weight and length), and F_1 survival (96 hours) using CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 10/20/2015.

F_1 96-hr survival was analyzed via the Fisher Exact/Bonferroni-Holm test and was not subjected to analyses of normality and homogeneity of variance. Other endpoints were tested for normality using the Shapiro-Wilk's test ($\alpha = 0.01$) and for homogeneity of variance using either Bartlett's or Levene's tests ($\alpha = 0.01$). Female dry weight data did not meet both assumptions but was monotonic and was analyzed using the non-parametric Jonckheere-Terpstra step-down test.

Female length, number of offspring per surviving female, and male dry weight and length data met both assumptions and were monotonic (or roughly monotonic as was the case for time to first brood); therefore, the data were analyzed using William's multiple comparison test.

F_0 survival pre-pairing data did not meet both assumptions and was non-monotonic; therefore, the data were analyzed using the Mann-Whitney U two-sample test. F_0 post-pairing survival met both assumptions but were not monotonic; therefore, the data were analyzed using Dunnett's multiple comparison test.

All analyses were conducted using the mean-measured concentrations (refer to copy of Excel worksheet in Appendix III), and all tests were conducted at $\alpha = 0.05$ unless specified otherwise.

Most sensitive endpoint: F_0 Male dry weight and length

Endpoint	Method	NOAEC (µg a.i./L)	LOAEC (µg a.i./L)
F_0 Pre-pairing survival	Mann-Whitney U two-sample t-test	157	>157
F_0 Post-pairing survival	Dunnett's	157	>157
Offspring/female*	Jonckheere-Terpstra Step-Down*	10	21
Time to first brood	William's	39	76
F_0 male total length	William's	<10	10
F_0 female total length	William's	21	39
F_0 male dry weight	William's	<10	10
F_0 female dry weight	Jonckheere-Terpstra Step-Down	39	76
F_1 96-hr survival	Fisher Exact/Bonferroni-Holm	84	>84

* The solvent control was statistically significantly different from the negative control (p=0.04), with 159% more offspring/female produced in the solvent control. A clear dose-response relationship was also observed when comparing the treatment groups to the solvent control as opposed to the negative control. Therefore, the reviewer determined that comparisons for this endpoint should be made against the solvent control.

General Comments:

1. The reviewer's results for female dry weight, and F_0 and F_1 survival were in agreement. However, for the remaining growth endpoints and number of offspring per surviving female, the reviewer's results differed from the study authors. The reviewer's NOAEC and LOAEC values were an entire test level lower than the study authors. For these growth endpoints, this was most likely due to the study authors using a non-parametric test for monotonic data (Jonckheere-Terpstra) whereas the reviewer used parametric tests for monotonic data (William's) as the assumptions of equal variance and normality were met. For the number of offspring per surviving female, the study authors interpreted the data as non-monotonic, whereas the reviewer interpreted the data as monotonic. Additionally, the study authors did

not analyze time to first brood, which was an affected endpoint. Additionally, for time to first brood, despite not being statistically significantly different, the NOAEC and LOAEC was determined observationally using the William's test to be 39 and 76 µg a.i./L, respectively. The reviewer's results are reported in the Conclusions section of this DER.

2. The study authors only reported the mean number of offspring per surviving female, they did not report the number of offspring per surviving female for each replicate. The reviewer independently calculated the endpoint and entered those values into CETIS (Appendix I). As a result, the reviewer's replicate means differed slightly from the study authors'. But it had not impact on the results for this endpoint. Additionally, the reviewer determined that there were statistically significantly more offspring produced per female in the solvent control than the negative control (p=0.04, Equal Variance t Two-Sample Test using a two-tailed test). Additionally, the reviewer observed a clear dose-response relationship compared to the solvent control that could not be observed compared to the negative control. Therefore, the reviewer determined endpoints for this effect by comparing treatment groups to the solvent control. Due to the statistically significant differences, the study author evaluated results against the negative control (rather than the pooled controls) and determined statistically significant effects only at the highest dose, while the reviewer, comparing against the solvent control determined statistically significant dose-responsive effects at all doses above the lowest dose. The study author did not present results from the study range-finder to see if this trend in solvent and treatment groups for offspring production was observable prior to the definitive test. The reviewer's results are reported in the Conclusions section of this DER.

The in-life portion of the definitive toxicity test was conducted from August 28, 2013 to September 29, 2013.

Only the total ***live*** young produced was provided, a count of the total number young produced per day comparing the number of dead versus live young produced was not provided. A total of dead vs. live young that were initially observed, was never quantified by the study authors and so it is not clear how many were actually lost to cannibalism by the G1 parents or G2 cohorts or if they may have actually been lost due to treatment. However based on the information that was provided in the Appendices from the study report the total number of dead F1 young in the negative and solvent controls, 85, 87, respectively, as compared to 74, 51, 35, and 2 in the mean-measured 10, 21, 39, 76, and 157 µg a.i./L treatment groups, respectively (Appendix II). Given the difference in young produced and live young available for the G2 phase of the experiment this information is especially important (Appendix 17 vs. Appendix 20 in the study report, respectively). Again, the study authors provided no rationale; however it can be postulated that the difference was attributed to potential cannibalism (either from G1 mysids or G2 cohorts), which is a possibility; however, given the great difference, this seems may not be the only reason. Although not explicitly stated, with the information presented for G2 live young for the highest test concentration, young were indeed produced (only 2 young, in one replicate); however, no ***live*** G2 mysids were collected for the highest test concentration, indicating that the two young that were produced died before the G2 phase began. Based on the information that was provided in the Appendices from the study report the total number of dead F1 young in the negative and solvent controls and treatment groups was estimated by the reviewer (Appendix II).

13. CONCLUSIONS:

This study **is scientifically sound** and is classified as **supplemental and may be used for risk characterization**. After 28 days reproductive and growth effects were observed in all of the treatment groups. However, there were no effects on survival for the F_0 (G1 mysids) or F_1 (G2 mysids) exposure groups. Based on the observed effects, the NOAEC/LOAEC value <10/10 µg a.i./L using the mean-measured concentrations, and the most sensitive endpoints, F_0 Male dry weight and length.

Although no significant differences were observed between the solvent and negative controls, there was a potential slight interaction with the solvent and the test substance for the F0 male dry weight endpoint (13% decrease in solvent control compared to negative control; p = 0.07) and a statistically significant difference between the number of offspring per surviving female produced in the solvent control compared to the negative control (159% increase in solvent control compared to negative control; p= 0.04), resulting in uncertainty that the solvent may have had an impact on these endpoints.

Since a definitive NOAEC could not be established in the study as dose-responsive effects on male weight and length were observed at all doses, additional data may be necessary including test concentrations less than the lowest test concentration used in this study, in order to establish a definitive NOAEC. It is also recommended that any new test consider whether a different solvent may be more appropriate, as there may be an interaction between the test substance and the solvent.

NOAEC: <10 µg a.i./L
LOAEC: 10 µg a.i./L

Endpoint(s) Affected: All reproductive and growth endpoints (*e.g.,* F_0 Male dry weight and length; Offspring/female; Time to first brood; F_0 Female dry weight and length)
Most sensitive endpoint(s): F_0 Male dry weight and length

14. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.1350: Mysid Chronic Toxicity Test.

American Society for Testing and Materials. 2008. ASTM Standard E1191-03a: Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids.

The SAS System for Windows. 1999-2001. Version 8.2. The SAS Institute, Inc., Gary, North Carolina.

APPENDIX I. COPY OF EXCEL SPREADSHEET CALCULATION OF OFFSPRING PER FEMALE:

C-µg ai/L	Code	Rep	n F0 Survivec	No. young produced	N females	**n Offspring Per Female**	Mean offspring per femal
0	N	1	9	36	5	7.2	5.2
0	N	2	9	30	5	6	
0	N	3	6	8	5	1.6	
0	N	4	10	29	5	5.8	
0	S	1	8	78	5	15.6	13.4
0	S	2	9	88	5	17.6	
0	S	3	10	26	5	5.2	
0	S	4	7	45	3	15	
10		1	10	31	5	6.2	7.1
10		2	4	22	3	7.3	
10		3	9	32	5	6.4	
10		4	9	42	5	8.4	
21		1	10	20	5	4	3.9
21		2	10	20	5	4	
21		3	10	29	5	5.8	
21		4	8	9	5	1.8	
39		1	8	18	4	4.5	3.9
39		2	8	15	4	3.75	
39		3	8	22	5	4.4	
39		4	8	15	5	3	
76		1	6	13	4	3.25	2.7
76		2	9	10	4	2.5	
76		3	8	15	4	3.75	
76		4	8	7	5	1.4	
157		1	8	2	5	0.4	0.1
157		2	9	0	4	0	
157		3	4	0	3	0	
157		4	8	0	0		

APPENDIX II. COPY OF EXCEL SPREADSHEET CALCULATION OF TOTAL DEAD F1 YOUNG

C-µg ai/L	Code	Rep	n F1 Exposed		Total No. young p	Total n F1 Exposed	Total dead
0	N	1	18		103	18.0	85.0
0	N	2	0				
0	N	3	0				
0	N	4	0.0				
0	S	1	51		237	150	87
0	S	2	40				
0	S	3	49				
0	S	4	10				
10		1	47		127		
10		2	30				
10		3	0				
10		4	0				
21		1	4		78	4	74
21		2	0				
21		3	0				
21		4	0				
39		1	19		70	19	51
39		2	0				
39		3	0				
39		4	0				
76		1	10		45	10	35
76		2	0				
76		3	0				
76		4	0				
157		1	0		2	0	2
157		2	0				
157		3	0				
157		4	0				

APPENDIX III. COPY OF EXCEL SPREADSHEET WITH MEASURED CONCENTRATIONS

F_0 (G1, 1st generation)

	Day									
Nominal Concentrations (mg ai/L)	**0**	**7**	**14**	**15**	**16**	**21**	**28**	**Mean (ug ai/L)**	**S.D.**	**CV (%)**
Negative Control								***<7.50***	N/A	N/A
Solvent control								***<7.50***	N/A	N/A
13	11.9	10.3	12.9	7.96	9.21	9.11	10.4	***10.3***	1.7	17
25	23.1	18.8	25.3	18.7	17.9	17.9	22	***20.5***	2.9	14
50	42.5	45.3	42.2	34	32.8	33.6	39.3	***38.5***	5.1	13
100	84.5	79.1	88.4	69.5	62.4	70.8	77.5	***76.0***	9.1	12
200	165	191	175	132	134	155	148	***157***	21.5	14

F_1 (G2, 2nd generation)

	Day						
Nominal Concentrations (mg ai/L)	**0**	**6**	**13**	**15**	**Mean (ug ai/L)**	**S.D.**	**CV (%)**
Negative Control					***<7.50***	N/A	N/A
Solvent control					***<7.50***	N/A	N/A
13	11	11	12.5	11.6	***11.5***	0.7	6
25	22.8	23	23.8	24.3	***23.5***	0.7	3
50	45.6	44.5	45.6	44.8	***45.1***	0.6	1
100	83.8	82.6	87.2	83.7	***84.3***	2.0	2
200	195	177	169	160	***175***	14.9	8