Data Evaluation Report on the Toxicity of Novaluron Technical to Sheepshead Minnows (*Cyprinodon variegatus*), Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 50610210

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	447937
	OECD Data Point	{.....}
	EPA MRID	50610210
	EPA Guideline	850.1400

Test Material: Novaluron **Purity (%):** 98.8%
Common name: Novaluron Technical
Chemical name: IUPAC: (RS)-1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea
CAS name: N-[[[3-chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl]amino]carbonyl]-2,6-difluorobenzamide
CAS No.: 116714-46-6

Primary Reviewer: David A. McEwen **Signature:** David A. McEwen
Staff Scientist, CDM Smith/CSS-Dynamac JV **Date:** 1/13/2019

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Secondary Reviewer(s): N.E. Federoff **Signature:**
{Wildlife Biologist/USEPA/OPP/EFED/ERB2 **Date:** 8/9/2019

EPA PC Code 124002

CITATION: Urann, K. 2018. Novaluron - Early Life-Stage Toxicity Test with Sheepshead Minnow, *Cyprinodon variegatus*, under Flow-Through Conditions. Unpublished study performed by Smithers Viscient, Wareham, Massachusetts. Laboratory Study No. 14125.6108. Study sponsored by ADAMA Makhteshim Ltd., Beer-Sheva, Israel. Study initiated February 28, 2017 and completed May 14, 2018.

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 34-day chronic toxicity of Novaluron technical to the early life-stage of the sheepshead minnow (*Cyprinodon variegatus*) was studied under flow-through conditions. Fertilized eggs/embryos (120/level, <20 hours old) were exposed to Novaluron at nominal concentrations of 0 (negative and solvent controls), 0.19, 0.38, 0.75, 1.5, and 3.0 µg ai/L. The mean-measured concentrations were <0.050 (<MDL, controls), 0.18, 0.34, 0.70, 1.3, and 2.8 µg ai/L (%CV=5.6-14%), respectively. The test system was maintained at 24 to 26°C and a pH of 7.4 to 7.7.

No treatment-related effects were observed on hatching success, time to hatch, post-hatch survival, or growth (length and dry weight) at any treatment level compared to the negative control. The 34-day NOAEC and LOAEC values were 2.8 and >2.8 µg ai/L, respectively, using mean-measured concentrations.

This study is scientifically sound and is classified as acceptable.

Results Synopsis

Test Organism Size/Age: Embryos, <20 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

NOAEC: 2.8 µg ai/L
LOAEC: >2.8 µg ai/L

Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the U.S. EPA Ecological Effects Test Guideline OCSPP 850.1400: *Fish Early-Life Stage Toxicity Test* (draft, 1996a); OECD 210 - Fish Early-Life Stage Toxicity Test (2013); and ASTM E: 1241-05 - Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes (2013). The following deviations from OCSPP 850.1400 and OECD 210 were observed:

1. The physiochemical properties of the test substance were not reported.
2. The concentrations of several dilution water parameters (particulate matter, ammonia, and chlorine) were not reported.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with GLP Standards as published by the U.S. EPA (40 CFR Part 160), with the following exceptions: routine water and food contaminant screening analyses were performed by GeoLabs, Inc. (Braintree, MA) using standard validated methods. This exception had no impact on the study results.

A. MATERIALS:

1. Test Material: Novaluron technical

Description: Not reported

Lot No./Batch No.: 96869065 (Lot No.)

Purity: 98.8%

Stability of compound under test conditions: The reported mean-measured concentrations were 89-93% of nominal with coefficient of variance ranging from 5.6 to 14%.

Storage conditions of test chemicals: Room temperature

Physicochemical properties of Novaluron.

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: Sheepshead minnow (*Cyprinodon variegatus*)
EPA recommends rainbow trout (Oncorhynchus mykiss), bluegill sunfish (Lepomis macrochirus) or fathead minnow (Pimephales promelas) for freshwater, and sheepshead minnow (Cyprinodon variegatus) for estuarine/marine. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.

Age /embryonic stage at test initiation: Embryos, <20 hours old
EPA recommends fish embryo age at test initiation is as soon as possible after the eggs have been fertilized.

Method of collection of the fertilized eggs: Fertilized embryos were collected from approximately 6-month old brood stock.

Source: In-house laboratory brood stock

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 20-day (14 days post-hatch) preliminary study was conducted with 30 embryos per replicate and four replicates per level, at nominal concentrations of 0 (negative and solvent control), 0.19, 0.38, 0.75, 1.5, and 3.0 µg ai/L. Mean hatching success was 71-78% in the controls and all treatment groups. Following hatch, larvae were thinned to 20 larvae per replicate. The percent normal larvae at hatch averaged 94-100% in all groups, including controls. The percent larval survival averaged 89-99% in the control and all treatment groups. The total length averaged 11.14 and 11.80 mm in the negative and solvent controls and 11.32, 11.51, 11.62, 11.57, and 11.68 mm in the nominal 0.19, 0.38, 0.75, 1.5, and 3.0 µg ai/L groups, respectively. The wet weights averaged 0.0036 and 0.0044 g in the negative and solvent controls and 0.0038, 0.0041, 0.0043, 0.0043, and 0.0046 g in the nominal 0.19, 0.38, 0.75, 1.5, and 3.0 µg ai/L groups, respectively. Based on these results and consultation with the Study Sponsor, the definitive nominal concentrations of 0.19, 0.38, 0.75, 1.5, and 3.0 µg ai/L were selected.

b. Definitive study: In-life test dates were November 22 to December 26, 2017.

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions (same as test or not): Feeding (type, source, amount given, frequency): Health (any mortality observed):	14 days Similar to test conditions (19 to 25 ºC and dissolved oxygen range of 85 to 100% of saturation) Not reported No mortality was observed the 48 hours prior to test initiation.	*EPA recommends embryos be immersed in the test solutions before cleavage of the blastodisc commences, or as close as possible after this stage.*
Number of fertilized eggs/embryos in each treatment at test initiation:	120 embryos/treatment level, divided into 30 embryos/chamber, and 4 replicate chambers/treatment	Thinned to 20 alevins/replicate on Day 6. *Each treatment should include a minimum of 60 embryos, divided equally between at least 2 replicate test chambers. OECD also recommends at least 60 eggs, divided between at least 2 replicates.*
Concentration of test material Nominal: Mean-measured:	0 (negative and solvent controls), 0.19, 0.38, 0.75, 1.5, and 3.0 µg ai/L <0.050 (<MDL, controls), 0.18, 0.34, 0.70, 1.3, and 2.8 µg ai/L	The reported concentrations were adjusted for the purity of the test substance. The %CV range was 5.6 to 14%. *A minimum of 5 concentrations, spaced by a constant factor not exceeding 3.2, and a control, all replicated, plus solvent control if appropriate should be used.* *- Toxicant concentration should be measured at least 5 times at regular intervals, and at least once per week for studies longer than one month.* *- Concentrations of test substance in solution should be within ± 20% of the mean measured values during study.* *- One concentration should adversely affect a life stage and one concentration should not affect any life stage.* *OECD also recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2 and concentrations be within ± 20% of the mean measured values.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	Acetone (6 µL/L)	*The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, and ethanol.* *OECD recommends that the solvent not have an effect on survival nor produce any other adverse effects; concentration should not be greater than 0.1 ml/L.*
<u>Number of replicates</u> Negative control: Solvent control: Treatments:	 4 4 4/level	*At least 2 replicates per test concentration and control.* *A solvent control should be used in conjunction with a solubilizing agent.*
<u>Test condition</u> Static renewal/flow-through: Type of dilution system for flow through method: Flow rate: Renewal rate for static renewal:	 Flow-through Proportional intermittent-flow diluter *ca.* 7.1 volume additions per day N/A	The diluter system was calibrated prior to exposure initiation and calibration was confirmed at exposure termination. The function of the diluter system (e.g., flow rate, stock solution consumption) was monitored daily and a visual check of the system's operation was performed twice daily. The exposure system was functioning properly for 20 days prior to exposure initiation. *Flow-through systems are generally recommended, including a system which continually dispenses and dilutes a stock solution of the test substance (e.g. metering pump, proportional diluter, or saturator system). EPA recommends that flow rate equal at least 5 test chamber volumes per 24 hours, and not vary by more than 10% throughout the test (OECD also recommends 5 test chamber volumes/24 hours). For flow through tests, biomass loading rate should not exceed 0.5 g/L/24 h and 5 g/L solution at any time. For static-renewal, EPA recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water.*
Aeration, if any	No aeration was reported.	*Aeration is not recommended.*

Parameter	Details	Remarks *Criteria*
Duration of the test	34 days: 6-day hatching period and 28-day post-hatch period	*EPA recommended test duration is 32 days (or 28 days post-hatch) for fathead minnow and sheepshead minnow; 30 days post-hatch for zebra fish and ricefish; 2 weeks after controls are free-feeding (or 60 days post-hatch) for rainbow trout; and 28 to 60 days for other species. OECD recommendations for test duration are species specific and range from 28-60 days.*
<u>Embryo cups, if used</u> type/material (glass/stainless steel): size: fill volume:	Glass jars with 475-µm Nitex® mesh screen bottoms 5 cm diameter and 8 cm high Not reported	Incubation cups gently oscillated using rocker arm at 1 rpm. *Incubation cups were suspended in each replicate vessel and were oscillated via a rocker arm at 1 rpm.*
<u>Test vessel</u> type/material: (glass/stainless steel) size: fill volume:	Glass 30 × 14.5 × 20 cm with 12.5 cm high side drains 5.5 L	During the 34-day exposure, the aquaria were brushed and siphoned when necessary (at least once per week) to remove excess food and fecal matter. *EPA recommends any glass, stainless steel, or other chemically inert vessels can be used.*
Source of dilution water: <u>Quality of dilution water</u> Particulate matter: TOC: Un-ionized ammonia: Residual chlorine: Total organophosphorus pesticides: Total organochlorine pesticides + PCBs: Organic chlorine:	Natural sea water was collected from the Cape Cod Canal (Bourne, MA) from about 1-4 m offshore at a depth of *ca*. 0.5 m. The seawater was diluted to a salinity of approximately 20±3‰ with laboratory well water and filtered (50- and 1-µm). Not reported 1.7 and 1.3 mg/L (November and December 2017) Not reported Not reported Not detected Not detected Not reported	Representative samples of the dilution water source were analyzed periodically for the presence of pesticides, PCBs, and toxic metals by GeoLabs, Inc. (Braintree, Massachusetts). None of these compounds have been detected at concentrations that are considered toxic in any samples analyzed. *Any water with test species control survival meeting % hatching success and % post-hatch success in guideline is suitable as a test water. Maximum allowable concentrations for water quality parameters are provided in EPA's 850.1400 guideline (<u>http://www.epa.gov/ocspp/pubs/frs/home/draftguidelines.htm</u>). EPA also recommends testing for heavy metals (e.g. Cu, Pb, Zn, Hg, Cd, Ni), major anions and cations (e.g. Ca, Mg, Na, K, Cl, sulfate), and suspended solids. OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*

Parameter	Details	Remarks *Criteria*
Water quality during testing Hardness: pH: Dissolved oxygen: Temperature(s) (record all the temperatures used for different life stages): Salinity (for marine or estuarine species): Other measurements: Photoperiod: Interval of water quality measurements:	Not determined 7.4 to 7.7 5.22 to 7.39 mg/L (70.8-99.9% saturation) Daily: 24 to 25°C Continuous: 24 to 26°C 19 to 21‰ None 16-hour light/8-hour dark with 15- to 30-minute transition periods. Light intensity was 540-990 lux. Temperature, dissolved oxygen, salinity, and pH were measured in each aquarium at test initiation and daily in alternating replicates thereafter. Additionally, temperature was continuously measured in replicate A of the control group.	 *EPA Recommendations:* *Dissolved Oxygen: 60 - 100% saturation. OECD recommends that DO concentration be between 60 - 90% saturation.* *Temperature for fathead minnow, zebra fish and sheepshead minnow: 25±2°C; rainbow trout: 10-12±2°C, depending on life stage; and ricefish: 23-24±1-2°C, depending on life stage. Temperature should not deviate by more than ±1.5 °C between test chambers or between successive days during test.* *Salinity for sheepshead minnow: 15-30 ‰ ±2%; salinity for silverside: 20 ‰.* *Recommended photoperiod: 12-16 hours, depending on species.* *EPA recommends DO, salinity (if relevant), and temperature be measured weekly, and pH and hardness be measured at the beginning and end of the test. Temperature should preferably be monitored continuously in at least one test vessel.* *OECD also recommends at a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test.*
Post-hatch details When the post-hatch period began: Number of hatched eggs (alevins)/ treatment released to the test chamber: On what day, the alevins were released from the incubation cups to the test chamber:	 Day 5 20 alevins/replicate (total of 80 alevins/group) Day 6	Mean hatching success was 87 and 83% for the negative and solvent controls, respectively. *Hatching success in each control should be ≥ 66% for rainbow trout and fathead minnow; >75% for sheepshead minnow; and >66 or >80% for other species as shown.* *Post-Hatch success in each control should be ≥ 70% in rainbow trout, fathead minnow and zebra fish; ≥ 80% in ricefish and sheepshead minnow; and ≥ 60 to ≥ 80% for other species as shown.*

Parameter	Details	Remarks *Criteria*
Post-hatch Feeding Start date: Type/source of feed: amount given: Frequency of feeding:	 Day 7 Live brine shrimp nauplii (*Artemia salina*) Food was provided three times daily. At each feeding, larvae were fed *ad libitum* such that all larvae were afforded equal access to food. Larvae were not fed during the 24 hours prior to study termination.	*EPA's 850.1400 guidelines for feeding and handling recommendations for brood and test animals of recommended species and other species are at (https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0154-0033).*
Stability of chemical in the test system	The mean-measured concentrations were 89-93% of nominal with coefficient of variance ranging from 5.6 to 14%.	
Recovery of chemical: Frequency of measurement: LOD: MDL:	89 to 93% of nominal Days 0, 6, 13, 20, 27, and 34 Not reported 0.050 µg ai/L	Recoveries based on mean-measured concentrations. The QC sample recoveries were 94.1-112% of nominal.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Fertilization success study, if any Number of eggs used: On what day the eggs were removed to check the embryonic development:	 30 embryos At test initiation	
Other parameters, if any	Biomass loading, based on controls at test termination did not exceed 0.045 g/L under the exposure's flow-through conditions or 0.32 g/L at any time in any replicate exposure aquarium.	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	- Embryonic development - Hatching success - Time to hatch - Survival (post-hatch and overall [Day 34]) - Growth (length and dry weight) - Behavior and appearance	*Recommended parameters measured include:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and juveniles;* *- Time to swim-up (if appropriate);* *- Measurement of growth (length and weight); dry weight (24 hours at 60°C) is preferred to wet weight;* *- Incidence and description of morphological abnormalities and behavioral effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g. alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects:	 Daily Daily Daily N/A Day 34 (Day 28 post-hatch) At test initiation Daily	*Observations on hatching and survival should be made at least once daily; length and weight at end of test; and morphological abnormalities and behavioral effects at adequate intervals, depending on duration of test.*
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY: Hatching success averaged 84, 85, 88, 88, and 86% in the mean-measured 0.18, 0.34, 0.70, 1.3, and 2.8 µg ai/L groups, respectively, compared to 87 and 83% in the negative and solvent controls (Table 3). No significant effects in hatching success were observed. The NOAEC and LOAEC values for hatching success were 2.8 and >2.8 µg ai/L, respectively.

No treatment-related effects on survival at Day 28 post-hatch (91-99% in all groups, including the controls) were observed. The NOAEC and LOAEC values for survival were 2.8 and >2.8 mg ai/L, respectively.

Table 3: Effect of Novaluron technical on Egg Hatching and Survival at Different Life Stages of Sheepshead Minnow (*Cyprinodon variegatus*). [a]

Mean-measured (and Nominal) Concentrations (µg ai/L)	Eggs hatched/embryo viability			Day to Hatch Start (Days)	Juvenile-survival on Day 28 post-hatch [b]	
	No. of eggs at study initiation	Hatch/embryo viability				
		No.	%		No.	% Survival
Neg. Control (<MDL) [c]	120	104	87	5.7	77	96
Solv. Control (<MDL)	120	100	83	5.9	79	99
0.18 (0.19)	120	101	84	5.9	73	91
0.34 (0.38)	120	102	85	5.6	77	96
0.70 (0.75)	120	105	88	5.7	75	94
1.3 (1.5)	119	105	88	5.7	78	98
2.8 (3.0)	120	103	86	5.8	78	98
NOAEC	2.8 µg ai/L			2.8 µg ai/L	2.8 µg ai/L	
LOAEC	>2.8 µg ai/L			>2.8 µg ai/L	>2.8 µg ai/L	

a Data were obtained from Table 4 on page 37-38 and Appendix 4 on pages 120-126 of the study report. The reviewer calculated the number of hatched embryos and the number for juvenile survival (see Appendix I, excel calculations).
b The number of larvae was thinned to 20 per replicate (80/dose) on Day 6.
c MDL = 0.050 µg ai/L

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Time to hatch: No treatment-related effect on the time to hatch was observed (started on Day 5) and all viable embryos were hatched by Day 6 in all groups (including the controls). The NOAEC and LOAEC for time to hatch were 2.8 and >2.8 µg ai/L, respectively.

Clinical signs of toxicity: For embryonic development determined at test initiation, the mean observation for this test population was stage 12 (early gastrula), the median stage was 13 (one-quarter epiboly), and the range of the developmental stages was 11 to 13 (flat blastula to one-quarter epiboly). The observed range of developmental stages was generally consistent with previous *C. variegatus* early life-stage tests performed at Smithers Viscient.

At exposure termination, all surviving fish appeared normal and were visibly comparable in size with most of the control fish.

Growth: Total lengths averaged 19.04, 18.73, 18.91, 18.93, and 19.00 mm in the mean-measured 0.18, 0.34, 0.70, 1.3, and 2.8 µg ai/L groups, respectively, compared to 18.79 and 18.82 mm in the negative and solvent controls (Table 4). The dry weights averaged 0.0223, 0.0217, 0.0220, 0.0219, and 0.0228 g in the mean-measured 0.18, 0.34, 0.70, 1.3, and 2.8 µg ai/L groups, respectively, compared to 0.0214 and 0.0216 g in the negative and solvent controls. No significant reductions in length or dry weight were observed at any concentration compared to the negative control. The NOAEC and LOAEC for growth were 2.8 and >2.8 µg ai/L, respectively.

Table 4: Effect of Novaluron technical on Growth of Juvenile Sheepshead Minnow (*Cyprinodon variegatus*). [a]

Mean-measured (and Nominal) Concentrations (µg ai/L)	Swim-up (days)			Growth-length (mm ± SD)	Growth-wet weight (g ± SD)
	day x1	day x2	day xn		
Neg. Control (<MDL) [b]	N/A	N/A	N/A	18.79 ± 0.29	0.0214 ± 0.0016
Solv. Control (<MDL)	N/A	N/A	N/A	18.82 ± 0.24	0.0216 ± 0.0010
0.18 (0.19)	N/A	N/A	N/A	19.04 ± 0.43	0.0223 ± 0.0023
0.34 (0.38)	N/A	N/A	N/A	18.73 ± 0.32	0.0217 ± 0.0016
0.70 (0.75)	N/A	N/A	N/A	18.91 ± 0.36	0.0220 ± 0.0020
1.3 (1.5)	N/A	N/A	N/A	18.93 ± 0.31	0.0219 ± 0.0014
2.8 (3.0)	N/A	N/A	N/A	19.00 ± 0.24	0.0228 ± 0.0012
NOAEC	N/A	N/A	N/A	2.8 µg ai/L	2.8 µg ai/L
LOAEC	N/A	N/A	N/A	>2.8 µg ai/L	>2.8 µg ai/L

a Data were obtained from Table 4 on page 37-38 of the study report.
b MDL = 0.050 µg ai/L

C. REPORTED STATISTICS:

Data that were statistically analyzed included time-to-hatch, embryo hatching success, post-hatch survival, overall survival, and larval growth at exposure termination (total length and dry weight). The negative control and solvent control data were compared using an Equal Variance Two-Sample t-Test. For this study, there was no significant difference determined between the negative control and solvent control for all of the endpoints; therefore, the negative control was used to determine treatment effects for all endpoints. All data were checked for normality using Shapiro-Wilk's test ($p<0.01$) and for homogeneity of variance using Bartlett's Equality of Variance test or a Variance Ratio F test ($p<0.01$). Data for all endpoints were normally distributed and met the assumption of homogeneity of variance; therefore, Dunnett's Multiple Comparison Test was used to establish treatment effects.

The LOAEC and NOAEC values were estimated based on significance of the data. As there were no significant effects on any endpoint evaluated, no EC_x values were calculated. All statistical tests were performed using mean-measured concentrations (µg/L) and CETIS™ version 1.8 (2013) statistical software.

Hatching success
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Days to Hatch
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Post-hatch survival
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Overall survival

NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Total length
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Dry weight
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer analyzed hatching success, time-to-hatch, post-hatch survival, and growth (length and dry weight) using CETIS version 1.9.5.3 statistical software with backend database settings updated by EFED on 07/25/17. The negative and solvent control data were compared using an Equal Variance t Two-Sample test. No significant differences were observed and all subsequent analyses were conducted by comparing treatment level data to the negative control only.

Data for all endpoints were tested for normality using the Shapiro-Wilk's test ($\alpha = 0.01$) and for homogeneity of variance using Levene's or Bartlett's tests ($\alpha = 0.01$). The data for all endpoints met both assumptions and were analyzed using analysis of variance followed by parametric Dunnett's multiple comparison test. All analyses were conducted $\alpha = 0.05$ unless specified otherwise, and all toxicity values are based on the mean-measured concentrations.

Hatching success
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Days to Hatch
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Post-hatch survival
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Total length
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Dry weight
NOAEC: 2.8 µg/L
LOAEC: >2.8 µg/L

Endpoints affected: None

E. STUDY DEFICIENCIES:

There were no deviations and/or deficiencies from OCSPP guidance affecting the scientific soundness or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's statistical conclusions were in agreement with those reported by the study author. The agreed upon results are reported in the Executive Summary and Conclusions sections of this DER.

The validity criteria that were met included hatching success of ≥75%, control post-hatch survival of ≥80%, dissolved oxygen content of ≥60%, water temperature did not differ by more than ± 1°C between test chambers or between successive days at any time during the test, the solvent (acetone) had no significant effect on compared to the negative control in any endpoint, and the concentrations of the test substance were within ±20% of the mean measured values throughout the study.

G. CONCLUSIONS:

This study is scientifically sound and is classified as acceptable. No treatment-related effects were observed on hatching success, time to hatch, post-hatch survival, or growth (length and dry weight) at any treatment level compared to the negative control. The 34-day NOAEC and LOAEC values were 2.8 and >2.8 µg ai/L, respectively, using mean-measured concentrations.

NOAEC: 2.8 µg ai/L
LOAEC: >2.8 µg ai/L

Endpoints affected: None

III. REFERENCES:

None; other than standard guidelines and methodologies.

APPENDIX I: Reviewer Excel Calculations

Mean-measured concentration (µg/L)	Number of embryos treated	Number hatched per replicate	Total hatched	No. exposed post-hatch	% Survival Day 28 Post-hatch	No. survived Day 28 post-hatch	Total survived
0	30	26		20	0.95	19	
0	30	27		20	1.00	20	
0	30	25		20	1.00	20	
0	30	26	**104**	20	0.90	18	**77**
0	30	29		20	1.00	20	
0	30	23		20	0.95	19	
0	30	24		20	1.00	20	
0	30	24	**100**	20	1.00	20	**79**
0.18	30	23		20	0.87	17	
0.18	30	26		20	0.96	19	
0.18	30	25		20	0.80	16	
0.18	30	27	**101**	20	1.00	20	**73**
0.34	30	25		20	0.95	19	
0.34	30	26		20	1.00	20	
0.34	30	26		20	1.00	20	
0.34	30	25	**102**	20	0.90	18	**77**
0.7	30	27		20	0.87	17	
0.7	30	26		20	0.95	19	
0.7	30	27		20	1.00	20	
0.7	30	25	**105**	20	0.95	19	**75**
1.3	30	26		20	0.95	19	
1.3	30	25		20	1.00	20	
1.3	30	29		20	1.00	20	
1.3	30	25	**105**	20	0.95	19	**78**
2.8	30	28		20	1.00	20	
2.8	30	25		20	0.95	19	

2.8	30	27		20	1.00	20	
2.8	30	23	**103**	20	0.95	19	**78**

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**			
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater		
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**			
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture	**Age:**	
Sample ID:	06-2241-8621	**Code:**	50610210	**Project:**	Insecticide		
Sample Date:	22 Nov-17	**Material:**	Novaluron	**Source:**	ADAMA Makhteshim, Ltd		
Receipt Date:		**CAS (PC):**		**Station:**			
Sample Age:	n/a	**Client:**	CDM Smith - E. Krupka				

PC Code 124002 MRID 50610210 mean-measured concentrations of active ingredient

SIngle Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
01-3982-0816	Hatching Success	Equal Variance t Two-Sample Test	0.5060	Solvent Blank passed hatching success	1
03-3423-2187	Mean Dry Weight	Equal Variance t Two-Sample Test	0.8629	Solvent Blank passed mean dry weight	1
12-3085-3572	Mean Length	Equal Variance t Two-Sample Test	0.8874	Solvent Blank passed mean length	1
21-2742-0753	Post Hatch Survival	Equal Variance t Two-Sample Test	0.3903	Solvent Blank passed post hatch survival	1
01-7435-6699	Time to Hatch	Equal Variance t Two-Sample Test	0.1110	Solvent Blank passed time to hatch	1

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	TU	PMSD	S
08-1703-3300	Hatching Success	Dunnett Multiple Comparison Test		2.8	>2.8	n/a		9.76%	1
09-0252-4195	Hatching Success	Williams Multiple Comparison Test		2.8	>2.8	n/a		7.57%	1
06-3601-9418	Mean Dry Weight	Dunnett Multiple Comparison Test		2.8	>2.8	n/a		13.6%	1
21-3229-5165	Mean Dry Weight	Williams Multiple Comparison Test		2.8	>2.8	n/a		10.6%	1
05-3177-2605	Mean Length	Dunnett Multiple Comparison Test		2.8	>2.8	n/a		2.99%	1
19-9353-2948	Mean Length	Williams Multiple Comparison Test		2.8	>2.8	n/a		2.32%	1
08-3750-0390	Post Hatch Survival	Dunnett Multiple Comparison Test		2.8	>2.8	n/a		9.83%	1
00-4043-5795	Post Hatch Survival	Williams Multiple Comparison Test		2.8	>2.8	n/a		7.62%	1
08-6444-2776	Time to Hatch	Dunnett Multiple Comparison Test		2.8	>2.8	n/a		5.71%	1
06-1068-0564	Time to Hatch	Williams Multiple Comparison Test		2.8	>2.8	n/a		4.43%	1

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Hatching Success Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	0.8333	0.6897	0.9770	0.7667	0.9667	0.0451	0.0903	10.83%	0.00%
0	N	4	0.8667	0.8234	0.9100	0.8333	0.9000	0.0136	0.0272	3.14%	-4.00%
0.18		4	0.8417	0.7511	0.9323	0.7667	0.9000	0.0285	0.0569	6.76%	-1.00%
0.34		4	0.8500	0.8194	0.8806	0.8333	0.8667	0.0096	0.0193	2.26%	-2.00%
0.7		4	0.8750	0.8242	0.9258	0.8333	0.9000	0.0160	0.0319	3.65%	-5.00%
1.3		4	0.8750	0.7746	0.9754	0.8333	0.9667	0.0316	0.0631	7.21%	-5.00%
2.8		4	0.8583	0.7407	0.9759	0.7667	0.9333	0.0370	0.0739	8.61%	-3.00%

Mean Dry Weight Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	0.0216	0.01996	0.02324	0.0204	0.0228	0.000516	0.001033	4.78%	0.00%
0	N	4	0.02142	0.01881	0.02404	0.0199	0.0233	0.000822	0.001644	7.67%	0.81%
0.18		4	0.0223	0.01866	0.02594	0.0208	0.0257	0.001145	0.002291	10.27%	-3.24%
0.34		4	0.02172	0.0192	0.02425	0.0201	0.0238	0.000793	0.001586	7.30%	-0.58%
0.7		4	0.022	0.01888	0.02512	0.0198	0.0245	0.000981	0.001961	8.91%	-1.85%
1.3		4	0.02188	0.01966	0.02409	0.0206	0.0238	0.000695	0.001389	6.35%	-1.27%
2.8		4	0.02278	0.02086	0.02469	0.0216	0.024	0.000601	0.001201	5.27%	-5.44%

Mean Length Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	18.82	18.44	19.2	18.58	19.15	0.1194	0.2387	1.27%	0.00%
0	N	4	18.79	18.34	19.25	18.45	19.12	0.1428	0.2856	1.52%	0.15%
0.18		4	19.04	18.36	19.73	18.56	19.6	0.2146	0.4291	2.25%	-1.18%
0.34		4	18.73	18.22	19.24	18.4	19.14	0.1605	0.3209	1.71%	0.48%
0.7		4	18.91	18.34	19.48	18.45	19.29	0.1787	0.3575	1.89%	-0.48%
1.3		4	18.93	18.44	19.43	18.59	19.34	0.1562	0.3123	1.65%	-0.60%
2.8		4	19	18.62	19.39	18.74	19.32	0.121	0.242	1.27%	-0.98%

Post Hatch Survival Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	0.9875	0.9477	1.0000	0.9500	1.0000	0.0125	0.0250	2.53%	0.00%
0	N	4	0.9625	0.8863	1.0000	0.9000	1.0000	0.0239	0.0479	4.97%	2.53%
0.18		4	0.9000	0.7547	1.0000	0.8000	1.0000	0.0456	0.0913	10.14%	8.86%
0.34		4	0.9625	0.8863	1.0000	0.9000	1.0000	0.0239	0.0479	4.97%	2.53%
0.7		4	0.9375	0.8374	1.0000	0.8500	1.0000	0.0315	0.0629	6.71%	5.06%
1.3		4	0.9750	0.9291	1.0000	0.9500	1.0000	0.0144	0.0289	2.96%	1.27%
2.8		4	0.9750	0.9291	1.0000	0.9500	1.0000	0.0144	0.0289	2.96%	1.27%

Time to Hatch Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	5.925	5.773	6.077	5.8	6	0.04787	0.09574	1.62%	0.00%
0	N	4	5.675	5.277	6.073	5.4	6	0.125	0.25	4.41%	4.22%
0.18		4	5.875	5.636	6.114	5.7	6	0.075	0.15	2.55%	0.84%
0.34		4	5.65	5.191	6.109	5.3	6	0.1443	0.2887	5.11%	4.64%
0.7		4	5.725	5.645	5.805	5.7	5.8	0.025	0.05	0.87%	3.38%
1.3		4	5.75	5.545	5.955	5.6	5.9	0.06455	0.1291	2.25%	2.95%
2.8		4	5.75	5.474	6.026	5.6	6	0.0866	0.1732	3.01%	2.95%

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Hatching Success Detail

Conc-ug/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	0.9667	0.7667	0.8000	0.8000
0	N	0.8667	0.9000	0.8333	0.8667
0.18		0.7667	0.8667	0.8333	0.9000
0.34		0.8333	0.8667	0.8667	0.8333
0.7		0.9000	0.8667	0.9000	0.8333
1.3		0.8667	0.8333	0.9667	0.8333
2.8		0.9333	0.8333	0.9000	0.7667

Mean Dry Weight Detail

Conc-ug/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	0.0204	0.022	0.0212	0.0228
0	N	0.0199	0.0202	0.0223	0.0233
0.18		0.0216	0.0208	0.0257	0.0211
0.34		0.022	0.021	0.0201	0.0238
0.7		0.0214	0.0198	0.0223	0.0245
1.3		0.0219	0.0238	0.0206	0.0212
2.8		0.0216	0.024	0.0219	0.0236

Mean Length Detail

Conc-ug/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	18.58	18.79	18.76	19.15
0	N	18.7	18.45	18.9	19.12
0.18		19.06	18.56	19.6	18.95
0.34		18.81	18.57	18.4	19.14
0.7		18.84	18.45	19.06	19.29
1.3		18.96	19.34	18.59	18.84
2.8		18.74	19.03	18.93	19.32

Post Hatch Survival Detail

Conc-ug/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	1.0000	0.9500	1.0000	1.0000
0	N	0.9500	1.0000	1.0000	0.9000
0.18		0.8500	0.9500	0.8000	1.0000
0.34		0.9500	1.0000	1.0000	0.9000
0.7		0.8500	0.9500	1.0000	0.9500
1.3		0.9500	1.0000	1.0000	0.9500
2.8		1.0000	0.9500	1.0000	0.9500

Time to Hatch Detail

Conc-ug/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	5.9	5.8	6	6
0	N	5.4	5.6	6	5.7
0.18		5.7	5.8	6	6
0.34		5.3	5.6	6	5.7
0.7		5.7	5.7	5.8	5.7
1.3		5.8	5.9	5.7	5.6
2.8		5.7	6	5.7	5.6

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Analysis ID:	01-3982-0816	**Endpoint:**	Hatching Success	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:02	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed hatching success	13.31%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.7071	2.447	0.115	6	CDF	0.5060	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0022222	0.0022222	1	0.5	0.5060	Non-Significant Effect
Error	0.0266667	0.0044444	6			
Total	0.0288889		7			

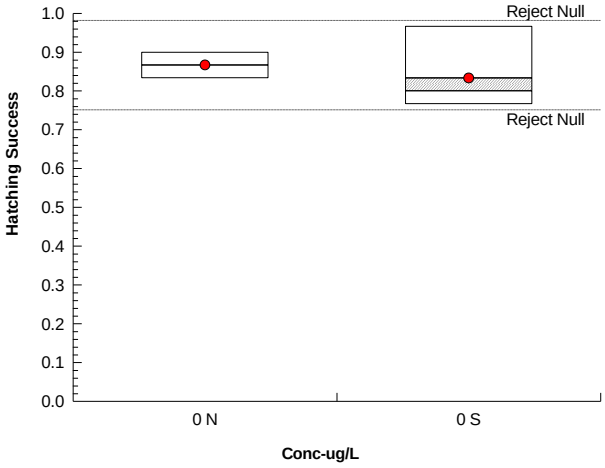
ANOVA Assumptions Tests

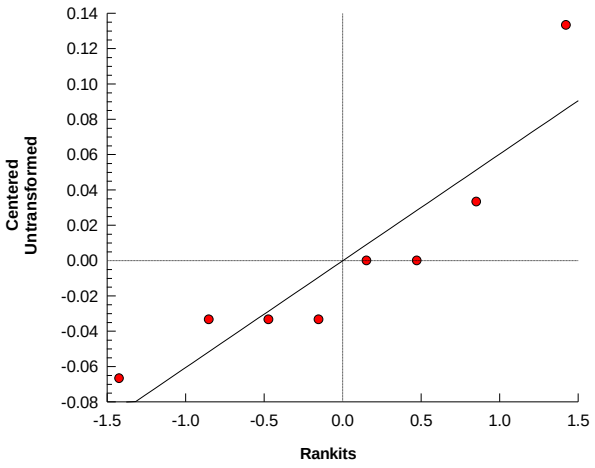
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	11	47.47	0.0796	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.8417	0.6451	0.0784	Normal Distribution

Hatching Success Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	0.8333	0.6897	0.9770	0.8000	0.7667	0.9667	0.0451	10.83%	0.00%
0	N	4	0.8667	0.8234	0.9100	0.8667	0.8333	0.9000	0.0136	3.14%	-4.00%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Smithers Viscient**

Analysis ID:	08-1703-3300	**Endpoint:**	Hatching Success	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:06	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	2.8	>2.8	n/a		9.76%

Dunnett Multiple Comparison Test

Control vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.18	0.7115	2.407	0.085	6	CDF	0.5527	Non-Significant Effect
	0.34	0.4743	2.407	0.085	6	CDF	0.6586	Non-Significant Effect
	0.7	-0.2372	2.407	0.085	6	CDF	0.8937	Non-Significant Effect
	1.3	-0.2372	2.407	0.085	6	CDF	0.8937	Non-Significant Effect
	2.8	0.2372	2.407	0.085	6	CDF	0.7542	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0037037	0.0007407	5	0.3	0.9065	Non-Significant Effect
Error	0.0444444	0.0024691	18			
Total	0.0481481		23			

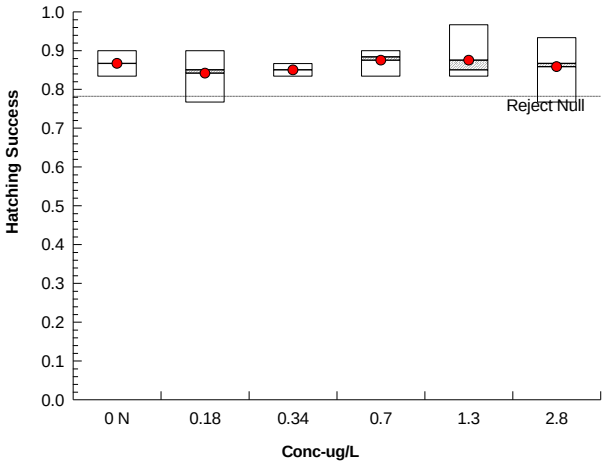
ANOVA Assumptions Tests

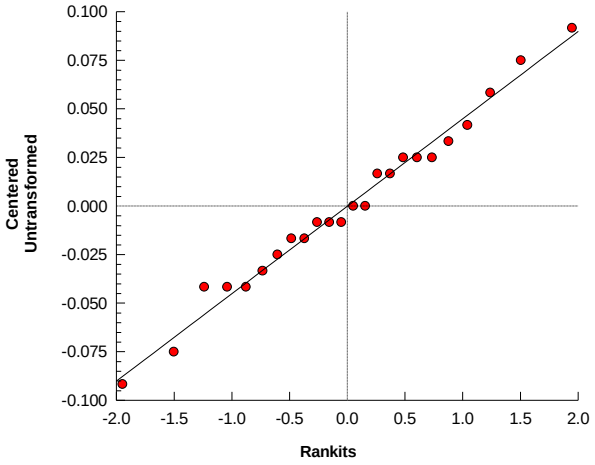
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.487	15.09	0.2616	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9861	0.884	0.9774	Normal Distribution

Hatching Success Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.8667	0.8234	0.9100	0.8667	0.8333	0.9000	0.0136	3.14%	0.00%
0.18		4	0.8417	0.7511	0.9323	0.8500	0.7667	0.9000	0.0285	6.76%	2.88%
0.34		4	0.8500	0.8194	0.8806	0.8500	0.8333	0.8667	0.0096	2.26%	1.92%
0.7		4	0.8750	0.8242	0.9258	0.8833	0.8333	0.9000	0.0160	3.65%	-0.96%
1.3		4	0.8750	0.7746	0.9754	0.8500	0.8333	0.9667	0.0316	7.21%	-0.96%
2.8		4	0.8583	0.7407	0.9759	0.8667	0.7667	0.9333	0.0370	8.61%	0.96%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Smithers Viscient**

Analysis ID:	09-0252-4195	**Endpoint:**	Hatching Success	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:06	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	2.8	>2.8	n/a		7.57%

Williams Multiple Comparison Test

Control	vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.18	0.7115	1.734	0.061	6	CDF	>0.05	Non-Significant Effect
		0.34	0.5929	1.818	0.064	6	CDF	>0.05	Non-Significant Effect
		0.7	0.3162	1.845	0.065	6	CDF	>0.05	Non-Significant Effect
		1.3	0.1779	1.859	0.065	6	CDF	>0.05	Non-Significant Effect
		2.8	0.2372	1.867	0.066	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0037037	0.0007407	5	0.3	0.9065	Non-Significant Effect
Error	0.0444444	0.0024691	18			
Total	0.0481481		23			

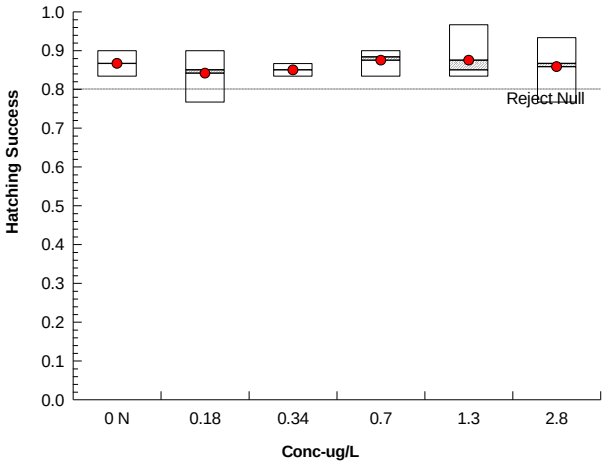
ANOVA Assumptions Tests

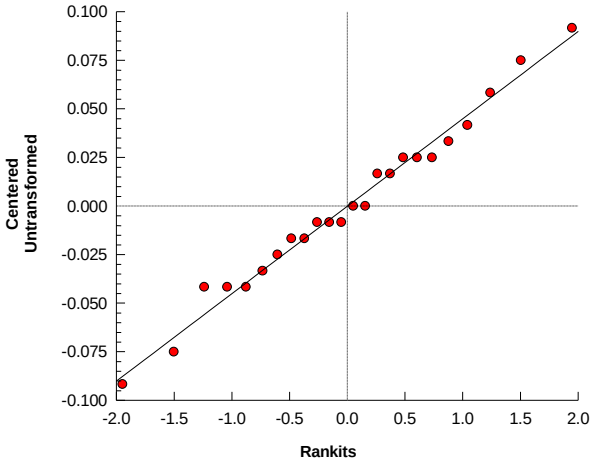
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.487	15.09	0.2616	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9861	0.884	0.9774	Normal Distribution

Hatching Success Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.8667	0.8234	0.9100	0.8667	0.8333	0.9000	0.0136	3.14%	0.00%
0.18		4	0.8417	0.7511	0.9323	0.8500	0.7667	0.9000	0.0285	6.76%	2.88%
0.34		4	0.8500	0.8194	0.8806	0.8500	0.8333	0.8667	0.0096	2.26%	1.92%
0.7		4	0.8750	0.8242	0.9258	0.8833	0.8333	0.9000	0.0160	3.65%	-0.96%
1.3		4	0.8750	0.7746	0.9754	0.8500	0.8333	0.9667	0.0316	7.21%	-0.96%
2.8		4	0.8583	0.7407	0.9759	0.8667	0.7667	0.9333	0.0370	8.61%	0.96%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Smithers Viscient**

Analysis ID:	03-3423-2187	**Endpoint:**	Mean Dry Weight	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:02	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed mean dry weight	11.09%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.1803	2.447	0.002	6	CDF	0.8629	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.125E-08	6.125E-08	1	0.0325	0.8629	Non-Significant Effect
Error	1.131E-05	1.885E-06	6			
Total	1.137E-05		7			

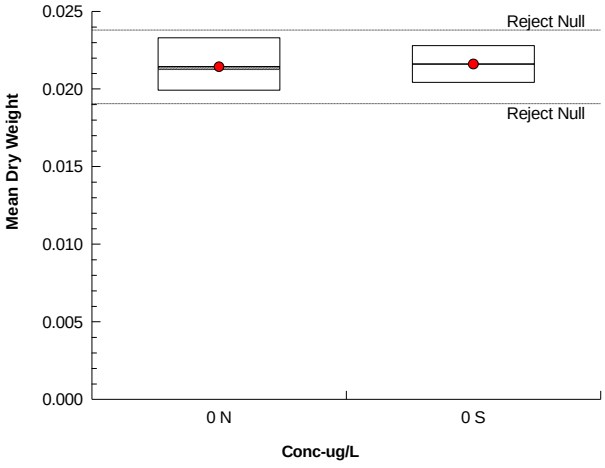
ANOVA Assumptions Tests

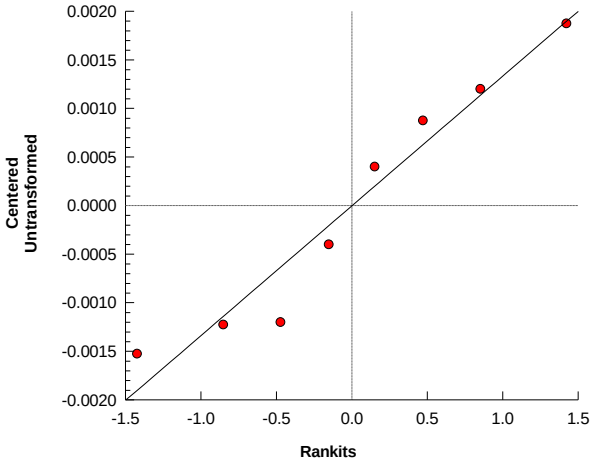
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	2.534	47.47	0.4653	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9242	0.6451	0.4652	Normal Distribution

Mean Dry Weight Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	0.0216	0.01996	0.02324	0.0216	0.0204	0.0228	0.000516	4.78%	0.00%
0	N	4	0.02142	0.01881	0.02404	0.02125	0.0199	0.0233	0.000822	7.67%	0.81%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Analysis ID:	06-3601-9418	**Endpoint:**	Mean Dry Weight	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:06	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	2.8	>2.8	n/a		13.64%

Dunnett Multiple Comparison Test

Control vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.18	-0.7208	2.407	0.003	6	CDF	0.9643	Non-Significant Effect
	0.34	-0.2471	2.407	0.003	6	CDF	0.8958	Non-Significant Effect
	0.7	-0.4737	2.407	0.003	6	CDF	0.9359	Non-Significant Effect
	1.3	-0.3707	2.407	0.003	6	CDF	0.9195	Non-Significant Effect
	2.8	-1.112	2.407	0.003	6	CDF	0.9873	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.443E-06	8.887E-07	5	0.3015	0.9056	Non-Significant Effect
Error	5.305E-05	2.947E-06	18			
Total	5.749E-05		23			

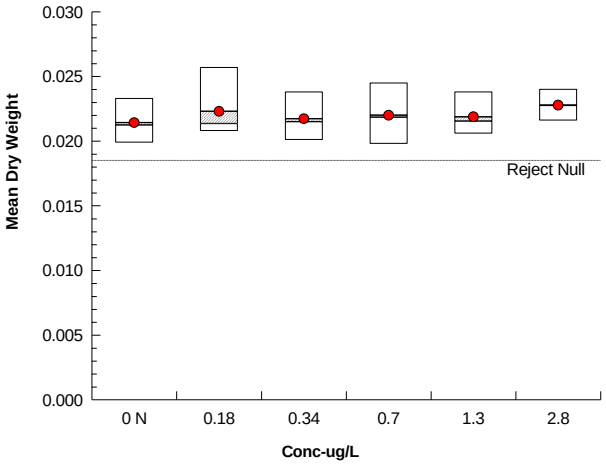
ANOVA Assumptions Tests

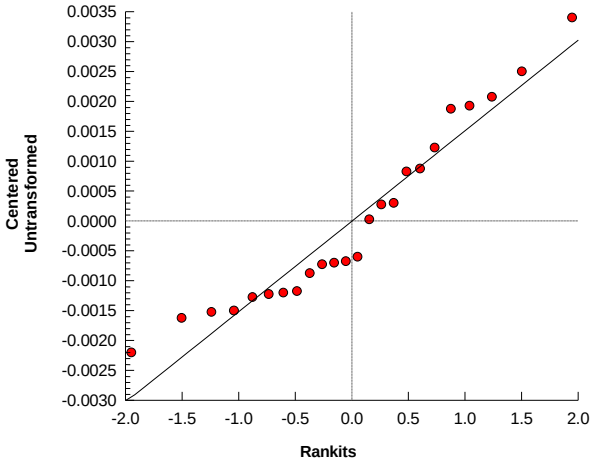
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	1.434	15.09	0.9205	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9274	0.884	0.0851	Normal Distribution

Mean Dry Weight Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.02142	0.01881	0.02404	0.02125	0.0199	0.0233	0.000822	7.67%	0.00%
0.18		4	0.0223	0.01866	0.02594	0.02135	0.0208	0.0257	0.001145	10.27%	-4.08%
0.34		4	0.02172	0.0192	0.02425	0.0215	0.0201	0.0238	0.000793	7.30%	-1.40%
0.7		4	0.022	0.01888	0.02512	0.02185	0.0198	0.0245	0.000981	8.91%	-2.68%
1.3		4	0.02188	0.01966	0.02409	0.02155	0.0206	0.0238	0.000695	6.35%	-2.10%
2.8		4	0.02278	0.02086	0.02469	0.02275	0.0216	0.024	0.000601	5.27%	-6.30%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Analysis ID:	21-3229-5165	**Endpoint:**	Mean Dry Weight	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:06	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	2.8	>2.8	n/a		10.58%

Williams Multiple Comparison Test

Control vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.18	-0.7208	1.734	0.002	6	CDF	>0.05	Non-Significant Effect
	0.34	-0.2471	1.818	0.002	6	CDF	>0.05	Non-Significant Effect
	0.7	-0.3604	1.845	0.002	6	CDF	>0.05	Non-Significant Effect
	1.3	-0.3638	1.859	0.002	6	CDF	>0.05	Non-Significant Effect
	2.8	-0.5509	1.867	0.002	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.443E-06	8.887E-07	5	0.3015	0.9056	Non-Significant Effect
Error	5.305E-05	2.947E-06	18			
Total	5.749E-05		23			

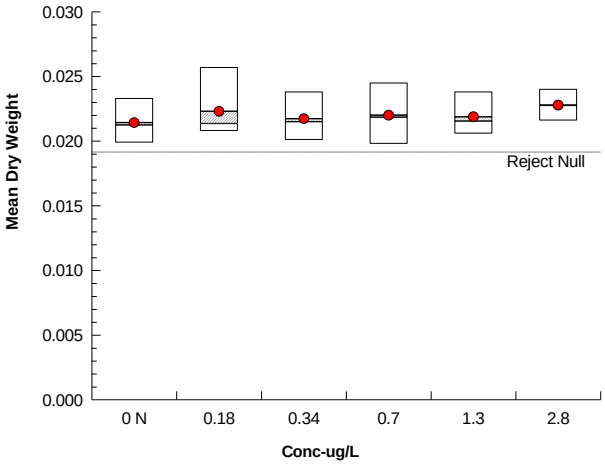
ANOVA Assumptions Tests

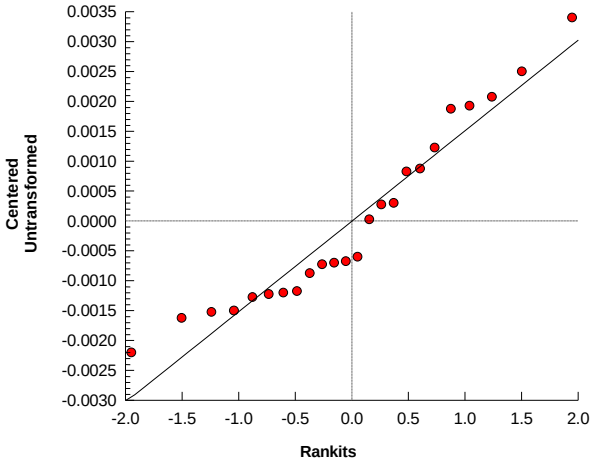
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	1.434	15.09	0.9205	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9274	0.884	0.0851	Normal Distribution

Mean Dry Weight Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.02142	0.01881	0.02404	0.02125	0.0199	0.0233	0.000822	7.67%	0.00%
0.18		4	0.0223	0.01866	0.02594	0.02135	0.0208	0.0257	0.001145	10.27%	-4.08%
0.34		4	0.02172	0.0192	0.02425	0.0215	0.0201	0.0238	0.000793	7.30%	-1.40%
0.7		4	0.022	0.01888	0.02512	0.02185	0.0198	0.0245	0.000981	8.91%	-2.68%
1.3		4	0.02188	0.01966	0.02409	0.02155	0.0206	0.0238	0.000695	6.35%	-2.10%
2.8		4	0.02278	0.02086	0.02469	0.02275	0.0216	0.024	0.000601	5.27%	-6.30%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Smithers Viscient**

Analysis ID:	12-3085-3572	**Endpoint:**	Mean Length	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:02	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed mean length	2.42%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.1478	2.447	0.455	6	CDF	0.8874	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0015125	0.0015125	1	0.02183	0.8874	Non-Significant Effect
Error	0.415675	0.0692792	6			
Total	0.417188		7			

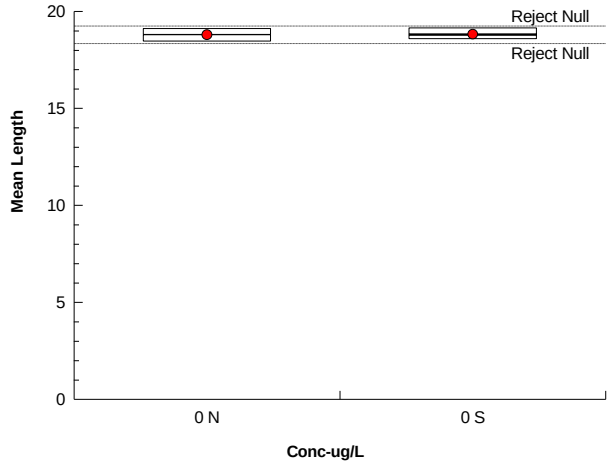
ANOVA Assumptions Tests

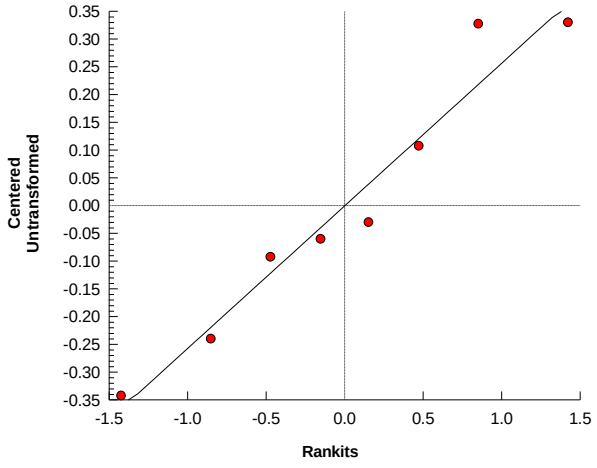
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	1.431	47.47	0.7755	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9341	0.6451	0.5541	Normal Distribution

Mean Length Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	18.82	18.44	19.2	18.78	18.58	19.15	0.1194	1.27%	0.00%
0	N	4	18.79	18.34	19.25	18.8	18.45	19.12	0.1428	1.52%	0.15%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Analysis ID:	05-3177-2605	**Endpoint:**	Mean Length	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:06	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	2.8	>2.8	n/a		2.99%

Dunnett Multiple Comparison Test

Control vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.18	-1.072	2.407	0.561	6	CDF	0.9858	Non-Significant Effect
	0.34	0.268	2.407	0.561	6	CDF	0.7426	Non-Significant Effect
	0.7	-0.5038	2.407	0.561	6	CDF	0.9401	Non-Significant Effect
	1.3	-0.6003	2.407	0.561	6	CDF	0.9522	Non-Significant Effect
	2.8	-0.9112	2.407	0.561	6	CDF	0.9781	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.291671	0.0583342	5	0.5363	0.7462	Non-Significant Effect
Error	1.95792	0.108774	18			
Total	2.2496		23			

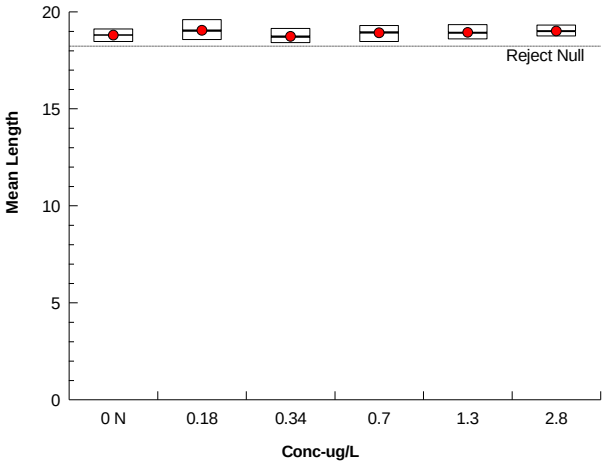
ANOVA Assumptions Tests

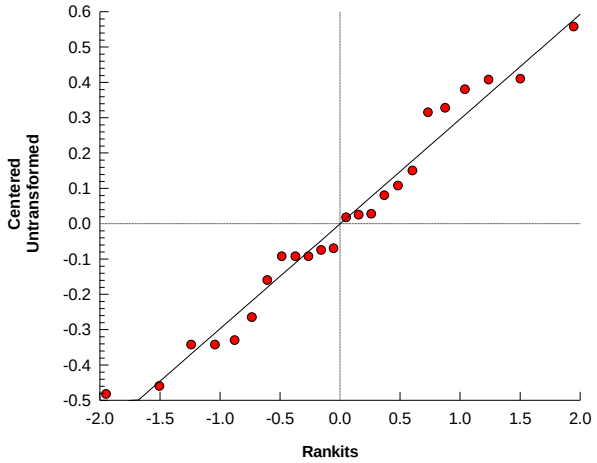
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	1.016	15.09	0.9612	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9622	0.884	0.4847	Normal Distribution

Mean Length Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	18.79	18.34	19.25	18.8	18.45	19.12	0.1428	1.52%	0.00%
0.18		4	19.04	18.36	19.73	19.01	18.56	19.6	0.2146	2.25%	-1.33%
0.34		4	18.73	18.22	19.24	18.69	18.4	19.14	0.1605	1.71%	0.33%
0.7		4	18.91	18.34	19.48	18.95	18.45	19.29	0.1787	1.89%	-0.63%
1.3		4	18.93	18.44	19.43	18.9	18.59	19.34	0.1562	1.65%	-0.74%
2.8		4	19.01	18.62	19.39	18.98	18.74	19.32	0.121	1.27%	-1.13%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Smithers Viscient**

Analysis ID:	19-9353-2948	**Endpoint:**	Mean Length	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:06	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	2.8	>2.8	n/a		2.32%

Williams Multiple Comparison Test

Control	vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.18	-1.072	1.734	0.404	6	CDF	>0.05	Non-Significant Effect
		0.34	0.268	1.818	0.424	6	CDF	>0.05	Non-Significant Effect
		0.7	-0.1179	1.845	0.430	6	CDF	>0.05	Non-Significant Effect
		1.3	-0.2787	1.859	0.434	6	CDF	>0.05	Non-Significant Effect
		2.8	-0.4368	1.867	0.435	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.291671	0.0583342	5	0.5363	0.7462	Non-Significant Effect
Error	1.95792	0.108774	18			
Total	2.2496		23			

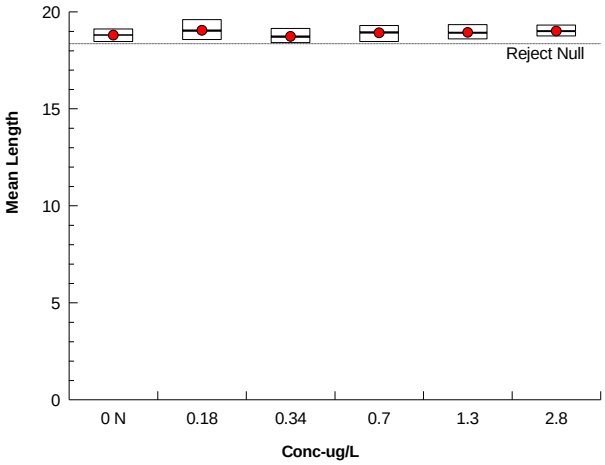
ANOVA Assumptions Tests

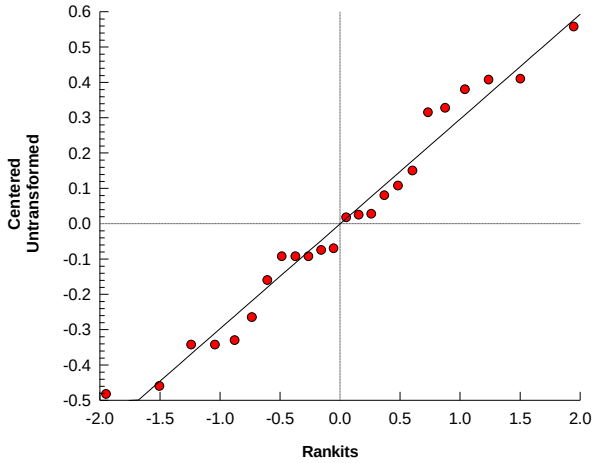
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	1.016	15.09	0.9612	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9622	0.884	0.4847	Normal Distribution

Mean Length Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	18.79	18.34	19.25	18.8	18.45	19.12	0.1428	1.52%	0.00%
0.18		4	19.04	18.36	19.73	19.01	18.56	19.6	0.2146	2.25%	-1.33%
0.34		4	18.73	18.22	19.24	18.69	18.4	19.14	0.1605	1.71%	0.33%
0.7		4	18.91	18.34	19.48	18.95	18.45	19.29	0.1787	1.89%	-0.63%
1.3		4	18.93	18.44	19.43	18.9	18.59	19.34	0.1562	1.65%	-0.74%
2.8		4	19.01	18.62	19.39	18.98	18.74	19.32	0.121	1.27%	-1.13%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Smithers Viscient**

Analysis ID:	21-2742-0753	**Endpoint:**	Post Hatch Survival	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:02	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed post hatch survival	6.86%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.9258	2.447	0.066	6	CDF	0.3903	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.00125	0.00125	1	0.8571	0.3903	Non-Significant Effect
Error	0.00875	0.0014583	6			
Total	0.01		7			

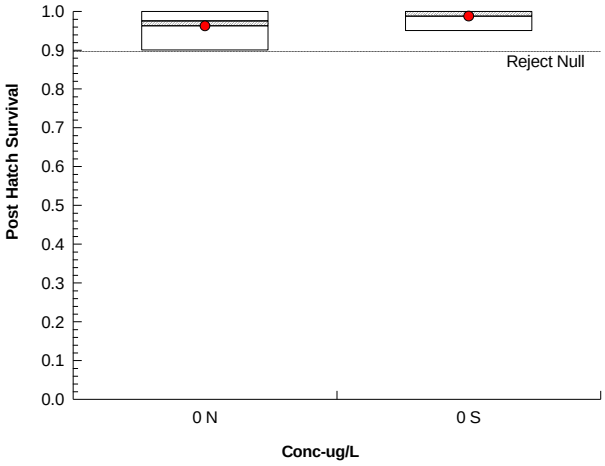
ANOVA Assumptions Tests

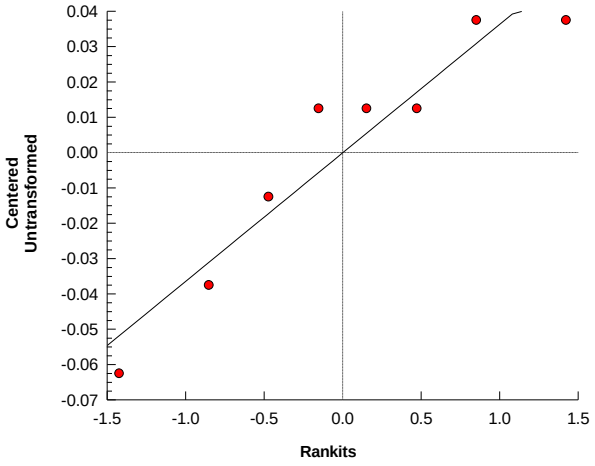
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	3.667	47.47	0.3142	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.8973	0.6451	0.2730	Normal Distribution

Post Hatch Survival Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	0.9875	0.9477	1.0000	1.0000	0.9500	1.0000	0.0125	2.53%	0.00%
0	N	4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	2.53%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Analysis ID:	08-3750-0390	**Endpoint:**	Post Hatch Survival	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:06	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	2.8	>2.8	n/a		9.83%

Dunnett Multiple Comparison Test

Control vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.18	1.59	2.407	0.095	6	CDF	0.2003	Non-Significant Effect
	0.34	0	2.407	0.095	6	CDF	0.8333	Non-Significant Effect
	0.7	0.636	2.407	0.095	6	CDF	0.5871	Non-Significant Effect
	1.3	-0.318	2.407	0.095	6	CDF	0.9100	Non-Significant Effect
	2.8	-0.318	2.407	0.095	6	CDF	0.9100	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0167708	0.0033542	5	1.085	0.4016	Non-Significant Effect
Error	0.055625	0.0030903	18			
Total	0.0723958		23			

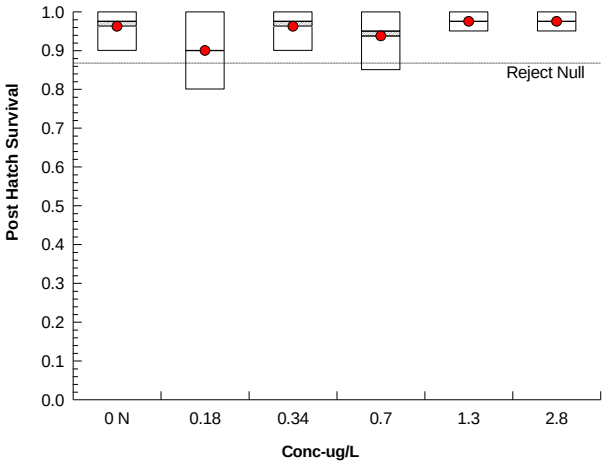
ANOVA Assumptions Tests

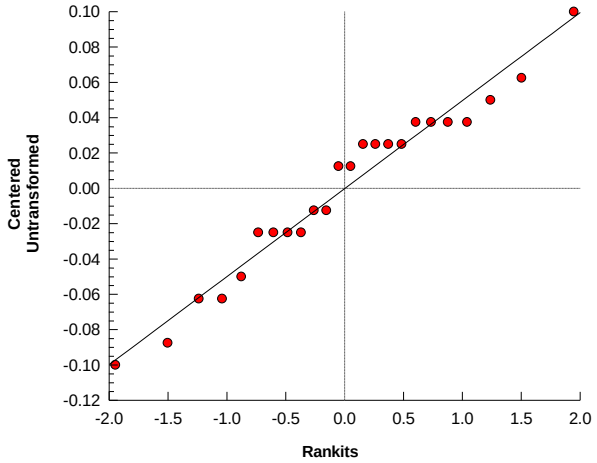
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.257	15.09	0.3853	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9646	0.884	0.5374	Normal Distribution

Post Hatch Survival Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	0.00%
0.18		4	0.9000	0.7547	1.0000	0.9000	0.8000	1.0000	0.0456	10.14%	6.49%
0.34		4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	0.00%
0.7		4	0.9375	0.8374	1.0000	0.9500	0.8500	1.0000	0.0315	6.71%	2.60%
1.3		4	0.9750	0.9291	1.0000	0.9750	0.9500	1.0000	0.0144	2.96%	-1.30%
2.8		4	0.9750	0.9291	1.0000	0.9750	0.9500	1.0000	0.0144	2.96%	-1.30%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Smithers Viscient**

Analysis ID:	00-4043-5795	**Endpoint:**	Post Hatch Survival	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:06	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	2.8	>2.8	n/a		7.62%

Williams Multiple Comparison Test

Control	vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.18	1.59	1.734	0.068	6	CDF	>0.05	Non-Significant Effect
		0.34	0.795	1.818	0.071	6	CDF	>0.05	Non-Significant Effect
		0.7	0.742	1.845	0.073	6	CDF	>0.05	Non-Significant Effect
		1.3	0.477	1.859	0.073	6	CDF	>0.05	Non-Significant Effect
		2.8	0.318	1.867	0.073	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0167708	0.0033542	5	1.085	0.4016	Non-Significant Effect
Error	0.055625	0.0030903	18			
Total	0.0723958		23			

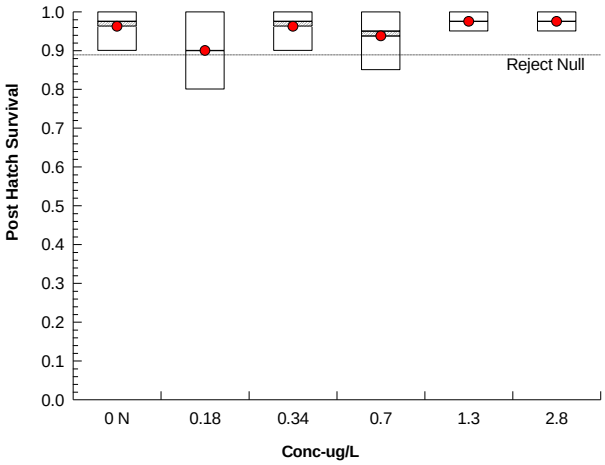
ANOVA Assumptions Tests

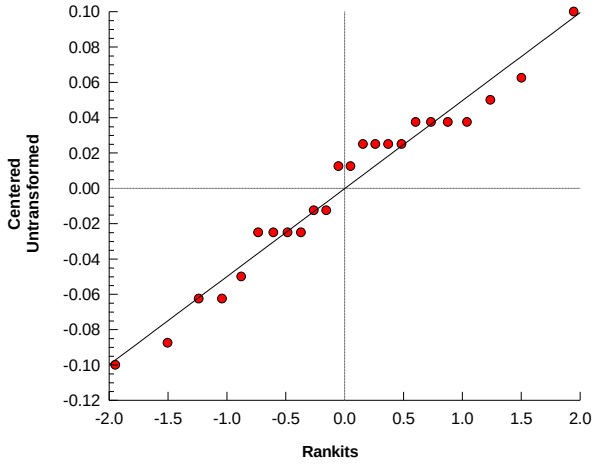
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.257	15.09	0.3853	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9646	0.884	0.5374	Normal Distribution

Post Hatch Survival Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	0.00%
0.18		4	0.9000	0.7547	1.0000	0.9000	0.8000	1.0000	0.0456	10.14%	6.49%
0.34		4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	0.00%
0.7		4	0.9375	0.8374	1.0000	0.9500	0.8500	1.0000	0.0315	6.71%	2.60%
1.3		4	0.9750	0.9291	1.0000	0.9750	0.9500	1.0000	0.0144	2.96%	-1.30%
2.8		4	0.9750	0.9291	1.0000	0.9750	0.9500	1.0000	0.0144	2.96%	-1.30%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Smithers Viscient**

Analysis ID:	01-7435-6699	**Endpoint:**	Time to Hatch	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:02	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed time to hatch	5.77%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	1.868	2.447	0.328	6	CDF	0.1110	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.125	0.125	1	3.488	0.1110	Non-Significant Effect
Error	0.215	0.0358333	6			
Total	0.34		7			

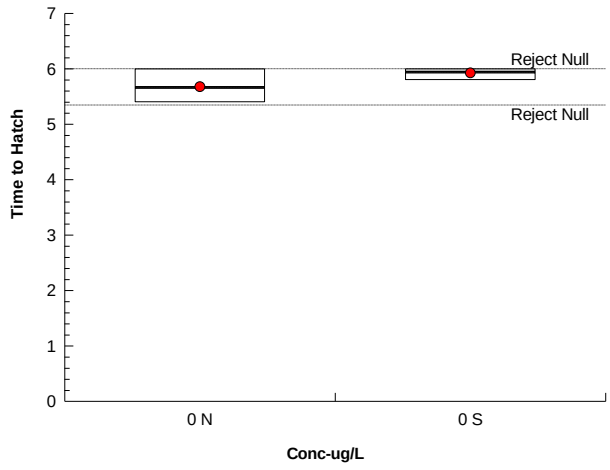
ANOVA Assumptions Tests

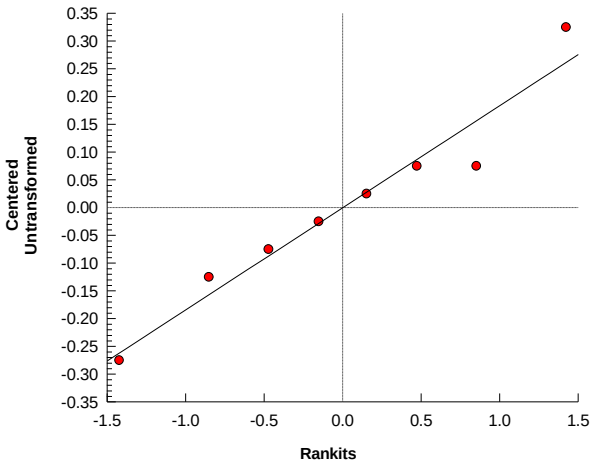
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	6.818	47.47	0.1492	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9645	0.6451	0.8521	Normal Distribution

Time to Hatch Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	5.925	5.773	6.077	5.95	5.8	6	0.04787	1.62%	0.00%
0	N	4	5.675	5.277	6.073	5.65	5.4	6	0.125	4.41%	4.22%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Analysis ID:	08-6444-2776	**Endpoint:**	Time to Hatch	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:07	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	2.8	>2.8	n/a		5.71%

Dunnett Multiple Comparison Test

Control vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.18	1.486	2.407	0.324	6	CDF	0.2326	Non-Significant Effect
	0.34	-0.1857	2.407	0.324	6	CDF	0.8822	Non-Significant Effect
	0.7	0.3714	2.407	0.324	6	CDF	0.7018	Non-Significant Effect
	1.3	0.5571	2.407	0.324	6	CDF	0.6225	Non-Significant Effect
	2.8	0.5571	2.407	0.324	6	CDF	0.6225	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.12375	0.02475	5	0.6828	0.6424	Non-Significant Effect
Error	0.6525	0.03625	18			
Total	0.77625		23			

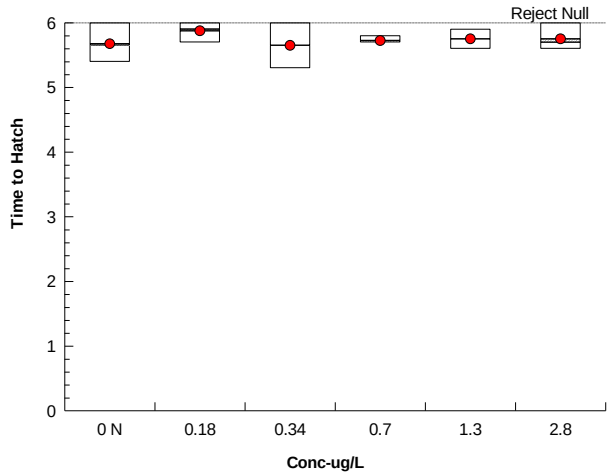
ANOVA Assumptions Tests

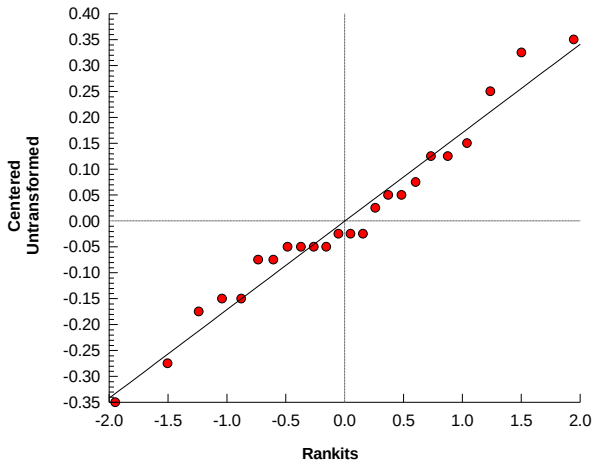
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	7.277	15.09	0.2008	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9681	0.884	0.6195	Normal Distribution

Time to Hatch Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	5.675	5.277	6.073	5.65	5.4	6	0.125	4.41%	0.00%
0.18		4	5.875	5.636	6.114	5.9	5.7	6	0.075	2.55%	-3.52%
0.34		4	5.65	5.191	6.109	5.65	5.3	6	0.1443	5.11%	0.44%
0.7		4	5.725	5.645	5.805	5.7	5.7	5.8	0.025	0.87%	-0.88%
1.3		4	5.75	5.545	5.955	5.75	5.6	5.9	0.06455	2.25%	-1.32%
2.8		4	5.75	5.474	6.026	5.7	5.6	6	0.0866	3.01%	-1.32%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Smithers Viscient**

Analysis ID:	06-1068-0564	**Endpoint:**	Time to Hatch	**CETIS Version:**	CETISv1.9.5
Analyzed:	09 Feb-19 19:07	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	07-7250-9000	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	22 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Filtered seawater
Ending Date:	26 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	34d 0h	**Taxon:**	Actinopterygii	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	2.8	>2.8	n/a		4.43%

Williams Multiple Comparison Test

Control vs	Conc-ug/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.18	1.486	1.734	0.233	6	CDF	>0.05	Non-Significant Effect
	0.34	-0.1857	1.818	0.245	6	CDF	>0.05	Non-Significant Effect
	0.7	0.09285	1.845	0.248	6	CDF	>0.05	Non-Significant Effect
	1.3	0.2476	1.859	0.250	6	CDF	>0.05	Non-Significant Effect
	2.8	0.325	1.867	0.251	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.12375	0.02475	5	0.6828	0.6424	Non-Significant Effect
Error	0.6525	0.03625	18			
Total	0.77625		23			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	7.277	15.09	0.2008	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9681	0.884	0.6195	Normal Distribution

Time to Hatch Summary

Conc-ug/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	5.675	5.277	6.073	5.65	5.4	6	0.125	4.41%	0.00%
0.18		4	5.875	5.636	6.114	5.9	5.7	6	0.075	2.55%	-3.52%
0.34		4	5.65	5.191	6.109	5.65	5.3	6	0.1443	5.11%	0.44%
0.7		4	5.725	5.645	5.805	5.7	5.7	5.8	0.025	0.87%	-0.88%
1.3		4	5.75	5.545	5.955	5.75	5.6	5.9	0.06455	2.25%	-1.32%
2.8		4	5.75	5.474	6.026	5.7	5.6	6	0.0866	3.01%	-1.32%

Graphics