Joint Exhibit 88

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

Data Evaluation Report on the Chronic Toxicity of 2,3,5,6-Tetrachloroterephthalic acid (degradate of DCPA) to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number {.....} EPA MRID No. 51235101

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	460199
	OECD Data Point	{.....}
	EPA MRID	51235101
	EPA Guideline	850.1300

Test Material: 2,3,5,6-Tetrachloroterephthalic acid (degradate of DCPA) **Purity (%):** 97.3%
Common name: TCTPAc
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Primary Reviewer: Elizabeth Krupka **Signature:**
Environmental Scientist, CDM/CSS-Dynamac JV **Date:** 01/04/2021

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EPA/OPP/EFED/ERB2/Senior Scientist **Date:** 11/16/2021

Reference/Submission No. {.....}

Company Code	{.....}	[For PMRA]
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Date Evaluation Completed: 16-11-2021

CITATION: Goudie, O. J., 2019. 2,3,5,6-Tetrachloroterephthalic acid (TCTPAc): Chronic Toxicity Test with the Cladoceran, *Daphnia magna*, Conducted Under Flow-Through Conditions. Unpublished study performed by Eurofins EAG Agroscience, LLC, Columbia, Missouri. Laboratory Study No. 86482. Study sponsored by Amvac Chemical Corporation, Newport Beach, CA. Study initiated May 23, 2018 and completed January 23, 2019.

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

EXECUTIVE SUMMARY:

The 21-day chronic toxicity of **2,3,5,6-Tetrachloroterephthalic acid, TPA (degradate of DCPA)** to *Daphnia magna* was studied under flow-through conditions. Daphnids were exposed to the test substance at nominal concentrations of 0 (negative control), 0.31, 0.63, 1.3, 2.5, and 5.0 mg a.i./L. The mean-measured concentrations were <0.0300 (<LOD, negative control), 0.290, 0.596, 1.23, 2.53, and 4.96 mg a.i./L.

Adult survival at 21 days averaged 98% in the negative control and ranged from 90 to 100% in the first four treatment levels. Survival was significantly reduced to 73% (a maximum effect of 26%) in the highest treatment level ($p = 0.0071$). No significant effects were observed for any other endpoint tested. The overall NOAEC and LOAEC values were determined to be 2.53 and 4.96 mg a.i./L, respectively.

This study **is scientifically sound** and is classified as **acceptable**.

Results Synopsis

Test Organism Age (eg. 1st instar): Neonates, <24 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

Parental Survival
NOAEC: 2.53 mg a.i./L
LOAEC: 4.96 mg a.i./L

Endpoints affected: Survival
Most sensitive endpoint: Survival

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based upon procedures outlined in OECD Guideline No. 211: *Daphnia magna* Reproduction Test (2012) and U.S. EPA OCSPP Guideline No. 850.1300: Daphnid Chronic Toxicity Test (2016). The following deficiencies from OCSPP 850.1300 and/or OECD 211 were noted:

1. The physicochemical properties of the test substance were not reported. This is considered to be a minor deficiency.
2. Incomplete dilution water parameters (TOC, particulate matter, pH, conductivity, alkalinity, etc.) were reported. OCSPP guideline recommend that these parameters are measured and that these water quality characteristics meet EPA specifications. Although pH was slightly outside the recommended OCSPP guideline range of 6.0 – 8.5, at 8.5 to 8.6 during the test. But because the measured pH was within the range recommended by the OECD 211 guideline (6 – 9), this is considered to be a minor deficiency. The solubility (175.4 mg/L) of TPA in water would not result in an underestimation of toxicity; and the full preparation details of the dilution water were presented in Appendix 1 of the study report (pg. 25-26).
3. No information is provided to determine if the culture used for testing is first-brood progeny. This is considered a study deficiency.
4. Dry weight measurements were not recorded or analyzed. OCSPP guidance (850.1300) indicates that adult growth be determined and prefers that both dry weight and length be measured to evaluate effects on growth. However, tests conducted according to the 850.1300 guideline are considered acceptable so long as either length or dry weight are measured. OECD guidance recommends that growth measurements be included but does not specify weight as an endpoint. Total length is the only growth parameter that was measured. This is considered a minor deficiency.

These deficiencies *do not* impact the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with all pertinent U.S. EPA GLPs as published by the U.S. EPA (40 CFR Part 160, 1989), which are compatible with OECD Principles of GLP [C(97)186/Final] with the following exceptions: latest food and water characterizations, and characterization of the test substance and positive control substance.

A. MATERIALS:

1. Test Material:	2,3,5,6-Tetrachloroterephthalic acid (degradate of DCPA)
Description:	White solid
Lot No./Batch No.:	T/H-1-166 (Lot No.)
Purity:	97.3%
Stability of compound under test conditions:	Stable. The %CV ranged from 3 to 9%.
Storage conditions of test chemicals:	Refrigerated.

Physicochemical properties of 2,3,5,6-Tetrachloroterephthalic acid (degradate of DCPA).

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test Organism:

Species: *Daphnia magna*
Age at test initiation: <24 hours old
Source: In-house laboratory culture

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A 14-day preliminary range-finding study was performed at nominal concentrations of 0 (negative and solvent (50 µL DMF/L) controls), 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.i./L. Each treatment was replicated twice with 10 daphnids each.

Parental survival was 90 and 100% in the negative and solvent controls, respectively, and ranged from 80 to 95% in all treatment groups. The mean number of young produced per reproductive day ranged from 6.9 to 9.3 in the treatment groups compared to 5.5 and 7.2 in the negative and solvent controls, respectively. Results of the range-finding test did not appear to be dose-responsive. Range-finding results and discussion with the Sponsor were used to aid in the selection of the definitive test concentrations.

b. Definitive Study: Definitive test was conducted September 12 to October 3, 2018.

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period:	N/A - continuously cultured in-house.	Approximately one day prior to neonate selection, adult daphnids were isolated by transferring adults to fresh culture with a water/food suspension. Adults producing the young for the definitive test were *ca.* 16 days old.
Conditions: (same as test or not)	Same temperature (20°C), dilution water, and lighting as test (no further details provided).	*The recommended acclimation period for brood daphnids, in 100-percent dilution water at dilution temperature, is a minimum of 48 hours prior to start of test. Daphnids should be fed the same food as used for the definitive test [for automatic feeding devices, a suggested rate is 5 to 7 µg food (either solids or algal cells, dry weight) per liter of dilution water or test solution; and for manual once-a-day feeding, a suggested*
Feeding:	Fed a suspension of the algal species *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) at least once a day supplemented by a prepared artificial diet consisting of a yeast, wheat grass (cereal leaves), and	

Parameter	Details	Remarks *Criteria*
	salmon starter (trout feed) suspension (termed YCT).	*rate is 15 µg food (dry weight) per liter of dilution water or test solution].*
Health (any mortality observed):	There were no signs of stress, presence of males, delay in first brood production, ephippia, disease, or physical damage. No adult mortality occurred during the 48-hour period prior to production of neonates used to initiate the definitive test.	*Cultures should not contain ephippia and should produce young before Day 12 and produce at least 3 young per adult per day within 7 days prior to test.* *Pretest mortality should be <20% 48 hours prior to testing.*
Test condition: Static renewal/flow-through:	Flow-through	Dilutor stock solutions were prepared on 27, 29, 31 August, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29 September and 01 October 2018. The diluter stock solution was prepared at a target concentration of 10 mg/L be weight 2.0555 g (2.0000 g corrected for 97.3% purity). A stainless-steel barrel was filled with 200 L of dilution water and approx. 1 L removed for rinsing use. An overhead mixer with stainless-steel shaft was placed on the barrel and stirring initiated. The container containing the pre-weighed test substance was filled approximately half full with an aliquot of the previously removed dilution water, stirred with a glass rod, then poured into the barrel. The solution stirred for approx. 15 minutes. Each time the diluter system cycled, the pump introduced 2,000 mL of the diluter stock solution to the chemical mixing cell of the diluter system, where the solution was diluted with approx. 2,000 mL of dilution water to generate test solution for the highest treatment. The four lower treatment solutions were generated by mixing appropriate volumes of the highest test substance treatment solution with dilution water at each cycle of the proportional diluter system. Operation of the diluter system and delivery of the test solution was initiated on 28 August 2018.
Type of dilution system- for flow through method:	A 2-L proportional equal solvent diluter system was used to deliver each concentration of the test substance and negative control to the test chambers. Approximately 2 L of test solution was delivered to four replicate test chambers, with each replicate receiving approximately 500 mL per cycle. The diluter cycle rate during the definitive test was maintained at approximately 2.2 cycles per hour. The cycle rate was sufficient to provide approximately five volume additions to each test chamber over a 24-hour period. The accuracy of the diluter was verified by volumetric measurement of replicate delivery volumes before test initiation and at test termination, and measurement of treatment delivery volumes during the test. The diluter was allowed to operate for approximately 15 days prior to initiating the definitive test. Proper operation of the proportional diluter and all mechanical systems was verified twice each day during the definitive test.	*EPA recommends consistent flow rate of ≥ 5 vol/24 hours, meter systems calibrated before study and checked twice daily during test period. Flow rates should not vary >10% between test chambers.* *For static-renewal: test dilution water should be replaced at least once every 3 days.*
Renewal rate for static renewal:	N/A	

Parameter	Details	Remarks *Criteria*
Aeration, if any:	No aeration was provided during testing.	*EPA recommends if aeration is needed to achieve DO level, it should be done before the addition of the test substance, and all treatment and control chambers should be given the same aeration treatment.*
Duration of the test:	21 days	*Recommended duration is 21 days.*
<u>Test vessel</u> Material (glass/stainless steel): Size (for growth and reproduction/survival test): Fill volume:	Glass aquaria (15 cm wide, 22 cm long, and 24 cm high) Not reported 5 L (14 cm)	Daphnids were maintained in retention baskets within the replicate test chambers to facilitate daily observations. Each retention basket consisted of a glass petri dish (1.5 x 10 cm) with a stainless steel screen collar (approximate mesh opening of 381 µm), attached with translucent silicone sealant. Aquaria were arranged in a temperature controlled water bath. *EPA recommends for static tests: 250 ml jars* *For flow-through tests: glass or stainless-steel containers with stainless steel or nylon screen bottoms and can be constructed using 250–mL beakers or other suitable containers equipped with screened overflow holes, standpipes, or V-shaped notches. Daphnids should always be submerged in at least 5 cm of test solution. OECD guideline recommends that parent animals be maintained individually; one per vessel, with 50 - 100 ml of medium in each vessel.*
Source of dilution water: <u>Quality of dilution water</u> Particulate matter: TOC or COD: Un-ionized ammonia: Residual chlorine:	Moderately hard freshwater prepared by blending naturally hard well water with well water that was demineralized by reverse osmosis. The dilution water was passed through a sediment filter prior to use in the definitive test. Not reported Not reported < Reporting limit < Reporting limit	Well water used to prepare dilution water was characterized and screened over 3 years (2015, 2016 and 2017), results were presented in Appendix B in the study report pg. 60-63 *Recommended source of dilution water is: surface or ground water, reconstituted water or dechlorinated tap water if daphnids will survive in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress. Maximum allowable concentrations for water quality parameters are provided in EPA's 850.1300 guideline*

Parameter	Details	Remarks *Criteria*
Total organophosphorus pesticides: Total organochlorine pesticides + PCBs: Organic chlorine: Hardness as $CaCO_3$: Specific conductivity: pH: Dissolved Oxygen: Alkalinity:	< Reporting limit or not available < Reporting limit Not reported 140 to 160 mg/L as CaCO3 Not reported Not reported Not reported Not reported	*(https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines).*
Water quality during testing pH: Dissolved oxygen: Temperature: Other measurements: Photoperiod: Interval of water quality measurements:	 8.5 to 8.6 7.8 to 8.5 mg/L (90 to 96% of sat.) 19.2 to 20.6 ºC Hardness: 146-152 mg/L as $CaCO_3$ Alkalinity: 160-164 mg/L as $CaCO_3$ Conductivity 343-351 µS/cm 16 hours light:8 hours dark, with 30-minute transition periods; light intensity of 639 to 683 lux at test initiation Total hardness, total alkalinity, and conductivity were measured in alternating replicates of the negative control and highest treatment level on days 0, 7 ,14, and 21. Temperature, dissolved oxygen concentration, and pH were measured in each replicate of the control and each treatment level with surviving organisms at initiation and once each week following initiation.	 EPA Recommendations: *pH: OECD recommends that pH range be 6-9 and does not vary more than 1.5 units in any one test.* *Dissolved oxygen: 60-105% saturation.* *Temperature: 20±1°C. OECD recommends a range of 18 - 22°C; temperature should not vary > ±2°C.* *Photoperiod: 16 hours light and 8 hours darkness, with a 15 to 30–min transition period.* *DO, temperature, and pH should be measured at the beginning of the test and on days 7, 14, and 21 in at least two chambers of the high, middle, low, and control test concentrations.*
Number of replicates Negative control: Solvent control: Treatments:	 4 N/A 4/level	Daphnids were impartially added. *Static-renewal: 10 or more replicates of one daphnid each.* *Flow-through: four replicates of equal*

Parameter	Details	Remarks *Criteria*
		number of daphnids.
Number of organisms per replicate:		Total of 40 daphnids per level
For growth and reproduction: For survival test:	10 10	*For each test concentration and control group (negative and solvent, if used);* *For static tests, EPA and OECD recommend a minimum of 10 daphnids held individually.* *For flow-through tests, 20 daphnids total divided equally into four replicates at each concentration and control.*
Treatment Concentrations: Nominal: Mean-measured:	0 (negative control), 0.31, 0.63, 1.3, 2.5, and 5.0 mg a.i./L < 0.0300 (<LOD, control), 0.290, 0.596, 1.23, 2.53, and 4.96 mg a.i./L	Concentrations of the test item were measured on Day -13, and days 0, 7, 14, and 21. At each sample point, samples were collected from a single replicate of the control and each test substance treatment. Alternating replicates were sampled during each event. Mean-measured values do not include the day -13 results. The control and all test substance solutions were clear and colorless with no visible particulates, surface film, undissolved test substance, or precipitate throughout the test.
		Treatment concentrations should include a geometric series at a separation factor of 1.5 to 2 of at least five concentrations plus a control/solvent control. The variability of measured concentrations between replicates of the same concentration should not exceed ±20%. *Concentration of test substance in each test chamber should be measured at a minimum before the test and on days 7, 14 and 21, and in the appropriate chamber after a malfunction.* *OECD recommends that at least 5 test concentrations and a control be used in a geometric series with a separation factor not exceeding 3.2.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used):	N/A	*Solvent concentration should not exceed 0.1 ml/L. Recommended solvents include dimethylformamide and triethylene glycol, but acetone and ethanol can be used if necessary.* *OECD recommends <0.1 ml/L of solvent.*
Recovery of the chemical: Frequency of determination: Level of Quantitation: Level of Detection:	88 to 108% of nominal Days -13, 0, 7, 14, and 21 0.100 mg/L 0.0300 mg/L	Mean-measured values do not include the day -13 results. A method validation in freshwater was performed on 07 August 2018 (pg. 20-21; Tables 1-4 in study report). Due to heavy degradation of the analytical column, the instrument conditions were revised from those used for the method validation. The revision to the method was confirmed as being suitable for quantifying the test substance in freshwater by the acceptable QC recoveries analyzed concurrently with the definitive test samples. Measured concentrations from QC samples during the definitive test in freshwater ranged from 73 to 102% of nominal at a concentration of 0.100 mg/L and from 92 to 98% of nominal at a concentration of 32.6 mg/L
Positive control {if used, indicate the chemical and concentrations}	Potassium Chloride (99.5%; Batch No. SLBV6834) 0 (control), 120, 240, 500, 1000, and 2000 mg a.i./L Study conducted 25 to 27 June 2018.	24-hour EC_{50} (95% CIs) = 930 (770 to 1100) mg a.i./L 48-hour EC_{50} (95% CIs) = 640 (570 to 710) mg a.i./L Appendix F; pg. 99-101 in study report.
Other parameters, if any	During exposure daphnids were offered an algal suspension and a prepared invertebrate food solution (YCT daphnid food mixture) containing between 2.42 and 2.48 g of suspension solids/L. Five to 8 mL of a $6.0x10^7$ cells/mL *P. subcapitata* algal suspension were manually added to each replicate chamber four times daily (*e.g.*, morning, early mid-day, late midday, and afternoon), except for day 0 where a 5.0-mL volume was added at early mid-day and an 8.0-mL volume was added late mid-day and afternoon and day	

Parameter	Details	Remarks *Criteria*
	21 where an 8.0-mL volume was added in the morning only. With the exception of day 21, a 4.0-mL volume of invertebrate food solution was manually added to each replicate chamber two times daily (*e.g.,* morning and afternoon). On day 21 a 4.0-mL volume of invertebrate food solution was manually added to each replicate chamber in the morning only.	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	- Parental immobility (mortality) - Other parental sublethal effects - Time of first brood release - Offspring production - Survival of first-generation daphnids - Length of surviving P-generation daphnia	Immobile daphnids, defined as those organisms not able to swim within 15 seconds after gentle agitation of the test vessel or gentle disturbance of the individual, were discarded; therefore, immobility was synonymous with mortality. *Recommended endpoints measured:* *- survival of first-generation daphnids (F_0);* *- number of offspring produced per female;* *- time to first brood;* *- dry weight and length (optional) of each first generation daphnid alive at the end of the test (F_0);* *- survival of offspring (F_1) and successful birthrate;* *- incidence and description of morphological abnormalities and behavioral effects;* *- observations of other effects or clinical signs.*
Observation intervals	All test vessels were examined daily. Growth measurements were determined on Day 21.	*The number of immobilized daphnids in each chamber should be recorded on day 21 of the test. After offspring are produced, they should be counted and removed from the test chambers every 2 or 3 days.*
Were raw data included?	Yes, however no weight measurements were provided.	

Parameters	Details	Remarks
		Criteria
Other observations, if any	None	

II. RESULTS AND DISCUSSION

A. MORTALITY AND SUB-LETHAL EFFECTS:

Adult survival at 21 days was 98% in the negative control and ranged from 90 to 100% in the first four treatment levels. Survival was reduced to 73% at the highest treatment level, which was determined to be significant by the study author via Williams' test ($p = 0.05$). No other sublethal effects were observed.

Table 3: Effect of 2,3,5,6-Tetrachloroterephthalic acid (degradate of DCPA) on Survival, Growth, and Reproduction of *Daphnia* sp.[a]

Mean-measured (and Nominal) Concentration, mg a.i./L	Adult Survival (Day 21)		Mean Time to First Brood (Days)[b]	Mean Young per Adult Reproductive Day (Successful Birthrate)	Mean No. of Offspring Released per Surviving Female[e]	Mean First Brood Production Rate	Mean Length of Surviving Adults (mm)	Dry Weight of Surviving Adults (mg)
	No.	%						
Negative Control (<LOQ)[c]	38[d]	98	7	11.5	171	0.14872	4.4	N/A
0.290 (0.31)	38	95	7	12.3	194	0.14872	4.5	N/A
0.596 (0.63)	40	100	8	11.0	164	0.13967	4.4	N/A
1.23 (1.3)	39	98	8	11.8	181	0.13967	4.4	N/A
2.53 (2.5)	36	90	8	12.5	202	0.14359	4.5	N/A
4.96 (5.0)	29	73*	8	12.0	194	0.13333	4.5	N/A
NOAEC, mg ai/L	2.53		Not calculated	4.96	Not calculated	4.96	4.96	N/A
LOAEC, mg ai/L	4.96		Not calculated	>4.96	Not calculated	>4.96	>4.96	N/A
EC_{50} (95% CI), mg ai/L	>4.96		Not calculated	Not calculated	Not calculated	>4.96	Not calculated	N/A

[a] Data were obtained from Table 6 through 11 on page 32 and 38 of the study report.
[b] Reviewer-calculated using data reported on page 34 of the study report.
[c] LOQ = 0.0300 mg a.i./L
[d] One control daphnid was accidentally killed during change over process on Day 14. This daphnid was excluded by the reviewer from the number of daphnids exposed at test initiation.
[e] Reviewer-calculated using data reported in Appendix E (page 95-98) of the study report.
* Significant reduction in survival as compared to the control (Williams' test, $p = 0.05$).

B. EFFECT ON REPRODUCTION AND GROWTH: The mean time to first brood averaged 7 days in the negative control and ranged from 7 to 8 days in the groups exposed to the test material. Successful birthrate (reported as mean young per adult reproductive day) averaged 11.5 in the negative control and ranged from 11.0 to 12.5 days in the treatment groups. Time to first brood was not evaluated by the study author. No significant effects on successful birth rate were determined.

Mean total body length (helmet-to-spine) averaged 4.4 mm in the negative control and ranged from 4.4 to 4.5 mm in the treatment groups. The weights at test termination were not measured by the study author. No significant effects on length were observed.

C. REPORTED STATISTICS:

Test endpoints analyzed statistically were survival, reproduction (young per parent at study start, young per adult reproductive day, young per adult at study start and 1st production rate), and growth (total length). All statistical analyses were performed using SAS software (version 9.3) and Microsoft Excel 2016.

Data were analyzed using a one-way analysis of variance (ANOVA) and one-tailed Dunnett's and Williams' tests. Prior to the Dunnett's and Williams' tests, Shapiro-Wilk's and Levene's test were conducted to test for normality and homogeneity of variance, respectively. The Shapiro-Wilk's and Levene's tests indicated the assumptions of normality and homogeneity of variance were not met for survival, number of young per adult reproductive day, time to first brood, and first brood production rate data. Therefore, these parameters were analyzed with a non-parametric ANOVA, and Dunnett's and William's tests on the ranks of the values. Assumptions of normality and homogeneity of variance were met for number of young per parent at study start and length data; therefore, these parameters were analyzed with a parametric ANOVA, followed by Dunnett's and Williams' tests on the untransformed data. EC_{50} values, where possible, were visually estimated to be greater than the highest test level.

Parental Survival
LC_{50} (21 d): >4.96 mg ai/L 95% C.I.: N/A
NOAEC: 2.53 mg ai/L
LOAEC: 4.96 mg ai/L

Production Rate of First Brood
EC_{50} (21 d): >4.96 mg ai/L 95% C.I.: N/A
NOAEC: 4.96 mg ai/L
LOAEC: >4.96 mg ai/L

Reproduction/Successful Birthrate (Young/Adult Reproductive Day)
NOAEC: 4.96 mg ai/L
LOAEC: >4.96 mg ai/L

Length
NOAEC: 4.96 mg ai/L
LOAEC: >4.96 mg ai/L

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer assessed the parental survival, growth, and reproduction endpoints using CETIS version 1.9.6.12 statistical software with database backend settings updated by EFED on 07/25/17. Mean-measured test concentrations were used for analysis and reporting.

The statistical endpoints included F_0 mortality/survival, F_0 growth (length), and reproduction (number of live offspring, successful birth rate, and time to first brood). The survival, reproductive and growth endpoints were assessed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively.

The number of live offspring and successful birth rate endpoints met both assumptions but were not monotonic, so were analyzed using the Dunnett Multiple Comparison Test. Length and time to first brood did not meet one or both assumptions, were non-monotonic, and were subsequently analyzed using the Mann-Whitney U Two-Sample test. Survival data also did not meet both assumptions but were generally monotonically decreasing and therefore analyzed using the Jonckheere-Terpstra Step-Down Test.

The decrease in number of live offspring at the highest dose (16%↓) was narrowly not statistically significantly different from controls (p=0.10), but there was broad overlap in confidence intervals between this dose and the controls. Survival was also narrowly not statistically significantly different at the 2nd highest treatment (2.53 mg a.i./L, 8%↓), relative to controls (p=0.09), but this appears to be a result of a single poor performing replicate with 20% mortality.

Total Length
NOAEC: 4.96 mg ai/L
LOAEC: >4.96 mg ai/L

Parental Survival
NOAEC: 2.53 mg ai/L
LOAEC: 4.96 mg ai/L

No. of Live Offspring
NOAEC: 4.96 mg ai/L
LOAEC: >4.96 mg ai/L

Successful Birth Rate
NOAEC: 4.96 mg ai/L
LOAEC: >4.96 mg ai/L

Time to First Brood
NOAEC: 4.96 mg ai/L
LOAEC: >4.96 mg ai/L

Endpoints affected: Survival
Most sensitive endpoint: Survival

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were in complete agreement with regard to the NOAEC and LOAEC values for survival, growth (length), and reproduction (successful birth rate (young/adult reproductive day)). The study author did not determine a NOAEC for time to first brood or number of live offspring. The study author additionally determined a NOAEC for *production rate of first brood, which was something that the reviewer did not determine.* The reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

Following 21 days of exposure, the number of live offspring produced averaged 1630, 1837, 1638, 1766, 1809, and 1371 for the mean-measured 0 (negative control), 0.29, 0.596, 1.23, 2.53 and 4.96 mg a.i./L levels, respectively.

All validity requirements were met. Specifically, 1) ≤20% of the control organisms appeared to be immobilized, stressed, or diseased during the test; 2) each surviving control daphnid produced an average of >60 young (the

control the number of live young produced per surviving control daphnids ranged from 148-188); 3) no ephippia were produced by control animals; and 4) the coefficient of variation around the mean number of living offspring produced per parent animal in the control(s) was <25%.

The experimental phase of the definitive study was conducted from September 12 to October 3, 2018.

G. CONCLUSIONS:

This study **is scientifically sound** and is classified as **acceptable**. A significant effect on parental survival was observed at the highest test concentration, with a maximum effect of 26%. No significant effects were observed for any other endpoint. Therefore, the overall NOAEC and LOAEC values were determined to be 2.53 and 4.96 mg a.i./L, respectively.

III. REFERENCES:

Mount, D.I. and Brungs, W.A. 1967. A Simplified Dosing Apparatus for Fish Toxicological Studies. Water Res. 1: 21-29.

All other references were standard guidelines and methods.