Data Evaluation Report on the Chronic Toxicity of CPU (Novaluron degradate) to Freshwater Invertebrates - Daphnia sp.

PMRA Submission Number {.....} EPA MRID No. 50610204

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	447937
	OECD Data Point	{.....}
	EPA MRID	50610204
	EPA Guideline	850.1300

Test material: **CPU (Novaluron degradate)** **Purity (%):** 86.9%
Common name: Not reported
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: 1-[-3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]urea

Primary Reviewer: Elizabeth Krupka **Signature:**
Environmental Scientist, CDM/CSS-Dynamac JV **Date:** 12/28/2018

Secondary Reviewer: Moncie V. Wright, Ph.D. **Signature:**
Environmental Scientist, CDM/CSS-Dynamac JV **Date:** 2/8/2019

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

EPA PC Code 124002

CITATION: Shaw, A.C. 2018. CPU – Full Life-Cycle Toxicity Test with Water Fleas, Daphnia magna, Under Static-Renewal Conditions. Unpublished study performed by Smithers Viscient, Wareham, Massachusetts. Laboratory Study No. 14125.6118; Sponsor Protocol/Project No. R-38343. Study sponsored by ADAMA Makhteshim Ltd., Beer-Sheva, Israel. Study completed April 5, 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 21-day-chronic toxicity of CPU (Novaluron degradate) to *Daphnia magna* was studied under static-renewal conditions. Daphnids were exposed to nominal concentrations of 0 (negative control), 0.038, 0.075, 0.15, 0.30, and 0.60 mg ai/L. The mean-measured concentrations were <0.0072 (<MDL, control), 0.045, 0.092, 0.17, 0.34, and 0.69 mg ai/L.

Reproduction (number of live offspring and successful birth rate) and growth (length and dry weight) were significantly affected in the experiment. Reproduction and length was the most sensitive endpoints, resulting in an overall NOAEC and LOAEC of 0.17 and 0.34 mg ai/L, respectively.

This study is scientifically sound and is classified as acceptable.

Results Synopsis

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

NOAEC: 0.17 mg ai/L
LOAEC: 0.34 mg ai/L

Endpoints affected: Reproduction (number of live offspring and successful birth rate) and growth (length and dry weight)
Most sensitive endpoints: Reproduction (number of live offspring and successful birth rate) and Length

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based upon procedures outlined in OECD Guideline No. 211, Daphnia magna Reproduction Test (2008); U.S. EPA OCSPP Guideline No. 850.1300, Daphnid Chronic Toxicity Test; and U.S. EPA OCSPP No. 850.1000, Special Considerations for Conducting Aquatic Laboratory Studies (1996). The following deviations from OCSPP 850.1300 were noted:

1. The physicochemical properties of the test substance were not reported.

2. Dilution water parameters including particulate matter, total organic carbon, ammonia, chlorine, and pesticide concentrations were not reported.

3. The dissolved oxygen concentration range of 90 – 130 percent saturation fell outside of the recommended range of 60 – 105 percent saturation.

4. The pH in the spent solutions ranged from 7.9 - 9.0. OCSPP guidance recommends a maximum pH of 8.5.

These deviations do not impact the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. This study was conducted in accordance with U.S. EPA GLP Standards as published in 40 CFR, Part 160 with the following exception: routine water and food contaminant screening analyses. It was reported that since the analyses were conducted following

standard validated methods (U.S. EPA), this exception had no impact on the study results.

A. MATERIALS:

1. Test Material CPU (Novaluron degradate)

Description: Not reported

Lot No./Batch No. : 554-187-04 (Lot No.)

Purity: 86.9%

Stability of compound under test conditions: The test substance was stable under test conditions. The mean measured concentrations were 110 to 120% of nominal concentrations. The coefficients of variation ranged from 8 to 10%. *(OECD recommends stability in water and light)*

Physicochemical properties of CPU (Novaluron degradate).

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

Storage conditions of test chemicals: Room temperature in a dark, ventilated cabinet in the original container

2. Test Organism:

Species: *Daphnia magna*
EPA and OECD recommend Daphnia magna.. EPA also allows the use of D. pulex.

Age at test initiation: <24 hours old at exposure initiation

EPA recommends that daphnids are in their first instar (≤24 hrs old)

Source: Smithers Viscient culture
EPA recommends all test organisms be from the same laboratory-reared culture.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A 15-day preliminary range-finding test was conducted at Smithers Viscient under static-renewal conditions. The test was conducted at nominal concentrations of

0.016, 0.054, 0.18, 0.60 and 2.0 mg/L using daphnids <24 hours old, with five replicate test exposure vessels per test concentration and one daphnid per replicate. The 18-day preliminary study resulted in a lack of mortality at all concentrations tested.

Based on the results of preliminary testing, nominal concentrations of 0.038, 0.075, 0.15, 0.30 and 0.60 mg/L and a control were selected for the definitive exposure.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period:	Continuously cultured; sexually mature daphnids isolated for 24 hours prior to test initiation	*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 mg 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 rate is 15 mg food (dry weight) per liter of dilution water or test solution].* *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.*
Conditions: (same as test or not)	Similar to test conditions (fortified laboratory well water, pH 7.7-8.3, 16L:8D photoperiod, temperature 21-22°C, dissolved oxygen 6.7-9.1 mg/L)	
Feeding:	Unicellular green algae, *Ankistrodesmus falcatus* (4×10^7 cells/mL) a suspension of YCT (yeast, cereal leaves, and flaked fish food)	
Health (any mortality observed):	Mortality was <20% days prior to initiation, no ephippia were observed, offspring were released in culture prior to day 12, offspring were not used in a previous test or first brood progeny, and parent daphnids produced ≥3 offspring/adult in the 7 days prior to test initiation.	
Test condition:		*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*
static renewal/flow-through:	Static-renewal	
Type of dilution system- for flow through method.	N/A	
Renewal rate for static renewal	Every 48 or 72 hours	

Parameter	Details	Remarks *Criteria*
		days.
Aeration, if any	None	*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 beakers 100 mL 80 mL	*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: Total organophosphorus pesticides: Total organochlorine pesticides + PCBs: Organic chlorine: Hardness as $CaCO_3$: Specific conductivity: pH:	Laboratory well water Not reported 1.0-1.3 mg/L (October-November 2018) Not reported Not reported Not reported Not reported Not reported 170 – 180 mg/L 710 to 830 µS/cm 7.3 to 8.2	Representative samples of the dilution water source were analyzed periodically for the presence of pesticides, PCBs, and toxic metals by Eurofins Lancaster Laboratories Environmental, Lancaster, Pennsylvania. None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed. *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*

Parameter	Details	Remarks *Criteria*
		signs of stress. Maximum allowable concentrations for water quality parameters are provided in EPA's 850.1300 guideline (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:	 Fresh: 7.7 - 8.2 Spent: 7.9 - 9.0 90 – 130% Saturation 19 - 22°C 16 hours light and 8 hours dark with 15- to 30-minute transition periods At test initiation and weekly thereafter	Light intensity was 16 to 20 µE m-2 s-1 measured with a Licor Model LI-250A photometer 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:	 10 N/A 10	 *Static-renewal: 10 or more replicates of one daphnid each.* *Flow-through: four replicates of equal number of daphnids.*
Number of organisms per replicate: For growth and reproduction: For survival test:	 1 1	 *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:		*Treatment concentrations should include*

Parameter	Details	Remarks *Criteria*
nominal: measured:	0 (negative control), 0.038, 0.075, 0.15, 0.30, and 0.60 mg ai/L <0.0072 (<MDL, control), 0.045, 0.092, 0.17, 0.34, and 0.69 mg ai/L	*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.*
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	 0.0001 mg ai/L 0.0072 mg ai/L (MDL)	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured	- Parental immobility (mortality)	

Parameters	Details	Remarks *Criteria*
including the sublethal effects/toxicity symptoms	- Other parental sub-lethal effects - Time of first brood release - Offspring production - Survival of first-generation daphnids - Terminal length and dry weight of surviving P-generation daphnia	*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 for survival and sublethal effects. Offspring were removed, counted, and discarded at the first renewal interval after the first observation of brood release in any exposure vessel and daily throughout the remainder of the test. 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	
Other observations, if any	None	

II. RESULTS AND DISCUSSION

A. MORTALITY AND SUB-LETHAL EFFECTS:

Survival was 100% in the negative control, as compared to survival ranging from 80 to 100% in the groups exposed to the test material.

Mean total body length averaged 4.84 in the negative control, and ranged from 2.77 to 4.79 mm in the treatment groups.

Mean total dry weight averaged 1.14 mg in the negative control, as compared to weight in the treatment groups ranging from 0.23 to 1.16 mg.

Table 3: Effect of CPU (Novaluron degradate) on Growth, Reproduction, and Survival of Daphnia sp.[a]

Mean Measured (and Nominal) Treatment Concentrations (mg ai/L)	Adult Survival		Time to First Brood (Days)	Mean Total No. of Offspring per Surviving Female ± SD (Day 21)	Mean Total No. of Offspring per Female per Reproductive	Mean Total Length of Surviving Adults ± SD (mm)	Mean Dry Weight of Surviving Adults ± SD (mg)
	No.	%					

					Day ± SD (Day 21)		
Negative Control (<MDL)[b]	10	100	8.1	158 ± 14	11 ± 0.94	4.84 ± 0.094	1.14 ± 0.10
0.045 (0.038)	10	100	8.2	159 ± 28	11 ± 1.9	4.77 ± 0.14	1.16 ± 0.085
0.092 (0.075)	8	80	8.7	166 ± 17	10 ± 1.7	4.74 ± 0.15	1.09 ± 0.13
0.17 (0.15)	9	90	8.0	157 ± 6.4	9.4 ± 3.3	4.79 ± 0.085	1.15 ± 0.044
0.34 (0.30)	10	100	8.0	146 ± 8.7	9.7 ± 0.58*	4.54 ± 0.11**	1.07 ± 0.077
0.69 (0.60)	8	80	N/A	0***	0*	2.77 ± 0.15**	0.23 ± 0.077**
NOAEC, mg ai/L	0.69		N/A	0.34	0.17	0.17	0.34
LOAEC, mg ai/L	>0.69			0.69	0.34	0.34	0.69
EC_{50} (95% CI), mg ai/L	>0.69			0.49 (0.47 - 0.49)	0.49 (0.45 - 0.49)	>0.69	0.54 (0.52 - 0.55)

[a] Data reported in Table 12 on page 45 of the study report.
[b] MDL = <0.0072 mg ai/L
*Significant reduction compared to the control, based on Wilcoxon's Test with Bonferroni-Holm's Adjustment.
**Significant reduction compared to the control, based on Dunnett's Multiple Comparison Test.
***Significant reduction compared to the control, based on Dunnett's T3 Multiple Comparison Test.

B. EFFECT ON REPRODUCTION: The reported first brood release occurred on Days 8-9 across the control and all exposure groups except the highest. No brood release occurred in the 0.69 mg ai/L treatment level.

The mean number of offspring per surviving female averaged 159, 166, 157, 146, and 0 for the mean-measured 0.045, 0.092, 0.17, 0.34, and 0.69 mg ai/L treatment levels, respectively, compared to 158 in the negative control.

The mean number of offspring per female per reproductive day averaged 11, 10, 9.4, 9.7, and 0 for the mean-measured 0.045, 0.092, 0.17, 0.34, and 0.69 mg ai/L treatment levels, respectively, compared to 11 in the negative control.

C. REPORTED STATISTICS: Data that were statistically analyzed included organism survival (i.e., immobilization), reproduction (cumulative number of offspring produced per female), distribution of first broods, and growth (as total body length and dry weight).

All statistical analyses were conducted at the 95% level of certainty, except tests to assess normality and homogeneity of variance, where the 99% level of certainty was applied. The highest test concentration that elicited no statistically significant difference between the exposed organisms and the control (NOAEC) was

determined. The lowest test concentration that elicited a statistically significant effect on organism performance is defined as the LOAEC.

If at least one test concentration caused a ≥ 10, 20, or 50% reduction of survival, reproduction, or growth in the test population, then the ECx values and 95% confidence intervals were calculated. If no test concentration caused ≥ 10, 20, or 50% reduction of survival, reproduction, or growth of the test population, then ECx values were empirically estimated to be greater than the highest mean measured concentration tested.

CETIS Version 1.8 was used to perform the statistical computations.

Parental Survival
LC_{50} (21 d): >0.69 mg ai/L 95% C.I.: N/A
NOAEC: 0.69 mg ai/L
LOAEC: >0.69 mg ai/L

Reproduction (offspring/surviving female)
EC_{50} (21 d): 0.49 mg ai/L 95% C.I.: 0.47 to 0.49 mg ai/L
NOAEC: 0.34 mg ai/L
LOAEC: 0.69 mg ai/L

Reproduction (offspring/per female per reproductive day)
EC_{50} (21 d): 0.49 mg ai/L 95% C.I.: 0.45 to 0.49 mg ai/L
NOAEC: 0.17 mg ai/L
LOAEC: 0.34 mg ai/L

Production Rate of first broods
EC_{50} (21 d): >0.34 mg ai/L 95% C.I.: N/A
NOAEC: 0.34 mg ai/L
LOAEC: >0.34 mg ai/L

Total Length
EC_{50} (21 d): >0.69 mg ai/L 95% C.I.: N/A
NOAEC: 0.17 mg ai/L
LOAEC: 0.34 mg ai/L

Dry Weight
EC_{50} (21 d): 0.54 mg ai/L 95% C.I.: 0.52 to 0.55 mg ai/L
NOAEC: 0.34 mg ai/L
LOAEC: 0.69 mg ai/L

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer assessed the parental survival, growth, and reproduction endpoints using CETIS version 1.9.5.3 statistical software with database backend settings updated by EFED on 07/25/17. The mean-measured concentrations were used for the analyses.

The statistical endpoints included F_0 mortality/survival, F_0 growth (dry weight and length), and reproduction (number of live offspring, successful birth rate, and time to first brood).

The parental survival data were analyzed using the Fisher Exact/Bonferroni-Holm test. The parental growth (weight and length) data were confirmed to be normally distributed and have homogeneous variances using Shapiro-Wilk's and Bartlett's tests, respectively, and were therefore analyzed using ANOVA followed by Dunnett's test.

Time to first brood and number of live offspring data had non-normal distribution and unequal variances using Shapiro-Wilk's and Levene's tests, respectively, and were therefore analyzed using ANOVA followed by the Mann-Whitney U two-sample test. Successful birth rate data had non-normal distribution but equal variances using Shapiro-Wilk's and Levene's tests, respectively, and were therefore analyzed using ANOVA followed by the Mann-Whitney U two-sample test.

Parental Survival
NOAEC: 0.69 mg ai/L
LOAEC: >0.69 mg ai/L

No. of Live Offspring
NOAEC: 0.17 mg ai/L
LOAEC: 0.34 mg ai/L

Total Length
NOAEC: 0.17 mg ai/L
LOAEC: 0.34 mg ai/L

Dry Weight
NOAEC: 0.34 mg ai/L
LOAEC: 0.69 mg ai/L

Successful Birth Rate
NOAEC: 0.17 mg ai/L
LOAEC: 0.34 mg ai/L

Time to First Brood
NOAEC: 0.34 mg ai/L
LOAEC: >0.34 mg ai/L

Endpoints affected: Reproduction (number of live offspring and successful birth rate) and growth (length and dry weight)
Most sensitive endpoints: Reproduction (number of live offspring and successful birth rate) and Length

E. STUDY DEFICIENCIES:

There were no deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's and the study author's results generally agreed. The reviewer's results are reported in the Executive Summary and Conclusions sections of this DER. Only the NOAEC and LOAEC values were reported as they are the only toxicity values required by the EPA for this guideline.

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; 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 October 25 to November 15, 2017.

G. CONCLUSIONS: Reproduction (number of live offspring and successful birth rate) and growth (length and dry weight) were significantly affected in the experiment. Reproduction and length were the most sensitive endpoints, resulting in an overall NOAEC and LOAEC of 0.17 and 0.34 mg ai/L, respectively.

III. REFERENCES:

None with the exception of standard guidelines and methodologies.

OPPTS 850.1300 Chronic Invert (Daphnid) **Smithers Viscient**

Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**		
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water	
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**		
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture	**Age:**
Sample ID:	01-6967-2164	**Code:**	50610204	**Project:**	Insecticide	
Sample Date:	25 Oct-17	**Material:**	CPU (novaluron degradate)	**Source:**	ADAMA Makhteshim, Ltd	
Receipt Date:		**CAS (PC):**		**Station:**		
Sample Age:	n/a	**Client:**	CDM Smith - E. Krupka			

PC Code 124002 MRID 50610204 mean-measured concentrations

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	TU	PMSD	S
03-4704-4634	F0 Dry Weight	Dunnett Multiple Comparison Test		0.34	0.69	0.4844		8.42%	1
07-4901-1858	F0 Dry Weight	Williams Multiple Comparison Test		0.17	0.34	0.2404		6.52%	1
06-1043-7669	F0 Length	Dunnett Multiple Comparison Test		0.17	0.34	0.2404		2.72%	1
10-2015-3280	F0 Length	Williams Multiple Comparison Test		0.17	0.34	0.2404		2.11%	1
12-1453-1650	F0 Survival	Fisher Exact/Bonferroni-Holm Test		0.69	>0.69	n/a		n/a	1
03-7363-9027	n Live Offspring	Jonckheere-Terpstra Step-Down Test		0.17	0.34	0.2404		n/a	1
11-3169-0721	n Live Offspring	Mann-Whitney U Two-Sample Test		0.17	0.34	0.2404		15.6%	1
20-9002-5662	Successful Birth Rate	Jonckheere-Terpstra Step-Down Test		0.17	0.34	0.2404		n/a	1
01-3083-4384	Successful Birth Rate	Mann-Whitney U Two-Sample Test		0.17	0.34	0.2404		14.4%	1
03-8460-4303	Time to First Brood	Jonckheere-Terpstra Step-Down Test		0.34	>0.34	n/a		n/a	1
11-3781-3407	Time to First Brood	Mann-Whitney U Two-Sample Test	✓	0.045	0.092	0.06434		5.12%	1

OPPTS 850.1300 Chronic Invert (Daphnid) **Smithers Viscient**

F0 Dry Weight Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	1.143	1.072	1.214	1.02	1.29	0.03155	0.09978	8.73%	0.00%
0.045		10	1.159	1.098	1.22	1.02	1.26	0.02702	0.08543	7.37%	-1.40%
0.092		8	1.086	0.9797	1.193	0.95	1.3	0.04508	0.1275	11.74%	4.97%
0.17		9	1.148	1.114	1.181	1.08	1.21	0.01451	0.04353	3.79%	-0.42%
0.34		10	1.068	1.013	1.123	0.95	1.18	0.02439	0.07714	7.22%	6.56%
0.69		8	0.2312	0.1666	0.2959	0.12	0.35	0.02735	0.07736	33.45%	79.77%

F0 Length Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	4.835	4.767	4.903	4.7	4.95	0.02986	0.09443	1.95%	0.00%
0.045		10	4.77	4.67	4.87	4.6	5	0.04422	0.1398	2.93%	1.34%
0.092		8	4.744	4.623	4.865	4.6	5.05	0.05127	0.145	3.06%	1.89%
0.17		9	4.794	4.729	4.859	4.7	4.95	0.02819	0.08457	1.76%	0.84%
0.34		10	4.535	4.46	4.61	4.3	4.65	0.03337	0.1055	2.33%	6.20%
0.69		8	2.769	2.646	2.891	2.55	3	0.0517	0.1462	5.28%	42.74%

F0 Survival Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0.045		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0.092		10	0.8000	0.4984	1.0000	0.0000	1.0000	0.1333	0.4216	52.70%	20.00%
0.17		10	0.9000	0.6738	1.0000	0.0000	1.0000	0.1000	0.3162	35.14%	10.00%
0.34		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0.69		10	0.8000	0.4984	1.0000	0.0000	1.0000	0.1333	0.4216	52.70%	20.00%

n Live Offspring Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	158	147.9	168.1	127	180	4.477	14.16	8.96%	0.00%
0.045		10	159.3	139	179.6	112	210	8.979	28.39	17.82%	-0.82%
0.092		10	143.2	108	178.4	51	183	15.58	49.26	34.40%	9.37%
0.17		10	141.7	105.8	177.6	0	167	15.86	50.16	35.40%	10.32%
0.34		10	145.5	139.3	151.7	128	156	2.738	8.657	5.95%	7.91%
0.69		10	0	0	0	0	0	0	0		100.00%

Successful Birth Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	10.54	9.851	11.23	8.5	12	0.3045	0.9629	9.14%	0.00%
0.045		10	10.6	9.234	11.97	7.5	14	0.6039	1.91	18.02%	-0.57%
0.092		10	10.38	9.128	11.63	6.8	12	0.5535	1.75	16.86%	1.52%
0.17		10	9.5	7.086	11.91	0	11	1.067	3.375	35.52%	9.87%
0.34		10	9.61	9.251	9.969	8.5	10	0.1588	0.5021	5.22%	8.82%
0.69		8	0	0	0	0	0	0	0		100.00%

Time to First Brood Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	8.1	7.694	8.506	7	9	0.1795	0.5676	7.01%	0.00%
0.045		10	8.2	7.636	8.764	7	10	0.2494	0.7888	9.62%	-1.23%
0.092		10	8.7	8.217	9.183	8	10	0.2134	0.6749	7.76%	-7.41%
0.17		9	8	8	8	8	8	0	0	0.00%	1.23%
0.34		10	8	8	8	8	8	0	0	0.00%	1.23%

OPPTS 850.1300 Chronic Invert (Daphnid) **Smithers Viscient**

F0 Dry Weight Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1.15	1.07	1.05	1.28	1.02	1.08	1.18	1.07	1.29	1.24
0.045		1.13	1.26	1.17	1.23	1.21	1.14	1.26	1.14	1.03	1.02
0.092		0.96	1.09	1.3	0.95	1.07	1.01	1.06	1.25		
0.17		1.19	1.21	1.14	1.1	1.12		1.19	1.15	1.08	1.15
0.34		1.09	0.97	1	1.07	1.18	1.15	1.14	1.08	1.05	0.95
0.69		0.25		0.23	0.28		0.35	0.18	0.29	0.15	0.12

F0 Length Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	4.95	4.9	4.75	4.9	4.95	4.75	4.9	4.8	4.75	4.7
0.045		5	4.65	4.85	4.6	4.7	4.95	4.65	4.8	4.85	4.65
0.092		4.65	4.85	4.65	5.05	4.6	4.75	4.7	4.7		
0.17		4.75	4.9	4.8	4.8	4.95		4.8	4.7	4.75	4.7
0.34		4.6	4.3	4.55	4.55	4.6	4.55	4.65	4.6	4.55	4.4
0.69		2.85		2.85	2.8		3	2.8	2.7	2.55	2.6

F0 Survival Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.045		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.092		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000
0.17		1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
0.34		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.69		1.0000	0.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000

n Live Offspring Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	170	159	148	158	127	180	165	164	153	156
0.045		173	129	210	153	112	181	134	170	165	166
0.092		179	146	151	180	169	174	183	142	51	57
0.17		154	167	152	164	158	0	150	158	150	164
0.34		152	138	128	144	140	151	142	152	156	152
0.69		0	0	0	0	0	0	0	0	0	0

Successful Birth Rate Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	11	11	9.9	11	8.5	12	11	11	10	10
0.045		12	8.6	14	10	7.5	12	8.9	11	11	11
0.092		12	9.7	10	12	11	12	12	9.5	6.8	8.8
0.17		10	11	10	11	11	0	10	11	10	11
0.34		10	9.2	8.5	9.6	9.3	10	9.5	10	10	10
0.69		0		0	0		0	0	0	0	0

Time to First Brood Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	8	8	8	9	9	8	8	8	8	7
0.045		8	10	8	7	8	8	9	8	8	8
0.092		8	10	9	8	9	8	8	9	9	9
0.17		8	8	8	8	8		8	8	8	8
0.34		8	8	8	8	8	8	8	8	8	8
0.69											

OPPTS 850.1300 Chronic Invert (Daphnid) — **Smithers Viscient**

Analysis ID:	03-4704-4634	**Endpoint:**	F0 Dry Weight	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.34	0.69	0.4844		8.42%

Dunnett Multiple Comparison Test

Control vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.045	-0.4064	2.305	0.091	18	CDF	0.9326	Non-Significant Effect
	0.092	1.359	2.305	0.096	16	CDF	0.2736	Non-Significant Effect
	0.17	-0.1181	2.305	0.093	17	CDF	0.8746	Non-Significant Effect
	0.34	1.905	2.305	0.091	18	CDF	0.1123	Non-Significant Effect
	0.69*	21.84	2.305	0.096	16	CDF	9.3E-07	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.48405	1.09681	5	141.5	<1.0E-37	Significant Effect
Error	0.379691	0.0077488	49			
Total	5.86374		54			

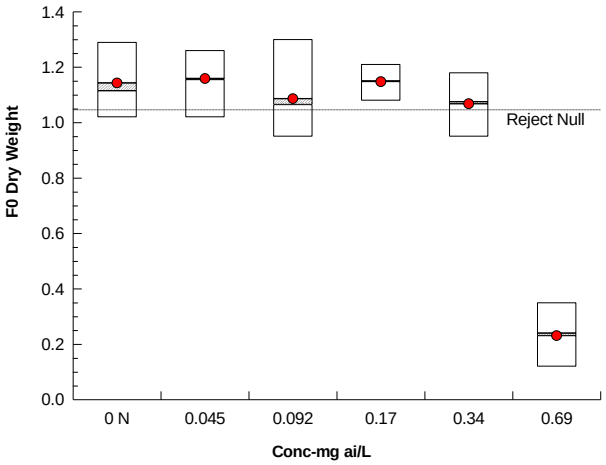
ANOVA Assumptions Tests

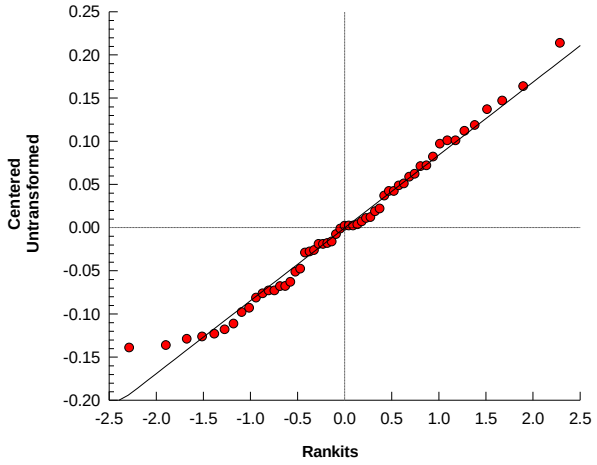
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	8.156	15.09	0.1478	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9787	0.9417	0.4356	Normal Distribution

F0 Dry Weight Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	1.143	1.072	1.214	1.115	1.02	1.29	0.03155	8.73%	0.00%
0.045		10	1.159	1.098	1.22	1.155	1.02	1.26	0.02702	7.37%	-1.40%
0.092		8	1.086	0.9797	1.193	1.065	0.95	1.3	0.04508	11.74%	4.97%
0.17		9	1.148	1.114	1.181	1.15	1.08	1.21	0.01451	3.79%	-0.42%
0.34		10	1.068	1.013	1.123	1.075	0.95	1.18	0.02439	7.22%	6.56%
0.69		8	0.2313	0.1666	0.2959	0.24	0.12	0.35	0.02735	33.45%	79.77%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) — **Smithers Viscient**

Analysis ID:	07-4901-1858	**Endpoint:**	F0 Dry Weight	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.17	0.34	0.2404		6.52%

Williams Multiple Comparison Test

Control vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.045	-0.4064	1.677	0.066	18	CDF	>0.05	Non-Significant Effect
	0.092	1.359	1.747	0.073	16	CDF	>0.05	Non-Significant Effect
	0.17	0.5978	1.773	0.072	17	CDF	>0.05	Non-Significant Effect
	0.34*	1.905	1.788	0.070	18	CDF	<0.05	Significant Effect
	0.69*	21.84	1.785	0.075	16	CDF	<0.05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.48405	1.09681	5	141.5	<1.0E-37	Significant Effect
Error	0.379691	0.0077488	49			
Total	5.86374		54			

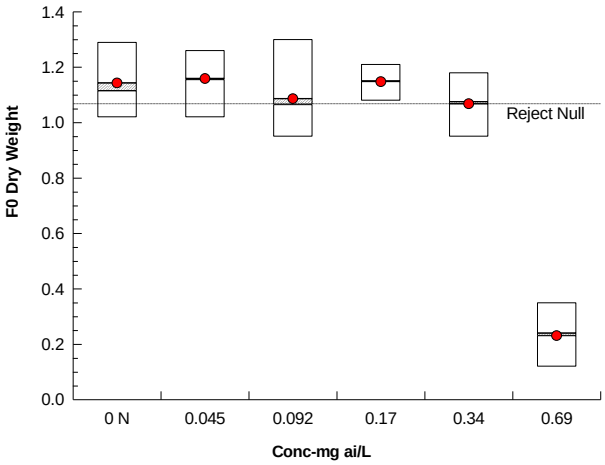
ANOVA Assumptions Tests

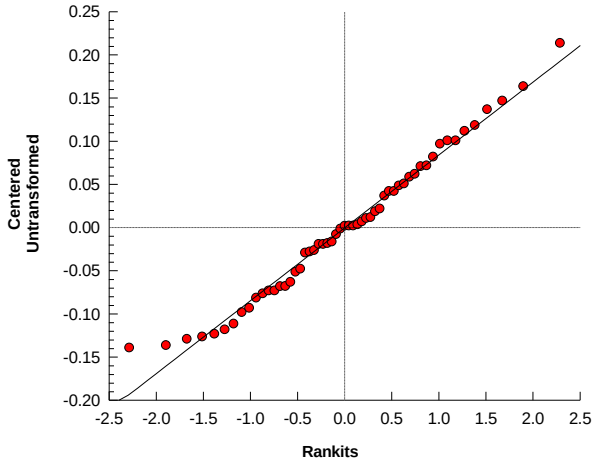
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	8.156	15.09	0.1478	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9787	0.9417	0.4356	Normal Distribution

F0 Dry Weight Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	1.143	1.072	1.214	1.115	1.02	1.29	0.03155	8.73%	0.00%
0.045		10	1.159	1.098	1.22	1.155	1.02	1.26	0.02702	7.37%	-1.40%
0.092		8	1.086	0.9797	1.193	1.065	0.95	1.3	0.04508	11.74%	4.97%
0.17		9	1.148	1.114	1.181	1.15	1.08	1.21	0.01451	3.79%	-0.42%
0.34		10	1.068	1.013	1.123	1.075	0.95	1.18	0.02439	7.22%	6.56%
0.69		8	0.2313	0.1666	0.2959	0.24	0.12	0.35	0.02735	33.45%	79.77%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) — **Smithers Viscient**

Analysis ID:	06-1043-7669	**Endpoint:**	F0 Length	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.17	0.34	0.2404		2.72%

Dunnett Multiple Comparison Test

Control vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.045	1.207	2.305	0.124	18	CDF	0.3347	Non-Significant Effect
	0.092	1.597	2.305	0.132	16	CDF	0.1915	Non-Significant Effect
	0.17	0.733	2.305	0.128	17	CDF	0.5509	Non-Significant Effect
	0.34*	5.57	2.305	0.124	18	CDF	3.5E-06	Significant Effect
	0.69*	36.17	2.305	0.132	16	CDF	9.3E-07	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	26.9492	5.38984	5	371.7	<1.0E-37	Significant Effect
Error	0.710597	0.014502	49			
Total	27.6598		54			

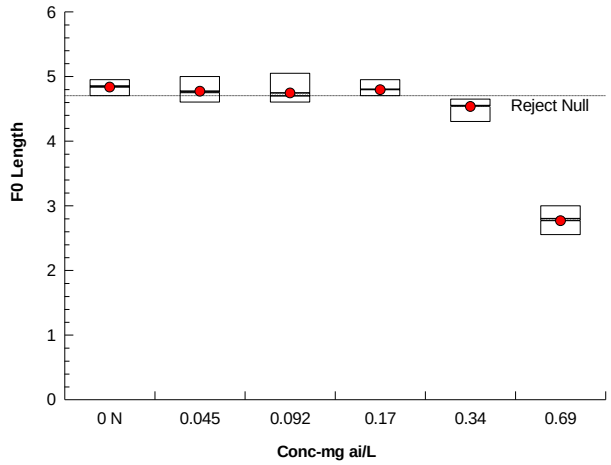
ANOVA Assumptions Tests

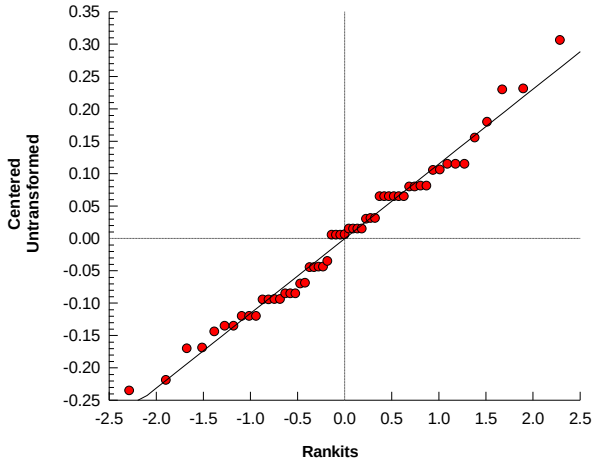
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	4.194	15.09	0.5218	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9823	0.9417	0.5904	Normal Distribution

F0 Length Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	4.835	4.767	4.903	4.85	4.7	4.95	0.02986	1.95%	0.00%
0.045		10	4.77	4.67	4.87	4.75	4.6	5	0.04422	2.93%	1.34%
0.092		8	4.744	4.623	4.865	4.7	4.6	5.05	0.05127	3.06%	1.89%
0.17		9	4.794	4.729	4.859	4.8	4.7	4.95	0.02819	1.76%	0.84%
0.34		10	4.535	4.46	4.61	4.55	4.3	4.65	0.03338	2.33%	6.20%
0.69		8	2.769	2.646	2.891	2.8	2.55	3	0.0517	5.28%	42.74%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) — **Smithers Viscient**

Analysis ID:	10-2015-3280	**Endpoint:**	F0 Length	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.17	0.34	0.2404		2.11%

Williams Multiple Comparison Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.045	1.207	1.677	0.090	18	CDF	>0.05	Non-Significant Effect
		0.092	1.597	1.747	0.1	16	CDF	>0.05	Non-Significant Effect
		0.17	1.168	1.773	0.098	17	CDF	>0.05	Non-Significant Effect
		0.34*	5.57	1.788	0.096	18	CDF	<0.05	Significant Effect
		0.69*	36.17	1.785	0.102	16	CDF	<0.05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	26.9492	5.38984	5	371.7	<1.0E-37	Significant Effect
Error	0.710597	0.014502	49			
Total	27.6598		54			

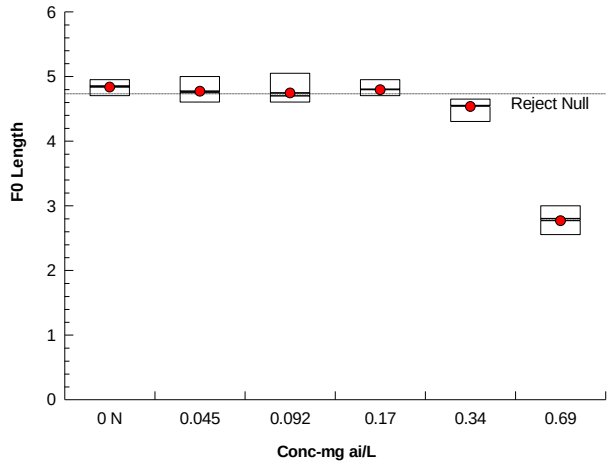
ANOVA Assumptions Tests

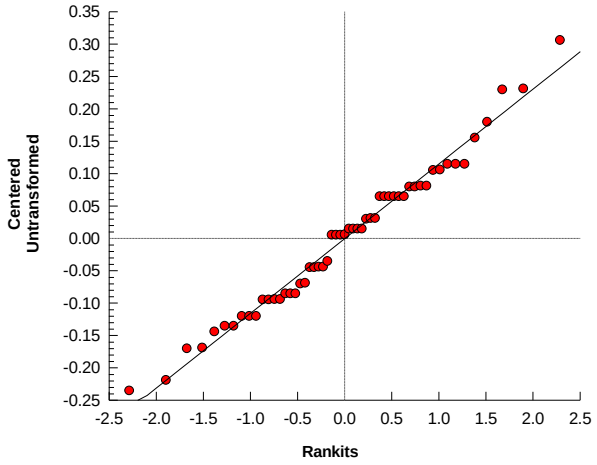
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	4.194	15.09	0.5218	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9823	0.9417	0.5904	Normal Distribution

F0 Length Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	4.835	4.767	4.903	4.85	4.7	4.95	0.02986	1.95%	0.00%
0.045		10	4.77	4.67	4.87	4.75	4.6	5	0.04422	2.93%	1.34%
0.092		8	4.744	4.623	4.865	4.7	4.6	5.05	0.05127	3.06%	1.89%
0.17		9	4.794	4.729	4.859	4.8	4.7	4.95	0.02819	1.76%	0.84%
0.34		10	4.535	4.46	4.61	4.55	4.3	4.65	0.03338	2.33%	6.20%
0.69		8	2.769	2.646	2.891	2.8	2.55	3	0.0517	5.28%	42.74%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) — **Smithers Viscient**

Analysis ID:	11-3169-0721	**Endpoint:**	n Live Offspring	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	Nonparametric-Two Sample	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture

Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.17	0.34	0.2404		15.55%

Mann-Whitney U Two-Sample Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.045	42.5	n/a	3	18	Exact	0.7171	Non-Significant Effect
		0.092	50.5	n/a	1	18	Exact	0.4939	Non-Significant Effect
		0.17	61	n/a	2	18	Exact	0.2113	Non-Significant Effect
		0.34*	83.5	n/a	1	18	Exact	0.0047	Significant Effect
		0.69*	100	n/a	0	18	Exact	5.4E-06	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	189200	37840	5	37.69	<1.0E-37	Significant Effect
Error	54210.3	1003.89	54			
Total	243410		59			

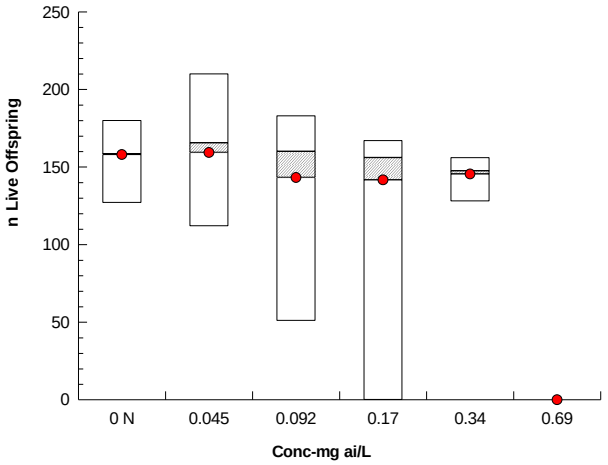
ANOVA Assumptions Tests

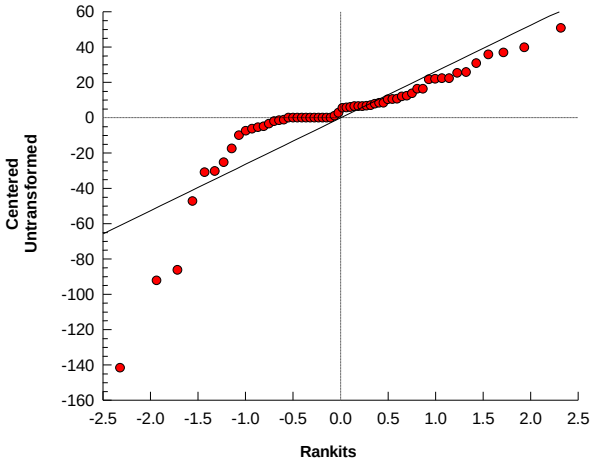
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	3.795	3.377	0.0051	Unequal Variances
	Mod Levene Equality of Variance Test	2.072	3.377	0.0831	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.7443	0.9459	7.0E-09	Non-Normal Distribution

n Live Offspring Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	158	147.9	168.1	158.5	127	180	4.477	8.96%	0.00%
0.045		10	159.3	139	179.6	165.5	112	210	8.979	17.82%	-0.82%
0.092		10	143.2	108	178.4	160	51	183	15.58	34.40%	9.37%
0.17		10	141.7	105.8	177.6	156	0	167	15.86	35.40%	10.32%
0.34		10	145.5	139.3	151.7	147.5	128	156	2.738	5.95%	7.91%
0.69		10	0	0	0	0	0	0	0		100.00%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) — **Smithers Viscient**

Analysis ID:	03-7363-9027	**Endpoint:**	n Live Offspring	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C > T	0.17	0.34	0.2404	

Jonckheere-Terpstra Step-Down Test

Control vs	Conc-mg ai/L	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control	0.045	-0.5676	1.645	3	Asymp	0.7148	Non-Significant Effect
	0.092	-0.1902	1.645	4	Asymp	0.5754	Non-Significant Effect
	0.17	0.6763	1.645	7	Asymp	0.2494	Non-Significant Effect
	0.34*	2.106	1.645	11	Asymp	0.0176	Significant Effect
	0.69*	4.785	1.645	12	Asymp	8.5E-07	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	189200	37840	5	37.69	<1.0E-37	Significant Effect
Error	54210.3	1003.89	54			
Total	243410		59			

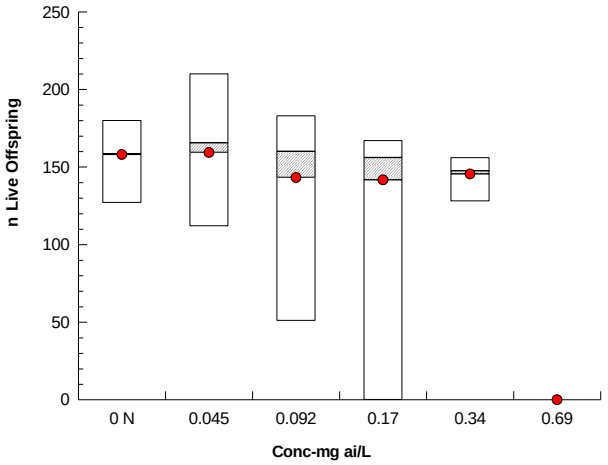
ANOVA Assumptions Tests

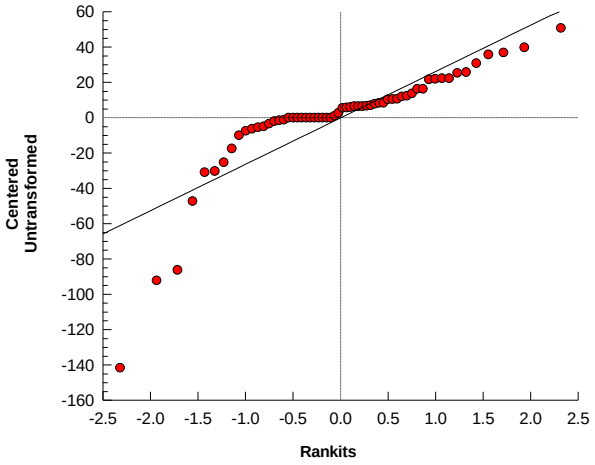
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	3.795	3.377	0.0051	Unequal Variances
	Mod Levene Equality of Variance Test	2.072	3.377	0.0831	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.7443	0.9459	7.0E-09	Non-Normal Distribution

n Live Offspring Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	158	147.9	168.1	158.5	127	180	4.477	8.96%	0.00%
0.045		10	159.3	139	179.6	165.5	112	210	8.979	17.82%	-0.82%
0.092		10	143.2	108	178.4	160	51	183	15.58	34.40%	9.37%
0.17		10	141.7	105.8	177.6	156	0	167	15.86	35.40%	10.32%
0.34		10	145.5	139.3	151.7	147.5	128	156	2.738	5.95%	7.91%
0.69		10	0	0	0	0	0	0	0		100.00%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) — **Smithers Viscient**

Analysis ID:	01-3083-4384	**Endpoint:**	Successful Birth Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	Nonparametric-Two Sample	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.17	0.34	0.2404		14.35%

Mann-Whitney U Two-Sample Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.045	46.5	n/a	3	18	Exact	0.6129	Non-Significant Effect
		0.092	49.5	n/a	3	18	Exact	0.5213	Non-Significant Effect
		0.17	55.5	n/a	2	18	Exact	0.3309	Non-Significant Effect
		0.34*	80.5	n/a	2	18	Exact	0.0077	Significant Effect
		0.69*	80	n/a	0	16	Exact	2.3E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	718.331	143.666	5	43.06	<1.0E-37	Significant Effect
Error	173.509	3.33671	52			
Total	891.84		57			

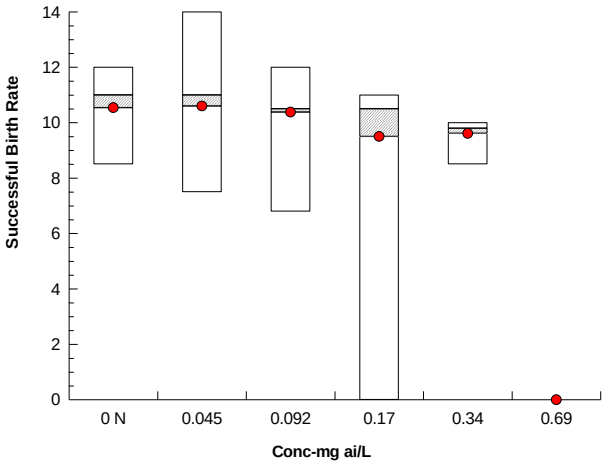
ANOVA Assumptions Tests

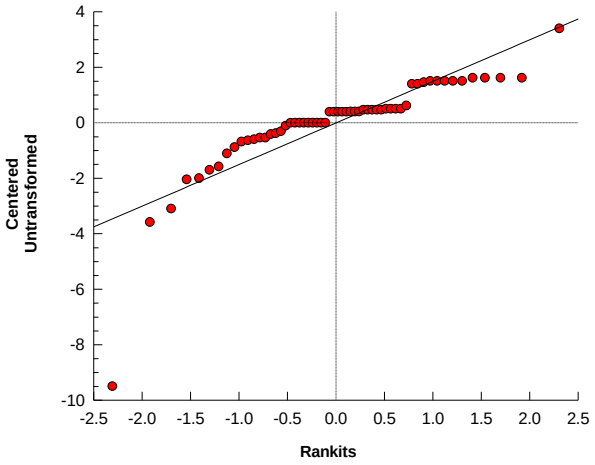
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	2.886	3.392	0.0225	Equal Variances
	Mod Levene Equality of Variance Test	1.602	3.392	0.1760	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.7379	0.9443	7.7E-09	Non-Normal Distribution

Successful Birth Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	10.54	9.851	11.23	11	8.5	12	0.3045	9.14%	0.00%
0.045		10	10.6	9.234	11.97	11	7.5	14	0.6039	18.02%	-0.57%
0.092		10	10.38	9.128	11.63	10.5	6.8	12	0.5535	16.86%	1.52%
0.17		10	9.5	7.086	11.91	10.5	0	11	1.067	35.52%	9.87%
0.34		10	9.61	9.251	9.969	9.8	8.5	10	0.1588	5.22%	8.82%
0.69		8	0	0	0	0	0	0	0		100.00%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) **Smithers Viscient**

Analysis ID:	20-9002-5662	**Endpoint:**	Successful Birth Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C > T	0.17	0.34	0.2404	

Jonckheere-Terpstra Step-Down Test

Control vs	Conc-mg ai/L	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control	0.045	-0.2742	1.645	3	Asymp	0.6080	Non-Significant Effect
	0.092	-0.1166	1.645	3	Asymp	0.5464	Non-Significant Effect
	0.17	0.3854	1.645	3	Asymp	0.3500	Non-Significant Effect
	0.34*	1.968	1.645	5	Asymp	0.0245	Significant Effect
	0.69*	4.274	1.645	6	Asymp	9.6E-06	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	718.331	143.666	5	43.06	<1.0E-37	Significant Effect
Error	173.509	3.33671	52			
Total	891.84		57			

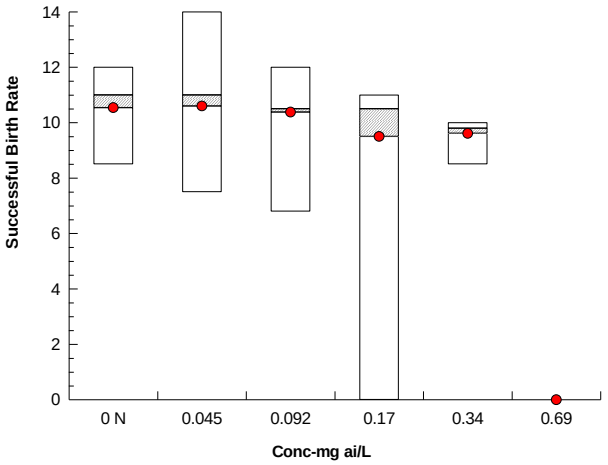
ANOVA Assumptions Tests

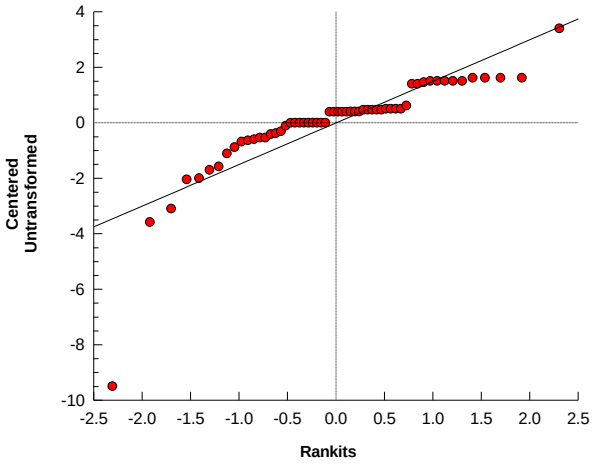
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	2.886	3.392	0.0225	Equal Variances
	Mod Levene Equality of Variance Test	1.602	3.392	0.1760	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.7379	0.9443	7.7E-09	Non-Normal Distribution

Successful Birth Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	10.54	9.851	11.23	11	8.5	12	0.3045	9.14%	0.00%
0.045		10	10.6	9.234	11.97	11	7.5	14	0.6039	18.02%	-0.57%
0.092		10	10.38	9.128	11.63	10.5	6.8	12	0.5535	16.86%	1.52%
0.17		10	9.5	7.086	11.91	10.5	0	11	1.067	35.52%	9.87%
0.34		10	9.61	9.251	9.969	9.8	8.5	10	0.1588	5.22%	8.82%
0.69		8	0	0	0	0	0	0	0		100.00%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) **Smithers Viscient**

Analysis ID:	11-3781-3407	**Endpoint:**	Time to First Brood	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:39	**Analysis:**	Nonparametric-Two Sample	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	0.045	0.092	0.06434		5.12%

Mann-Whitney U Two-Sample Test

Control vs	Conc-mg ai/L	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.045	51	n/a	4	18	Exact	0.4892	Non-Significant Effect
	0.092*	73	n/a	3	18	Exact	0.0471	Significant Effect
	0.17	40.5	n/a	1	17	Exact	0.8607	Non-Significant Effect
	0.34	45	n/a	1	18	Exact	0.8684	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.35918	0.839796	4	2.933	0.0311	Significant Effect
Error	12.6	0.286364	44			
Total	15.9592		48			

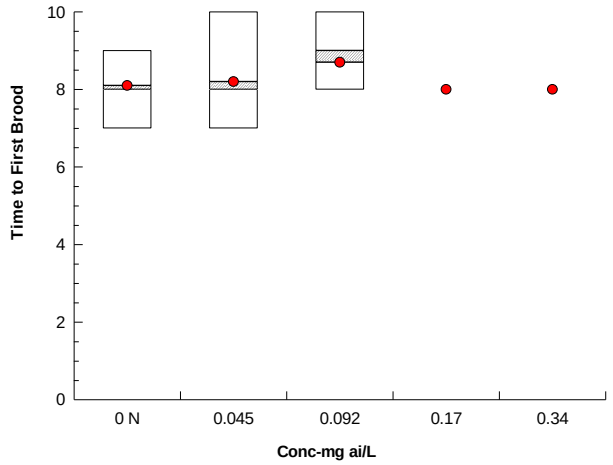
ANOVA Assumptions Tests

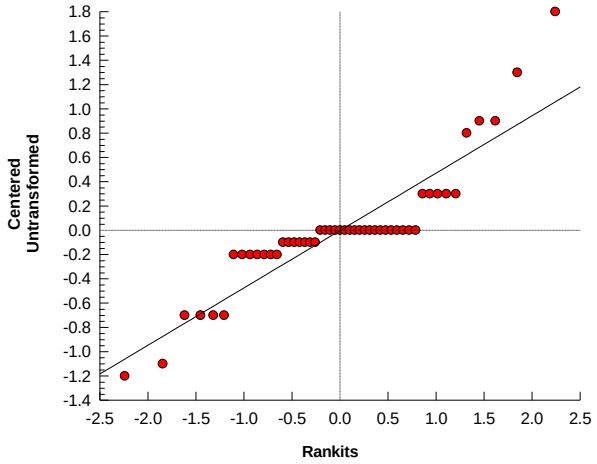
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	5.847	3.778	7.3E-04	Unequal Variances
	Mod Levene Equality of Variance Test	2.389	3.79	0.0657	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.837	0.9356	8.3E-06	Non-Normal Distribution

Time to First Brood Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	8.1	7.694	8.506	8	7	9	0.1795	7.01%	0.00%
0.045		10	8.2	7.636	8.764	8	7	10	0.2494	9.62%	-1.23%
0.092		10	8.7	8.217	9.183	9	8	10	0.2134	7.76%	-7.41%
0.17		9	8	8	8	8	8	8	0	0.00%	1.23%
0.34		10	8	8	8	8	8	8	0	0.00%	1.23%

Graphics





OPPTS 850.1300 Chronic Invert (Daphnid) **Smithers Viscient**

Analysis ID:	03-8460-4303	**Endpoint:**	Time to First Brood	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:39	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C < T	0.34	>0.34	n/a	

Jonckheere-Terpstra Step-Down Test

Control vs	Conc-mg ai/L	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control	0.045	0.09346	1.645	3	Asymp	0.7740	Non-Significant Effect
	0.092	1.915	1.645	3	Asymp	0.7740	Non-Significant Effect
	0.17	0.2474	1.645	3	Asymp	0.7740	Non-Significant Effect
	0.34	-0.7521	1.645	3	Asymp	0.7740	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.35918	0.839796	4	2.933	0.0311	Significant Effect
Error	12.6	0.286364	44			
Total	15.9592		48			

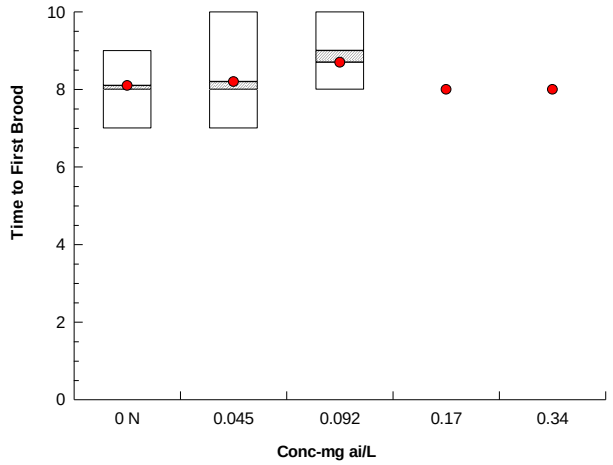
ANOVA Assumptions Tests

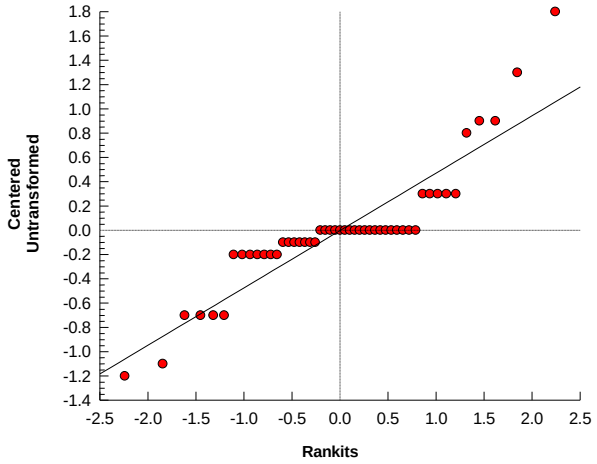
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	5.847	3.778	7.3E-04	Unequal Variances
	Mod Levene Equality of Variance Test	2.389	3.79	0.0657	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.837	0.9356	8.3E-06	Non-Normal Distribution

Time to First Brood Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	8.1	7.694	8.506	8	7	9	0.1795	7.01%	0.00%
0.045		10	8.2	7.636	8.764	8	7	10	0.2494	9.62%	-1.23%
0.092		10	8.7	8.217	9.183	9	8	10	0.2134	7.76%	-7.41%
0.17		9	8	8	8	8	8	8	0	0.00%	1.23%
0.34		10	8	8	8	8	8	8	0	0.00%	1.23%

Graphics





CETIS Analytical Report

OPPTS 850.1300 Chronic Invert (Daphnid) **Smithers Viscient**

Analysis ID:	12-1453-1650	**Endpoint:**	F0 Survival	**CETIS Version:**	CETISv1.9.5
Analyzed:	06 Feb-19 18:38	**Analysis:**	STP 2xK Contingency Tables	**Status Level:**	1
Batch ID:	01-2358-1558	**Test Type:**	Chronic Daphnia (21-d)	**Analyst:**	
Start Date:	25 Oct-17	**Protocol:**	OPPTS 850.1300 Chronic Invert (Daphnid	**Diluent:**	Fortified well water
Ending Date:	15 Nov-17	**Species:**	Daphnia magna	**Brine:**	
Test Length:	21d 0h	**Taxon:**	Branchiopoda	**Source:**	Lab In-House Culture **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C > T	0.69	>0.69	n/a	

Fisher Exact/Bonferroni-Holm Test

Control	vs	Group	Test Stat	P-Type	P-Value	Decision(α:5%)
Negative Control		0.045	1.0000	Exact	1.0000	Non-Significant Effect
		0.092	0.2368	Exact	1.0000	Non-Significant Effect
		0.17	0.5000	Exact	1.0000	Non-Significant Effect
		0.34	1.0000	Exact	1.0000	Non-Significant Effect
		0.69	0.2368	Exact	1.0000	Non-Significant Effect

Data Summary

Conc-mg ai/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	N	10	0	10	1	0	0.0%
0.045		10	0	10	1	0	0.0%
0.092		8	2	10	0.8	0.2	20.0%
0.17		9	1	10	0.9	0.1	10.0%
0.34		10	0	10	1	0	0.0%
0.69		8	2	10	0.8	0.2	20.0%

Graphics