

## Jennifer Cline

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**From:** Jennifer Cline <jcline@cityofmolalla.com>  
**Sent:** Thursday, March 03, 2016 2:21 PM  
**To:** COLE David; 'HEINS Pat'  
**Cc:** Jason Clifford  
**Subject:** Molalla Bioassay  
**Attachments:** Cover Letter for fathead minnow.docx; Molalla December Bioassay(2)3474(fathead minnow #2).PDF; Molalla December Bioassay3459.pdf

Hello,

Please find attached a letter from Jason Clifford and electronic copies of two Bioassay Priority Pollutant reports. As Jason mentions in his letter, both reports are the same with the exception the 1<sup>st</sup> report (dated February 17<sup>th</sup>) is the WA DOE alternate method fathead minnow fungus test. The first WET test (dated January 21<sup>st</sup>) had failed the flathead minnow fungus requirement. Hard copies have been sent by mail.

Please let Jason 503-793-5283 or myself know if you have any questions.

### Jennifer Cline, P.E. | Public Works Director

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2/05/2016

Molalla WWTP Priority Pollutants per Permit 101514

Attn: Dave Cole, Pat Heins

Dear Sirs,

There are (2) Whole Effluent Toxicity reports this session. The reports are the same with the exception of this report that is attached to this letter; this report is the Washington DOE alternate method fathead minnow fungus test. The first WET test failed the fathead minnow fungus. Brett Muckey, Biologist/Bioassay Laboratory Manager from CH2M Applied Sciences Laboratory contacted Lori Pillsbury about our fungus fail. She concurred that the City could perform a second test using the Washington DOE alternate method. This report is the result of the second test, which passed. I am sending you both reports, completing the Whole Effluent Toxicity requirement stated in City permit #101514. If you have any questions, do not hesitate to call me at 503-793-5283, Tuesday-Friday 0700-1730.

Respectfully,

Jason Clifford

Lead Operator

City of Molalla WWTP

**BIOASSAY REPORT  
CHRONIC AND ACUTE  
BIOASSAYS CONDUCTED  
December 15 through 22, 2015**

Prepared for

CITY OF MOLALLA  
MOLALLA, OREGON

Prepared by



**CH2MHILL**  
*Applied Sciences Laboratory (ASL)*

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NELAC #OR100022

Report Date: January 21, 2016  
Lab I.D. No. B3459

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## INTRODUCTION

CH2M Applied Sciences Laboratory (CH2M-ASL) conducted chronic and acute dual-endpoint bioassays from December 15 through 22, 2015, on samples provided by the City of Molalla, Molalla, Oregon. The tests were conducted using the water flea (*Ceriodaphnia dubia*), the fathead minnow (*Pimephales promelas*), and algae (*Raphidocelis subcapitata*, formerly known as *Selenastrum capricornutum*).

## OVERVIEW OF REGULATORY GUIDANCE

The following provides an overview and excerpts of applicable permit specifics, regulatory guidance, and other relevant information. This is intended only as a helpful guide, from a laboratory perspective, for understanding test outcomes. The final responsibility for interpretation of results remains with the client and/or regulatory agency.

The following guidance is taken from CH2M's reading of the NPDES permit for the City of Molalla STP facility (permit #OR00101514, effective June 1, 2014, to June 1, 2019).

### Acute toxicity:

- *Acute Permit Limit:* "An acute WET test will be considered to show toxicity if there is a statistically significant difference in survival between the control and 10% effluent reported as the NOEC  $\leq$  10 percent effluent."
- Please note: There is an apparent conflict within the above quote (taken from Section 10.b.iv. of the permit)
  - Statistical fact: In a test where the NOEC value is exactly equal to 10%, no statistically significant difference between the control and 10% effluent exists.
  - Using the first portion of the quote, referencing 'a statistically significant difference', would infer that toxicity was not shown.
  - Using the last portion of the quote, referencing 'NOEC  $\leq$  10%', would infer that toxicity was shown.
  - ASL's believes that this conflict is the result of what likely is a typographic error in the permit and that a "< 10" was intended rather than the " $\leq$  10" as written. However, this should be referred to OR DEQ for clarification.
- Acute toxicity data may be obtained as Dual End-Point Tests.

### Chronic toxicity:

- *Chronic Permit Limit:* "A chronic WET test will be considered to show toxicity if the IC<sub>25</sub> ... occurs at dilutions equal to or less than the dilution known to occur at the edge of the mixing zone, that is, IC<sub>25</sub>  $\leq$  4%"

### Evaluation of Causes and Exceedances:

- "If any test exhibits toxicity, ... another test ... shall be conducted within two weeks".
- "If two consecutive WET tests indicate acute and/or chronic toxicity ... the permittee shall immediately notify the Department of the results."

## SUMMARY OF TEST RESULTS

Exhibits 1 and 2 provide a summary of the final test results.

### EXHIBIT 1

#### EXHIBIT 1

#### Summary of Acute Dual-Endpoint Test Results

Species	NOEC (%)	LOEC (%)	Is there a statistically significant difference between control and 10% ?
<i>C. dubia</i>	100	> 100	No
<i>P. promelas</i>	nr <sup>a</sup>	nr <sup>a</sup>	nr <sup>a</sup>

Note: acronyms are as defined below Exhibit 2.

<sup>a</sup> Indicates the test results were confounded by pathogenic interference (fungus) and therefore questionable. See further discussion under Tables 2 and 4.

From the NPDES permit: “An acute WET test will be considered to show toxicity if there is a statistically significant difference in survival between the control and 10% effluent reported as the NOEC  $\leq$  10 percent effluent.”

More detailed information is provided in the Results and Discussion sections.

### EXHIBIT 2

#### Summary of Chronic Test Results

Species	NOEC (%)	LOEC (%)	IC <sub>25</sub> (%)
<i>C. dubia</i>	40	100	55.6
<i>P. promelas</i>	nr <sup>a</sup>	nr <sup>a</sup>	nr <sup>a</sup>
<i>R. subcapitata</i>	40	100	57.6

Note: acronyms are as defined below.

nr <sup>a</sup> Indicates the test results were confounded by pathogenic interference (fungus) and therefore “not reliable”. See further discussion under Table 4.

From the NPDES permit: “A chronic WET test will be considered to show toxicity if the IC<sub>25</sub> ... occurs at dilutions equal to or less than the dilution known to occur at the edge of the mixing zone, that is, IC<sub>25</sub>  $\leq$  4%”.

More detailed information is provided in the Chronic Results and Data Interpretation sections.

## ACRONYM DEFINITIONS (from EPA guidance):

NOEC = No Observed Effect Concentration: The highest test concentration that causes no observable adverse effects on the test organisms (i.e. no statistically significant reduction from the control).

LOEC = Low Observed Effect Concentration: The lowest test concentration that does cause an observable adverse effect on the test organisms (i.e. is statistically significant reduction from the control).

IC<sub>25</sub> = Inhibition Concentration (25%): A point estimate of the test concentration that would cause a 25 percent reduction of a non-quantal biological measurement (i.e. growth, reproduction, etc.) for the test population.

## METHODS AND MATERIALS

### TEST METHODS

The acute test methods were performed according to: *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, USEPA Office of Water (2002), EPA-821-R-02-012.

The chronic test methods were performed according to: *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, Fourth Edition, (EPA 2002), EPA-821-R-02-013.

Additional guidance was provided by:

- *Reasonable Potential Analysis for Toxic Pollutants, Internal Management Directive*, Oregon Department of Environmental Quality (February 2005).

### DEVIATIONS FROM PROTOCOLS

Deviations from required procedures in the test methods:

- None noted.

Deviations from recommended procedures in the test methods:

- The *P. promelas* chronic reference toxicant (reftox) test conducted concurrently fell outside the cusum chart control limits. Follow up reftox testing initiated on Dec 29, 2015, was within cusum chart range and provides demonstration of ongoing laboratory performance. See Reference Toxicant Tests section for further information.

## **TEST ORGANISMS**

The *C. dubia* were obtained from CH2M's in-house cultures and were less than 24 hours old and within an 8-hour age range for the chronic test. The *P. promelas* were obtained from Aquatox Inc., Hot Springs, Arkansas, and were less than 48 hours old and within a 24-hour age range at test initiation. The *R. subcapitata* was obtained from CH2M's in-house cultures and were cultured and acclimated to test conditions for 4 days prior to test initiation. All organisms tested were fed and maintained during culturing, acclimation, and testing as prescribed by the EPA (2002). The test organisms appeared vigorous and in good condition prior to testing.

## **DILUTION WATER**

The dilution water used was reconstituted, moderately hard water with a total hardness of 80 to 100 mg/L as CaCO<sub>3</sub> and an alkalinity of 60 to 70 mg/L as CaCO<sub>3</sub>. The sample used for the *R. subcapitata* test had nutrients added, including EDTA, and was passed through a 0.45 micron filter prior to test initiation.

## **TEST CONCENTRATIONS**

The concentrations for the acute dual-end point and chronic tests were 2, 4, 10, 40, and 100 percent sample with dilution water for the control. For the chronic *C. dubia* test, 10 organisms per concentration were used with 10 test vessels per concentration and one organism per vessel. For the *P. promelas* chronic test, 10 organisms per chamber, with four chambers per concentration for a total of 40 organisms per concentration were used. The *R. subcapitata* test was run with four replicates per test concentration using an initial cell density in each test chamber of 10,000 cells/ml.

## **SAMPLE COLLECTION**

Samples were collected by City of Molalla personnel on December 14, 16, and 18, 2015. The samples were accepted as scheduled by CH2M's Applied Sciences Laboratory. All samples were received within the EPA recommended 0 to 6 °C range.

All samples were initially used for test initiation or test solution renewal within the EPA recommended maximum holding time of 36 hours of sample collection. All subsequent uses of a sample occurred within the EPA recommended maximum holding time of 72 hours past the time of initial use of that sample.

Following receipt, the samples were stored in the dark at 0 to 6 °C until test solutions were prepared and tested. Chain of Custody for sample collection is provided in Appendix C.



## **SAMPLE PREPARATION**

Samples used during these tests were filtered through a 60 µm net upon arrival and temperature was adjusted prior to test initiation and each daily renewal. The sample used for the *R. subcapitata* test had nutrients, including EDTA, added, and was 0.45 micron filtered prior to test initiation.

## **MONITORING OF BIOASSAYS**

Samples were monitored on arrival for hardness, total residual chlorine, ammonia, and temperature.

For the *P. promelas* and *C. dubia* chronic tests, pre- and post-renewal solutions were monitored for dissolved oxygen and pH daily in all the concentrations. Conductivity was measured in each new sample (100 percent sample) and in the control. *C. dubia* survival and neonate production was measured daily in the chronic tests. According to EPA, *C. dubia* tests should be terminated when 60 percent or more of the surviving female *C. dubia* in the controls have produced their third brood and at least 15 neonates per adult are produced. *P. promelas* mortality was measured daily and fish growth was measured by dry weight analysis at the conclusion of the *P. promelas* chronic test. Temperature was monitored in pre-renewal solutions daily.

For the *R. subcapitata* chronic test, solutions were monitored for pH and temperature daily in the control and all concentrations tested. Conductivity and dissolved oxygen was measured in the control and all concentrations tested at test initiation. Total hardness and alkalinity were measured in the control, low, middle, and high test concentrations at test initiation. *R. subcapitata* growth was determined by cell counts using a Coulter counter. Temperature was monitored in a surrogate chamber daily.

## **DATA ANALYSIS**

The effects measured during the *C. dubia* acute dual-end point test included survival data taken from the chronic test over the initial 48 hour exposure period. The effects measured during the *P. promelas* acute dual-end point acute test included survival data taken from the chronic test over the initial 96 hour exposure period. The statistical analyses performed were those outlined in *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, USEPA Office of Water (2002), EPA-821-R-02-012, using CETIS. The acute NOEC and LOEC values were established by hypothesis testing as follows: Dunnett's Procedure, Bonferroni's T-Test, or homoscedastic (equal variance) T-test was used to compare the survival data between the control and each sample concentration. When the assumptions of normality or homogeneity of variance necessary for Dunnett's Procedure or T-test with Bonferroni Adjustment could not be met, Steel's Many-One Rank Test, Wilcoxon Rank Sum with Bonferroni Adjustment, heteroscedastic T-test or Wilcoxon Two-Sample Test was used to analyze the data.

The effects measured during the *C. dubia* chronic test included survival and reproduction over the exposure period. The effects measured during the *P. promelas* chronic test included survival and growth over the exposure period. The effect measured during the *R. subcapitata* test was algae growth response. The statistical analyses performed were those outlined in *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, Fourth Edition, (EPA 2002), EPA-821-R-02-013, CETIS. The chronic NOEC and LOEC values were established by hypothesis testing as follows: Dunnett's Procedure or Bonferroni's T-Test was used to compare the survival, fecundity, or growth data between the control and each sample concentration. When the assumptions of normality or homogeneity of variance necessary for Dunnett's Procedure or T-test with Bonferroni Adjustment could not be met, Steel's Many-One Rank Test or Wilcoxon Rank Sum with Bonferroni Adjustment was used to analyze the data. The chronic point estimate values (IC<sub>25</sub> values) were calculated for growth, reproduction, and/or fecundity effects by use of the Linear Interpolation method.

## RESULTS AND DISCUSSION

The raw data sheets for all tests are presented in Appendix A.

### ACUTE BIOASSAYS

Table 1 summarizes the survival data for the *C. dubia* acute dual-endpoint test.

<b>Table 1</b>			
<b>Summary of Acute Dual-Endpoint Results</b>			
<b>Percent Survival</b>			
Concentration (%)	0 hr	24 hr	48 hr
<i>C. dubia</i>			
Control	100	100	100
2	100	100	100
4	100	100	100
10	100	100	100
40	100	100	100
100	100	100	100

The *C. dubia* acute dual-endpoint test indicated no statistically significant reduction in survival at any of the concentrations tested when compared to the control. By EPA definition, the NOEC and the LOEC were 100 and greater than 100 percent sample, respectively.

From the NPDES permit: “An acute WET test will be considered to show toxicity if there is a statistically significant difference in survival between the control and 10% effluent reported as the NOEC  $\leq$  10 percent effluent.”

Dissolved oxygen concentrations remained at 4.0 mg/L or greater throughout the test period. Test temperatures remained in the range of 25 $\pm$ 1°C.

The test meets Test Acceptability Criteria (TAC) of a minimum 90 percent control survival. Unless referenced above, the tests proceeded without any noted deviations or interruptions that could have affected test results. The testing should be considered “valid”.

Table 2 summarizes the survival data for the *P. promelas* acute dual-endpoint test.

<b>Table 2</b> <b>Summary of Acute Dual-Endpoint Results</b> <i>P. promelas</i> <b>Percent Survival</b>					
Concentration (%)	0 hr	24 hr	48 hr	72 hr	96 hr
Control	100	100	100	100	100
2	100	100	97.5	90.0	85.0 <sup>a</sup>
4	100	100	100	82.5	77.5
10	100	97.5	97.5	62.5	55.0
40	100	97.5	97.5	97.5	97.5
100	100	100	100	100	97.5

<sup>a</sup> Indicates a statistically significant reduction from the control at alpha = 0.05.

The *P. promelas* acute dual-endpoint test results indicated a statistically significant reduction in survival only at the 2 percent test concentration when compared to the control. By EPA definition, the NOEC and the LOEC were 100 and 2 percent sample, respectively.

Note: Fungus was noted in seven of the 24 test chambers by the 96 hour observations (the control and the 40% effluent chambers did not show fungus development). These pathogenic effects often have a confounding effect and make test interpretation difficult.

EPA 821-B-00-004, page 4-13, identifies this data set as an interrupted concentration response with a non-significant effect bracketed by significant effects. Additionally, EPA-821-R-02-013, states that tests that show an interrupted dose response “should be used with extreme caution”. Consultation with OR-DEQ indicates that, given the fungus and the high variability, the test should be repeated. See Jan 12, 2016, email communication with Lori Pillsbury included in Appendix A.

From the NPDES permit: “An acute WET test will be considered to show toxicity if there is a statistically significant difference in survival between the control and 10% effluent reported as the NOEC  $\leq$  10 percent effluent.”

Dissolved oxygen concentrations remained at 4.0 mg/L or greater throughout the test period. Test temperatures remained in the range of 25±1°C.

The test meets Test Acceptability Criteria (TAC) of a minimum 90 percent control survival. While this technically makes the test “valid”, the confounding effect of the fungus makes reliable interpretation of test results difficult. As per communication with ORDEQ, the test should be considered “anomalous” or inconclusive and not used for regulatory compliance.

## CHRONIC BIOASSAYS

Table 3 summarizes the survival and reproduction data for the *C. dubia* chronic test.

<b>Concentration (%)</b>	<b>Percent Survival</b>	<b>Ave. No. Young Per Adult</b>
Control	100	25.1
2	100	27.3
4	100	24.9
10	100	26.0
40	80	23.3
100	100	13.1 <sup>a</sup>

<sup>a</sup> Indicates a statistically significant reduction from the control

The *C. dubia* test results indicated no statistically significant reduction in survival at any sample concentration tested and a statistically significant reduction in reproduction only at the 100 percent sample concentration when compared to the control. By EPA definition, the NOEC and the LOEC were 40 and 100 percent sample, respectively.

The IC<sub>25</sub> value calculated on *C. dubia* reproduction was 55.6 percent sample.

From the NPDES permit: "A chronic WET test shall be considered to show toxicity if the IC<sub>25</sub> (25% inhibition concentration) occurs at dilutions equal to or less than the dilution that is known to occur at the edge of the mixing zone, i.e. IC<sub>25</sub> ≤ 4%".

The dissolved oxygen levels in the chronic tests remained above 4.0 mg/L. Test temperatures remained at 25±1°C.

The test meets Test Acceptability Criteria (TAC) for a minimum 80 percent control survival and a minimum 15 young produced per surviving control adult. Unless referenced above, the tests proceeded without any noted deviations or interruptions that could have affected test results. The testing should be considered "valid".

Table 4 summarizes the survival and growth data for the *P. promelas* chronic test.

<b>Table 4</b> <b>Summary of Chronic Results</b> <i>P. promelas</i>		
<b>Sample Concentration (%)</b>	<b>Percent Survival</b>	<b>Mean Dry Weight Per Fish (mg)</b>
Control	100	0.715
2	75.0	0.620
4	65.0	0.488
10	42.5 <sup>a</sup>	0.366
40	92.5	0.605
100	95.0	0.590 <sup>a</sup>

<sup>a</sup> Indicates a statistically significant reduction from control at alpha = 0.05

The *P. promelas* test results indicated a statistically significant reduction in survival only at the 10 percent sample concentration and a statistically significant reduction in growth only at the 100 percent test concentration when compared to the control. By EPA definition, the NOEC and the LOEC were 40 and 10 percent sample, respectively.

Note: Fungus was noted in nine of the 24 test chambers by the Day 7 test termination (the control and the 40% effluent chambers did not show fungus development). These pathogenic effects often have a confounding effect and make test interpretation difficult.

EPA 821-B-00-004, page 4-13, identifies this data set as an interrupted concentration response with a non-significant effect bracketed by significant effects. Additionally, EPA-821-R-02-013, states that tests that show an interrupted dose response “should be used with extreme caution”. Consultation with OR-DEQ indicates that, given the fungus and the high variability, the test should be repeated. See Jan 12, 2016, email communication with Lori Pillsbury included in Appendix A.

The IC<sub>25</sub> value on *P. promelas* growth was calculated as 3.5 percent sample. However, it should be noted that, in cases where the data is not monotonically increasing (i.e. an increasing effect with increasing sample concentration, as seen here), the linear interpolation statistical program used for IC<sub>25</sub> calculations “smooths” the data to meet required statistical assumptions. In this case, the growth data from the 4, 10, 40 and 100% sample concentrations were averaged. This then limits the IC<sub>25</sub> calculation to a value of less than 4% or greater than 100%, thereby limiting the accuracy of the calculation.

From the NPDES permit: “A chronic WET test shall be considered to show toxicity if the IC<sub>25</sub> (25% inhibition concentration) occurs at dilutions equal to or less than the dilution that is known to occur at the edge of the mixing zone, i.e. IC<sub>25</sub> ≤ 4%”.

The dissolved oxygen levels in the chronic tests remained above 4.0 mg/L.

The *P. promelas* chronic test meets Test Acceptability Criteria (TAC) for a minimum 80 percent control survival and a minimum weight of 0.250 mg per surviving control organism. Unless referenced above, the tests proceeded without any noted deviations or interruptions that could have affected test results. The testing should be considered “conditionally acceptable”.

The *P. promelas* chronic reference toxicant test conducted concurrently was outside of cusum chart range for both the survival and growth endpoints. Specifically, the test results were above the cusum chart range indicating that the test organisms were potentially insensitive. EPA 821-R-02-013 section 4.16.6 recognizes that “reference toxicant test results should not be used as a *de facto* criterion for rejection of individual effluent or receiving water tests.” Rather, “the reviewer should consider the degree to which the reference toxicant test result fell outside of control chart limits, the width of the limits, the direction of the deviation ... , the test conditions ..., and the objective of the test.”

Repeat reftox testing initiated on Dec 29, 2015, resulted in survival and growth rates within cusum chart range. This provides the requirement of demonstrating satisfactory laboratory performance for the test method as indicated in EPA 821-R-02-013, section 4.16.6.

The dissolved oxygen levels in the chronic tests remained above 4.0 mg/L. Test temperatures remained at 25±1°C.

The *P. promelas* test meets Test Acceptability Criteria (TAC) for a minimum 80 percent control survival and a minimum weight of 0.250 mg per surviving control organism. While this technically makes the test “valid”, the confounding effect of the fungus makes reliable interpretation of test results difficult. As per communication with ORDEQ, the test should be considered “anomalous” or inconclusive and not used for regulatory compliance.

Table 5 summarizes the algae growth data for the *R. subcapitata* chronic test.

<b>Table 5</b> <b>Summary of Chronic Results</b> <i>R. subcapitata</i>	
<b>Concentration</b> <b>(%)</b>	<b>Growth</b> <b>(Cells/ml x 10<sup>6</sup>)</b>
Control	2.606
2	2.753
4	3.562
10	3.297
40	3.372
100	1.152 <sup>a</sup>

<sup>a</sup> Indicates a statistically significant reduction from the control at p equal to 0.05.

The *R. subcapitata* test results indicated a statistically significant reduction in growth only at the 100 percent sample concentration when compared to the control. By EPA definition, the NOEC and the LOEC were 40 and 100 percent sample, respectively.

The IC<sub>25</sub> value calculated on *R. subcapitata* growth was 57.6 percent sample.

From the NPDES permit: “A chronic WET test shall be considered to show toxicity if the IC<sub>25</sub> (25% inhibition concentration) occurs at dilutions equal to or less than the dilution that is known to occur at the edge of the mixing zone, i.e. IC<sub>25</sub> ≤ 4%”.

The dissolved oxygen levels in the chronic tests remained above 4.0 mg/L. Test temperatures remained at 25±1°C.

The test meets Test Acceptability Criteria (TAC) for a minimum 1.0 x 10<sup>6</sup> cells/ml control growth and a maximum coefficient of variance (CV%) of 20% in the controls. Unless referenced above, the tests proceeded without any noted deviations or interruptions that could have affected test results. The testing should be considered “valid”.



## REFERENCE TOXICANT TESTS

Reference toxicant (reftox) testing is performed to document both initial and ongoing laboratory performance of the test method(s). While the health of the test organisms is primarily evaluated by the performance of the laboratory control, reftox test results also may be used to assess the health and sensitivity of the test organisms. Reftox test results within their respective cumulative summary (cusum) chart limits are indicative of consistent laboratory performance and normal test organism sensitivity.

The results of the reftox tests for *C. dubia* and *R. subcapitata* indicate that the test organisms were within their respective cusum chart limits based on EPA guidelines. This demonstrates ongoing laboratory proficiency of the test methods and suggests normal test organism sensitivity in the associated client testing.

The *P. promelas* chronic reference toxicant test conducted concurrently was outside of cusum chart range for both the survival and growth endpoints. Repeat reftox testing initiated on Dec 29, 2015, resulted in survival and growth rates within cusum chart range. This provides the requirement of demonstrating satisfactory laboratory performance for the test method as indicated in EPA 821-R-02-013, section 4.16.6.

The *C. dubia*, *P. promelas*, and *R. subcapitata* reftox tests were conducted using sodium chloride. The data sheets for the reference toxicant tests are provided in Appendix B.

Table 6 summarizes the reference toxicant test results and Cusum chart limits.

<b>Species</b>	<b>IC<sub>25</sub></b>	<b>Cusum Chart Limits</b>
<i>C. dubia</i> (survival)	1.20	0.79 to 2.09
<i>C. dubia</i> (reproduction)	0.74	0.22 to 1.14
<i>P. promelas</i> (survival) Dec. 15, 2015	3.94	1.63 to 3.88
<i>P. promelas</i> (growth) Dec. 15, 2015	3.081	1.907 to 3.080
<i>P. promelas</i> (survival) Dec. 29, 2015	2.3	1.6 to 4.1
<i>P. promelas</i> (growth) Dec. 29, 2015	2.2	1.9 to 3.2
<i>R. subcapitata</i> (growth)	1.17	0.61 to 1.80

**APPENDIX A**  
**RAW DATA SHEETS**



FRESHWATER TOXICITY TEST: SAMPLE AND DILUTION WATER DATA

Client: City of Molalla

SDG # B 3459

Test Initiation: Date 12-15-15

Contact: Jason Clifford (503) 829-5407

Test Termination: Date 12-22-15

Sample ID Number	Field ID	Collected Date (mm/dd/yy)	Time (Pacific Zone)	Date Received	Temp (°C) as Rcv'd	Total Residual Chlorine (mg/l) <input type="checkbox"/> Dechlorination allowed as Rcv'd / after Dechlor.	Ammonia NH <sub>3</sub> -N mg/l as Rcv'd	Hardness mg/l as CaCO <sub>3</sub> as Rcv'd	Alkalinity mg/l as CaCO <sub>3</sub> as Rcv'd	DO (mg/L) as Rcv'd	pH as Rcv'd	Cond. (uS) as Rcv'd	60 um filtered prior to use?
03489 -01	FIND Effluent	12/14/15	06:00	12/14/15	3.6	<0.021	8.59	56	-	-	-	-	<input checked="" type="checkbox"/>
-02	↓	12/16/15	06:00	12/16/15	5.7	<0.021	7.70	#	-	-	-	-	<input checked="" type="checkbox"/>
-03	↓	12/18/15	07:00	12/18/15	4.7	<0.021	7.74	55	-	-	-	-	<input checked="" type="checkbox"/>
						/							
						/							
						/							
						/							
						/							
						/							
						/							
					Reporting Limits:	0.02 mg/L	0.10 mg/L	4 mg/L	4 mg/L	na	na	na	na

Note: "-" Indicates data collection or dechlorination not needed. Any other adjustments to samples prior to use are documented in Comments below or on Dilutions page.

Dilution Water	ID#	Hardness mg/l as CaCO <sub>3</sub>	Alkalinity mg/l as CaCO <sub>3</sub>	Comments:
Recon MH (Algae)	4300	104	82	<input checked="" type="checkbox"/> Indicates the action was taken, (□= action not taken): " - " = sample not dechlorinated, or analyte not collected/needed.
Recon MH (FHM)	4303	88	66	<input checked="" type="checkbox"/> Subsample for algae test had nutrients added and was 0.45 um filtered
↓	4304	96	66	* Hardness subsample at -02 sample not filtered or analysed for. See 1-15-16
↓	4306	86	66	

Water Quality Meters Used/ID#: Dissolved Oxygen # 3 pH # 11 Conductivity # 2

Client City of Molalla

Sample Designation (SDG): B 3459

<b>Test Species Information</b>	Cd # <u>3139</u> <i>Ceriodaphnia dubia</i> Chronic	FHM # <u>1849</u> <i>Pimephales promelas</i> Chronic	Rs # <u>757</u> <i>Raphidocelis subcapitata</i> Chronic		
Organism Age at Initiation	<24 hrs, all within an 8 hr window	<48 hrs, all within a 24 hour window	na		
Test Container Size	30 ml	800 ml	125 ml		
Test Volume	15 ml	500 ml	50 ml		
Feeding: Type and Amount	0.10 ml Algae and 0.10 ml YCT daily	0.15 ml <i>Artemia</i> , 2 x Daily	1 ml/L Nutrients, incl. EDTA, added		
Aeration:	<input checked="" type="checkbox"/> None <input type="checkbox"/> Prior to use	<input checked="" type="checkbox"/> None <input type="checkbox"/> Prior to use <input type="checkbox"/> @ _____ hrs	<input checked="" type="checkbox"/> None <input type="checkbox"/> Prior to use		
In Test Chambers via Slow Bubble :					
Acclimation Period	<24 hrs	<24 hrs	4 Days		
Organism Source	In-House	<u>Aquatox</u>	In-House		
Size	-	-	-		
Loading Rate	-	-	-		

Dissolved Oxygen aeration justifications (in test chambers):

Test(s):  All  \_\_\_\_\_  
Date:

Comments:

# Test Solution Preparation and Dilution Record

Client: City of Molalla

Note:  Indicates task not done,  Indicates task was done. Temp adj. = Temperature adjusted to ambient or test temp  
 Ditto marks ( ' ' ) indicate that the same SDG, batch of dilution water, or food as the previous day's entry was used.

## *Ceriodaphnia dubia* - Chronic

Test Concentration (%)	Sample Volume (mls)	Final Volume (mls)
Control	0.00 →	200
2.0	4.00 →	200
4.0	8.00 →	200
10	20.0 →	200
40	80.0 →	200
100	200 →	200

Total Sample volume needed per day = 312 mls

## Fathead minnow - Chronic

Test Concentration (%)	Sample Volume (mls)	Final Volume (mls)
Control	0.00 →	2000
2.0	40.0 →	2000
4.0	80.0 →	2000
10	200 →	2000
40	800 →	2000
100	2,000 →	2000

Total Sample volume needed per day = 3120 mls

## Algae - Chronic

Test Concentration (%)	Sample Volume (mls)	Final Volume (mls)
Control	0.00 →	500
2.0	10.0 →	500
4.0	20.0 →	500
10	50.0 →	500
40	200 →	500
100	500 →	500

Test Day	Sample ID Used	Daily Sample Preparation (prior to dilution)	Dilution Water Used	YCT ID Used	Algae ID Used	Date	Time	Initials
0 (Initiation)	B3459-01	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H303	# 1062	# 1073	12/15/2015	07:00	DW
1	B - 01	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H304	# 1062	# 1073	12/16/15	07:35	DW
2	B - 02	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H304	# 1062	# 1073	12/17/15	09:00	KS
3	B - 02	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H304	# 1072	# 1073	12/18/15	07:30	DW
4	B - 03	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H304	# 1072	# 1073	12/19/15	09:20	DW
5	B - 03	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H306	# 1072	# 1073	12/20/15	09:20	DW
6	B -	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID#	#	#	1/1"	:	:
7	B -	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID#	#	#	1/1"	:	:

Test Day	Sample ID Used	Daily Sample Preparation (prior to dilution)	Dilution Water Used	Date	Time	Initials
0 (Initiation)	B3459-01	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H303	12/15/2015	07:00	DW
1	B - 01	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H304	12/16/15	07:35	DW
2	B - 02	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H304	12/17/15	08:45	KT
3	B - 02	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H304	12/18/15	07:30	DW
4	B - 03	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H304	12/19/15	07:40	S
5	B - 03	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H306	12/20/15	09:20	DW
6	B - 03	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID# H306	12/21/15	07:00	DW

Test Day	Sample ID Used	Daily Sample Preparation (prior to dilution)	Dilution Water Used	Date	Time	Initials
0 (Initiation)	B3459-01	<input checked="" type="checkbox"/> Temperature adjusted <input type="checkbox"/> Aerated	ID# H300	12/19/2015	09:30	S

Nutrients added (incl. EDTA)  
 0.45 um filtered Lot# T50163  
 1.60 um filtered (WDOE clients only)

Total Sample volume needed = 630 mls



***Ceriodaphnia dubia***  
**Survival and Reproduction**  
**Test Data Summary**

Client City of Molalla Test Start Date 12-15-2015  
 Sample Description \_\_\_\_\_ Initial Sample ID# B 3459  
 Data summarized by D.W.

Percent or Concentration	Total Live Young Produced in First 3 Broods per Replicate										# Alive Adults	Total Live Young
	A	B	C	D	E	F	G	H	I	J		
Control	30	14	28	26	30	29	23	27	28	16	10	251
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
2.0%	30	17	31	25	30	32	32	33	27	16	10	273
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
4.0%	25	16	27	30	13	31	33	33	26	15	10	249
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
10%	29	18	21	31	31	27	34	32	24	13	10	260
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
40%	6	24	27	27	24	31	12	35	21	26	8	233
	AD? ✓	AD?	AD?	AD?	AD?	AD?	AD? ✓	AD?	AD?	AD?		
100%	10	16	8	13	7	12	17	18	20	10	10	131
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		

Test Organism Mortality (Adult dead) =  AD?  ✓ # of Alive Adults = Number of test organism alive at termination  
 Test Organism identified as Male =  AD?  M Total Live Young = Total neonates produced in first 3 broods  
 Test Organism Injured during test =  AD?  I

Footnote: As per EPA-600-4-91-002 and EPA-821-R-02-013, *Ceriodaphnia dubia* test should be terminated when 60% of the surviving control organisms have produced their third brood, or at the end of eight days, whichever occurs first.

Also as per EPA-821-R-02-013 (13.10.9.1), "In this three-brood test, offspring from fourth or higher broods should not be counted and should not be included in the total number of neonates produced during the test."

**CERIODAPHNIA CHRONIC SURVIVAL AND REPRODUCTION DATA**

Neo's obtained from  
Culture Board ID:  
Slot #:

A	B	C	D	E	F	G	H	I	J
G	G	G	G	G	I	I	I	I	H
20	30	31	51	59	25	32	35	43	39

Incubator Used: # 6  
Random Template  
Used: 6 conc# 19

Client City of Molalla

Test Initiation: Date: 12/15/2015 Time: 09:00

Sample Description \_\_\_\_\_ Initial Sample ID # B3459 -

Termination: Date: 12/21/2015 Time: 09:15

Technician Day 0 DW Day 1 DW Day 2 SW Day 3 MS Day 4 DW Day 5 DW Day 6 DW Day 7 \_\_\_\_\_ Day 8 \_\_\_\_\_

Time Day 0 0900 Day 1 0900 Day 2 1155 Day 3 1025 Day 4 1040 Day 5 1040 Day 6 0915 Day 7 \_\_\_\_\_ Day 8 \_\_\_\_\_

Cerio ID: Cd 3139

Percent	Day	Daily Number of Live Young for each Replicate										No. Live Adults	Daily Total Live Young
		A	B	C	D	E	F	G	H	I	J		
Control	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	4	0	0	0	5	5	5	6	5	0	10	30
	4	0	4	4	5	0	0	7	7	0	5	20	32
	5	12	10	11	10	13	10	0	0	11	11	10	88
	6	14	0	13	11	12	14	11	14	12	0	10	101
	7												
	8												
2.0%	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	5	4	5	4	4	5	4	0	10	31
	4	5	6	0	0	5	12	13	0	0	5	10	46
	5	10	11	12	10	6/0	0	0	13	11	11	10	84
	6	15	0	14	11	14	16	15	15	12	0	10	112
	7												
	8												
4.0%	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	4	4	0	5	4	5	5	0	10	27
	4	7	6	0	0	4	0	0	13	0	5	10	35
	5	6	10	9	11	9	12	12	0	9	10	10	88
	6	12	0	14	15	0	14	17	15	12	0	10	99
	7												
	8												
10%	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	3	5	5	4	5	5	4	0	10	31
	4	6	7	0	10	0	11	0	11	0	0	10	35
	5	9	11	7	0	11	0	12	0	11	0	10	61
	6	14	0	11	16	15	12	17	16	(13)	9	10	123 (110)
	7												
	8												
40%	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	4	0	0	5	4	5	2	0	10	20
	4	AD 6	4	0	4	5	11	6	0	0	4	10	40
	5		9	10	11	10	0	2/AD	11	7	9	8	69
	6		11	13	12	9	15		19	12	13	8	104
	7												
	8												
100%	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	0	0	0	0	0	0	0	0	10	0
	4	6	5	2	3	0	0	3	4	5	3	10	31
	5	0	11	6	6	5	5	8	7	8	7	10	63
	6	4	0	0	4	2	7	6	7	7	0	10	37
	7												
	8												

"AD" = Adult Dead, "AY" = Aborted young, "M" = male organism, "F" = Female, "R" = Adult releasing young, "/" = split brood ( carry-over brood / current day brood ),  
"Inj" = Adult Injured during test solution renewal, replicate removed from analysis. "AM" = Adult missing, remove from analysis. A circled neonate count = 4th brood

Footnote: As per EPA, C. dubia tests should be terminated when 60% of the surviving control organisms have produced their third brood, or at eight days, whichever occurs first.



CERIODAPHNIA WATER QUALITY DATA

Client: City of Molalla Initiated Date: 1/20 Time: 09:00 Adults Isolated Date: 12/14/2015 Time: 10:55  
 Sample Description: DW Initial Sample ID #: B3459 Day 3: DW Day 4: M Day 5: DW Day 6: DW Day 7: DW Day 8: DW  
 Tech: Day 0: DW Day 1: DW Day 2: DW Day 3: 08:50 Day 4: 10:25 Day 5: 10:40 Day 6: 09:15 Day 7: 09:15 Day 8: 09:15  
 Time: Day 0: 09:00 Day 1: 09:00 Day 2: 14:15 Day 3: 184 Day 4: 184 Day 5: 184 Day 6: 184 Day 7: 184 Day 8: 184  
 Therm. Day 0 #: 217 Day 1 #: 184 Day 2 #: 184 Day 3 #: 184 Day 4 #: 184 Day 5 #: 184 Day 6 #: 184 Day 7 #: 184 Day 8 #: 184

%	Dissolved Oxygen (mg/l)									pH									Temperature (°C) / Conductivity (µS) (1 <sup>st</sup> use of each sample only)								
	0	1	2	3	4	5	6	7	8	0	1	2	3	4	5	6	7	8	0	1	2	3	4	5	6	7	8
Control	8.2	7.2	8.2	7.6	7.9	7.8	7.8	7.8	7.8	7.8	7.9	7.9	7.7	7.8	7.8	7.9	7.9	7.9	24.8	24.9	25.3	25.0	24.1	24.7	25.4	25.4	24.9
2.0%	8.0	7.3	7.9	7.6	7.8	7.8	7.8	7.8	7.8	7.8	7.9	8.0	7.7	7.8	7.8	7.9	7.9	7.9	24.9	25.4	25.4	24.7	24.7	24.5	24.9	24.9	24.9
4.0%	8.2	7.4	8.1	7.9	7.9	7.8	7.8	7.8	7.8	7.9	7.9	8.0	7.9	7.9	7.8	7.9	7.8	7.8	24.9	25.1	25.2	24.3	24.5	25.1	25.5	24.9	24.9
10%	8.0	7.9	7.5	7.9	7.8	7.8	7.7	7.8	7.8	8.0	7.9	8.0	7.9	7.8	7.8	7.9	7.8	7.8	24.9	25.5	25.1	25.5	24.9	24.7	25.4	24.9	24.9
40%	8.0	7.9	7.1	7.7	7.9	7.9	7.9	7.8	7.8	7.9	7.7	7.7	7.9	7.8	7.8	7.8	7.8	7.8	24.8	25.8	24.7	24.7	24.5	24.9	25.1	24.9	24.9
100%	8.1	8.2	7.5	8.1	7.9	7.9	7.8	7.8	7.8	7.9	7.7	7.7	7.9	7.8	7.7	7.7	7.7	7.7	24.7	25.6	24.5	25.2	24.4	25.2	25.4	24.9	24.9
	8.0	7.1	7.8	7.8	7.5	8.1	8.1	8.1	8.1	7.8	7.6	7.7	7.4	7.5	7.5	7.5	7.5	7.5	24.1	23.9	23.9	23.7	23.7	23.7	23.7	23.7	23.7

COMMENTS: Temperatures taken just prior to test solution renewals. DO, pH, and Conductivity taken following organism transfer.

23.8

= Temp out of recom. range

Note: All Day 0 data represents conditions at initiation. All other days: numerator represents pre-renewal conditions, denominator represents post-renewal conditions.



# CETIS Summary Report

Report Date: 21 Dec-15 14:15 (p 1 of 2)  
 Test Code: B345901cdc | 10-6318-2911

## Ceriodaphnia 7-d Survival and Reproduction Test

CH2M HILL - ASL

<b>Batch ID:</b> 21-4633-9217	<b>Test Type:</b> Reproduction-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 15 Dec-15 09:00	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 21 Dec-15 09:15	<b>Species:</b> Ceriodaphnia dubia	<b>Brine:</b>
<b>Duration:</b> 6d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> <24h
<b>Sample ID:</b> 13-3002-0090	<b>Code:</b> B3459-01	<b>Client:</b>
<b>Sample Date:</b> 14 Dec-15 06:00	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 14 Dec-15 10:20	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 27h (3.6 °C)	<b>Station:</b>	

### Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-6366-6176	2d Survival Rate	100	>100	N/A	N/A	1	Fisher Exact/Bonferroni-Holm Test
08-5194-3947	6d Survival Rate	100	>100	N/A	N/A	1	Fisher Exact/Bonferroni-Holm Test
18-9938-5528	Reproduction	40	100	63.25	27.3%	2.5	Steel Many-One Rank Test

### Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
06-6315-0578	2d Survival Rate	EC50	>100	N/A	N/A	<1	Linear Interpolation (ICPIN)
16-3375-5926	Reproduction	IC25	55.61	19.05	66.48	1.798	Linear Interpolation (ICPIN)

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
16-3375-5926	Reproduction	Control Resp	25.1	15 - NL	Yes	Passes Acceptability Criteria ✓
18-9938-5528	Reproduction	Control Resp	25.1	15 - NL	Yes	Passes Acceptability Criteria ✓
18-9938-5528	Reproduction	PMSD	0.2732	0.13 - 0.47	Yes	Passes Acceptability Criteria ✓

### 2d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
2		10	1	1	1	1	1	0	0	0.0%	0.0%
4		10	1	1	1	1	1	0	0	0.0%	0.0%
10		10	1	1	1	1	1	0	0	0.0%	0.0%
40		10	1	1	1	1	1	0	0	0.0%	0.0%
100		10	1	1	1	1	1	0	0	0.0%	0.0%

### 6d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
2		10	1	1	1	1	1	0	0	0.0%	0.0%
4		10	1	1	1	1	1	0	0	0.0%	0.0%
10		10	1	1	1	1	1	0	0	0.0%	0.0%
40		10	0.8	0.6426	0.9574	0	1	0.1333	0.4216	52.7%	20.0%
100		10	1	1	1	1	1	0	0	0.0%	0.0%

### Reproduction Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	25.1	22.96	27.24	14	30	1.81	5.724	22.81%	0.0%
2		10	27.3	24.99	29.61	16	33	1.955	6.183	22.65%	-8.77%
4		10	24.9	22.06	27.74	13	33	2.401	7.593	30.49%	0.8%
10		10	26	23.44	28.56	13	34	2.165	6.848	26.34%	-3.59%
40		10	23.3	20.09	26.51	6	35	2.716	8.59	36.87%	7.17%
100		10	13.1	11.44	14.76	7	20	1.41	4.458	34.03%	47.81%

**CETIS Summary Report**

Report Date: 21 Dec-15 14:15 (p 2 of 2)  
 Test Code: B345901cdc | 10-6318-2911

**Ceriodaphnia 7-d Survival and Reproduction Test**

**CH2M HILL - ASL**

**2d Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	1	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
40		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1

**6d Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	1	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
40		0	1	1	1	1	1	0	1	1	1
100		1	1	1	1	1	1	1	1	1	1

**Reproduction Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	30	14	28	26	30	29	23	27	28	16
2		30	17	31	25	30	32	32	33	27	16
4		25	16	27	30	13	31	33	33	26	15
10		29	18	21	31	31	27	34	32	24	13
40		6	24	27	27	24	31	12	35	21	26
100		10	16	8	13	7	12	17	18	20	10

**CETIS Analytical Report**

Report Date: 21 Dec-15 14:15 (p 1 of 3)  
 Test Code: B345901cdc | 10-6318-2911

**Ceriodaphnia 7-d Survival and Reproduction Test** **CH2M HILL - ASL**

Analysis ID: 06-6315-0578      Endpoint: 2d Survival Rate      CETIS Version: CETISv1.8.1  
 Analyzed: 21 Dec-15 14:15      Analysis: Linear Interpolation (ICPIN)      Official Results: Yes

Batch ID: 21-4633-9217      Test Type: Reproduction-Survival (7d)      Analyst:  
 Start Date: 15 Dec-15 09:00      Protocol: EPA/821/R-02-013 (2002)      Diluent: Mod-Hard Synthetic Water  
 Ending Date: 21 Dec-15 09:15      Species: Ceriodaphnia dubia      Brine:  
 Duration: 6d 0h      Source: In-House Culture      Age: <24h

Sample ID: 13-3002-0090      Code: B3459-01      Client:  
 Sample Date: 14 Dec-15 06:00      Material: Unknown      Project:  
 Receive Date: 14 Dec-15 10:20      Source: Molalla, city of (OR0101514)  
 Sample Age: 27h (3.6 °C)      Station:

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.015E+09	200	Yes	Two-Point Interpolation

**Point Estimates**

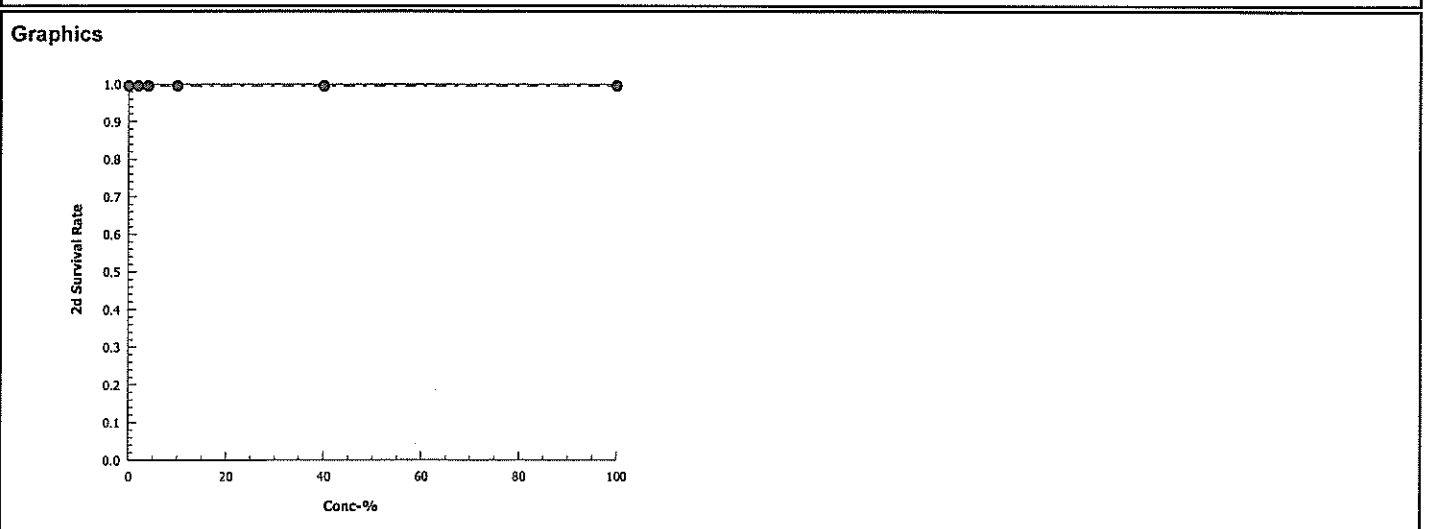
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC50	>100	N/A	N/A	<1	N/A	N/A

**2d Survival Rate Summary** **Calculated Variate(A/B)**

Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	10	1	1	1	0	0	0.0%	0.0%	10	10
2		10	1	1	1	0	0	0.0%	0.0%	10	10
4		10	1	1	1	0	0	0.0%	0.0%	10	10
10		10	1	1	1	0	0	0.0%	0.0%	10	10
40		10	1	1	1	0	0	0.0%	0.0%	10	10
100		10	1	1	1	0	0	0.0%	0.0%	10	10

**2d Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	1	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
40		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1



**CETIS Analytical Report**

Report Date: 21 Dec-15 14:15 (p 1 of 2)  
 Test Code: B345901cdc | 10-6318-2911

**Ceriodaphnia 7-d Survival and Reproduction Test** **CH2M HILL - ASL**

Analysis ID: 14-6366-6176      Endpoint: 2d Survival Rate      CETIS Version: CETISv1.8.1  
 Analyzed: 21 Dec-15 14:14      Analysis: STP 2x2 Contingency Tables      Official Results: Yes

Batch ID: 21-4633-9217      Test Type: Reproduction-Survival (7d)      Analyst:  
 Start Date: 15 Dec-15 09:00      Protocol: EPA/821/R-02-013 (2002)      Diluent: Mod-Hard Synthetic Water  
 Ending Date: 21 Dec-15 09:15      Species: Ceriodaphnia dubia      Brine:  
 Duration: 6d 0h      Source: In-House Culture      Age: <24h

Sample ID: 13-3002-0090      Code: B3459-01      Client:  
 Sample Date: 14 Dec-15 06:00      Material: Unknown      Project:  
 Receive Date: 14 Dec-15 10:20      Source: Molalla, city of (OR0101514)  
 Sample Age: 27h (3.6 °C)      Station:

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU
Untransformed		C > T	Not Run	100	>100	N/A	1

**Fisher Exact/Bonferroni-Holm Test**

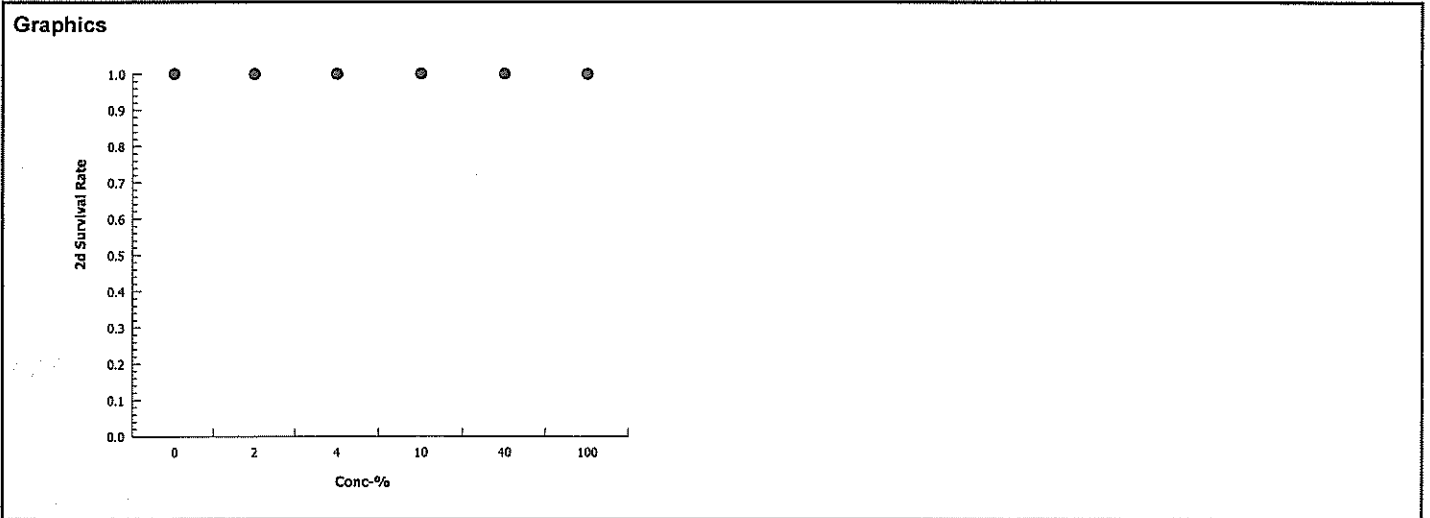
Control	vs	Conc-%	Test Stat	P-Value	Decision(0.05)
Dilution Water		2	1	1.0000	Non-Significant Effect
		4	1	1.0000	Non-Significant Effect
		10	1	1.0000	Non-Significant Effect
		40	1	1.0000	Non-Significant Effect
		100	1	1.0000	Non-Significant Effect

**Data Summary**

Conc-%	Control Type	No-Resp	Resp	Total
0	Dilution Water	10	0	10
2		10	0	10
4		10	0	10
10		10	0	10
40		10	0	10
100		10	0	10

**2d Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	1	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
40		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1



**CETIS Analytical Report**

Report Date: 21 Dec-15 14:15 (p 2 of 2)  
 Test Code: B345901cdc | 10-6318-2911

**Ceriodaphnia 7-d Survival and Reproduction Test** CH2M HILL - ASL

Analysis ID: 08-5194-3947      Endpoint: 6d Survival Rate      CETIS Version: CETISv1.8.1  
 Analyzed: 21 Dec-15 14:13      Analysis: STP 2x2 Contingency Tables      Official Results: Yes

Batch ID: 21-4633-9217      Test Type: Reproduction-Survival (7d)      Analyst:  
 Start Date: 15 Dec-15 09:00      Protocol: EPA/821/R-02-013 (2002)      Diluent: Mod-Hard Synthetic Water  
 Ending Date: 21 Dec-15 09:15      Species: Ceriodaphnia dubia      Brine:  
 Duration: 6d 0h      Source: In-House Culture      Age: <24h

Sample ID: 13-3002-0090      Code: B3459-01      Client:  
 Sample Date: 14 Dec-15 06:00      Material: Unknown      Project:  
 Receive Date: 14 Dec-15 10:20      Source: Molalla, city of (OR0101514)  
 Sample Age: 27h (3.6 °C)      Station:

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU
Untransformed		C > T	Not Run	100	>100	N/A	1

**Fisher Exact/Bonferroni-Holm Test**

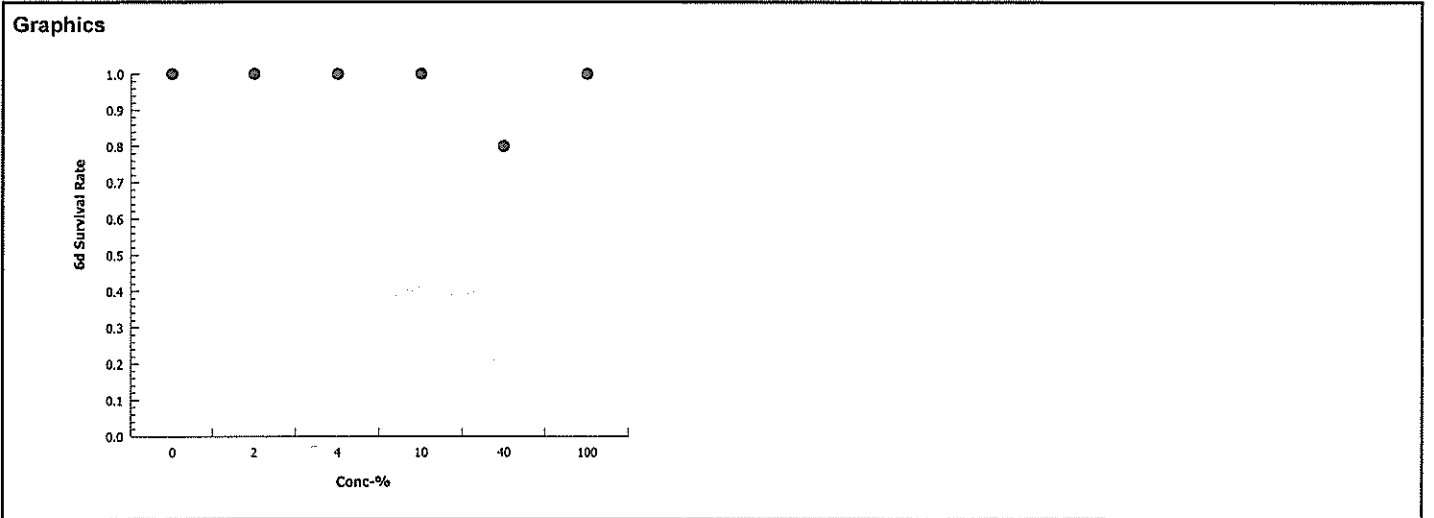
Control	vs	Conc-%	Test Stat	P-Value	Decision(0.05)
Dilution Water		2	1	1.0000	Non-Significant Effect
		4	1	1.0000	Non-Significant Effect
		10	1	1.0000	Non-Significant Effect
		40	0.2368	1.0000	Non-Significant Effect
		100	1	1.0000	Non-Significant Effect

**Data Summary**

Conc-%	Control Type	No-Resp	Resp	Total
0	Dilution Water	10	0	10
2		10	0	10
4		10	0	10
10		10	0	10
40		8	2	10
100		10	0	10

**6d Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	1	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
40		0	1	1	1	1	1	0	1	1	1
100		1	1	1	1	1	1	1	1	1	1



**CETIS Analytical Report**

Report Date: 21 Dec-15 14:15 (p 1 of 2)  
 Test Code: B345901cdc | 10-6318-2911

**Ceriodaphnia 7-d Survival and Reproduction Test** **CH2M HILL - ASL**

<b>Analysis ID:</b> 18-9938-5528	<b>Endpoint:</b> Reproduction	<b>CETIS Version:</b> CETISv1.8.1
<b>Analyzed:</b> 21 Dec-15 14:13	<b>Analysis:</b> Nonparametric-Control vs Treatments	<b>Official Results:</b> Yes

<b>Batch ID:</b> 21-4633-9217	<b>Test Type:</b> Reproduction-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 15 Dec-15 09:00	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 21 Dec-15 09:15	<b>Species:</b> Ceriodaphnia dubia	<b>Brine:</b>
<b>Duration:</b> 6d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> <24h

<b>Sample ID:</b> 13-3002-0090	<b>Code:</b> B3459-01	<b>Client:</b>
<b>Sample Date:</b> 14 Dec-15 06:00	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 14 Dec-15 10:20	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 27h (3.6 °C)	<b>Station:</b>	

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	0	C > T	Not Run	40	100	63.25	2.5	27.3%

**Steel Many-One Rank Test**

Control	vs Conc-%	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
Dilution Water	2	124	75	18	4	0.9965	Non-Significant Effect
	4	107.5	75	18	5	0.8837	Non-Significant Effect
	10	114	75	18	2	0.9629	Non-Significant Effect
	40	95.5	75	18	2	0.5455	Non-Significant Effect
	100*	62.5	75	18	1	0.0030	Significant Effect

**Auxiliary Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	0	2.7	3.2	0.3277	No Outliers Detected

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1331.283	266.2567	5	5.934	0.0002	Significant Effect
Error	2422.9	44.86852	54			
Total	3754.183	311.1252	59			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.281	15.09	0.5097	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9264	0.9459	0.0014	Non-normal Distribution

**Reproduction Summary**

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	25.1	22.92	27.28	14	30	1.81	5.724	22.81%	0.0%
2		10	27.3	24.95	29.65	16	33	1.955	6.183	22.65%	-8.77%
4		10	24.9	22.01	27.79	13	33	2.401	7.593	30.49%	0.8%
10		10	26	23.4	28.6	13	34	2.165	6.848	26.34%	-3.59%
40		10	23.3	20.03	26.57	6	35	2.716	8.59	36.87%	7.17%
100		10	13.1	11.4	14.8	7	20	1.41	4.458	34.03%	47.81%

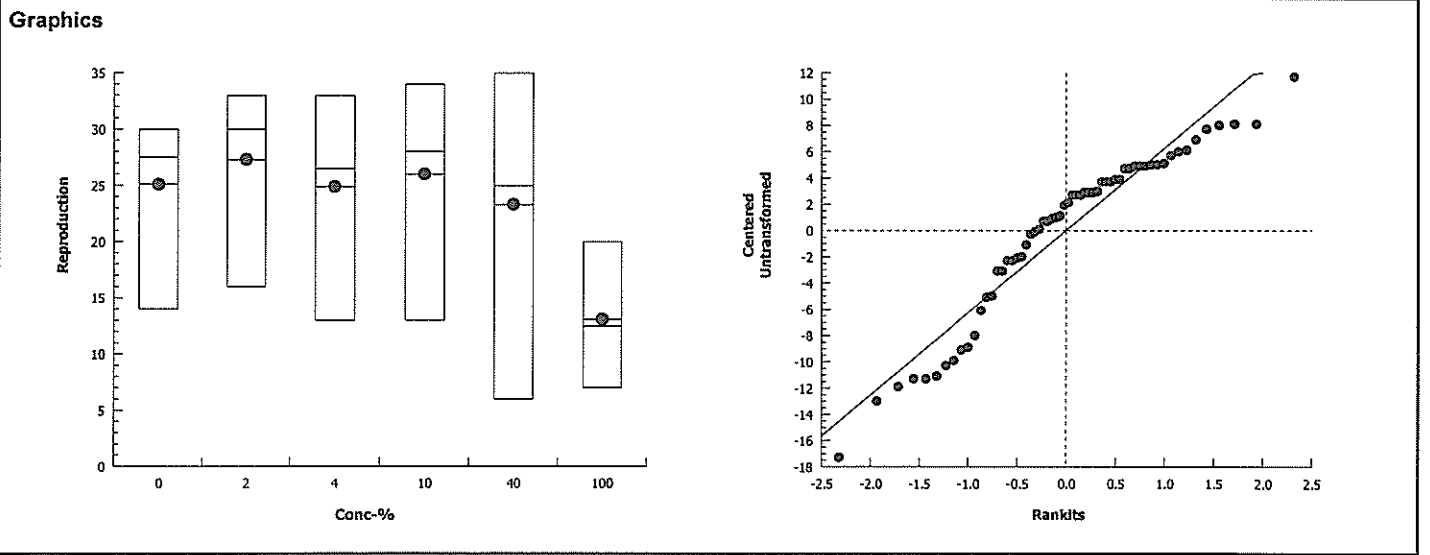
# CETIS Analytical Report

Report Date: 21 Dec-15 14:15 (p 2 of 2)  
 Test Code: B345901cdc | 10-6318-2911

**Ceriodaphnia 7-d Survival and Reproduction Test** **CH2M HILL - ASL**

Analysis ID: 18-9938-5528      Endpoint: Reproduction      CETIS Version: CETISv1.8.1  
 Analyzed: 21 Dec-15 14:13      Analysis: Nonparametric-Control vs Treatments      Official Results: Yes

Reproduction Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	30	14	28	26	30	29	23	27	28	16
2		30	17	31	25	30	32	32	33	27	16
4		25	16	27	30	13	31	33	33	26	15
10		29	18	21	31	31	27	34	32	24	13
40		6	24	27	27	24	31	12	35	21	26
100		10	16	8	13	7	12	17	18	20	10



**CETIS Analytical Report**

Report Date: 21 Dec-15 14:15 (p 2 of 3)  
 Test Code: B345901cdc | 10-6318-2911

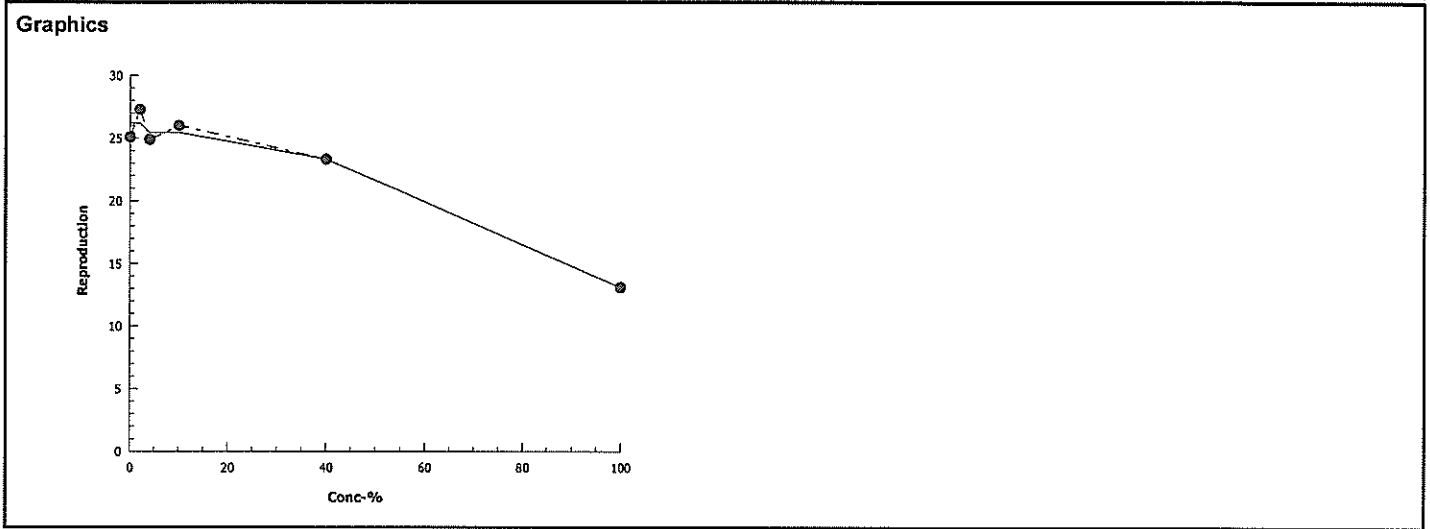
Ceriodaphnia 7-d Survival and Reproduction Test						CH2M HILL - ASL					
Analysis ID: 16-3375-5926		Endpoint: Reproduction		CETIS Version: CETISv1.8.1		Analyzed: 21 Dec-15 14:13		Analysis: Linear Interpolation (ICPIN)		Official Results: Yes	
Batch ID: 21-4633-9217		Test Type: Reproduction-Survival (7d)		Analyst:		Start Date: 15 Dec-15 09:00		Protocol: EPA/821/R-02-013 (2002)		Diluent: Mod-Hard Synthetic Water	
Ending Date: 21 Dec-15 09:15		Species: Ceriodaphnia dubia		Brine:		Duration: 6d 0h		Source: In-House Culture		Age: <24h	
Sample ID: 13-3002-0090		Code: B3459-01		Client:		Sample Date: 14 Dec-15 06:00		Material: Unknown		Project:	
Receive Date: 14 Dec-15 10:20		Source: Molalla, city of (OR0101514)				Sample Age: 27h (3.6 °C)		Station:			
Linear Interpolation Options											
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method						
Log(X+1)	Linear	1.064E+09	200	Yes	Two-Point Interpolation						
Residual Analysis											
Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)						
Extreme Value	Grubbs Extreme Value	2.7	3.2	0.3277	No Outliers Detected						
Point Estimates											
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL					
IC25	55.61	19.05	66.48	1.798	1.504	5.25					
Reproduction Summary			Calculated Variate								
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect		
0	Dilution Water	10	25.1	14	30	1.81	5.724	22.81%	0.0%		
2		10	27.3	16	33	1.955	6.183	22.65%	-8.77%		
4		10	24.9	13	33	2.401	7.593	30.49%	0.8%		
10		10	26	13	34	2.165	6.848	26.34%	-3.59%		
40		10	23.3	6	35	2.716	8.59	36.87%	7.17%		
100		10	13.1	7	20	1.41	4.458	34.03%	47.81%		
Reproduction Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	30	14	28	26	30	29	23	27	28	16
2		30	17	31	25	30	32	32	33	27	16
4		25	16	27	30	13	31	33	33	26	15
10		29	18	21	31	31	27	34	32	24	13
40		6	24	27	27	24	31	12	35	21	26
100		10	16	8	13	7	12	17	18	20	10



# CETIS Analytical Report

Report Date: 21 Dec-15 14:15 (p 3 of 3)  
Test Code: B345901cdc | 10-6318-2911

<b>Ceriodaphnia 7-d Survival and Reproduction Test</b>		<b>CH2M HILL - ASL</b>
<b>Analysis ID:</b> 16-3375-5926	<b>Endpoint:</b> Reproduction	<b>CETIS Version:</b> CETISv1.8.1
<b>Analyzed:</b> 21 Dec-15 14:13	<b>Analysis:</b> Linear Interpolation (ICPIN)	<b>Official Results:</b> Yes



Random Template Used: 6 conc. x 4 reps. # 11 Waterbath/incubator Used: \_\_\_\_\_ Date Initiated 12/15/2015 Time 14:20  
 Initial sample ID B 3459 # 4 Date Terminated 12/22/2015 Time 10:08

Client City of Molalla Sample Description \_\_\_\_\_

Tech: Day 0 MC Day 1 KS/DW Day 2 2 Day 3 2 Day 4 2 Day 5 MS Day 6 MS Day 7 KS/2  
 Time Day 0 1420 Day 1 1100 Day 2 1130 Day 3 1235 Day 4 1100 Day 5 1305 Day 6 1015 Day 7 1000

Conc. or Percent	Day	Number of Live Organisms				Dissolved O <sub>2</sub> (mg/l)		pH		Temp. (°C)	Therm. ID #	Conductivity (µS) Post (1 <sup>st</sup> use)
		A	B	C	D	Pre	Post	Pre	Post	Pre		
Control	0	10	10	10	10		7.9		8.3	Post: 24.5	177	301
	1	10	10	10	10	7.0	7.9	7.8	8.2	25.5	184	
	2	10	10	10	10	6.9	7.5	7.6	8.0	24.2	159	322
	3	10	10	10	10	7.1	7.9	7.5	8.0	24.8	159	
	4	10	10	10	10	7.3	7.7	7.5	8.0	25.0	277	324
	5	10	10	10	10	7.2	7.8	7.5	8.0	25.2	117	
	6	10	10	10	10	7.4	7.5	7.3	7.9	25.1	159	
	7	10	10	10	10	7.2		7.8		24.7	159	
2.0%	0	10	10	10	10		8.1		8.2	Post: 24.6		
	1	10	10	10	10	7.0	7.9	7.7	8.2	25.4		
	2	9	10	10	10	7.0	7.6	7.6	8.0	24.8		
	3	9	8 F	9 F	10	7.1	8.1	7.6	8.1	24.9		
	4	9	7	9	9 F	7.3	8.0	7.5	8.0	25.0		
	5	9	6 F	8 F	9 F	7.1	7.9	7.6	8.0	25.2		
	6	9	6	7 F	6 F	7.4	7.7	7.3	7.9	25.2		
	7	9	6	7	8	7.7		7.4		24.8		
4.0%	0	10	10	10	10		8.1		8.2	Post: 24.6		
	1	10	10	10	10	7.0	7.9	7.8	8.1	25.4		
	2	10	10	10	10	6.9	7.6	7.6	8.0	24.8		
	3	9 F	10	4	10	7.1	8.1	7.6	8.1	24.9		
	4	8 F	10	3	10	7.4	8.1	7.6	8.0	25.0		
	5	10 F	10	1 F	10	7.2	7.9	7.6	8.1	24.7		
	6	5 F	10	1	10	7.3	7.8	7.3	7.9	24.6		
	7	5	10	1	10	7.7		7.5		24.7		
10%	0	10	10	10	10		8.2		8.0	Post: 24.6		
	1	10	10	9	10	7.0	7.9	7.8	8.1	25.4		
	2	10	10	9	10	7.0	7.6	7.6	7.9	24.7		
	3	6 F	3 F	6 F	10	7.1	8.2	7.7	8.0	24.8		
	4	5	2 F	5 F	10	7.5	8.2	7.6	7.9	25.0		
	5	5	1 F	5	10	7.2	8.0	7.6	8.1	24.4		
	6	4	1	5	10	7.1	7.8	7.3	7.9	24.9		
	7	3	0	4	10	7.6		7.4		24.8		
40%	0	10	10	10	10		8.3		8.7.7	Post: 24.7		1260 µC 1265 µS
	1	10	10	10	9	7.0	8.1	7.8	7.7	25.4		
	2	10	10	10	9	6.9	7.8	7.6	7.4	24.8		
	3	10	10	10	9	7.2	8.3	7.6	7.6	24.9		
	4	10	10	10	9	7.6	8.2	7.6	7.6 MS	25.0		
	5	10	10	10	9	7.4	7.8	7.6	8.0 7.8	25.2		
	6	10	10	10	9	7.2	7.6	7.2	7.8	24.7		
	7	9	10	9	9	7.5		7.5		24.7		
100%	0	10	10	10	10		8.1		7.4	Post: 24.6		241
	1	10	10	10	10	7.0	8.0	7.7	7.5	25.3		
	2	10	10	10	10	6.9	8.3	7.5	7.0	25.0		237
	3	10	10	10	10	7.2	8.3	7.5	7.1	25.0		
	4	10	9	10	10	7.6	8.2	7.5	7.1	25.0		233
	5	10	9	10	10	7.5	8.2	7.4	7.2	25.4		
	6	10	8 F	10	10	6.9	7.9	7.2	7.2	25.1		
	7	10	8	10	10	7.0		7.4		24.7		

✓ Indicates one organism inadvertently poured off during solution renewal, replaced into container. Pre = Pre-renewal solutions. Post = Post-renewal solutions.  
 "M" = organism missing, start count reduced. "Inj" = organism injured, remove from stats. Day 0 Temperatures = Post-renewals  
 "F" = fungus noted on dead organisms. Therm ID# = Thermometer ID used for all measurements that day.  
 Aeration in test chambers begun @ \_\_\_\_\_ (Note observations on Test Organism Info sheet) 23.8 = Temp. out of recommended range

**FATHEAD MINNOW 7-DAY GROWTH DATA**

Client City of Molalla Tins Labeled As: MOLALLA (12/15)  
 Lab ID: B3459 Start Date: 12/15/2015

Sample Description: \_\_\_\_\_

Technician:	<u>MJO</u>	<u>KJ</u>
Date:	<u>1/5/2015</u>	<u>12/3/2015</u>
Balance Serial #:	<u>B328543647</u>	<u>B328543647</u>

Percent	Replicate	Total Weight (mg)	Tare Weight (mg)	No. of Fish
Control	A	1149.73	1142.60	10
	B	1139.06	1131.81	10
	C	1154.98	1148.23	10
	D	1154.70	1147.23	10
2.0 %	A	1151.48	1144.50	9
	B	1155.52	1149.83	6
	C	1169.64	1164.25	7
	D	1191.83	1185.08	8
4.0 %	A	1106.45	1102.26	5
	B	1160.13	1153.16	10
	C	1146.07	1145.05	1
	D	1147.40	1140.07	10
10 %	A	1149.02	1146.59	3
	B	1176.83	1176.83	0
	C	1143.59	1139.47	4
	D	1142.84	1134.75	10
40 %	A	1151.99	1146.65	9
	B	1146.37	1139.76	10
	C	1150.43	1145.14	9
	D	1157.31	1150.36	9
100 %	A	1138.88	1132.62	10
	B	1182.87	1177.15	8
	C	1135.57	1130.10	10
	D	1176.81	1170.68	10

weigh to 0.01 mg

## FATHEAD MINNOW 7-DAY GROWTH DATA

Client City of Molalla Tins Labeled As: MOLALLA (12/15)

Lab ID: \_\_\_\_\_ Start Date: 12/15/2015

Sample Description: \_\_\_\_\_

Technician: \_\_\_\_\_ KJ  
 Date: \_\_\_\_\_ 12/3/2015  
 Balance Serial #: B328543647 B328543647

Percent	Replicate	Total Weight (mg)	Tare Weight (mg)	No. of Fish
Control	A		1142.60	10
	B		1131.81	10
	C		1148.23	10
	D		1147.23	10
2.0 %	A		1144.50	9
	B		1149.83	6
	C		1164.25	7
	D		1185.08	8
4.0 %	A		1102.26	5
	B		1153.16	10
	C		1145.05	1
	D		1140.07	10
10 %	A		1146.65	3
	B		1139.76	0
	C		1145.14	4
	D		1150.36	10
40 %	A		1146.59	9
	B		1176.83	10
	C		1139.47	9
	D		1134.75	9
100 %	A		1132.62	10
	B		1177.15	8
	C		1130.10	10
	D		1170.68	10

weigh to 0.01 mg

*\* The 10% labeled tins were used for the 40% fish, the 40% labeled tins were used for the 10% fish.*

*1-15-16*

# CETIS Summary Report

Report Date: 08 Jan-16 16:18 (p 1 of 2)  
 Test Code: B345901ppc | 07-0235-7410

## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

<b>Batch ID:</b> 00-7718-9771	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 15 Dec-15 14:20	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 22 Dec-15 10:00	<b>Species:</b> Pimephales promelas	<b>Brine:</b>
<b>Duration:</b> 6d 20h	<b>Source:</b> Aquatox, AR	<b>Age:</b> <24h
<b>Sample ID:</b> 13-3002-0090	<b>Code:</b> B3459-01	<b>Client:</b>
<b>Sample Date:</b> 14 Dec-15 06:00	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 14 Dec-15 10:20	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 32h (3.6 °C)	<b>Station:</b>	

### Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-9060-2152	4d Survival Rate	100	>100 2	NA	28.7%	1	Steel Many-One Rank Sum Test
00-3616-0815	7d Survival Rate	100	>100 10	NA	41.5%	1	Dunnett Multiple Comparison Test
03-0064-3435	Mean Dry Biomass-mg	40	100	63.25	45.3%	2.5	Steel Many-One Rank Sum Test

### Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
09-8051-0880	4d Survival Rate	EC50	>100	N/A	N/A	<1	Linear Interpolation (ICPIN)
17-7539-2400	Mean Dry Biomass-mg	IC25	3.459	1.896	N/A	28.91	Linear Interpolation (ICPIN)

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-3616-0815	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
03-0064-3435	Mean Dry Biomass-mg	Control Resp	0.715	0.25 - NL	Yes	Passes Acceptability Criteria
17-7539-2400	Mean Dry Biomass-mg	Control Resp	0.715	0.25 - NL	Yes	Passes Acceptability Criteria
03-0064-3435	Mean Dry Biomass-mg	PMSD	0.4531	0.12 - 0.3	Yes	Above Acceptability Criteria

### 4d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
2		4	0.85	0.6909	1	0.7	0.9	0.05	0.1	11.76%	15.0%
4		4	0.775	0.2493	1	0.3	1	0.1652	0.3304	42.63%	22.5%
10		4	0.55	0.02225	1	0.2	1	0.1658	0.3317	60.3%	45.0%
40		4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	2.5%
100		4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	2.5%

### 7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
2		4	0.75	0.5446	0.9554	0.6	0.9	0.06455	0.1291	17.21%	25.0%
4		4	0.65	0	1	0.1	1	0.2179	0.4359	67.06%	35.0%
10		4	0.425	0	1	0	1	0.2097	0.4193	98.66%	57.5%
40		4	0.925	0.8454	1	0.9	1	0.025	0.05	5.41%	7.5%
100		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	5.0%

### Mean Dry Biomass-mg Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.715	0.667	0.763	0.675	0.747	0.01508	0.03015	4.22%	0.0%
2		4	0.6203	0.4961	0.7444	0.539	0.698	0.03902	0.07804	12.58%	13.25%
4		4	0.4877	0.02158	0.9539	0.102	0.733	0.1465	0.293	60.06%	31.78%
10		4	0.366	-0.1755	0.9075	0	0.809	0.1702	0.3403	92.98%	48.81%
40		4	0.6048	0.4683	0.7412	0.529	0.695	0.04287	0.08574	14.18%	15.42%
100		4	0.5895	0.5314	0.6476	0.547	0.626	0.01825	0.0365	6.19%	17.55%

# CETIS Summary Report

Report Date: 08 Jan-16 16:18 (p 2 of 2)  
 Test Code: B345901ppc | 07-0235-7410

## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

### 4d Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
2		0.9	0.7	0.9	0.9
4		0.8	1	0.3	1
10		0.5	0.2	0.5	1
40		1	1	1	0.9
100		1	0.9	1	1

### 7d Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
2		0.9	0.6	0.7	0.8
4		0.5	1	0.1	1
10		0.3	0	0.4	1
40		0.9	1	0.9	0.9
100		1	0.8	1	1

### Mean Dry Biomass-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.713	0.725	0.675	0.747
2		0.698	0.569	0.539	0.675
4		0.419	0.697	0.102	0.733
10		0.243	0	0.412	0.809
40		0.534	0.661	0.529	0.695
100		0.626	0.572	0.547	0.613

### 4d Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
2		9/10	7/10	9/10	9/10
4		8/10	10/10	3/10	10/10
10		5/10	2/10	5/10	10/10
40		10/10	10/10	10/10	9/10
100		10/10	9/10	10/10	10/10

### 7d Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
2		9/10	6/10	7/10	8/10
4		5/10	10/10	1/10	10/10
10		3/10	0/10	4/10	10/10
40		9/10	10/10	9/10	9/10
100		10/10	8/10	10/10	10/10

**CETIS Analytical Report**

Report Date: 08 Jan-16 16:18 (p 1 of 6)  
 Test Code: B345901ppc | 07-0235-7410

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

Analysis ID: 00-9060-2152	Endpoint: 4d Survival Rate	CETIS Version: CETISv1.8.1
Analyzed: 30 Dec-15 13:05	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes
Batch ID: 00-7718-9771	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 15 Dec-15 14:20	Protocol: EPA/821/R-02-013 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 22 Dec-15 10:00	Species: Pimephales promelas	Brine:
Duration: 6d 20h	Source: Aquatox, AR	Age: <24h
Sample ID: 13-3002-0090	Code: B3459-01	Client:
Sample Date: 14 Dec-15 06:00	Material: Unknown	Project:
Receive Date: 14 Dec-15 10:20	Source: Molalla, city of (OR0101514)	
Sample Age: 32h (3.6 °C)	Station:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	28.7%	100	>100	NA	1

**Steel Many-One Rank Sum Test**

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2*	10	10	0	6	0.0417		Significant Effect
		4	14	10	1	6	0.3451		Non-Significant Effect
		10	12	10	1	6	0.1424		Non-Significant Effect
		40	16	10	1	6	0.6105		Non-Significant Effect
		100	16	10	1	6	0.6105		Non-Significant Effect

**Auxiliary Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.607	2.802	0.1164	No Outliers Detected

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.8833552	0.176671	5	3.102	0.0342	Significant Effect
Error	1.02512	0.05695112	18			
Total	1.908475		23			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.777	4.248	0.1685	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8798	0.884	0.0082	Non-normal Distribution

**4d Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1	1	1	#Error	1	1	0	0.0%	0.0%
2		4	0.85	0.6909	1	#Error	0.7	0.9	0.05	11.76%	15.0%
4		4	0.775	0.2493	1	#Error	0.3	1	0.1652	42.63%	22.5%
10		4	0.55	0.02225	1	#Error	0.2	1	0.1658	60.3%	45.0%
40		4	0.975	0.8954	1	#Error	0.9	1	0.025	5.13%	2.5%
100		4	0.975	0.8954	1	#Error	0.9	1	0.025	5.13%	2.5%

**Angular (Corrected) Transformed Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.412	1.412	1.412	#Error	1.412	1.412	0	0.0%	0.0%
2		4	1.185	0.9794	1.39	#Error	0.9912	1.249	0.06447	10.89%	16.11%
4		4	1.128	0.503	1.752	#Error	0.5796	1.412	0.1963	34.82%	20.14%
10		4	0.8616	0.2298	1.493	#Error	0.4636	1.412	0.1985	46.08%	38.98%
40		4	1.371	1.242	1.501	#Error	1.249	1.412	0.04074	5.94%	2.89%
100		4	1.371	1.242	1.501	#Error	1.249	1.412	0.04074	5.94%	2.89%

# CETIS Analytical Report

Report Date: 08 Jan-16 16:18 (p 2 of 6)  
Test Code: B345901ppc | 07-0235-7410

## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

Analysis ID: 00-9060-2152      Endpoint: 4d Survival Rate      CETIS Version: CETISv1.8.1  
Analyzed: 30 Dec-15 13:05      Analysis: Nonparametric-Control vs Treatments      Official Results: Yes

### 4d Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
2		0.9	0.7	0.9	0.9
4		0.8	1	0.3	1
10		0.5	0.2	0.5	1
40		1	1	1	0.9
100		1	0.9	1	1

### Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.412
2		1.249	0.9912	1.249	1.249
4		1.107	1.412	0.5796	1.412
10		0.7854	0.4636	0.7854	1.412
40		1.412	1.412	1.412	1.249
100		1.412	1.249	1.412	1.412

### 4d Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	D				
2					
4					
10					
40					
100					



**CETIS Analytical Report**

Report Date: 08 Jan-16 16:18 (p 1 of 4)  
 Test Code: B345901ppc | 07-0235-7410

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

<b>Analysis ID:</b> 09-8051-0880	<b>Endpoint:</b> 4d Survival Rate	<b>CETIS Version:</b> CETISv1.8.1
<b>Analyzed:</b> 30 Dec-15 13:05	<b>Analysis:</b> Linear Interpolation (ICPIN)	<b>Official Results:</b> Yes
<b>Batch ID:</b> 00-7718-9771	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 15 Dec-15 14:20	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 22 Dec-15 10:00	<b>Species:</b> Pimephales promelas	<b>Brine:</b>
<b>Duration:</b> 6d 20h	<b>Source:</b> Aquatox, AR	<b>Age:</b> <24h
<b>Sample ID:</b> 13-3002-0090	<b>Code:</b> B3459-01	<b>Client:</b>
<b>Sample Date:</b> 14 Dec-15 06:00	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 14 Dec-15 10:20	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 32h (3.6 °C)	<b>Station:</b>	

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	158739362	200	Yes	Two-Point Interpolation

**Residual Analysis**

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.607	2.802	0.1164	No Outliers Detected

**Point Estimates**

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC50	>100	N/A	N/A	<1	NA	NA

**4d Survival Rate Summary**

C-%	Control Type	Count	Calculated Variate(A/B)								
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	40	40
2		4	0.85	0.7	0.9	0.05	0.1	11.76%	15.0%	34	40
4		4	0.775	0.3	1	0.1652	0.3304	42.63%	22.5%	31	40
10		4	0.55	0.2	1	0.1658	0.3317	60.3%	45.0%	22	40
40		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
100		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40

**4d Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
2		0.9	0.7	0.9	0.9
4		0.8	1	0.3	1
10		0.5	0.2	0.5	1
40		1	1	1	0.9
100		1	0.9	1	1

**4d Survival Rate Binomials**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
2		9/10	6/10	7/10	8/10
4		5/10	10/10	1/10	10/10
10		3/10	0/10	4/10	10/10
40		9/10	10/10	9/10	9/10
100		10/10	8/10	10/10	10/10

# CETIS Analytical Report

Report Date: 08 Jan-16 16:18 (p 2 of 4)  
Test Code: B345901ppc | 07-0235-7410

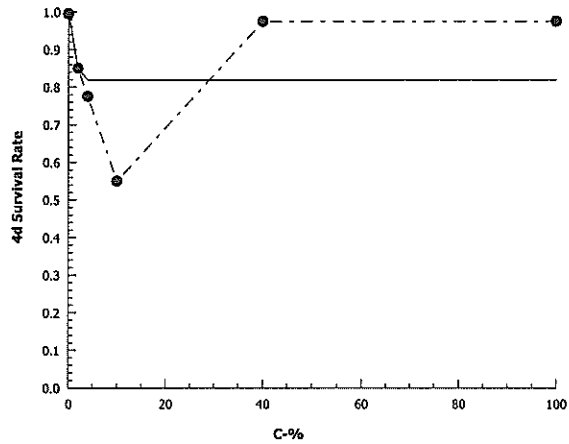
## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

Analysis ID: 09-8051-0880      Endpoint: 4d Survival Rate  
Analyzed: 30 Dec-15 13:05      Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.1  
Official Results: Yes

### Graphics



**CETIS Analytical Report**

Report Date: 08 Jan-16 16:18 (p 3 of 6)  
 Test Code: B345901ppc | 07-0235-7410

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

Analysis ID: 00-3616-0815	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.1
Analyzed: 30 Dec-15 13:06	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 00-7718-9771	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 15 Dec-15 14:20	Protocol: EPA/821/R-02-013 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 22 Dec-15 10:00	Species: Pimephales promelas	Brine:
Duration: 6d 20h	Source: Aquatox, AR	Age: <24h
Sample ID: 13-3002-0090	Code: B3459-01	Client:
Sample Date: 14 Dec-15 06:00	Material: Unknown	Project:
Receive Date: 14 Dec-15 10:20	Source: Molalla, city of (OR0101514)	
Sample Age: 32h (3.6 °C)	Station:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	41.5%	100	>100	NA	1

**Dunnett Multiple Comparison Test**

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	1.572	2.407	0.541	6	0.2055		Non-Significant Effect
		4	1.908	2.407	0.541	6	0.1217		Non-Significant Effect
		10*	3.127	2.407	0.541	6	0.0118		Significant Effect
		40	0.5434	2.407	0.541	6	0.6285		Non-Significant Effect
		100	0.3389	2.407	0.541	6	0.7149		Non-Significant Effect

**Auxiliary Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.499	2.802	0.1780	No Outliers Detected

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.40652	0.2813041	5	2.78	0.0496	Significant Effect
Error	1.82127	0.1011817	18			
Total	3.227791		23			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.421	4.248	0.0239	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9159	0.884	0.0474	Normal Distribution

**7d Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1	1	1	#Error	1	1	0	0.0%	0.0%
2		4	0.75	0.5446	0.9554	#Error	0.6	0.9	0.06455	17.21%	25.0%
4		4	0.65	0	1	#Error	0.1	1	0.2179	67.06%	35.0%
10		4	0.425	0	1	#Error	0	1	0.2097	98.66%	57.5%
40		4	0.925	0.8454	1	#Error	0.9	1	0.025	5.41%	7.5%
100		4	0.95	0.7909	1	#Error	0.8	1	0.05	10.53%	5.0%

**Angular (Corrected) Transformed Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.412	1.412	1.412	#Error	1.412	1.412	0	0.0%	0.0%
2		4	1.058	0.8102	1.306	#Error	0.8861	1.249	0.07796	14.73%	25.05%
4		4	0.9828	0.1386	1.827	#Error	0.3218	1.412	0.2653	53.98%	30.4%
10		4	0.7088	-0.1202	1.538	#Error	0.1588	1.412	0.2605	73.5%	49.8%
40		4	1.29	1.16	1.419	#Error	1.249	1.412	0.04074	6.32%	8.66%
100		4	1.336	1.093	1.578	#Error	1.107	1.412	0.07622	11.41%	5.4%

# CETIS Analytical Report

Report Date: 08 Jan-16 16:18 (p 4 of 6)  
 Test Code: B345901ppc | 07-0235-7410

## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

Analysis ID: 00-3616-0815      Endpoint: 7d Survival Rate      CETIS Version: CETISv1.8.1  
 Analyzed: 30 Dec-15 13:06      Analysis: Parametric-Control vs Treatments      Official Results: Yes

### 7d Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
2		0.9	0.6	0.7	0.8
4		0.5	1	0.1	1
10		0.3	0	0.4	1
40		0.9	1	0.9	0.9
100		1	0.8	1	1

### Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.412
2		1.249	0.8861	0.9912	1.107
4		0.7854	1.412	0.3218	1.412
10		0.5796	0.1588	0.6847	1.412
40		1.249	1.412	1.249	1.249
100		1.412	1.107	1.412	1.412

### 7d Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	D				
2					
4					
10					
40					
100					

# CETIS Analytical Report

Report Date: 08 Jan-16 16:18 (p 5 of 6)

Test Code: B345901ppc | 07-0235-7410

## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

<b>Analysis ID:</b> 03-0064-3435	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv1.8.1
<b>Analyzed:</b> 05 Jan-16 9:19	<b>Analysis:</b> Nonparametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 00-7718-9771	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 15 Dec-15 14:20	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 22 Dec-15 10:00	<b>Species:</b> Pimephales promelas	<b>Brine:</b>
<b>Duration:</b> 6d 20h	<b>Source:</b> Aquatox, AR	<b>Age:</b> <24h
<b>Sample ID:</b> 13-3002-0090	<b>Code:</b> B3459-01	<b>Client:</b>
<b>Sample Date:</b> 14 Dec-15 06:00	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 14 Dec-15 10:20	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 32h (3.6 °C)	<b>Station:</b>	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	45.3%	40	100	63.25	2.5

### Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	11.5	10	1	6	0.1083		Non-Significant Effect
		4	14	10	0	6	0.3451		Non-Significant Effect
		10	14	10	0	6	0.3451		Non-Significant Effect
		40	11	10	0	6	0.0805		Non-Significant Effect
		100*	10	10	0	6	0.0417		Significant Effect

### Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.631	2.802	0.1054	No Outliers Detected

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2931767	0.05863535	5	1.619	0.2057	Non-Significant Effect
Error	0.6519682	0.03622046	18			
Total	0.945145		23			

### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	22.15	15.09	0.0005	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9024	0.884	0.0243	Normal Distribution

### Mean Dry Biomass-mg Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.715	0.667	0.763	#Error	0.675	0.747	0.01508	4.22%	0.0%
2		4	0.6203	0.4961	0.7444	#Error	0.539	0.698	0.03902	12.58%	13.25%
4		4	0.4877	0.02158	0.9539	#Error	0.102	0.733	0.1465	60.06%	31.78%
10		4	0.366	-0.1755	0.9075	#Error	0	0.809	0.1702	92.98%	48.81%
40		4	0.6048	0.4683	0.7412	#Error	0.529	0.695	0.04287	14.18%	15.42%
100		4	0.5895	0.5314	0.6476	#Error	0.547	0.626	0.01825	6.19%	17.55%

### Mean Dry Biomass-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.713	0.725	0.675	0.747
2		0.698	0.569	0.539	0.675
4		0.419	0.697	0.102	0.733
10		0.243	0	0.412	0.809
40		0.534	0.661	0.529	0.695
100		0.626	0.572	0.547	0.613

# CETIS Analytical Report

Report Date: 08 Jan-16 16:18 (p 6 of 6)  
Test Code: B345901ppc | 07-0235-7410

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## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

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Analysis ID: 03-0064-3435	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.1
Analyzed: 05 Jan-16 9:19	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes



# CETIS Analytical Report

Report Date: 08 Jan-16 16:18 (p 4 of 4)  
Test Code: B345901ppc | 07-0235-7410

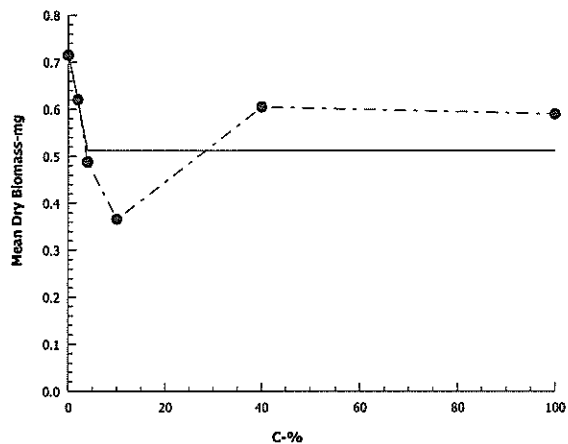
## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

Analysis ID: 17-7539-2400      Endpoint: Mean Dry Biomass-mg  
Analyzed: 05 Jan-16 9:19      Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.1  
Official Results: Yes

### Graphics





*Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*) TEST CONDITIONS

Client: City of Molalla

Sample Description: \_\_\_\_\_ Sample ID: B 3459 - 01

Test Start Date: 12/15/2015 Time: 10:45 Initials: [Signature]

Test End Date: 12/19/2015 Time: 10:00 Initials: [Signature]

**TEST CONDITIONS AT INITIATION:**

Species ID#: RS 793 Culturing period: 4 days Incubator ID#: 2

INOCULUM (Axenic Culture)		Culture Density = Mean # of Cells/Field x 250,000	
# Cells	# Fields		Culture Density
615	250		
MEAN	2.46	x 250,000 =	615,000

Filtration	0.45 um filter	X
	1.6 um filter	

Test Vol. / Replicate	50 mls
No. of Reps	4
Nutrient Dosage	1 ml per L

Test Start Density 10,000 x Test Volume 50 ml / Culture Density 615,000 = Inoculum Volume 0.81 ml

Confirming Counts			
	# Cells	# Fields	Cell Conc. x10 <sup>6</sup>
Flask #1	11	250	0.011
Flask #2	13	250	0.013
Flask #3	8	250	0.008

Light Intensity at Test Initiation (foot-candles)		
Location #1	Location #2	Location #3
390	393	401

Technician 0 hr [Signature] 24 hr [Signature] 48 hr [Signature] 72 hr [Signature] 96 hr [Signature]  
 Time 0 hr 10:45 24 hr 09:30 48 hr 08:50 72 hr 08:15 96 hr 10:00  
 Therm. ID# 0 hr # 213 24 hr # 156 48 hr # 213 72 hr # 213 96 hr # 215

Test Concn.	D.O. (mg/L)	pH					Temperature (°C)					Conductivity (umohs/cm)	Hard.	Alk.
		0 hr	24	48	72	96	0 hr	24	48	72	96			
Control	8.1	8.0	8.1	7.7	7.8	8.0	24.5	24.5	24.8	24.7	24.9	375	104	82
2.0 %	8.2	8.0	8.1	7.8	8.0	8.2	24.5	24.3	24.9	24.9	24.9	392	95	72
4.0 %	8.3	7.9	8.1	7.8	8.0	8.5	24.4	24.9	24.9	24.9	25.0	390		
10 %	8.2	7.8	8.1	7.8	8.2	8.7	24.4	24.9	24.9	24.9	25.0	384	99	71
40 %	8.1	7.7	8.1	7.8	8.3	8.8	24.4	24.8	24.9	24.9	25.0	358		
100 %	6.7	7.4	8.1	7.8	8.1	8.2	24.5	24.7	24.9	24.9	25.0	324	57	70

Comments: \_\_\_\_\_

(23.8) = Temp. out of recommended range

*Raphidocelis subcapitata* COULTER COUNTER WORKSHEET

Client City of Molalla

Sample Description \_\_\_\_\_

Lab ID B3459-01

Test Start Date 12/15/2015

Test Count Date 12/22/2015 Initials JW

Instrument Settings:

Aperture size 100 um  
 Aperture Kd value: 59.94

Set size 3.2 um

Gain: 256

Current: 1.414

Preamp Gain: 179.20

Dilution factor (DF) Default value: 51

Output: Concentration

Metered volume (ml): 0.5

Blank counts (use ISOTONE II):

Run	BLANK
DF	51
1	8692
2	5432
3	5266
4	5863
5	4012
6	3025

Note: Blank concentrations are Cells per ml ( not x 10<sup>6</sup>)

Dilutions: DF = 11 (1.0 ml sample to 10mL Isotone II)  
 DF = 51 (0.20 ml sample to 10mL Isotone II)  
 DF = 81 (0.25 ml sample to 20mL Isotone II)

Where: \* = Concentrations are in Cells per mL x 10<sup>6</sup>

TEST CONCENTRATION		Control		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				See Comments
1	2.575	2.826	2.375	
2	2.554	2.921	2.360	
3	2.561	2.933	2.345	
Replicate Average		2.563	2.893	2.360
CONCENTRATION MEAN # Cells / mL* =		2.606	CV % =	10.3%

TEST CONCENTRATION		2.0 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	2.823	2.678	2.574	3.260
2	2.393	2.624	2.552	3.258
3	2.364	2.672	2.584	3.250
Replicate Average		2.527	2.658	3.256
CONCENTRATION MEAN # Cells / mL* =		2.753	CV % =	12.4%

TEST CONCENTRATION		4.0 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	3.742	3.280	3.579	3.628
2	3.786	3.280	3.552	3.650
3	3.732	3.220	3.628	3.665
Replicate Average		3.753	3.260	3.586
CONCENTRATION MEAN # Cells / mL* =		3.562	CV % =	6.0%

TEST CONCENTRATION		10 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	3.342	2.941	3.318	3.240
2	3.334	3.922	3.280	3.230
3	3.350	3.018	3.324	3.261
Replicate Average		3.342	3.294	3.307
CONCENTRATION MEAN # Cells / mL* =		3.297	CV % =	1.2%

TEST CONCENTRATION		40 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	3.333	3.418	3.404	3.296
2	3.428	3.388	3.456	3.235
3	3.378	3.375	3.434	3.320
Replicate Average		3.380	3.394	3.431
CONCENTRATION MEAN # Cells / mL* =		3.372	CV % =	1.9%

TEST CONCENTRATION		100 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	1.182	1.140	1.116	1.157
2	1.175	1.153	1.123	1.163
3	1.143	1.178	1.126	1.172
Replicate Average		1.167	1.157	1.122
CONCENTRATION MEAN # Cells / mL* =		1.152	CV % =	1.8%

COMMENTS:  
 Control Rep D was not inoculated.  
 Control rep D will be removed for statistics.

*Raphidocelis subcapitata* COULTER COUNTER WORKSHEET

Client Molalla  
City of Molalla

TEST CONCENTRATION		Control			
Run	REP A	REP B	REP C	<del>REP D</del>	
DF (if other than default)				See Comment	
1	2.575	2.826	2.375	0.863	
2	2.554	2.921	2.360	0.854	
3	2.561	2.933	2.345	0.859	
Replicate Average					
CONCENTRATION MEAN # Cells / mL* =		CV% =			

Sample Description \_\_\_\_\_

Lab ID B3459-01

Test Start Date 12/15/2015

Test Count Date 12/22/2015 Initials JW

Instrument Settings:

Aperture size 100 um  
Aperture Kd value: 59.94

Set size 3.2 um

Gain: 256

Current: 1.414

Preamp Gain: 179.20

Dilution factor (DF) Default value: 51

Output: Concentration

Metered volume (ml): 0.5

Blank counts (use ISOTONE II):

Run	BLANK
DF	51
1	8692
2	5432
3	5286
4	5863
5	4012
6	3025

Note: Blank concentrations are Cells per ml ( not x 10<sup>6</sup>)

Dilutions: DF = 11 (1.0 ml sample to 10mL Isotone II)

DF = 51 (0.20 ml sample to 10mL Isotone II)

DF = 81 (0.25 ml sample to 20mL Isotone II)

Where: \* = Concentrations are in Cells per mL x 10<sup>6</sup>

TEST CONCENTRATION		2.0 %			
Run	REP A	REP B	REP C	REP D	
DF (if other than default)					
1	2.823	2.678	2.574	3.260	
2	2.393	2.642	2.552	3.258	
3	2.364	2.672	2.584	3.250	
Replicate Average					
CONCENTRATION MEAN # Cells / mL* =		CV% =			

TEST CONCENTRATION		4.0 %			
Run	REP A	REP B	REP C	REP D	
DF (if other than default)					
1	3.742	3.280	3.579	3.628	
2	3.786	3.280	3.552	3.650	
3	3.731	3.220	3.628	3.665	
Replicate Average					
CONCENTRATION MEAN # Cells / mL* =		CV% =			

TEST CONCENTRATION		10 %			
Run	REP A	REP B	REP C	REP D	
DF (if other than default)					
1	3.342	2.941	3.318	3.240	
2	3.334	2.922	3.328	3.230	
3	3.350	3.018	3.324	3.261	
Replicate Average					
CONCENTRATION MEAN # Cells / mL* =		CV% =			

TEST CONCENTRATION		40 %			
Run	REP A	REP B	REP C	REP D	
DF (if other than default)					
1	3.333	3.418	3.404	3.296	
2	3.428	3.388	3.456	3.235	
3	3.378	3.375	3.434	3.320	
Replicate Average					
CONCENTRATION MEAN # Cells / mL* =		CV% =			

TEST CONCENTRATION		100 %			
Run	REP A	REP B	REP C	REP D	
DF (if other than default)					
1	1.182	1.140	1.116	1.157	
2	1.175	1.153	1.123	1.163	
3	1.143	1.178	1.126	1.172	
Replicate Average					
CONCENTRATION MEAN # Cells / mL* =		CV% =			

COMMENTS: *\*\* If appears based on counts Control Rep D was not inoculated. Control rep D will be removed for statistics.*

**CETIS Summary Report**

Report Date: 22 Dec-15 14:54 (p 1 of 1)  
 Test Code: B345901rsc | 21-3592-1063

**Raphidocelis Growth Test**

**CH2M HILL - ASL**

Batch ID: 20-7591-2764	Test Type: Cell Growth	Analyst:
Start Date: 15 Dec-15 10:45	Protocol: EPA/821/R-02-013 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 19 Dec-15 10:00	Species: Raphidocelis subcapitata	Brine:
Duration: 95h	Source: In-House Culture	Age: 4d

Sample ID: 13-3002-0090	Code: B3459-01	Client:
Sample Date: 14 Dec-15 06:00	Material: Unknown	Project:
Receive Date: 14 Dec-15 10:20	Source: Molalla, city of (OR0101514)	
Sample Age: 29h (3.6 °C)	Station:	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-1721-6176	Cell Density	40	100	63.25	10.5%	2.5	Bonferroni Adj t Test

**Point Estimate Summary**

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
09-8858-3716	Cell Density	IC25	57.62	56.95	58.2	1.735	Linear Interpolation (ICPIN)

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-8858-3716	Cell Density	Control CV	0.1033	NL - 0.2	Yes	Passes Acceptability Criteria
17-1721-6176	Cell Density	Control CV	0.1033	NL - 0.2	Yes	Passes Acceptability Criteria
09-8858-3716	Cell Density	Control Resp	2.61E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
17-1721-6176	Cell Density	Control Resp	2.61E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
17-1721-6176	Cell Density	PMSD	0.105	0.091 - 0.29	Yes	Passes Acceptability Criteria

**Cell Density Summary**

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	3	2.606E+6	2.505E+6	2.706E+6	2.360E+6	2.893E+6	1.554E+5	2.692E+5	10.33%	0.0%
2		4	2.753E+6	2.626E+6	2.880E+6	2.527E+6	3.256E+6	1.700E+5	3.400E+5	12.35%	-5.65%
4		4	3.562E+6	3.482E+6	3.641E+6	3.260E+6	3.753E+6	1.064E+5	2.127E+5	5.97%	-36.7%
10		4	3.297E+6	3.281E+6	3.312E+6	3.244E+6	3.342E+6	2.039E+4	4.077E+4	1.24%	-26.52%
40		4	3.372E+6	3.349E+6	3.396E+6	3.284E+6	3.431E+6	3.143E+4	6.285E+4	1.86%	-29.42%
100		4	1.152E+6	1.145E+6	1.160E+6	1.122E+6	1.167E+6	1.042E+4	2.085E+4	1.81%	55.77%

**Cell Density Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	2.563E+6	2.893E+6	2.360E+6	
2		2.527E+6	2.658E+6	2.570E+6	3.256E+6
4		3.753E+6	3.260E+6	3.586E+6	3.648E+6
10		3.342E+6	3.294E+6	3.307E+6	3.244E+6
40		3.380E+6	3.394E+6	3.431E+6	3.284E+6
100		1.167E+6	1.157E+6	1.122E+6	1.164E+6

**CETIS Analytical Report**

Report Date: 22 Dec-15 14:54 (p 1 of 2)  
 Test Code: B345901rsc | 21-3592-1063

<b>Raphidocelis Growth Test</b>			<b>CH2M HILL - ASL</b>
Analysis ID: 17-1721-6176	Endpoint: Cell Density	CETIS Version: CETISv1.8.1	
Analyzed: 22 Dec-15 14:54	Analysis: Parametric-Multiple Comparison	Official Results: Yes	

Batch ID: 20-7591-2764	Test Type: Cell Growth	Analyst:
Start Date: 15 Dec-15 10:45	Protocol: EPA/821/R-02-013 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 19 Dec-15 10:00	Species: Raphidocelis subcapitata	Brine:
Duration: 95h	Source: In-House Culture	Age: 4d

Sample ID: 13-3002-0090	Code: B3459-01	Client:
Sample Date: 14 Dec-15 06:00	Material: Unknown	Project:
Receive Date: 14 Dec-15 10:20	Source: Molalla, city of (OR0101514)	
Sample Age: 29h (3.6 °C)	Station:	

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	0	C > T	Not Run	40	100	63.25	2.5	10.5%

Bonferroni Adj t Test								
Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Dilution Water	2		0.1825	2.583	4	292500	1.0000	Non-Significant Effect
	4		-9.028	2.583	5	273600	1.0000	Non-Significant Effect
	10		-6.525	2.583	5	273600	1.0000	Non-Significant Effect
	40		-7.237	2.583	5	273600	1.0000	Non-Significant Effect
	100*		13.72	2.583	5	273600	<0.0001	Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.571763E+13	3.143525E+12	5	163.4	<0.0001	Significant Effect
Error	3.077379E+11	19233620000	16			
Total	1.602536E+13	3.162759E+12	21			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	18.38	15.09	0.0025	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8969	0.8757	0.0258	Normal Distribution

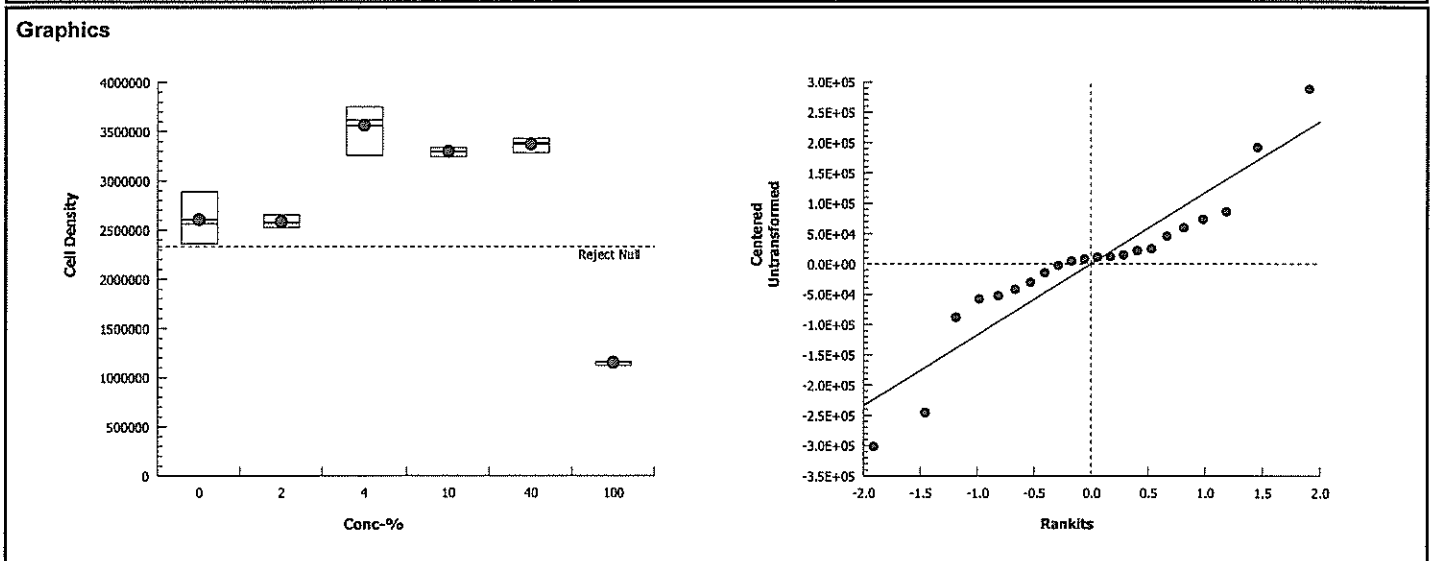
Cell Density Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	3	2.606E+6	2.503E+6	2.708E+6	2.360E+6	2.893E+6	1.554E+5	2.692E+5	10.33%	0.0%
2		3	2.585E+6	2.559E+6	2.610E+6	2.527E+6	2.658E+6	3.864E+4	6.692E+4	2.59%	0.79%
4		4	3.562E+6	3.481E+6	3.643E+6	3.260E+6	3.753E+6	1.064E+5	2.127E+5	5.97%	-36.7%
10		4	3.297E+6	3.281E+6	3.312E+6	3.244E+6	3.342E+6	2.039E+4	4.077E+4	1.24%	-26.52%
40		4	3.372E+6	3.348E+6	3.396E+6	3.284E+6	3.431E+6	3.143E+4	6.285E+4	1.86%	-29.42%
100		4	1.152E+6	1.144E+6	1.160E+6	1.122E+6	1.167E+6	1.042E+4	2.085E+4	1.81%	55.77%

# CETIS Analytical Report

Report Date: 22 Dec-15 14:54 (p 2 of 2)  
 Test Code: B345901rsc | 21-3592-1063

Raphidocelis Growth Test			CH2M HILL - ASL		
Analysis ID: 17-1721-6176	Endpoint: Cell Density	CETIS Version: CETISv1.8.1			
Analyzed: 22 Dec-15 14:54	Analysis: Parametric-Multiple Comparison	Official Results: Yes			

Cell Density Detail					
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	2.563E+6	2.893E+6	2.360E+6	
2		2.527E+6	2.658E+6	2.570E+6	Outlier
4		3.753E+6	3.260E+6	3.586E+6	3.648E+6
10		3.342E+6	3.294E+6	3.307E+6	3.244E+6
40		3.380E+6	3.394E+6	3.431E+6	3.284E+6
100		1.167E+6	1.157E+6	1.122E+6	1.164E+6



**CETIS Analytical Report**

Report Date: 22 Dec-15 14:54 (p 1 of 1)  
 Test Code: B345901rsc | 21-3592-1063

**Raphidocelis Growth Test** CH2M HILL - ASL

Analysis ID: 09-8858-3716      Endpoint: Cell Density      CETIS Version: CETISv1.8.1  
 Analyzed: 22 Dec-15 14:54      Analysis: Linear Interpolation (ICPIN)      Official Results: Yes

Batch ID: 20-7591-2764      Test Type: Cell Growth      Analyst:  
 Start Date: 15 Dec-15 10:45      Protocol: EPA/821/R-02-013 (2002)      Diluent: Mod-Hard Synthetic Water  
 Ending Date: 19 Dec-15 10:00      Species: Raphidocelis subcapitata      Brine:  
 Duration: 95h      Source: In-House Culture      Age: 4d

Sample ID: 13-3002-0090      Code: B3459-01      Client:  
 Sample Date: 14 Dec-15 06:00      Material: Unknown      Project:  
 Receive Date: 14 Dec-15 10:20      Source: Molalla, city of (OR0101514)  
 Sample Age: 29h (3.6 °C)      Station:

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	931651044	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	57.62	56.95	58.2	1.735	1.718	1.756

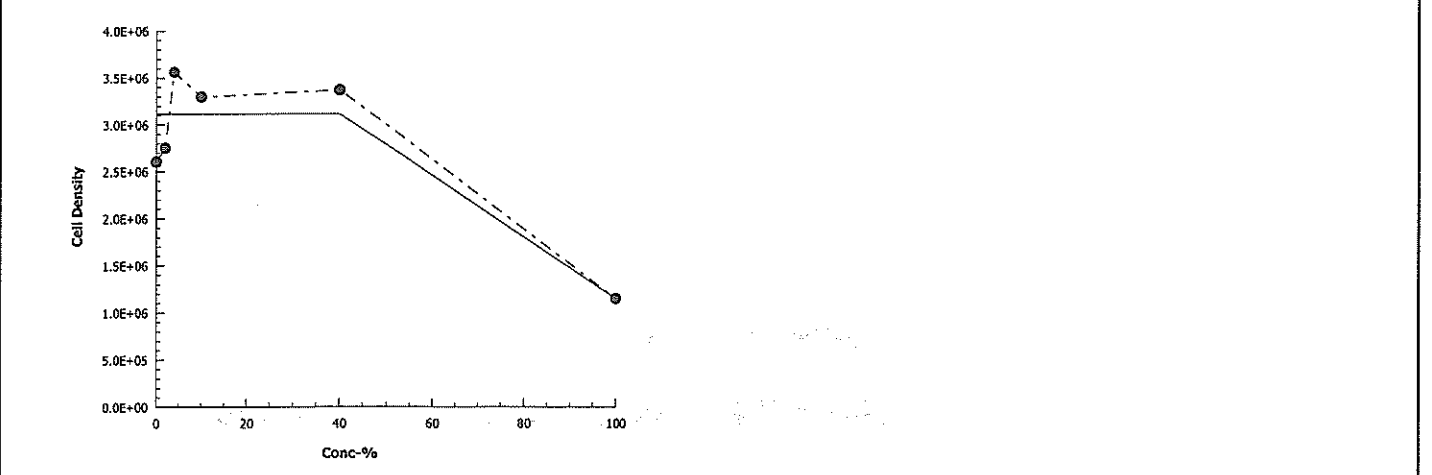
**Cell Density Summary** Calculated Variate

Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	3	2.606E+6	2.360E+6	2.893E+6	1.554E+5	2.692E+5	10.33%	0.0%
2		4	2.753E+6	2.527E+6	3.256E+6	1.700E+5	3.400E+5	12.35%	-5.65%
4		4	3.562E+6	3.260E+6	3.753E+6	1.064E+5	2.127E+5	5.97%	-36.7%
10		4	3.297E+6	3.244E+6	3.342E+6	2.039E+4	4.077E+4	1.24%	-26.52%
40		4	3.372E+6	3.284E+6	3.431E+6	3.143E+4	6.285E+4	1.86%	-29.42%
100		4	1.152E+6	1.122E+6	1.167E+6	1.042E+4	2.085E+4	1.81%	55.77%

**Cell Density Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	2.563E+6	2.893E+6	2.360E+6	
2		2.527E+6	2.658E+6	2.570E+6	3.256E+6
4		3.753E+6	3.260E+6	3.586E+6	3.648E+6
10		3.342E+6	3.294E+6	3.307E+6	3.244E+6
40		3.380E+6	3.394E+6	3.431E+6	3.284E+6
100		1.167E+6	1.157E+6	1.122E+6	1.164E+6

**Graphics**



**APPENDIX B**  
**REFERENCE TOXICANT DATA SHEETS**





***Ceriodaphnia dubia***  
**Survival and Reproduction**  
**Test Data Summary**

Client: QA/QC

Test Start Date 12-15-2015

Sample Description NaCl

Initial Sample ID# 2 B055-04X / 2B055-06

Data summarized by MS

Percent or Concentration	Total Live Young Produced in First 3 Broods per Replicate										# Alive Adults	Total Live Young
	A	B	C	D	E	F	G	H	I	J		
Control	26	27	26	25	25	27	24	26	25	12	9	238
	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input checked="" type="checkbox"/>		
0.25 g/L	24	28	27	26	31	29	28	25	31	26	10	275
	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>		
0.50 g/L	26	25	29	27	29	27	26	30	28	21	10	268
	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>		
1.0 g/L	10	10	16	15	15	14	11	12	12	13	10	132
	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>		
1.5 g/L	8	8	8	8	8	9	7	4	4	8	4	72
	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input type="checkbox"/>		
2.0 g/L	0	0	0	0	0	0	0	0	0	0	0	0
	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>		
4.0 g/L	0	0	0	0	0	0	0	0	0	0	0	0
	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>	AD? <input checked="" type="checkbox"/>		

Test Organism Mortality (Adult dead) =  AD?

# of Alive Adults = Number of test organism alive at termination

Test Organism identified as Male =  AD?  M

Total Live Young = Total neonates produced in first 3 broods

Test Organism Injured during test =  AD?  I

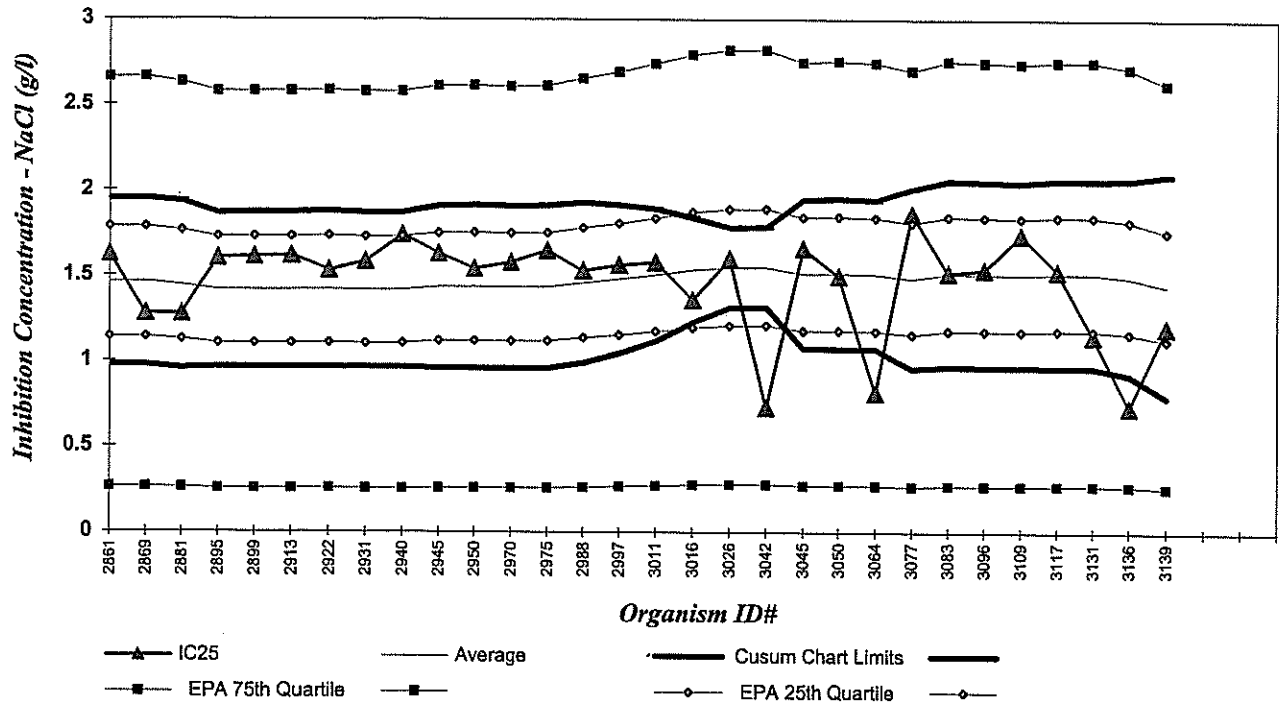
Footnote: As per EPA-600-4-91-002 and EPA-821-R-02-013, *Ceriodaphnia dubia* test should be terminated when 60% of the surviving control organisms have produced their third brood; or at the end of eight days, whichever occurs first.

Also as per EPA-821-R-02-013 (13.10.9.1), "In this three-brood test, offspring from fourth or higher broods should not be counted and should not be included in the total number of neonates produced during the test."

<u>Endpoint</u>	<u>IC25</u>	<u>Cusum Chart Limits</u>
Survival	<u>1.20</u>	<u>0.79 to 2.09</u>
Reproduction	<u>0.74</u>	<u>0.22 to 1.14</u>

Task Manager Karen Bresser  
 Project Manager Doug Dinn  
 QA Officer Singer Collins

**REFERENCE TOXICANT CUMULATIVE SUMMARY (CUSUM) CHART**  
*Ceriodaphnia dubia* Chronic Survival - IC25 Values



***Ceriodaphnia dubia* - Chronic (EPA Test Method 1002.0)**

**SODIUM CHLORIDE (g/L)**

Endpoint: Chronic Survival

Stats Method: Linear Interpolation

Test Conditions: Recon MH, 25 oC

From EPA 833-R-00-003:

10th Quartile CV (control limit) = 0.07

25th Quartile CV (warning limit) = 0.11

75th Quartile CV (warning limit) = 0.41

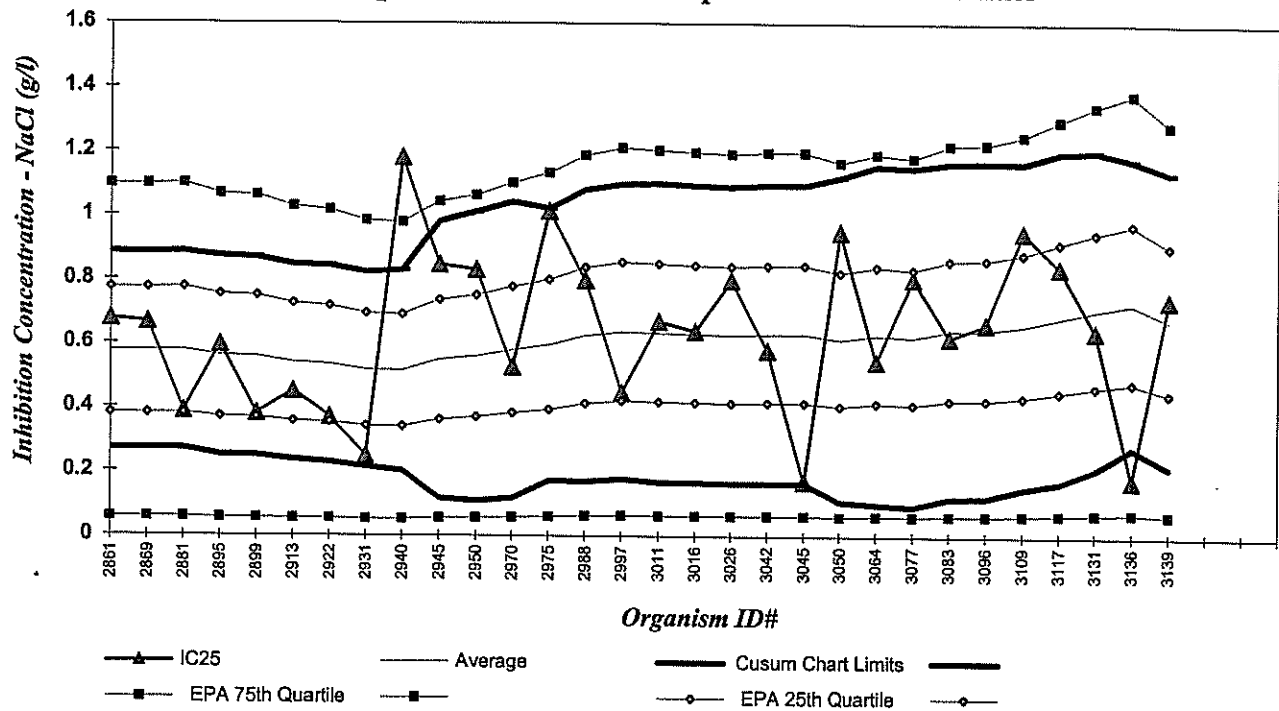
90th Quartile CV (control limit) = 0.81

*Intralab CV is compared to EPA Warning limits (25th and 75th CV's) and Control limits (10th and 90th CV's).*

*If lab CV is outside EPA Control limits, the EPA Control limits are used to set Cusum chart limits.*

Event #	Cerio ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
288	3064	05/12/15	0.82	1.51	0.22	1.07	1.94	0.18
289	3077	06/10/15	1.86	1.48	0.26	0.96	2.01	0.18
290	3083	07/07/15	1.52	1.51	0.27	0.97	2.06	0.18
291	3096	08/05/15	1.54	1.51	0.27	0.97	2.05	0.18
292	3109	09/09/15	1.74	1.51	0.27	0.97	2.05	0.18
293	3117	10/06/15	1.53	1.51	0.27	0.96	2.06	0.18
294	3131	11/17/15	1.15	1.51	0.27	0.96	2.06	0.19
295	3136	12/03/15	0.73	1.49	0.28	0.92	2.06	0.23
296	3139	12/15/15	1.20	1.44	0.32	0.79	2.09	0.23
297								
298								

**REFERENCE TOXICANT CUMULATIVE SUMMARY (CUSUM) CHART**  
*Ceriodaphnia dubia* Chronic Reproduction - IC25 Values



***Ceriodaphnia dubia* - Chronic (EPA Test Method 1002.0)**

**SODIUM CHLORIDE (g/L)**

Endpoint: Chronic Reproduction  
 Stats Method: Linear Interpolation  
 Test Conditions: Recon MH, 25 °C

**From EPA 833-R-00-003:**

10th Quartile CV (control limit) = 0.08  
 25th Quartile CV (warning limit) = 0.17  
 75th Quartile CV (warning limit) = 0.45  
 90th Quartile CV (control limit) = 0.62

*Intralab CV is compared to EPA Warning limits (25th and 75th CV's) and Control limits (10th and 90th CV's),*

*If lab CV is outside EPA Control limits, the EPA Control limits are used to set Cusum chart limits.*

Event #	Cerio ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
288	3064	5/12/2015	0.55	0.63	0.26	0.10	1.16	0.43
289	3077	6/10/2015	0.80	0.62	0.26	0.09	1.15	0.41
290	3083	7/7/2015	0.62	0.64	0.26	0.12	1.17	0.41
291	3096	8/5/2015	0.66	0.64	0.26	0.12	1.17	0.39
292	3109	9/9/2015	0.95	0.66	0.25	0.15	1.17	0.38
293	3117	10/6/2015	0.84	0.68	0.26	0.17	1.20	0.35
294	3131	11/17/2015	0.64	0.71	0.25	0.21	1.20	0.31
295	3136	12/3/2015	0.18	0.73	0.22	0.28	1.17	0.34
296	3139	12/15/2015	0.74	0.68	0.23	0.22	1.14	0.34
297								
298								

# BIOASSAY REFLOX AND CUSUM CHART EVALUATION FORM

Test Type: <i>Pronephroses Promelas</i> <input type="checkbox"/> Acute -OR- <input checked="" type="checkbox"/> Chronic Test Endpoint: <i>both Survival &amp; Growth</i> <input checked="" type="checkbox"/> Survival -OR- <input checked="" type="checkbox"/> Growth REFLOX test start date: <i>12-29-2015</i>	Analyst doing review: <i>Doug Wynn</i> Date: <i>12-23-15</i> Peer Reviewer: <i>B. Murray</i> Date: <i>1-6-16</i> Bioassay Section Lead: <i>B. Murray</i> Date: <i>1-6-16</i> QA Officer: _____ Date: _____
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	ASL limits within USEPA 25th Quartile	ASL limits between USEPA 25th Quartile and USEPA 75th Quartile	ASL limits outside of the USEPA 75th Quartile
<p><b>No USEPA Quartiles apply</b></p> <p><input type="checkbox"/> Cultured inhouse -OR-  <input type="checkbox"/> Reflux results from supplier available  <input type="checkbox"/> Test is Routine (10+ per year)  <input type="checkbox"/> Previous 19+ reflux tests in limits  <input type="checkbox"/> NOT subject to WDOE regulation</p> <p><b>IF ALL APPLY, reflux test need NOT be immediately repeated</b>                      Otherwise, REPEAT as soon as Practicable (preferably within same calendar month)</p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p><input type="checkbox"/> Cultured inhouse -OR-  <input type="checkbox"/> Reflux results from supplier available  <input type="checkbox"/> Test is Routine (10+ per year)  <input type="checkbox"/> Previous 9+ reflux tests in limits  <input type="checkbox"/> Cusum chart shows long term 95%+ of reflux tests being in range  <input type="checkbox"/> NOT subject to WDOE regulation</p> <p><b>IF ALL APPLY, reflux test need NOT be immediately repeated</b>                      Otherwise, REPEAT as soon as Practicable (preferably within same calendar month)</p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p><input type="checkbox"/> Cultured inhouse -OR-  <input type="checkbox"/> Reflux results from supplier available  <input checked="" type="checkbox"/> Test is Routine (10+ per year)  <input type="checkbox"/> Previous 19+ reflux tests in limits  <input type="checkbox"/> NOT subject to WDOE regulation</p> <p><b>IF ALL APPLY, reflux test need NOT be immediately repeated</b>                      Otherwise, REPEAT as soon as Practicable (preferably within same calendar month)</p> <p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p><b>REPEAT as soon as Practicable</b>                      (preferably within same calendar month)</p> <p>Does the reflux test need to be repeated?  <input checked="" type="checkbox"/> Yes</p>
<p><b>Root Cause Analysis:</b></p> <p>Correct Toxicant used? <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No                      Correct Water used? <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No                      Correct Temperature used? <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No                      DO, pH, Cond./Salinity OK? <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p> <p>If in disagreement with Analyst assessment, forward to Section Leader QA officer</p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p><b>Other Comments / Observations:</b></p> <p><i>- 2nd tested range.</i>  <i>Dec 29, 2015 test did meet cusum limits</i></p>		
<p><b>Peer Reviewer</b></p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p> <p>If in disagreement with Analyst assessment, forward to Section Leader QA officer</p> <p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p> <p>If in disagreement with Analyst assessment, forward to Section Leader QA officer</p> <p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>		
<p><b>QA Sect. Lead</b></p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>		

Random Template Used: 6 conc. x 4 reps. # 5  
 Stock Sol. ID 2B055-04X/2B055-06  
 Organism ID: FHM 1849

Waterbath/incubator Used: # 7

Date Initiated 12/15/2015 Time 14:00  
 Date Terminated 12/22/2015 Time 13:20

Test Container Size: 800 ml

Solution Volume / rep: 500 ml

Client: QA/QC - RefTox

Sample Description

NaCl (50 g/L stock)

Tech: Day 0 MC Day 1 K/DNA Day 2 e Day 3 e Day 4 e Day 5 MS Day 6 MS Day 7 KSLR  
 Time Day 0 1400 Day 1 1030 Day 2 1110 Day 3 1310 Day 4 1045 Day 5 1105 Day 6 0955 Day 7 1320

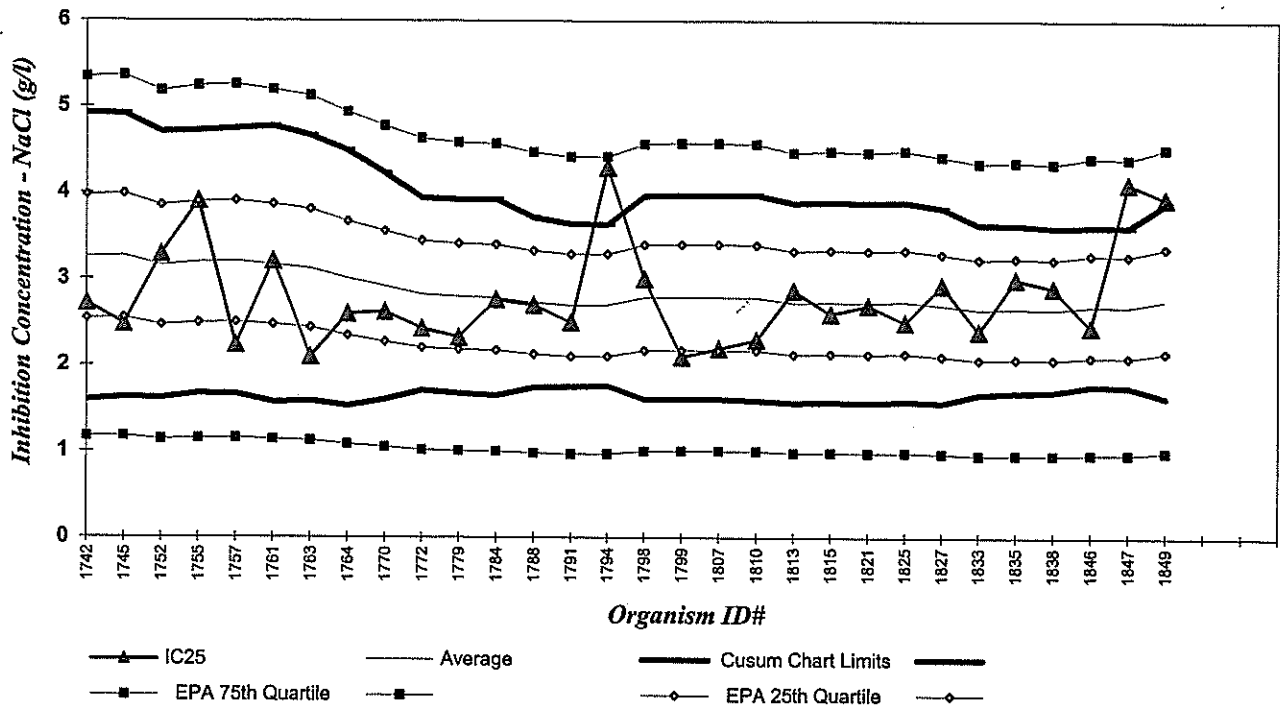
Conc. or Percent	Day	Number of Live Organisms				Dissolved O <sub>2</sub> (mg/l)		pH		Temp. (°C)	Therm ID #	Conductivity (µS)
		A	B	C	D	Pre	Post	Pre	Post			
Control	0	10	10	10	10		7.9		8.3	Post: 24.7	171	297
	1	10	10	10	10	8.0	8.1	7.6	8.2	24.9	184	328
	2	10	10	10	10	7.1	7.6	7.6	8.3	24.7	159	321
	3	10	10	10	10	7.2	7.8	7.6	8.2	24.6	159	321
	4	10	10	9	10	7.6	8.0	7.4	8.2	24.9	147	327
	5	10	10	9	10	7.9	8.0	7.3	8.2	25.0	177	330
	6	10	10	9	10	6.7	8.1	7.3	8.1	24.8	159	330
	7	10	10	9	10	6.7		7.4		24.5	159	
0.3 g/L	0	10	10	10	10		8.0		8.3	Post: 24.8		784
	1	10	10	10	10	8.0	8.1	8.8	8.3	25.1		828
	2	10	10	10	10	7.1	7.6	7.7	8.3	24.6		964
	3	10	10	10	10	7.3	7.8	7.8	8.2	24.6		913
	4	10	10	10	10	7.5	8.1	7.4	8.2	25.0		896
	5	10	10	10	10	7.8	8.0	7.4	8.2	25.1		890
	6	10	10	9	10	6.8	8.1	7.3	8.1	24.4		895
	7	10	10	9	9	6.7		7.4		24.3		
1.0 g/L	0	10	10	10	10		8.0		8.2	Post: 24.9		2200
	1	10	10	10	10	7.7	8.1	7.8	8.2	25.3		2050
	2	10	10	10	10	7.1	7.5	7.6	8.2	24.6		2180
	3	10	10	10	10	7.2	7.9	7.8	8.2	24.7		2120
	4	10	10	10	10	7.5	8.1	7.6	8.1	25.1		2180
	5	10	10	10	10	7.7	8.0	7.6	8.1	24.7		2210
	6	10	10	10	10	7.1	8.1	7.3	8.0	24.3		2210
	7	10	10	10	10	6.7		7.5		24.2		
2.0 g/L	0	10	10	10	10		8.0		8.1	Post: 24.9		3900
	1	10	10	10	10	7.7	8.1	7.9	8.2	25.1		3830
	2	10	10	10	10	7.1	7.5	7.7	8.2	24.7		3880
	3	10	10	10	10	7.2	7.9	7.8	8.2	24.7		3820
	4	10	10	10	10	7.5	8.0	7.6	8.1	25.0		3960
	5	10	10	10	10	7.6	8.0	7.6	8.1	24.8		4013
	6	10	10	10	10	7.2	8.1	7.5	8.0	24.4		4020
	7	10	10	9	10	6.7		7.5		24.4		
4.0 g/L	0	10	10	10	10		8.0		8.1	Post: 25.0		7120
	1	10	10	10	10	7.7	8.1	7.9	8.2	25.0		6720
	2	10	10	10	10	7.2	7.5	7.8	8.1	24.6		7240
	3	10	10	10	10	7.2	7.9	7.8	8.1	24.6		7220
	4	10	9	10	10	7.6	8.2	7.7	8.1	24.9		7140
	5	10	9	10	9	7.5	8.2	7.7	8.1	24.7		7220
	6	9	7	7	9	7.2	8.2	7.2	7.9	24.5		7140
	7	8	5	7	9	7.1		7.5		24.3		
8.0 g/L	0	10	10	10	10		8.0		8.0	Post: 25.0		12990
	1	9	9	10	10	7.7	8.1	7.9	8.1	25.3		13100
	2	0	0	0	0	7.2	7.5	7.7	8.0	24.7		12750
	3											
	4											
	5											
	6											
	7											

✓ Indicates one organism inadvertently poured off during solution renewal, replaced into container. Day 0 Temperatures = Post-renewals  
 "M" = organism missing, start count reduced. "Inj" = organism injured, remove from stats. Therm ID# = Thermometer ID used for all measurements that day.  
 "F" = fungus noted on dead organisms. Pre = Pre-renewal solutions. Post = Post-renewal solutions. 23.8 = Temp. out of recommended range

Endpoint IC25 Cusum Chart Limits  
 Survival - 3.937 \* 1.63 to 3.88  
 Growth 3.081 \* 1.907 to 3.080

Task Manager [Signature]  
 Project Manager [Signature]  
 QA Officer [Signature]

**REFERENCE TOXICANT CUMLATIVE SUMMARY (CUSUM) CHART**  
*Pimphales promelas* Chronic Survival - IC25 Values



***Pimphales promelas* - Chronic (EPA Test Method 1000.0)**

**SODIUM CHLORIDE (g/L)**

**From EPA 833-R-00-003:**

Endpoint: Chronic Survival

10th Quartile CV (control limit) = 0.03

Stats Method: Linear Interpolation

25th Quartile CV (warning limit) = 0.11

Test Conditions: Recon MH, 25 oC

75th Quartile CV (warning limit) = 0.32

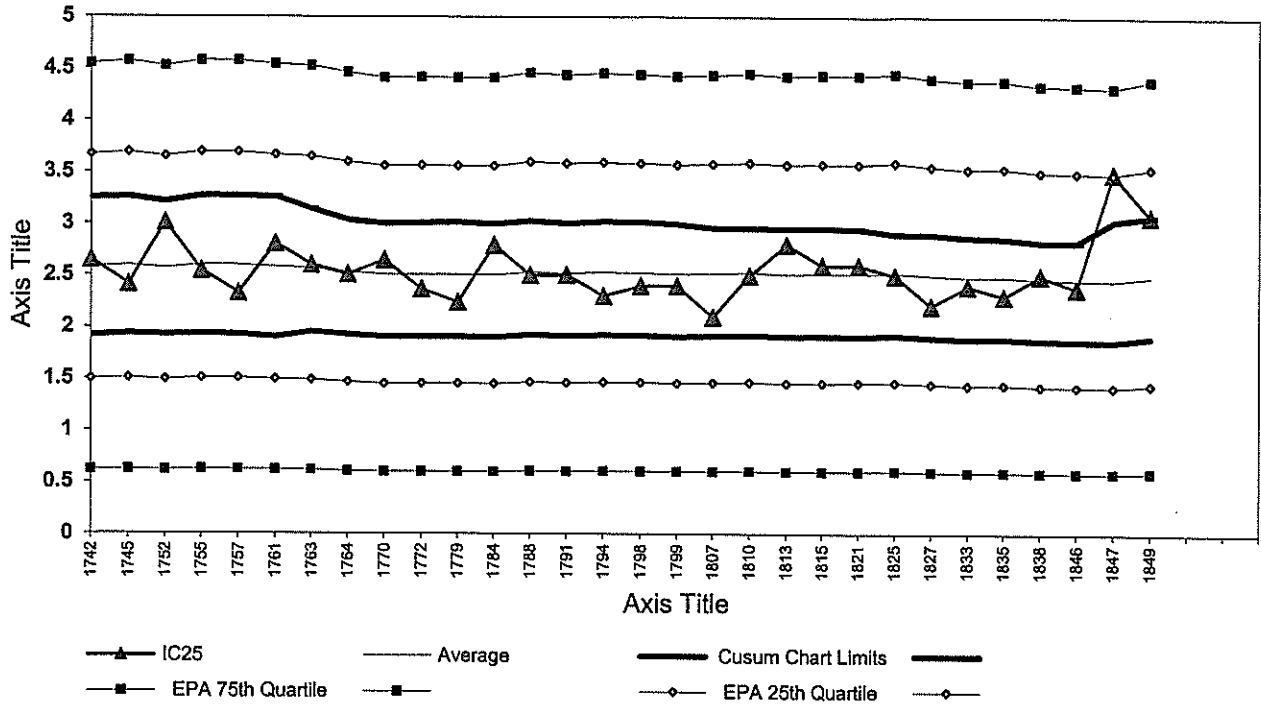
90th Quartile CV (control limit) = 0.52

*Intralab CV is compared to EPA Warning limits (25th and 75th CV's) and Control limits (10th and 90th CV's).*

*If lab CV is outside EPA Control limits, the EPA Control limits are used to set Cusum chart limits.*

Event #	FHM ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
316	1810	03/10/15	2.3	2.8	0.60	1.6	4.0	0.21
317	1813	03/31/15	2.9	2.7	0.58	1.6	3.9	0.21
318	1815	04/07/15	2.6	2.7	0.58	1.6	3.9	0.21
319	1821	05/19/15	2.7	2.7	0.58	1.6	3.9	0.21
320	1825	06/23/15	2.5	2.7	0.58	1.6	3.9	0.21
321	1827	07/07/15	2.9	2.7	0.57	1.6	3.8	0.19
322	1833	08/18/15	2.4	2.6	0.49	1.7	3.6	0.18
323	1835	09/15/15	3.0	2.7	0.49	1.7	3.6	0.18
324	1838	10/06/15	2.9	2.6	0.48	1.7	3.6	0.17
325	1846	11/17/15	2.4	2.7	0.46	1.8	3.6	0.17
326	1847	12/01/15	4.1	2.7	0.46	1.8	3.6	0.20
327	1849	12/15/15	3.937	2.8	0.56	1.63	3.88	0.22
328								

**REFERENCE TOXICANT CUMULATIVE SUMMARY (CUSUM) CHART**  
*Pimephales promelas* Chronic Biomass - IC25 Values



***Pimephales promelas* - Chronic (EPA Test Method 1000.0)**

**SODIUM CHLORIDE (g/L)**

Endpoint: Chronic Growth (Biomass)

Stats Method: Linear Interpolation

Test Conditions: Recon MH, 25 oC

**From EPA 833-R-00-003:**

10th Quartile CV (control limit) = 0.12

25th Quartile CV (warning limit) = 0.21

75th Quartile CV (warning limit) = 0.38

90th Quartile CV (control limit) = 0.45

*Intralab CV is compared to EPA Warning limits (25th and 75th CV's) and Control limits (10th and 90th CV's).*

*If lab CV is outside EPA Control limits, the EPA Control limits are used to set Cusum chart limits.*

Event #	FHM ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
318	1815	4/7/2015	2.6	2.5	0.22	1.9	3.0	0.09
319	1821	5/19/2015	2.6	2.5	0.22	1.9	3.0	0.09
320	1825	6/23/2015	2.5	2.5	0.22	1.9	2.9	0.07
321	1827	7/7/2015	2.2	2.5	0.19	1.9	2.9	0.08
322	1833	8/18/2015	2.4	2.5	0.20	1.9	2.9	0.08
323	1835	9/15/2015	2.3	2.5	0.19	1.9	2.9	0.07
324	1838	10/6/2015	2.5	2.5	0.18	1.9	2.8	0.07
325	1846	11/17/2015	2.4	2.5	0.18	1.9	2.8	0.07
326	1847	12/1/2015	3.5	2.5	0.18	1.9	3.0	0.12
327	1849	12/15/2015	3.081	2.5	0.29	1.907	3.080	0.13
328								
329								
330								

Random Template Used: 6 conc. x 4 reps. # 11 Waterbath/incubator Used: \_\_\_\_\_ Date Initiated 12/29/2015 Time 11:20  
 Stock Sol. ID 2B 055 - 06 # 4 Date Terminated 1/5/2016 Time 08:50  
 Organism ID: FHM 1851 Test Container Size: 800 ml Solution Volume / rep: 500 ml

Client: QA/QC - RefTox Sample Description: \_\_\_\_\_ NaCl (50 g/L stock): \_\_\_\_\_

Tech: Day 0 R Day 1 MC Day 2 R Day 3 MC Day 4 MC Day 5 MC Day 6 3m Day 7 MC  
 Time Day 0 1120 Day 1 0950 Day 2 1045 Day 3 1000 Day 4 1235 Day 5 1320 Day 6 165 Day 7 0850

Conc. or Percent	Day	Number of Live Organisms				Dissolved O <sub>2</sub> (mg/l)		pH		Temp. (°C)	Therm ID #	Conductivity (µS)
		A	B	C	D	Pre	Post	Pre	Post			
Control	0	10	10	10	10		7.8		7.7	Post: 25.1	177	310
	1	10	10	9	10	6.8	8.0	7.2	8.4	24.7	177	311
	2	10	10	9	9	6.4	7.6	7.4	8.4	25.5	172	318
	3	10	10	9	9	7.4	7.9	7.2	8.4	24.5	159	317
	4	10	10	9	9	7.0	8.0	7.4	8.4	24.4	177	320
	5	10	10 ✓	9	9	6.9	7.8	7.4	8.4	24.6	177	326
	6	10	10	9	9	7.0	7.7	7.5	8.4	24.3	189	319
	7	10	10	9	9	6.9		7.3		24.8	159	
0.3 g/L	0	10	10	10	10		7.7		7.8	Post: 25.2		866
	1	10	10	10	10	6.9	7.9	7.2	8.3	24.8		862
	2	9	9	9	9	6.5	7.9	7.5	8.4	25.5		871
	3	9	9	9	9	7.0	7.9	7.2	8.3	24.5		866
	4	9	9	9	9	7.1	8.0	7.4	8.4	24.6		871
	5	9	9	9	9	6.9	7.9	7.5	8.5	24.6		860
	6	9	9	9	9 ✓	7.0	7.8	7.5	8.3	24.3		889
	7	9	9	9	9	6.8		7.4		24.9		
1.0 g/L	0	10	10	10	10		7.7		7.9	Post: 25.3		2200
	1	10	10	10	10	6.9	7.9	7.5	8.3	24.7		2150
	2	10	10	10	7	6.4	7.9	7.5	8.3	25.4		2220
	3	10	10	10	7	7.0	8.0	7.3	8.3	24.4		2170
	4	10	10	10	7	7.3	8.0	7.5	8.3	24.5		2160
	5	10	10	10	7	7.0	7.8	7.5	8.4	24.9		2160
	6	10	10	10	7	6.7	8.0	7.5	8.3	24.4		2100
	7	10	10	10	7	6.7		7.4		24.6		
2.0 g/L	0	10	10	10	10		7.7		8.0	Post: 25.3		3960
	1	10	10	10	10	7.1	7.8	7.4	8.3	24.5		3940
	2	10	10	9	10	6.6	7.9	7.5	8.4	25.5		3980
	3	10	9	9	10	7.0	8.0	7.3	8.3	24.5		3980
	4	10	9	9	9	7.2	8.0	7.5	8.2	24.4		3910
	5	10	9	9	9	6.8	7.9	7.6	8.3	24.8		3930
	6	9	9	9	8	6.6	8.2	7.5	8.3	24.4		3820
	7	9	9	6	8	6.6		7.4		24.6		
4.0 g/L	0	10	10	10	10		7.7		8.3	Post: 25.5		7410
	1	10	9	10	9	6.9	7.8	7.5	8.3	24.5		7380
	2	10	6	10	8	6.6	7.9	7.6	8.4	25.4		7360
	3	10	5	8	7	7.1	8.1	7.5	8.2	24.5		7360
	4	7	3	6	7	7.3	8.0	7.6	8.1	24.6		7400
	5	3	2	5	6	6.9	7.8	7.6	8.2	24.8		7330
	6	1	2	5	4	6.8	7.9	7.6	8.2	24.4		7230
	7	1	2	4	4	6.8		7.6		24.8		
8.0 g/L	0	10	10	10	10		7.7		8.3	Post: 25.5		12878
	1	3	3	3	4	7.0	7.8	7.7	8.2	24.6		13300
	2	0	0	0	0	6.7	8.0	7.5	8.3	25.3		13050
	3											
	4											
	5											
	6											
	7											

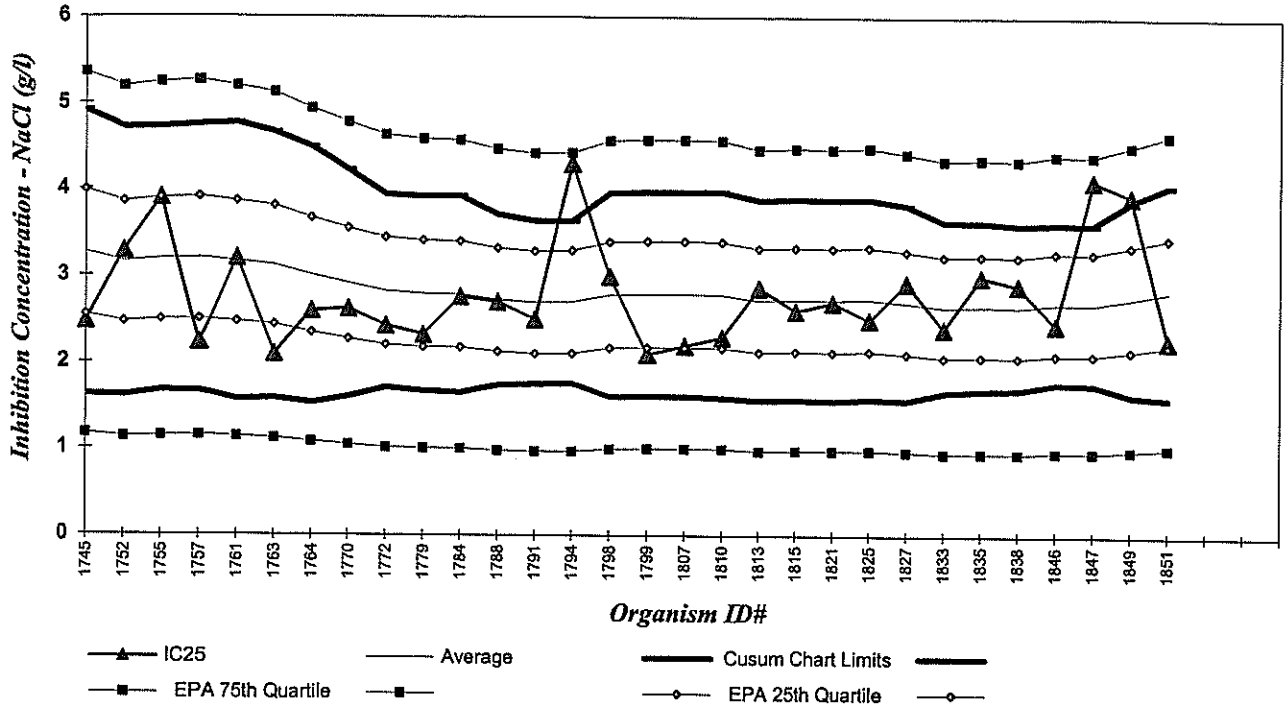
✓ Indicates one organism inadvertently poured off during solution renewal, replaced into container. Day 0 Temperatures = Post-renewals  
 "M" = organism missing, start count reduced; "Inj" = organism injured, remove from stats. Therm ID# = Thermometer ID used for all measurements that day.  
 "F" = fungus noted on dead organisms. Pre = Pre-renewal solutions. Post = Post-renewal solutions. (23.8) = Temp. out of recommended range

**Endpoint IC25 Cusum Chart Limits**  
 Survival: 2.3 to 4.1  
 Growth: 2.2 to 3.2

Task Manager: \_\_\_\_\_  
 Project Manager: \_\_\_\_\_  
 QA Officer: Winger Collins



**REFERENCE TOXICANT CUMLATIVE SUMMARY (CUSUM) CHART**  
*Pimphales promelas* Chronic Survival - IC25 Values



***Pimephales promelas* - Chronic (EPA Test Method 1000.0)**

**SODIUM CHLORIDE (g/L)**

Endpoint: Chronic Survival

Stats Method: Linear Interpolation

Test Conditions: Recon MH, 25 oC

From EPA 833-R-00-003:

10th Quartile CV (control limit) = 0.03

25th Quartile CV (warning limit) = 0.11

75th Quartile CV (warning limit) = 0.32

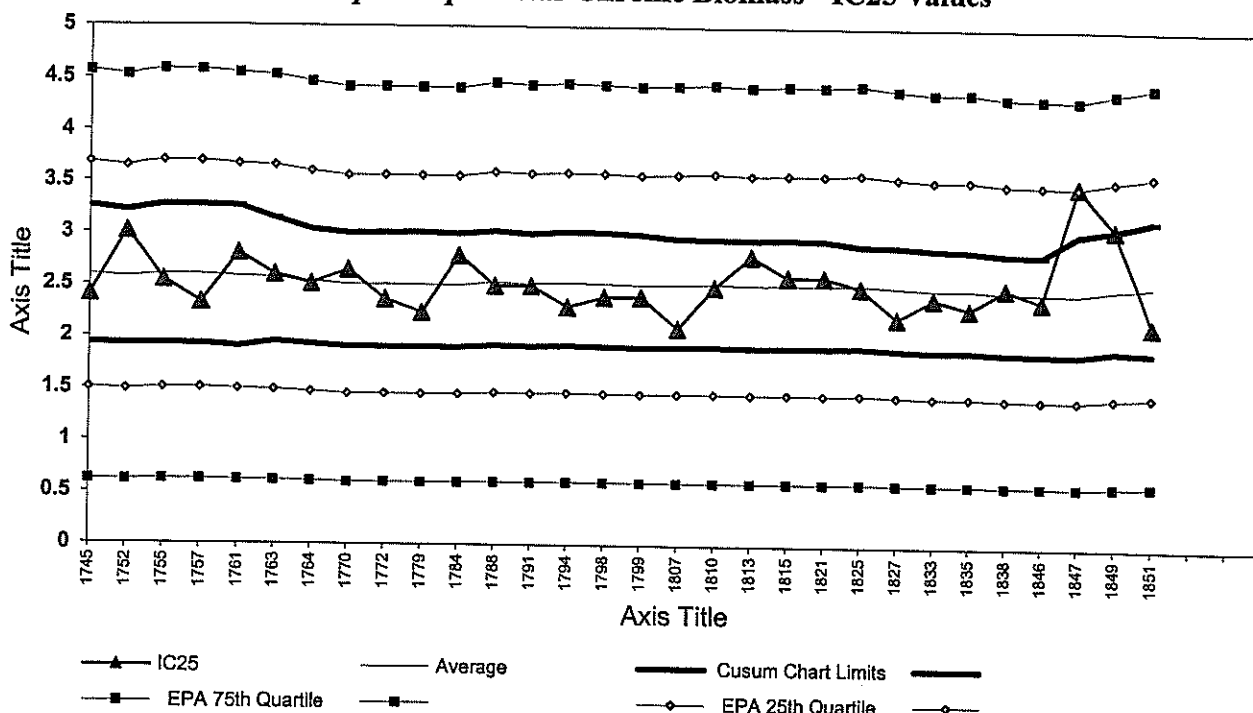
90th Quartile CV (control limit) = 0.52

*Intralab CV is compared to EPA Warning limits (25th and 75th CV's) and Control limits (10th and 90th CV's).*

*If lab CV is outside EPA Control limits, the EPA Control limits are used to set Cusum chart limits.*

Event #	FHM ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
318	1815	04/07/15	2.6	2.7	0.58	1.6	3.9	0.21
319	1821	05/19/15	2.7	2.7	0.58	1.6	3.9	0.21
320	1825	06/23/15	2.5	2.7	0.58	1.6	3.9	0.21
321	1827	07/07/15	2.9	2.7	0.57	1.6	3.8	0.19
322	1833	08/18/15	2.4	2.6	0.49	1.7	3.6	0.18
323	1835	09/15/15	3.0	2.7	0.49	1.7	3.6	0.18
324	1838	10/06/15	2.9	2.6	0.48	1.7	3.6	0.17
325	1846	11/17/15	2.4	2.7	0.46	1.8	3.6	0.17
326	1847	12/01/15	4.1	2.7	0.46	1.8	3.6	0.20
327	1849	12/15/15	3.937	2.8	0.56	1.63	3.88	0.22
328	1851	12/29/16	2.3	2.8	0.62	1.6	4.1	0.22
329								
330								

**REFERENCE TOXICANT CUMULATIVE SUMMARY (CUSUM) CHART**  
*Pimephales promelas* Chronic Biomass - IC25 Values



***Pimephales promelas* - Chronic (EPA Test Method 1000.0)**

**SODIUM CHLORIDE (g/L)**

From EPA 833-R-00-003:

Endpoint: Chronic Growth (Biomass)

10th Quartile CV (control limit) = 0.12

Stats Method: Linear Interpolation

25th Quartile CV (warning limit) = 0.21

Test Conditions: Recon MH, 25 oC

75th Quartile CV (warning limit) = 0.38

90th Quartile CV (control limit) = 0.45

*Intralab CV is compared to EPA Warning limits (25th and 75th CV's) and Control limits (10th and 90th CV's).*

*If lab CV is outside EPA Control limits, the EPA Control limits are used to set Cusum chart limits.*

Event #	FHM ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
318	1815	4/7/2015	2.6	2.5	0.22	1.9	3.0	0.09
319	1821	5/19/2015	2.6	2.5	0.22	1.9	3.0	0.09
320	1825	6/23/2015	2.5	2.5	0.22	1.9	2.9	0.07
321	1827	7/7/2015	2.2	2.5	0.19	1.9	2.9	0.08
322	1833	8/18/2015	2.4	2.5	0.20	1.9	2.9	0.08
323	1835	9/15/2015	2.3	2.5	0.19	1.9	2.9	0.07
324	1838	10/6/2015	2.5	2.5	0.18	1.9	2.8	0.07
325	1846	11/17/2015	2.4	2.5	0.18	1.9	2.8	0.07
326	1847	12/1/2015	3.5	2.5	0.18	1.9	3.0	0.12
327	1849	12/15/2015	3.081	2.5	0.29	1.907	3.080	0.13
328	1851	12/29/2015	2.2	2.5	0.32	1.9	3.2	0.13
329								
330								

Raphidocelis subcapitata (formerly Selenastrum capricornutum) TEST CONDITIONS

Client: \_\_\_\_\_

QA / QC

Sample Description: \_\_\_\_\_

NaCl

Reagent Log # 2B 055-04

Test Start Date: 12/3/2015

Time: 1540

Initials: JW/MC

Test End Date: 12-7-15

Time: 1300

Initials: MC

TEST CONDITIONS AT INITIATION:

Species ID#: R5791  
Age: 6

Dilution Water ID #: 4296  
Source Water: 4294  
EDTA Added?  Yes / No

INOCULUM (Axenic Culture)		Culture Density = Mean # of Cells/Field x 250,000	
# Cells	# Fields		Culture Density
475	200		
MEAN	2.375	x 250,000 =	593,750

Filtration	0.45 um filter	X
	1.6 um filter	

Test Vol. / Replicate	50 ml
No. of Reps	4
Nutrient Dosage	1 ml per L

Test Start Density x Test Volume / Culture Density = Inoculum Volume  
10,000 x 50 ml / 593,750 = 0.84 ml

Confirming Counts			
	# Cells	# Fields	Cell Conc. x 10 <sup>6</sup> (cells/ml)
Flask #1	7	250	0.007
Flask #2	10	250	0.010
Flask #3	11	250	0.011

Light Intensity at Test Initiation (foot-candles)		
Location #1	Location #2	Location #3
396	390	423

Technician 0 hr JW/MC 24 hr MC 48 hr h 72 hr h 96 hr MC  
Time 0 hr 1540 24 hr 0830 48 hr 1315 72 hr 1240 96 hr 1300  
Thermometer ID: 0 hr #186 24 hr #186 48 hr #186 72 hr #212 96 hr #2186  
RELOC 12-7-15

Test Concn. (g/L)	D.O. (mg/L)	pH					Temperature (°C)					Conductivity (umohs/cm)	Hard.	Alk.
		0 hr	24	48	72	96	0 hr	24	48	72	96			
Control	6.4	8.1	7.9	8.0	8.3	9.1	24.2	24.7	25.0	24.5	24.6	368	100	76
0.25	6.5	8.1	8.0	8.1	8.2	9.0	24.2	25.0	25.2	24.3	24.9	818	99	71
0.50	6.3	8.0	8.0	8.1	8.2	9.0	24.3	25.0	25.3	25.1	25.1	1266		
1.0	6.5	8.0	8.1	8.1	8.2	8.9	24.3	24.9	25.3	25.1	25.4	2140	103	70
2.0	6.5	8.0	8.1	8.1	8.1	8.6	24.2	24.6	25.2	25.0	25.5	3810		
4.0	6.7	7.9	8.0	8.1	8.0	8.4	24.2	24.6	25.1	24.9	25.3	6940	93	62

Task Manager [Signature]

Endpoint : Growth

Project Manager [Signature]

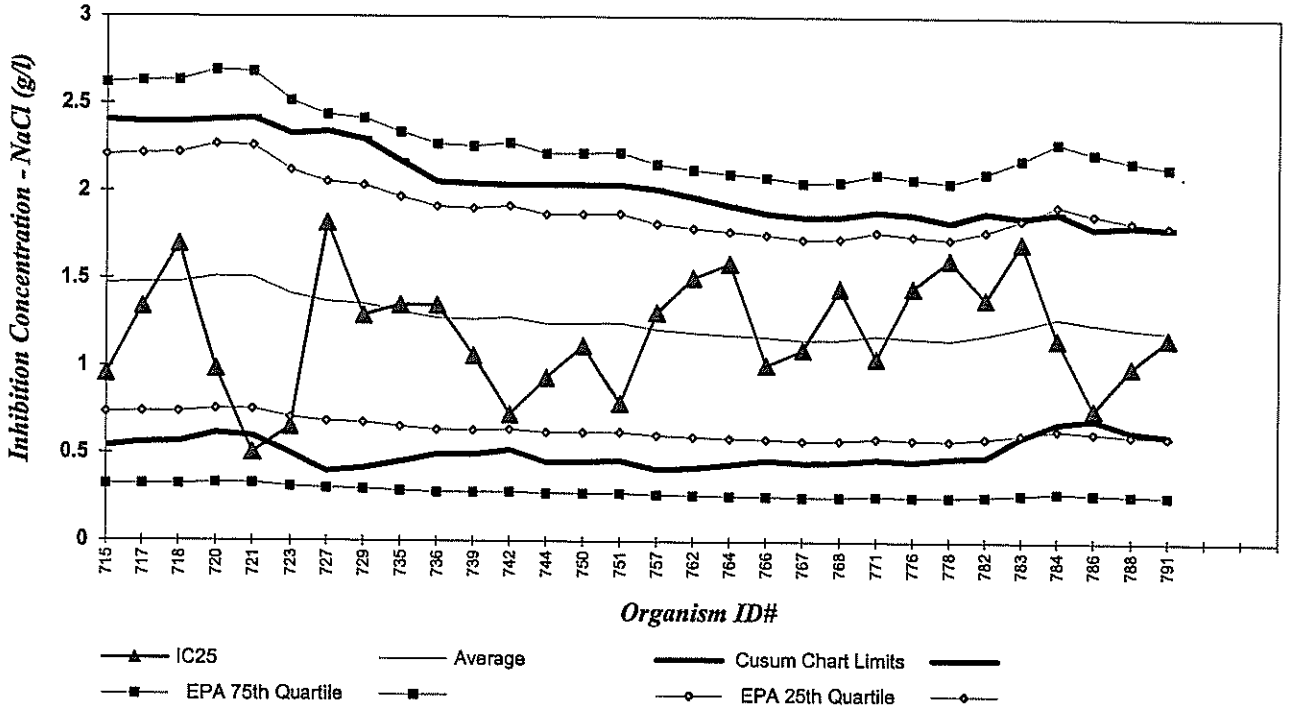
IC25 : 1.17

QA Officer [Signature]

Cusum Chart Limits : 0.61 to 1.80

Comments:

**REFERENCE TOXICANT CUMULATIVE SUMMARY (CUSUM) CHART**  
*Raphidocelis subcapitata* Chronic Growth - IC25 Values



***Raphidocelis subcapitata* - Chronic (EPA Test Method 1003.0)**

**SODIUM CHLORIDE (g/L)**

Endpoint: Chronic Growth

Stats Method: Linear Interpolation

Test Conditions: Recon MH, 25 oC

From EPA 833-R-00-003:

10th Quartile CV (control limit) = 0.02

25th Quartile CV (warning limit) = 0.25

75th Quartile CV (warning limit) = 0.39

90th Quartile CV (control limit) = 0.51

*As per EPA 833-R-00-003, section B.2.1, the quartiles listed above are from just a few labs (6) and therefore not to be considered typical or representative. Cusum limits are based on ASL data only.*

Event #	Algae ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
129	776	7/23/2015	1.45	1.17	0.35	0.46	1.87	0.29
130	778	8/11/2015	1.61	1.15	0.34	0.48	1.83	0.30
131	782	9/22/2015	1.39	1.18	0.35	0.48	1.88	0.26
132	783	9/29/2015	1.71	1.23	0.31	0.60	1.86	0.23
133	784	10/6/2015	1.16	1.28	0.30	0.68	1.88	0.22
134	786	11/10/2015	0.76	1.25	0.27	0.70	1.80	0.24
135	788	11/17/2015	1.00	1.22	0.29	0.63	1.81	0.25
136	791	12/3/2015	1.17	1.20	0.30	0.61	1.80	0.25
137								
138								
139								
140								
141								
142								

**APPENDIX C**  
**CHAIN OF CUSTODY**





Batch Number: B3159A

Date Received: 12/14/15 @ 1020

Client/Project: City of MOHAWA

Received By: JVP

Were custody seals intact?

Yes  No  N/A

Packing Material:

Ice  Blue Ice  Box

Temp OK? (<-6C) Therm ID: TH173 Exp. 01/16

3.6 °C  Yes  No  N/A

Was a Chain of Custody (CoC) Provided?

Yes  No  N/A

Was the CoC correctly filled out (If No, document below)

Yes  No  N/A

Were the sample containers in good condition (not broken or leaking)?

Yes  No  N/A

Are all samples within 36 hours of collection?

Yes  No  N/A

Method of Shipment:

Hand Delivered  FedEx  UPS  Greyhound  Other: \_\_\_\_\_  N/A

**Sample Exception Report** (The following exceptions were noted)

Client was notified on:

Client contact:

Resolution to Exception:







Batch Number: B 3459B

Date Received: 12/16/15

Client/Project: City of MOBILE

Received By: JRP

Were custody seals intact?

Yes  No  N/A

Packing Material:

Ice  Blue Ice  Box

Temp OK? (<6C) Therm ID: TH173 Exp. 0116

5.7°C  Yes  No  N/A

Was a Chain of Custody (CoC) Provided?

Yes  No  N/A

Was the CoC correctly filled out (If No, document below)

Yes  No  N/A

Were the sample containers in good condition (not broken or leaking)?

Yes  No  N/A

Are all samples within 36 hours of collection?

Yes  No  N/A

Method of Shipment:

Hand Delivered  FedEx  UPS  Greyhound  Other: \_\_\_\_\_  N/A

**Sample Exception Report** (The following exceptions were noted)

Client was notified on:

Client contact:

Resolution to Exception:

# CH2MHILL

## CHAIN OF CUSTODY RECORD FOR NPDES COMPLIANCE BIOMONITORING

Client City of Molalla  
 Address 1124 S. Toliver Road  
Molalla, OR 97038  
 Contact Person: Jason Clifford  
 Phone: 503-793-5283  
 E-mail: jclifford@cityofmolalla.com

Ship Samples to:  
 CH2M HILL - Applied Sciences Laboratory  
 Attention: Bioassay Lab  
 1100 NE Circle Blvd. Suite 300  
 Corvallis, OR 97330  
 Lab Phone: (541) 768-3160  
 Customer Service: (541) 768-3120

NPDES# 101514

Composite Sample Information:

Initiated: Date 12/17/15 Time 0700  
 Ended: Date 12/18/15 Time 0700  
 Chilled During Collection? Yes  No   
 Dechlorinated prior to shipping? Yes  No

CH2M HILL Project # / Purchase Order # \_\_\_\_\_

### Analysis Required / Comments

Sample ID	Date	Time	Sample Type		# of Containers	Lab ID#	Analysis Required									Concentration and/or Comments	
			Comp.	Grab			Fathead Acute	Fathead Chronic	Cerio Acute	Cerio Chronic	Green Algae	Trout Acute	Sheepshead Acute	Sheepshead Chronic	Menidia Acute		Menidia Chronic
Final Effluent	12-18-15		X		1	B3459 C											ICE 4.7°C

Sampled By & Title 	Jon Randall (Please sign and print name)	Date/Time 12/18/15	Relinquished By 	Jon Randall (Please sign and print name)	Date/Time 12/18/15
Received By 	(Please sign and print name)	Date/Time	Relinquished By (Please sign and print name)		Date/Time
Received By	(Please sign and print name)	Date/Time	Relinquished By (Please sign and print name)		Date/Time
Received By 	Pierrette Castro (Please sign and print name)	Date/Time 12/18/15 1043	Shipped Via	UPS <input type="checkbox"/> Bus <input type="checkbox"/> Fed-Ex <input type="checkbox"/> Hand <input type="checkbox"/> Other <input type="checkbox"/>	Shipping # B3459 C
Work Authorized By	(Please sign and print name)	Remarks			



Batch Number: B 3459 C  
Client/Project: City of Molalla

Date Received: 12/18/15  
Received By: PC

- Were custody seals intact?  Yes  No  N/A
- Packing Material:  Ice  Blue Ice  Box
- Temp OK? (<6C) Therm ID: TH173 Exp. 1/16 4.7 °C  Yes  No  N/A
- Was a Chain of Custody (CoC) Provided?  Yes  No  N/A
- Was the CoC correctly filled out (If No, document below)  Yes  No  N/A
- Were the sample containers in good condition (not broken or leaking)?  Yes  No  N/A
- Are all samples within 36 hours of collection?  Yes  No  N/A
- Method of Shipment:  Hand Delivered  FedEx  UPS  Greyhound  Other: \_\_\_\_\_  N/A

**Sample Exception Report** (The following exceptions were noted)

Client was notified on: \_\_\_\_\_ Client contact: \_\_\_\_\_

Resolution to Exception:

## **Muckey, Brett/CVO**

**From:** Muckey, Brett/CVO  
**Sent:** Tuesday, January 12, 2016 11:51 AM  
**To:** 'PILLSBURY Lori'  
**Cc:** Stanaway, Mike/CVO; COLE David  
**Subject:** RE: City of Molalla fathead minnow test - with fungus!

Thanks for the insight and feedback, Lori. (I had a great holiday, hope you did as well)

Yeah a bit of a mess. The previous 3 tests dating back to 2013 don't show any fungus ... so maybe this was just a onetime thing. I'll discuss the Ecology's alternate set up with Molalla and recommend that they are sure their tubing on the compositor is not fouled.

Brett

**From:** PILLSBURY Lori [mailto:PILLSBURY.Lori@deq.state.or.us]  
**Sent:** Tuesday, January 12, 2016 9:58 AM  
**To:** Muckey, Brett/CVO <Brett.Muckey@CH2M.com>  
**Cc:** Stanaway, Mike/CVO <Mike.Stanaway@CH2M.com>; COLE David <COLE.David@deq.state.or.us>; PILLSBURY Lori <PILLSBURY.Lori@deq.state.or.us>  
**Subject:** RE: City of Molalla fathead minnow test - with fungus!

Hi Brett

Good to hear from you. Hope you enjoyed your holidays.

The Molalla test seems to be quite a mess. I would require that they retest the fish given the problems and the high PMSD. Have they had issues with fungus before that you know of? If you feel it is necessary, go ahead and follow Ecology's alternative set-up. Please note this in your report.

They do not need to repeat the other two species.

Please let me know if you have other questions.

Thanks.

Lori

*Lori*

DEQ – Laboratory  
503-693-5735

**From:** [Brett.Muckey@CH2M.com](mailto:Brett.Muckey@CH2M.com) [<mailto:Brett.Muckey@CH2M.com>]  
**Sent:** Friday, January 08, 2016 5:02 PM  
**To:** PILLSBURY Lori  
**Cc:** [Mike.Stanaway@CH2M.com](mailto:Mike.Stanaway@CH2M.com)  
**Subject:** City of Molalla fathead minnow test - with fungus!

Lori,

We've run across another WET test issue and would like your take on whether the result is usable or not.

The situation is:

- Fungus running throughout a fathead chronic test.
  - Noted in 9 of 24 test chambers (basically everywhere but the control and the 40% effluent replicates).
  - Also, fungus was not noted in the other 3 chronic tests being done at the same time. (doesn't appear to be a fish supplier or lab water type of issue)
- The random die offs associated with the pathogenic effect are basically giving the statistical analysis fits.
  - 4 d acute dual endpoint shows an effect at the 2% (@ 85% survival), but not at any higher concentration ... including at the acute critical concentration of 10% (that showed only 55% survival)
  - 7 d chronic survival shows an interrupted dose response with significant reductions only at the 10% effluent
  - Growth shows a non-monotonically increasing response as well.
    - NOEC/LOEC come back at 40 and 100% ... which on the surface looks OK (except the 32% and 49% reductions in growth (at the 4% and 10 % effluent concentrations) don't show up as significant)
    - but the IC25 (listed to be used in the NPDES permit) ends up smoothing the data between the 4% and 100% concentrations. As you know, this limits the IC25 values to either less than 4% or greater than 100%. Stats say IC25 < 4% (a permit failure) ... but the actual reductions seen at the 40% and 100% were both less than 20% from control.
  - Overall PMSD of the growth endpoint is an unacceptably high 45. (vs. EPA bounds of 12 to 30) ... as such, the test is deemed insensitive (EPA chronic manual section 10.2.8.2.4.2)

In short ... the fungus has caused some serious issues here.

The questions are:

- Is there anything that can be reliably gleaned from this test?
- If so, is it enough to avoid making Molalla retest?? (concurrent Cerio and algae chronics show IC25 values of 55.6% and 57.6% vs the permit limit of 4%)
- Do we need to make a case for this test being considered anomalous (Washington DOE style)???
- And, if we need to retest, should we consider the WA DOE alternate method ????

I've briefly discussed this with the good folks at Molalla, but we wanted your input on it as well. I've attached the data sheets and the CETIS outputs for your viewing pleasure.

Please let me know what your thoughts are on this, (and I hope you had a good weekend!)

Thanks,

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*How is our Service? CH2MHILL's Applied Sciences Laboratory (ASL) would like your feedback. Please [Click Here](#) to leave your comments and suggestions*

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**BIOASSAY REPORT  
CHRONIC AND ACUTE  
BIOASSAYS CONDUCTED  
February 2 through 9, 2016**

Prepared for

CITY OF MOLALLA  
MOLALLA, OREGON

Prepared by



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State of Washington Department of Ecology (WDOE), Lab ID C1233

Report Date: February 17, 2016  
Lab I.D. No. B3474

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## INTRODUCTION

CH2M Applied Sciences Laboratory (CH2M-ASL) conducted chronic bioassay testing from February 2 through 9, 2016, on samples provided by the City of Molalla, Molalla, Oregon. The test was conducted using the fathead minnow (*Pimephales promelas*).

The testing was conducted as follow up on testing for the December 15, 2015 testing that showed pathogenic effects in the fathead minnow chronic test. This testing used the WDOE Fathead Minnow Survival and Growth (alternate version for samples having pathogens) test method.

## OVERVIEW OF REGULATORY GUIDANCE

The following provides an overview and excerpts of applicable permit specifics, regulatory guidance, and other relevant information. This is intended only as a helpful guide, from a laboratory perspective, for understanding test outcomes. The final responsibility for interpretation of results remains with the client and/or regulatory agency.

The following guidance is taken from CH2M's reading of the NPDES permit for the City of Molalla STP facility (permit #OR00101514, effective June 1, 2014, to June 1, 2019).

### Acute Toxicity:

- *Acute Permit Limit:* "An acute WET test will be considered to show toxicity if there is a statistically significant difference in survival between the control and 10% effluent reported as the NOEC  $\leq$  10 percent effluent."
- Please note: There is an apparent conflict within the above quote (taken from Section 10.b.iv. of the permit)
  - Statistical fact: In a test where the NOEC value is exactly equal to 10%, no statistically significant difference between the control and 10% effluent exists.
  - Using the first portion of the quote, referencing 'a statistically significant difference', would infer that toxicity was not shown.
  - Using the last portion of the quote, referencing 'NOEC  $\leq$  10%', would infer that toxicity was shown.
  - ASL's believes that this conflict is the result of what likely is a typographic error in the permit and that a "< 10" was intended rather than the " $\leq$  10" as written. However, this should be referred to OR DEQ for clarification.
- Acute toxicity data may be obtained as Dual End-Point Tests.

### Chronic toxicity:

- *Chronic Permit Limit:* "A chronic WET test will be considered to show toxicity if the IC<sub>25</sub> ... occurs at dilutions equal to or less than the dilution known to occur at the edge of the mixing zone, that is, IC<sub>25</sub>  $\leq$  4%"

**Evaluation of Causes and Exceedances:**

- “If any test exhibits toxicity ... another test ... shall be conducted within two weeks”.
- “If two consecutive WET tests indicate acute and/or chronic toxicity ... the permittee shall immediately notify the Department of the results.”

**SUMMARY OF TEST RESULTS**

**EXHIBIT 1**

**Summary of Acute Dual-Endpoint Test Results**

Species	NOEC (%)	LOEC (%)	LC <sub>50</sub> (%)
<i>P. promelas</i>	100	> 100	> 100

Note: acronyms are as defined below Exhibit 2.

From the NPDES permit: “ An acute WET test will be considered to show toxicity if there is a statistically significant difference in survival between the control and 10% effluent reported as the NOEC  $\leq$  10 percent effluent.”

More detailed information is provided in the Results and Discussion section.

Exhibit 2 provides a summary of the final test results.

**EXHIBIT 2**

**Summary of Chronic Test Results**

Species	NOEC (%)	LOEC (%)	IC <sub>25</sub> (%)
<i>P. promelas</i>	100	> 100	> 100

Note: acronyms are as defined below.

From the NPDES permit: “A chronic WET test will be considered to show toxicity if the IC<sub>25</sub> ... occurs at dilutions equal to or less than the dilution known to occur at the edge of the mixing zone, that is, IC<sub>25</sub>  $\leq$  4%”.

More detailed information is provided in the Results and Discussion section.

## ACRONYM DEFINITIONS (from EPA guidance):

NOEC = No Observed Effect Concentration: The highest test concentration that causes no observable adverse effects on the test organisms (i.e. no statistically significant reduction from the control).

LOEC = Low Observed Effect Concentration: The lowest test concentration that does cause an observable adverse effect on the test organisms (i.e. is statistically significant reduction from the control).

IC<sub>25</sub> = Inhibition Concentration (25%): A point estimate of the test concentration that would cause a 25 percent reduction of a non-quantal biological measurement (i.e. growth, reproduction, etc.) for the test population.

## METHODS AND MATERIALS

### TEST METHODS

The chronic test methods were performed according to:

- *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, Fourth Edition, (EPA 2002), EPA-821-R-02-013.
- *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria*, Washington State Department of Ecology (revised 2008) Pub# WQ-R-95-80.

Additional guidance was provided by:

- *Reasonable Potential Analysis for Toxic Pollutants, Internal Management Directive*, Oregon Department of Environmental Quality (February 2005).

### DEVIATIONS FROM PROTOCOLS

Deviations from required procedures in the test methods:

- None noted.

Deviations from recommended procedures in the test methods:

- Not all of the samples were within the EPA recommended holding temperature range of 0 to 6 °C upon arrival at the laboratory. See notation in Sample Collection below.
- The *P. promelas* chronic reference toxicant (reftox) test fell outside the cusum chart control limits. See Reference Toxicant Tests section for further information.

## TEST DESIGN

The following summarizes the conditions used for both overall testing and the specifics for each test (observations and notations can be found on the datasheets in Appendix A):

### Overall Test Design:

- Chronic tests: 2, 4, 10, 40, and 100 percent sample + dilution water for the control.

### Test Organism Conditions:

- All organisms tested were fed and maintained during culturing, acclimation, and testing as prescribed by the EPA (2002).
- The test organisms appeared vigorous and in good condition prior to testing.

### *P. promelas* chronic test (WDOE Alternate version):

- Source: Aquatox Inc., Hot Springs, Arkansas
- Age: Less than 48 hours old and within an 24 hour age range
- Design: Ten test vessels per concentration, two organisms per vessel
- Test Solution Renewal: Daily
- Monitoring:
  - Daily: Survival
  - Daily: DO and pH in pre and post-renewal solutions, all concentrations
  - Daily: Temperature in pre-renewal solutions, all concentrations
  - With each new sample: Conductivity in post-renewal solutions, control and highest sample concentration
- Termination: 7 days after test initiation.
- Endpoints: Survival and Growth (average dry weight per organism added @ initiation)
- Acute Dual-Endpoint: 96 hour Survival (from the 4 day chronic exposure data)

## DILUTION WATER

The dilution water used was the standard culture water used by CH2M-ASL:

- Reconstituted, moderately hard water (as per EPA protocol) with a total hardness of 80 to 100 mg/L as CaCO<sub>3</sub> and an alkalinity of 60 to 70 mg/L as CaCO<sub>3</sub>.

## SAMPLE COLLECTION AND STORAGE

Samples were collected by City of Molalla personnel. The samples were accepted as scheduled by CH2M's Applied Sciences Laboratory. Chain of Custody and Sample Receipt Records are provided in Appendix C.

- Not all samples were received within the EPA recommended 0 to 6 °C range.
  - The sample collected on February 1 was received at 7.5 °C which is outside of the EPA recommended 0 to 6 °C range.
    - However, it should be noted that the sample was received on ice, within 3 hours of collection.

- The February 3 and 5 samples were received in the 0 to 6 °C range.
- All samples were initially used for test initiation or test solution renewal within the EPA recommended maximum holding time of 36 hours of sample collection.
- All subsequent uses of a sample occurred within the EPA recommended maximum holding time of 72 hours past the time of initial use of that sample.
- Following receipt, the samples were stored in the dark at 0 to 6 °C until test solutions were prepared and tested.

## **SAMPLE PREPARATION**

Samples used during these tests were:

- Temperature adjusted prior to test initiation and each daily renewal.
- Filtered through a 60 µm net upon arrival.

## **DATA ANALYSIS**

The statistical analyses performed for the acute tests were those outlined in *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, USEPA Office of Water, Fifth Edition (2002), EPA-821-R-02-012, using CETIS.

The statistical analyses performed for the chronic tests were those outlined in *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, USEPA Office of Water, Fourth Edition (EPA 2002), EPA-821-R-02-013, CETIS.

Additional guidance was provided by *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria*, Washington State Department of Ecology (revised 2008) Pub# WQ-R-95-80.

- The specific statistical analysis and CETIS version used for each endpoint evaluation is listed with the statistical outputs included with each test in Appendix A.
- If any additional analysis methods were also used, an explanation of the rationale and reference to the source method is included with the presentation of those results below.

## RESULTS AND DISCUSSION

The raw data sheets for all tests are presented in Appendix A.

### ACUTE BIOASSAYS

Table 1 summarizes the survival data for the *P. promelas* acute dual-endpoint test.

<b>Table 1</b> <b>Summary of Acute Results</b> <i>P. promelas</i>	
<b>Sample Concentration (%)</b>	<b>Percent Survival (at 96 hours)</b>
Control	100
2.0	95
4.0	100
10.0	90
40.0	100
100	95

Statistical analysis in accordance with the EPA protocol and WDOE guidance results in:

- NOEC = 100 %
- LOEC > 100 %
- LC<sub>50</sub> > 100 %

From the NPDES permit: “An acute WET test will be considered to show toxicity if there is a statistically significant difference in survival between the control and 10% effluent reported as the NOEC  $\leq$  10 percent effluent.”

Dissolved oxygen concentrations remained at 4.0 mg/L or greater throughout the test period. Test temperatures remained in the range of 20 $\pm$ 1°C.

The test meets Test Acceptability Criteria (TAC) of a minimum 90 percent control survival. Unless referenced above, the tests proceeded without any noted deviations or interruptions that could have affected test results. The testing should be considered “valid”.

## CHRONIC BIOASSAYS

Table 2 summarizes the survival and growth data for the *P. promelas* chronic test.

<b>Sample Concentration (%)</b>	<b>Percent Survival</b>	<b>Mean Dry Weight Per Organism Added (mg)</b>
Control	100	0.537
2.0	95	0.532
4.0	100	0.455
10	90	0.506
40	95	0.476
100	95	0.523

Statistical analysis in accordance with the EPA protocol and WDOE guidance results in:

- NOEC = 100 %
- LOEC > 100 %
- IC<sub>25</sub> > 100 %

From the NPDES permit: “A chronic WET test will be considered to show toxicity if the IC<sub>25</sub> ... occurs at dilutions equal to or less than the dilution known to occur at the edge of the mixing zone, that is, IC<sub>25</sub> ≤ 4%”.

Note: The *P. promelas* chronic reference toxicant (reftox) test fell outside the cusum chart control limits. See Reference Toxicant Tests section for further information.

The dissolved oxygen levels in the chronic tests remained above 4.0 mg/L. Test temperatures remained at 25±1°C.

The *P. promelas* test meets Test Acceptability Criteria (TAC) for a minimum 80 percent control survival and a minimum weight of 0.250 mg per surviving control organism. Unless referenced above, the tests proceeded without any noted deviations or interruptions that could have affected test results. The testing should be considered “valid”.

## REFERENCE TOXICANT TESTS

Reference toxicant (reftox) testing is performed to document both initial and ongoing laboratory performance of the test method(s). While the health of the test organisms is primarily evaluated by the performance of the laboratory control, reftox test results also may be used to assess the health and sensitivity of the test organisms. Reftox test results within their respective cumulative summary (cusum) chart limits are indicative of consistent laboratory performance and normal test organism sensitivity.

The *P. promelas* chronic reference toxicant test, conducted concurrently, was outside of cusum chart range for the survival endpoint. Specifically, the test result was just below the cusum chart range for the chronic growth endpoint indicating that the test organisms were potentially overly sensitive. EPA 821-R-02-013, section 4.16.6 recognizes that “reference toxicant test results should not be used as a *de facto* criterion for rejection of individual effluent or receiving water tests.” Rather, “the reviewer should consider the degree to which the reference toxicant test result fell outside of control chart limits, the width of the limits, the direction of the deviation ... , the test conditions ... , and the objective of the test.” In this case, client test results that demonstrate no toxicity at the levels of regulatory concern using potentially overly sensitive test organisms should result in the test results being accepted.

The *P. promelas* reftox test was conducted using sodium chloride. The data sheets for the reference toxicant tests are provided in Appendix B.

Table 2 summarizes the reference toxicant test results and Cusum chart limits.

<b>Species</b>	<b>IC<sub>25</sub></b>	<b>Cusum Chart Limits</b>
<i>P. promelas</i> (survival)	1.6	1.4 to 4.1
<i>P. promelas</i> (growth)	1.4 *	1.6 to 3.2

\* Indicates a value outside of cusum chart limits.



**APPENDIX A**  
**RAW DATA SHEETS**

**FRESHWATER TOXICITY TEST: SAMPLE AND DILUTION WATER DATA**

Client City of Molalla      Test Initiation: Date 2-2-16  
 Contact Jason Clifford (503) 829-5407      Test Termination: Date 2-9-2016  
 SDG # B 3474

Sample ID Number	Field ID	Date Collected (mm/dd/yy)	Time (Pacific Zone)	Date Received	Temp (°C) as Rcvd	Total Residual Chlorine (mg/l) <input type="checkbox"/> as Rcvd / after Dechlor.	Ammonia NH <sub>3</sub> -N mg/l as Rcvd	Hardness mg/l as CaCO <sub>3</sub> as Rcvd	Alkalinity mg/l as CaCO <sub>3</sub> as Rcvd	DO (mg/L) as Rcvd	pH as Rcvd	Cond. (uS) as Rcvd	60 um filtered prior to USG?
B3474-01	Final Effluent	2/1/16	08:20	2/1/16	7.5	10.02 / -	7.23	50	-	-	-	-	<input checked="" type="checkbox"/>
-02	"	2/3/16	09:20	2/3/16	4.6	6.02 / -	7.14	51	-	-	-	-	<input checked="" type="checkbox"/>
-03	"	2/5/16	08:20	2/5/16	2.4	0.02 / -	7.14	48	-	-	-	-	<input checked="" type="checkbox"/>
						/							
						/							
						/							
						/							
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						/							
						/							
						/							
						/							

Note: "-" Indicates data collection or dechlorination not needed. Any other adjustments to samples prior to use are documented in Comments below or on Dilutions page.

Comments:  Indicates the action was taken, ( = action not taken):  
 Subsample for algae test had nutrients added and was 0.45 um filtered

Dilution Water	ID#	Hardness mg/l as CaCO <sub>3</sub>	Alkalinity mg/l as CaCO <sub>3</sub>
2062-16 Recon MH (Algae)	4316	110	76
Recon MH (FHM)	4319	88	64
"	4320	96	64

Water Quality Meters Used/ID#: Dissolved Oxygen # 3 pH # 11 Conductivity # 2

Client City of Molalla

Sample Designation (SDG): B 3474

Test Species Information	FHM # <u>1856</u> <i>Pimephales promelas</i> Chronic				
Organism Age at Initiation	<48 hrs, all within a 24 hour window				
Test Container Size	30 ml				
Test Volume	20 ml				
Feeding: Type and Amount	0.03 ml <i>Artemia</i> , 2 x Daily				
Aeration: In Test Chambers via Slow Bubble :	<input checked="" type="checkbox"/> None <input type="checkbox"/> Prior to use <input type="checkbox"/> @ _____ hrs				
Acclimation Period	<24 hrs				
Organism Source	<u>Aquatox</u>				
Size	-				
Loading Rate	-				

Dissolved Oxygen aeration justifications (in test chambers):

Test(s):  All  \_\_\_\_\_  
Date:

Comments:

# Test Solution Preparation and Dilution Record

Client: City of Molalla

Fathead minnow - Chronic

Test Concentration (%)	Sample Volume (mls)	Final Volume (mls)
Control	0.00 →	250
2.0	5.00 →	250
4.0	10.0 →	250
10	25.0 →	250
40	100 →	250
100	250 →	250

Total Sample volume needed per day = 390 mls

Note:  Indicates task not done,  Indicates task was done. Temp adj. = Temperature adjusted to ambient or test temp  
 Ditto marks ( ' ' ) indicate that the same SDG, batch of dilution water, or food as the previous day's entry was used.

Test Day	Sample ID Used	Daily Sample Preparation (prior to dilution)	Dilution Water Used	Date	Time	Initials
0 (Initiation)	B <del>37401</del>		ID # 4319	2/2/2016	07:30	DW
1	B 1-01	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID # 4319	2/3/2016	07:00	DW
2	B 1-02	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID # 4320	2/4/2016	07:25	DW
3	B 1-02	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID # 4320	2/5/2016	07:00	DW
4	B 1-03	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID # 4320	2/16/2016	06:55	JW
5	B 1-03	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID # 4320	2/17/2016	07:30	J
6	B 1-03	<input type="checkbox"/> Temp adj, <input type="checkbox"/> Aerated	ID # 4320	2/18/2016	08:55	MC

**FATHEAD MINNOW 7-DAY SURVIVAL AND WATER QUALITY DATA**

Protocol Template Used: Cerio board # 19

Waterbath/incubator Used: \_\_\_\_\_

Date Initiated 2/2/2016 Time 10:30

Initial sample ID B 3474

# 7

Date Terminated 2/9/2016 Time 10:20

Client City of Molalla

Sample Description \_\_\_\_\_

Tech: Day 0 DW Day 1 o Day 2 e Day 3 e Day 4 e Day 5 sw Day 6 MC Day 7 e

Time Day 0 1030 Day 1 1130 Day 2 1215 Day 3 1250 Day 4 0940 Day 5 0915 Day 6 1320 Day 7 1020

Conc. or Percent	Day	Number of Live Organisms										Dissolved O <sub>2</sub> (mg/l)		pH		Temp. (°C)	Therm. ID#	Conductivity (µS)
		A	B	C	D	E	F	G	H	I	J	Pre	Post	Pre	Post	Pre		Post (1 <sup>st</sup> use)
Control	0	2	2	2	2	2	2	2	2	2	2		8.0		7.8	Post: 24.7	217	316
	1	2	2	2	2	2	2	2	2	2	2	6.1	7.8	7.2	7.9	25.0	186	322
	2	2	2	2	2	2	2	2	2	2	2	6.3	8.2	7.2	8.1	25.0	186	295
	3	2	2	2	2	2	2	2	2	2	2	5.5	8.0	6.9	8.1	25.0	184	283
	4	2	2	2	2	2	2	2	2	2	2	5.5	7.9	6.7	8.0	25.0	186	296
	5	2	2	2	2	2	2	2	2	2	2	4.5*	7.8	6.9	8.0	25.3	184	284
	6	2	2	2	2	2	2	2	2	2	2	6.1	7.8	7.1	8.0	24.9	177	
2.0 %	0	2	2	2	2	2	2	2	2	2	2		8.0		7.8	Post: 24.9		
	1	2	2	2	2	2	2	2	2	2	2	6.2	7.8	7.3	7.9	25.0		
	2	2	2	2	2	2	2	2	2	2	2	6.3	8.3	7.1	8.1	24.9		
	3	2	2	2	2	2	2	1	2	2	2	5.2	7.9	7.0	8.1	25.0		
	4	2	2	2	2	2	2	1	2	2	2	5.7	7.9	6.9	8.1	24.9		
	5	2	2	2	2	2	2	1	2	2	2	4.5*	8.0	6.9	8.0	25.3		
	6	2	2	2	2	2	2	1	2	2	2	6.2	8.1	7.1	8.0	25.2		
4.0 %	0	2	2	2	2	2	2	2	2	2	2		8.0		7.8	Post: 25.2		
	1	2	2	2	2	2	2	2	2	2	2	6.4	7.8	7.4	7.9	25.0		
	2	2	2	2	2	2	2	2	2	2	2	6.2	8.1	7.1	8.0	24.8		
	3	2	2	2	2	2	2	2	2	2	2	5.1	7.9	7.0	8.0	24.9		
	4	2	2	2	2	2	2	2	2	2	2	5.1	7.8	6.9	8.1	24.8		
	5	2	2	2	2	2	2	2	2	2	2	4.3*	8.1	6.9	7.9	25.3		
	6	2	2	2	2	2	2	2	2	2	2	6.0	8.2	7.1	8.0	25.2		
10 %	0	2	2	2	2	2	2	2	2	2	2		8.0		7.8	Post: 25.2		
	1	2	2	2	2	2	2	2	2	1	2	6.4	7.8	7.4	7.9	24.7		
	2	2	2	2	2	2	2	2	2	1	2	6.3	8.0	7.1	7.9	24.8		
	3	2	2	2	2	2	2	2	2	1	2	5.5	7.9	7.0	8.0	24.9		
	4	2	1	2	2	2	2	2	2	1	2	5.4	7.9	7.0	8.1	24.9		
	5	2	1	2	2	2	2	2	2	1	2	4.6*	8.1	7.0	7.8	25.0		
	6	2	1	2	2	2	1	2	2	1	2	6.0	8.2	7.2	7.9	25.4		
40 %	0	2	2	2	2	2	2	2	2	2	2		8.1		7.4	Post: 25.4		
	1	2	2	2	2	2	2	2	2	2	2	6.5	8.0	7.3	7.7	25.0		
	2	2	2	2	2	2	2	2	2	2	2	6.3	8.1	7.1	7.7	24.8		
	3	2	2	2	2	2	2	2	2	2	2	5.5	7.8	7.1	7.7	24.8		
	4	2	2	2	2	2	2	2	2	2	2	5.5	8.0	7.0	7.7	24.7		
	5	2	2	2	2	2	2	2	1	2	2	4.7*	8.1	7.0	7.375	25.7		
	6	2	2	2	2	2	2	2	1	2	2	6.1	8.2	7.3	7.6	25.3		
100 %	0	2	2	2	2	2	2	2	2	2	2		8.3		7.0	Post: 25.3		192
	1	2	2	2	2	2	2	2	2	2	2	6.2	8.3	7.4	7.4	24.4		178
	2	2	2	2	2	2	1	2	2	2	2	6.3	8.3	7.2	7.5	24.8		196
	3	2	2	2	2	2	1	2	2	2	2	5.4	8.1	7.1	7.4	24.9		182
	4	2	2	2	2	2	1	2	2	2	2	4.7*	8.2	7.1	7.2	24.9		200
	5	2	2	2	2	2	1	2	2	2	2	4.7*	8.1	7.0	7.3	25.7		203
	6	2	2	2	2	2	1	2	2	2	2	6.1	7.8.3	7.3	7.3	25.3		

✓ Indicates one organism inadvertently poured off during solution renewal, replaced into container. Pre = Pre-renewal solutions. Post = Post-renewal solutions.  
 "M" = organism missing, start count reduced. "Inj" = organism injured, remove from stats. Day 0 Temperatures = Post-renewals  
 "F" = fungus noted on dead organisms. Therm ID# = Thermometer ID used for all measurements that day.  
 Aeration in test chambers begun @ \_\_\_\_\_ (Note observations on Test Organism Info sheet) (23.8) = Temp. out of recommended range

\* 2/7/2016 DO dropped quickly as samples set during change

## FATHEAD MINNOW 7-DAY GROWTH DATA

Client City of Molalla Tins Labeled As: MOLALLA (2/2)  
 Lab ID: B3474 Start Date 2/2/2016

Sample Description: \_\_\_\_\_

Technician:	<u>KJ</u>	<u>KJ</u>
Date:	<u>2/10/2016</u>	<u>1/29/2016</u>
Balance Serial #:	<u>B328543647</u>	<u>B328543647</u>

Percent	Replicate	Total Weight (mg)	Tare Weight (mg)	No. of Fish
Control	A	1140.02	1138.43	2
	B	1128.70	1127.70	2
	C	1139.20	1138.10	2
	D	1137.22	1136.05	2
	E	1145.74	1144.98	2
	F	1146.98	1146.00	2
	G	1155.53	1154.21	2
	H	1149.73	1148.70	2
	I	1136.36	1135.57	2
	J	1122.43	1121.44	2
2.0%	A	1162.76	1161.66	2
	B	1140.42	1139.38	2
	C	1142.63	1141.65	2
	D	1171.72	1170.45	2
	E	1148.46	1147.35	2
	F	1128.35	1127.18	2
	G	1138.87	1137.92	1
	H	1175.60	1174.72	2
	I	1140.52	1139.52	2
	J	1177.89	1176.76	2
4.0%	A	1160.16	1159.40	2
	B	1159.08	1157.85	2
	C	1168.66	1167.66	2
	D	1156.28	1155.15	2
	E	1150.45	1149.07	2
	F	1136.16	1135.52	2
	G	1167.91	1167.10	2
	H	1134.04	1133.54	2
	I	1165.67	1165.01	2
	J	1165.03	1164.05	2

weigh to 0.01 mg

**FATHEAD MINNOW 7-DAY GROWTH DATA**

Client City of Molalla Tins Labeled As: MOLALLA (2/2)  
 Lab ID: B B3474 Start Date 2/2/2016

Sample Description: \_\_\_\_\_

Technician:	<u>KJ</u>	<u>KJ</u>
Date:	<u>2/10/2016</u>	<u>1/29/2016</u>
Balance Serial #:	<u>50309851</u>	<u>50309851</u>

Percent	Replicate	Total Weight (mg)	Tare Weight (mg)	No. of Fish
10%	A	1141.45	1140.41	2
	B	1167.56	1166.59	1
	C	1127.47	1126.63	2
	D	1169.18	1167.76	2
	E	1161.12	1159.90	2
	F	1121.51	1121.02	1 of 1
	G	1136.43	1135.57	2
	H	1176.99	1176.09	2
	I	1165.20	1164.07	2
	J	1169.77	1169.02	1
40%	A	1166.21	1165.25	2
	B	1142.21	1141.17	2
	C	1164.72	1163.82	2
	D	1135.86	1134.89	2
	E	1140.79	1139.50	2
	F	1165.26	1164.30	2
	G	1142.97	1141.98	2
	H	1151.83	1151.13	2
	I	1135.96	1135.36	1
	J	1148.05	1146.95	2
100%	A	1137.74	1136.62	2
	B	1161.75	1160.59	2
	C	1151.76	1150.82	2
	D	1142.55	1141.44	2
	E	1142.94	1141.84	2
	F	1133.22	1132.52	1
	G	1130.39	1129.33	2
	H	1160.16	1159.10	2
	I	1159.89	1158.68	2
	J	1149.29	1148.29	2

weigh to 0.01 mg

## FATHEAD MINNOW 7-DAY GROWTH DATA

Client City of Molalla Tins Labeled As: MOLALLA (2/2)  
 Lab ID: B Start Date 2/2/2016  
 Sample Description: \_\_\_\_\_

Technician: \_\_\_\_\_ KJ  
 Date: \_\_\_\_\_ 1/29/2016  
 Balance Serial #: B328543647 B328543647

Percent	Replicate	Total Weight (mg)	Tare Weight (mg)	No. of Fish
Control	A		1138.43	2
	B		1127.70	2
	C		1138.10	2
	D		1136.05	2
	E		1144.98	2
	F		1146.00	2
	G		1154.21	2
	H		1148.70	2
	I		1135.57	2
	J		1121.44	2
2.0%	A		1161.66	2
	B		1139.38	2
	C		1141.65	2
	D		1170.45	2
	E		1147.35	2
	F		1127.18	2
	G		1137.92	1
	H		1174.72	2
	I		1139.52	2
	J		1176.76	2
4.0%	A		1159.40	2
	B		1157.85	2
	C		1167.66	2
	D		1155.15	2
	E		1149.07	2
	F		1135.52	2
	G		1167.10	2
	H		1133.54	2
	I		1165.01	2
	J		1164.05	2

weigh to 0.01 mg



**FATHEAD MINNOW 7-DAY GROWTH DATA**

Client City of Molalla Tins Labeled As: MOLALLA (2/2)  
 Lab ID: B Start Date 2/2/2016  
 Sample Description: \_\_\_\_\_

Technician: \_\_\_\_\_ KJ  
 Date: \_\_\_\_\_ 1/29/2016  
 Balance Serial #: 50309851 50309851

Percent	Replicate	Total Weight (mg)	Tare Weight (mg)	No. of Fish
10%	A		1140.41	2
	B		1166.59	1
	C		1126.63	2
	D		1167.76	2
	E		1159.90	2
	F		1121.02	1 / 1
	G		1135.57	2
	H		1176.09	2
	I		1164.07	2
	J		1169.02	1
40%	A		1165.25	2
	B		1141.17	2
	C		1163.82	2
	D		1134.89	2
	E		1139.50	2
	F		1164.30	2
	G		1141.98	2
	H		1151.13	2
	I		1135.36	1
	J		1146.95	2
100%	A		1136.62	2
	B		1160.59	2
	C		1150.82	2
	D		1141.44	2
	E		1141.84	2
	F		1132.52	1
	G		1129.33	2
	H		1159.10	2
	I		1158.68	2
	J		1148.29	2

weigh to 0.01 mg

**CETIS Summary Report**

Report Date: 17 Feb-16 13:55 (p 1 of 2)  
 Test Code: B347401ppc | 18-5116-9148

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

<b>Batch ID:</b> 04-7384-1677	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 02 Feb-16 10:30	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 09 Feb-16 08:35	<b>Species:</b> Pimephales promelas	<b>Brine:</b>
<b>Duration:</b> 6d 22h	<b>Source:</b> Aquatox, AR	<b>Age:</b> <48h
<b>Sample ID:</b> 04-7691-5455	<b>Code:</b> B3474-01	<b>Client:</b>
<b>Sample Date:</b> 01 Feb-16 08:20	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 01 Feb-16 13:03	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 26h (7.5 °C)	<b>Station:</b>	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-4355-4702	4d Survival Rate	100	>100	NA	20.5%	1	Steel Many-One Rank Sum Test
19-9049-5948	7d Survival Rate	100	>100	NA	21.0%	1	Wilcoxon/Bonferroni Adj Test
19-3355-7321	Mean Dry Biomass-mg	100	>100	NA	19.5%	1	Dunnett Multiple Comparison Test

**Point Estimate Summary**

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
13-1696-7502	4d Survival Rate	EC25	>100	N/A	N/A	<1	Linear Interpolation (ICPIN)
		EC50	>100	N/A	N/A	<1	
15-3454-7387	Mean Dry Biomass-mg	IC25	>100	N/A	N/A	<1	Linear Interpolation (ICPIN)
		IC50	>100	N/A	N/A	<1	

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
19-9049-5948	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria ✓
15-3454-7387	Mean Dry Biomass-mg	Control Resp	0.5365	0.25 - NL	Yes	Passes Acceptability Criteria ✓
19-3355-7321	Mean Dry Biomass-mg	Control Resp	0.5365	0.25 - NL	Yes	Passes Acceptability Criteria ✓
19-3355-7321	Mean Dry Biomass-mg	PMSD	0.1953	0.12 - 0.3	Yes	Passes Acceptability Criteria ✓

**4d Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
2		10	0.95	0.8369	1	0.5	1	0.05	0.1581	16.64%	5.0%
4		10	1	1	1	1	1	0	0	0.0%	0.0%
10		10	0.9	0.7492	1	0.5	1	0.06667	0.2108	23.42%	10.0%
40		10	1	1	1	1	1	0	0	0.0%	0.0%
100		10	0.95	0.8369	1	0.5	1	0.05	0.1581	16.64%	5.0%

**7d Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
2		10	0.95	0.8369	1	0.5	1	0.05	0.1581	16.64%	5.0%
4		10	1	1	1	1	1	0	0	0.0%	0.0%
10		10	0.9	0.7492	1	0.5	1	0.06667	0.2108	23.42%	10.0%
40		10	0.95	0.8369	1	0.5	1	0.05	0.1581	16.64%	5.0%
100		10	0.95	0.8369	1	0.5	1	0.05	0.1581	16.64%	5.0%

**Mean Dry Biomass-mg Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	0.5365	0.449	0.624	0.38	0.795	0.0387	0.1224	22.81%	0.0%
2		10	0.5315	0.4902	0.5728	0.44	0.635	0.01826	0.05774	10.86%	0.93%
4		10	0.4545	0.3533	0.5557	0.25	0.69	0.04474	0.1415	31.13%	15.28%
10		10	0.5055	0.4339	0.5771	0.375	0.71	0.03167	0.1001	19.81%	5.78%
40		10	0.4755	0.4064	0.5446	0.3	0.645	0.03055	0.0966	20.32%	11.37%
100		10	0.523	0.4716	0.5744	0.35	0.605	0.02272	0.07185	13.74%	2.52%

**CETIS Summary Report**

Report Date: 17 Feb-16 13:55 (p 2 of 2)  
 Test Code: B347401ppc | 18-5116-9148

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

**4d Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	0.5	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	0.5	1	1	1	1	1	1	1	0.5
40		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	0.5	1	1	1	1

**7d Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	0.5	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	0.5	1	1	1	1	1	1	1	0.5
40		1	1	1	1	1	1	1	1	0.5	1
100		1	1	1	1	1	0.5	1	1	1	1

**Mean Dry Biomass-mg Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	0.795	0.5	0.55	0.585	0.38	0.49	0.66	0.515	0.395	0.4951
2		0.55	0.52	0.49	0.635	0.555	0.585	0.475	0.44	0.5	0.565
4		0.38	0.615	0.5	0.565	0.69	0.32	0.405	0.25	0.33	0.49
10		0.52	0.485	0.42	0.71	0.61	0.49	0.4301	0.45	0.565	0.375
40		0.48	0.52	0.45	0.485	0.645	0.48	0.495	0.35	0.3	0.55
100		0.56	0.58	0.47	0.5551	0.55	0.35	0.53	0.53	0.605	0.5

**4d Survival Rate Binomials**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
2		2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2
4		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
10		2/2	1/2	2/2	2/2	2/2	1/1	2/2	2/2	2/2	1/2
40		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
100		2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2	2/2

**7d Survival Rate Binomials**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
2		2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2
4		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
10		2/2	1/2	2/2	2/2	2/2	1/1	2/2	2/2	2/2	1/2
40		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2
100		2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2	2/2

**CETIS Analytical Report**

Report Date: 17 Feb-16 13:55 (p 1 of 6)  
 Test Code: B347401ppc | 18-5116-9148

**Fathead Minnow 7-d Larval Survival and Growth Test** **CH2M HILL - ASL**

<b>Analysis ID:</b> 20-4355-4702	<b>Endpoint:</b> 4d Survival Rate	<b>CETIS Version:</b> CETISv1.8.8
<b>Analyzed:</b> 17 Feb-16 13:54	<b>Analysis:</b> Nonparametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 04-7384-1677	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 02 Feb-16 10:30	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 09 Feb-16 08:35	<b>Species:</b> Pimephales promelas	<b>Brine:</b>
<b>Duration:</b> 6d 22h	<b>Source:</b> Aquatox, AR	<b>Age:</b> <48h
<b>Sample ID:</b> 04-7691-5455	<b>Code:</b> B3474-01	<b>Client:</b>
<b>Sample Date:</b> 01 Feb-16 08:20	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 01 Feb-16 13:03	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 26h (7.5 °C)	<b>Station:</b>	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	20.5%	100	>100	NA	1

**Steel Many-One Rank Sum Test**

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	100	75	1	18	0.6974	Asymp	Non-Significant Effect ✓
		4	105	75	1	18	0.8333	Asymp	Non-Significant Effect ✓
		10	95	75	1	18	0.5278	Asymp	Non-Significant Effect ✓
		40	105	75	1	18	0.8333	Asymp	Non-Significant Effect ✓
		100	100	75	1	18	0.6974	Asymp	Non-Significant Effect ✓

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0804717	0.01609434	5	1.431	0.2282	Non-Significant Effect
Error	0.6074986	0.01124997	54			
Total	0.6879703		59			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	780.1	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.5681	0.9459	<0.0001	Non-normal Distribution

**4d Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	1	0	0.0%	0.0%
2		10	0.95	0.8369	1	1	0.5	1	0.05	16.64%	5.0%
4		10	1	1	1	1	1	1	0	0.0%	0.0%
10		10	0.9	0.7492	1	1	0.5	1	0.06667	23.42%	10.0%
40		10	1	1	1	1	1	1	0	0.0%	0.0%
100		10	0.95	0.8369	1	1	0.5	1	0.05	16.64%	5.0%

**Angular (Corrected) Transformed Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	10	1.209	1.209	1.21	1.209	1.209	1.209	0	0.0%	0.0%
2		10	1.167	1.071	1.263	1.209	0.7854	1.209	0.0424	11.49%	3.51%
4		10	1.209	1.209	1.21	1.209	1.209	1.209	0	0.0%	0.0%
10		10	1.108	0.9814	1.235	1.209	0.7854	1.209	0.05616	16.02%	8.35%
40		10	1.209	1.209	1.21	1.209	1.209	1.209	0	0.0%	0.0%
100		10	1.167	1.071	1.263	1.209	0.7854	1.209	0.0424	11.49%	3.51%

**CETIS Analytical Report**

Report Date: 17 Feb-16 13:55 (p 2 of 6)  
 Test Code: B347401ppc | 18-5116-9148

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

Analysis ID: 20-4355-4702      Endpoint: 4d Survival Rate      CETIS Version: CETISv1.8.8  
 Analyzed: 17 Feb-16 13:54      Analysis: Nonparametric-Control vs Treatments      Official Results: Yes

**4d Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	0.5	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	0.5	1	1	1	1	1	1	1	0.5
40		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	0.5	1	1	1	1

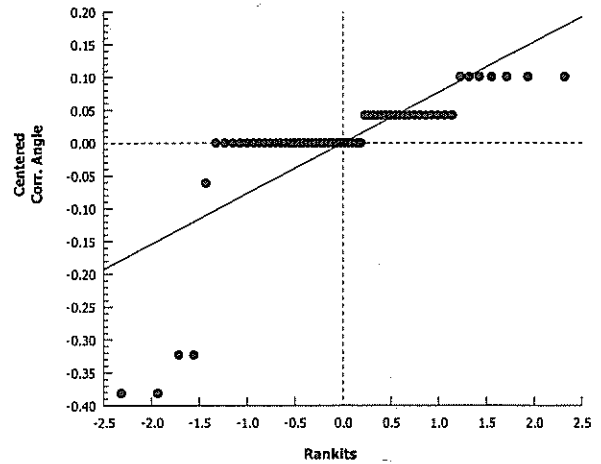
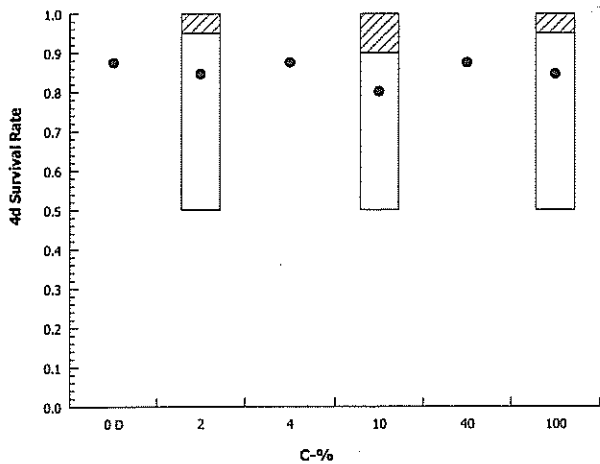
**Angular (Corrected) Transformed Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209
2		1.209	1.209	1.209	1.209	1.209	1.209	0.7854	1.209	1.209	1.209
4		1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209
10		1.209	0.7854	1.209	1.209	1.209	1.047	1.209	1.209	1.209	0.7854
40		1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209
100		1.209	1.209	1.209	1.209	1.209	0.7854	1.209	1.209	1.209	1.209

**4d Survival Rate Binomials**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
2		2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2
4		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
10		2/2	1/2	2/2	2/2	2/2	1/1	2/2	2/2	2/2	1/2
40		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
100		2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2	2/2

**Graphics**



# CETIS Analytical Report

Report Date: 17 Feb-16 13:55 (p 3 of 6)  
 Test Code: B347401ppc | 18-5116-9148

## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

<b>Analysis ID:</b> 19-9049-5948	<b>Endpoint:</b> 7d Survival Rate	<b>CETIS Version:</b> CETISv1.8.1
<b>Analyzed:</b> 10 Feb-16 10:55	<b>Analysis:</b> Nonparametric-Multiple Comparison	<b>Official Results:</b> Yes
<b>Batch ID:</b> 04-7384-1677	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 02 Feb-16 10:30	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 09 Feb-16 08:35	<b>Species:</b> Pimephales promelas	<b>Brine:</b>
<b>Duration:</b> 6d 22h	<b>Source:</b> Aquatox, AR	<b>Age:</b> <48h
<b>Sample ID:</b> 04-7691-5455	<b>Code:</b> B3474-01	<b>Client:</b>
<b>Sample Date:</b> 01 Feb-16 08:20	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 01 Feb-16 13:03	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 26h (7.5 °C)	<b>Station:</b>	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	21.0%	100	>100	NA	1

### Wilcoxon/Bonferroni Adj Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	100	NA	1	18	1.0000		Non-Significant Effect
		4	105	NA	1	18	1.0000		Non-Significant Effect
		10	95	NA	1	18	1.0000		Non-Significant Effect
		40	90	NA	1	17	1.0000		Non-Significant Effect
		100	100	NA	1	18	1.0000		Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.07949614	0.01589923	5	1.387	0.2441	Non-Significant Effect
Error	0.6074986	0.01146224	53			
Total	0.6869947		58			

### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	755.9	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.5708	0.9451	<0.0001	Non-normal Distribution

### 7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	1	0	0.0%	0.0%
2		10	0.95	0.8369	1	1	0.5	1	0.05	16.64%	5.0%
4		10	1	1	1	1	1	1	0	0.0%	0.0%
10		10	0.9	0.7492	1	1	0.5	1	0.06667	23.42%	10.0%
40		9	1	1	1	1	1	1	0	0.0%	0.0%
100		10	0.95	0.8369	1	1	0.5	1	0.05	16.64%	5.0%

### Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	10	1.209	1.209	1.21	1.209	1.209	1.209	0	0.0%	0.0%
2		10	1.167	1.071	1.263	1.209	0.7854	1.209	0.0424	11.49%	3.51%
4		10	1.209	1.209	1.21	1.209	1.209	1.209	0	0.0%	0.0%
10		10	1.108	0.9814	1.235	1.209	0.7854	1.209	0.05616	16.02%	8.35%
40		9	1.209	1.209	1.21	1.209	1.209	1.209	0	0.0%	0.0%
100		10	1.167	1.071	1.263	1.209	0.7854	1.209	0.0424	11.49%	3.51%

**CETIS Analytical Report**

Report Date: 17 Feb-16 13:55 (p 4 of 6)  
 Test Code: B347401ppc | 18-5116-9148

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

Analysis ID: 19-9049-5948      Endpoint: 7d Survival Rate      CETIS Version: CETISv1.8.1  
 Analyzed: 10 Feb-16 10:55      Analysis: Nonparametric-Multiple Comparison      Official Results: Yes

**7d Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	0.5	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	0.5	1	1	1	1	1	1	1	0.5
40		1	1	1	1	1	1	1	1	Outlier	1
100		1	1	1	1	1	0.5	1	1	1	1

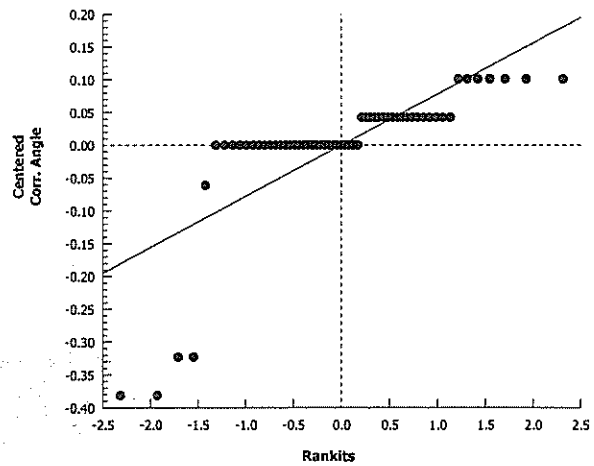
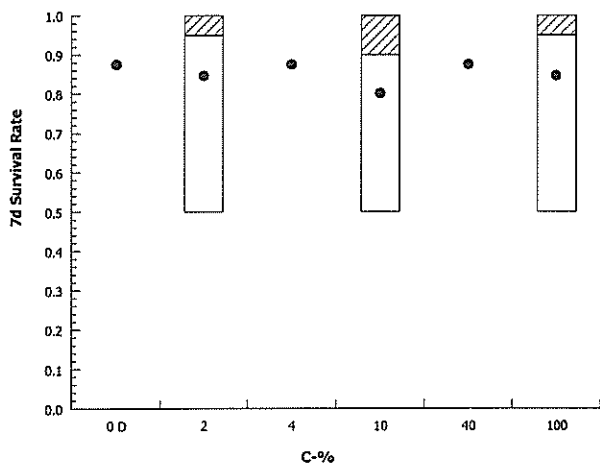
**Angular (Corrected) Transformed Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209
2		1.209	1.209	1.209	1.209	1.209	1.209	0.7854	1.209	1.209	1.209
4		1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209
10		1.209	0.7854	1.209	1.209	1.209	1.047	1.209	1.209	1.209	0.7854
40		1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209	1.209
100		1.209	1.209	1.209	1.209	1.209	0.7854	1.209	1.209	1.209	1.209

**7d Survival Rate Binomials**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
2		2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2
4		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
10		2/2	1/2	2/2	2/2	2/2	1/1	2/2	2/2	2/2	1/2
40		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2
100		2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2	2/2

**Graphics**



**CETIS Analytical Report**

Report Date: 17 Feb-16 13:55 (p 1 of 3)  
 Test Code: B347401ppc | 18-5116-9148

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

Analysis ID: 13-1696-7502	Endpoint: 4d Survival Rate	CETIS Version: CETISv1.8.8
Analyzed: 17 Feb-16 13:54	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 04-7384-1677	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 02 Feb-16 10:30	Protocol: EPA/821/R-02-013 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 09 Feb-16 08:35	Species: Pimephales promelas	Brine:
Duration: 6d 22h	Source: Aquatox, AR	Age: <48h
Sample ID: 04-7691-5455	Code: B3474-01	Client:
Sample Date: 01 Feb-16 08:20	Material: Unknown	Project:
Receive Date: 01 Feb-16 13:03	Source: Molalla, city of (OR0101514)	
Sample Age: 26h (7.5 °C)	Station:	

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	510830	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>100	N/A	N/A	<1	NA	NA
EC50	>100	N/A	N/A	<1	NA	NA

**4d Survival Rate Summary**

**Calculated Variate(A/B)**

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	10	1	1	1	0	0	0.0%	0.0%	20	20
2		10	0.95	0.5	1	0.05	0.1581	16.64%	5.0%	19	20
4		10	1	1	1	0	0	0.0%	0.0%	20	20
10		10	0.9	0.5	1	0.06667	0.2108	23.42%	10.0%	17	19
40		10	1	1	1	0	0	0.0%	0.0%	20	20
100		10	0.95	0.5	1	0.05	0.1581	16.64%	5.0%	19	20

**4d Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	0.5	1	1	1
4		1	1	1	1	1	1	1	1	1	1
10		1	0.5	1	1	1	1	1	1	1	0.5
40		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	0.5	1	1	1	1

**4d Survival Rate Binomials**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
2		2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2
4		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
10		2/2	1/2	2/2	2/2	2/2	1/1	2/2	2/2	2/2	1/2
40		2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
100		2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2	2/2



# CETIS Analytical Report

Report Date: 17 Feb-16 13:55 (p 2 of 3)  
Test Code: B347401ppc | 18-5116-9148

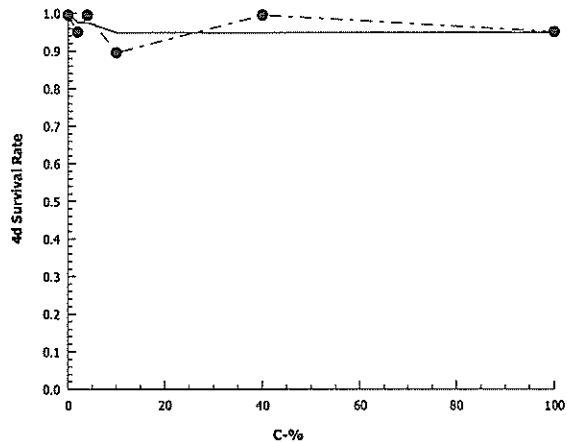
## Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

Analysis ID: 13-1696-7502      Endpoint: 4d Survival Rate  
Analyzed: 17 Feb-16 13:54      Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.8  
Official Results: Yes

### Graphics



**CETIS Analytical Report**

Report Date: 17 Feb-16 13:55 (p 5 of 6)  
 Test Code: B347401ppc | 18-5116-9148

**Fathead Minnow 7-d Larval Survival and Growth Test**

**CH2M HILL - ASL**

Analysis ID: 19-3355-7321	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.1
Analyzed: 10 Feb-16 10:55	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 04-7384-1677	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 02 Feb-16 10:30	Protocol: EPA/821/R-02-013 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 09 Feb-16 08:35	Species: Pimephales promelas	Brine:
Duration: 6d 22h	Source: Aquatox, AR	Age: <48h
Sample ID: 04-7691-5455	Code: B3474-01	Client:
Sample Date: 01 Feb-16 08:20	Material: Unknown	Project:
Receive Date: 01 Feb-16 13:03	Source: Molalla, city of (OR0101514)	
Sample Age: 26h (7.5 °C)	Station:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	19.5%	100	>100	NA	1

**Dunnett Multiple Comparison Test**

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	0.1095	2.289	0.105	18	0.7988		Non-Significant Effect ✓
		4	1.791	2.289	0.105	18	0.1342		Non-Significant Effect ✓
		10	0.6773	2.289	0.105	18	0.5658		Non-Significant Effect ✓
		40	1.333	2.289	0.105	18	0.2767		Non-Significant Effect ✓
		100	0.2948	2.289	0.105	18	0.7314		Non-Significant Effect ✓

**Auxiliary Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.64	3.2	0.4005	No Outliers Detected

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.05437499	0.010875	5	1.038	0.4048	Non-Significant Effect
Error	0.5656778	0.01047551	54			
Total	0.6200528		59			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.683	15.09	0.1224	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9786	0.9459	0.3708	Normal Distribution

**Mean Dry Biomass-mg Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	10	0.5365	0.449	0.624	0.5075	0.38	0.795	0.0387	22.81%	0.0%
2		10	0.5315	0.4902	0.5728	0.535	0.44	0.635	0.01826	10.86%	0.93%
4		10	0.4545	0.3533	0.5557	0.4475	0.25	0.69	0.04474	31.13%	15.28%
10		10	0.5055	0.4339	0.5771	0.4875	0.375	0.71	0.03167	19.81%	5.78%
40		10	0.4755	0.4064	0.5446	0.4825	0.3	0.645	0.03055	20.32%	11.37%
100		10	0.523	0.4716	0.5744	0.54	0.35	0.605	0.02272	13.74%	2.52%

**Mean Dry Biomass-mg Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	0.795	0.5	0.55	0.585	0.38	0.49	0.66	0.515	0.395	0.4951
2		0.55	0.52	0.49	0.635	0.555	0.585	0.475	0.44	0.5	0.565
4		0.38	0.615	0.5	0.565	0.69	0.32	0.405	0.25	0.33	0.49
10		0.52	0.485	0.42	0.71	0.61	0.49	0.4301	0.45	0.565	0.375
40		0.48	0.52	0.45	0.485	0.645	0.48	0.495	0.35	0.3	0.55
100		0.56	0.58	0.47	0.5551	0.55	0.35	0.53	0.53	0.605	0.5

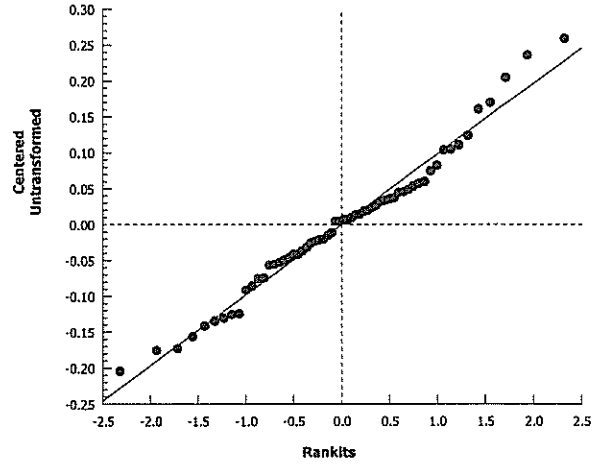
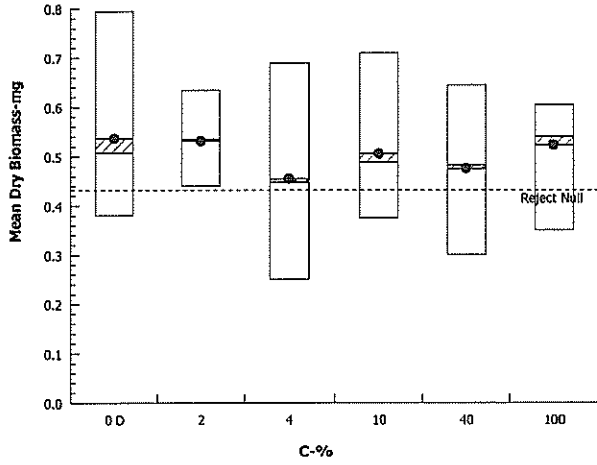
Fathead Minnow 7-d Larval Survival and Growth Test

CH2M HILL - ASL

Analysis ID: 19-3355-7321      Endpoint: Mean Dry Biomass-mg  
Analyzed: 10 Feb-16 10:55      Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.1  
Official Results: Yes

Graphics



**CETIS Analytical Report**

Report Date: 17 Feb-16 13:55 (p 3 of 3)  
 Test Code: B347401ppc | 18-5116-9148

**Fathead Minnow 7-d Larval Survival and Growth Test** **CH2M HILL - ASL**

<b>Analysis ID:</b> 15-3454-7387	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv1.8.8
<b>Analyzed:</b> 12 Feb-16 14:25	<b>Analysis:</b> Linear Interpolation (ICPIN)	<b>Official Results:</b> Yes
<b>Batch ID:</b> 04-7384-1677	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 02 Feb-16 10:30	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> Mod-Hard Synthetic Water
<b>Ending Date:</b> 09 Feb-16 08:35	<b>Species:</b> Pimephales promelas	<b>Brine:</b>
<b>Duration:</b> 6d 22h	<b>Source:</b> Aquatox, AR	<b>Age:</b> <48h
<b>Sample ID:</b> 04-7691-5455	<b>Code:</b> B3474-01	<b>Client:</b>
<b>Sample Date:</b> 01 Feb-16 08:20	<b>Material:</b> Unknown	<b>Project:</b>
<b>Receive Date:</b> 01 Feb-16 13:03	<b>Source:</b> Molalla, city of (OR0101514)	
<b>Sample Age:</b> 26h (7.5 °C)	<b>Station:</b>	

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1599875	200	Yes	Two-Point Interpolation

**Point Estimates**

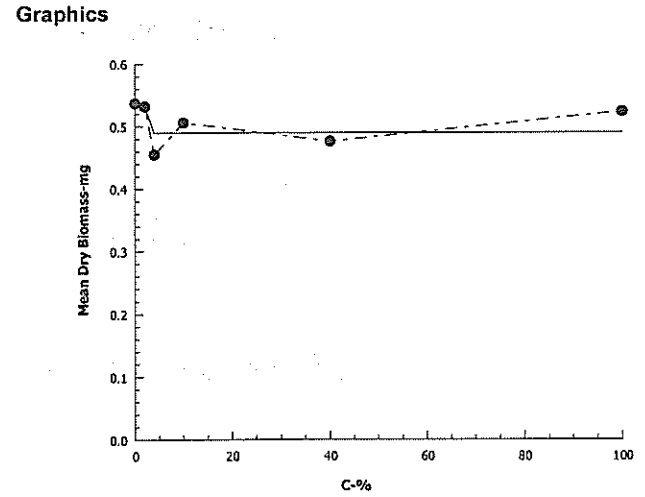
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	>100	N/A	N/A	<1	NA	NA
IC50	>100	N/A	N/A	<1	NA	NA

**Mean Dry Biomass-mg Summary**

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	0.5365	0.38	0.795	0.0387	0.1224	22.81%	0.0%
2		10	0.5315	0.44	0.635	0.01826	0.05774	10.86%	0.93%
4		10	0.4545	0.25	0.69	0.04474	0.1415	31.13%	15.28%
10		10	0.5055	0.375	0.71	0.03167	0.1001	19.81%	5.78%
40		10	0.4755	0.3	0.645	0.03055	0.0966	20.32%	11.37%
100		10	0.523	0.35	0.605	0.02272	0.07185	13.74%	2.52%

**Mean Dry Biomass-mg Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	0.795	0.5	0.55	0.585	0.38	0.49	0.66	0.515	0.395	0.4951
2		0.55	0.52	0.49	0.635	0.555	0.585	0.475	0.44	0.5	0.565
4		0.38	0.615	0.5	0.565	0.69	0.32	0.405	0.25	0.33	0.49
10		0.52	0.485	0.42	0.71	0.61	0.49	0.4301	0.45	0.565	0.375
40		0.48	0.52	0.45	0.485	0.645	0.48	0.495	0.35	0.3	0.55
100		0.56	0.58	0.47	0.5551	0.55	0.35	0.53	0.53	0.605	0.5



**APPENDIX B**  
**REFERENCE TOXICANT DATA SHEETS**

Random Template Used: 6 conc. x 4 reps. # 7  
 Stock Sol. ID Z B056-03  
 Organism ID: FHM 1856

Waterbath/incubator Used: # 7

Date Initiated 2/2/2016 Time 10:30  
 Date Terminated 2/9/2016 Time 08:35

Test Container Size: 800 ml Solution Volume / rep: 500 ml

Client QA/QC - RefTox

Sample Description NaCl (50 g/L stock)

Tech: Day 0 WJW Day 1 WJW Day 2 WJW Day 3 WJW Day 4 WJW Day 5 WJW Day 6 MC Day 7 WJW  
 Time Day 0 1030 Day 1 0910 Day 2 1100 Day 3 0945 Day 4 0900 Day 5 0750 Day 6 1305 Day 7 0835

Conc. or Percent	Day	Number of Live Organisms				Dissolved O <sub>2</sub> (mg/l)		pH		Temp. (°C) Pre	Therm ID #	Conductivity (µS) Post (daily)
		A	B	C	D	Pre	Post	Pre	Post			
Control	0	10	10	10	10		7.9		7.8	Post: 25.4	213	316
	1	10	10	10	10	6.8	7.5	7.5	8.3	25.5	184	348
	2	10	10	10	10	7.2	8.0	7.3	8.4	25.2	186	291
	3	10	10	10	10	7.3	7.6	7.3	8.5	24.9	177	294
	4	10	10	10	10	7.1	7.6	7.5	8.2	25.0	184	295
	5	10	10	10	9	6.6	7.8	7.2	8.4	25.0	186	292
	6	10	10	10	9	7.1	8.1	7.5	8.4	24.9	177	292
	7	10	10	10	9	7.2		7.2		24.7	159	
0.3 g/L	0	10	10	10	10		7.9		7.9	Post: 25.4		550
	1	10	10	10	10	7.0	7.6	7.5	8.3	25.4		549
	2	9	10	10	10	7.1	8.0	7.5	8.4	25.3		603
	3	9	10	10	10	7.4	7.8	7.5	8.4	24.9		866
	4	9	10	10	10	7.3	7.7	7.5	8.1	25.0		847
	5	9	10	10	10	7.1	7.7	7.3	8.3	25.1		843
	6	9	10	10	10	7.2	8.1	7.5	8.4	25.0		842
	7	9	10	10	10	7.3		7.3		24.7		
1.0 g/L	0	10	10	10	10		7.9		8.0	Post: 25.3		2200
	1	10	10	10	10	7.0	7.7	7.7	8.2	25.3		2190
	2	10	10	10	10	7.2	8.0	7.5	8.4	25.2		2150
	3	10	10	10	10	6.7	7.9	7.4	8.3	25.0		2090
	4	10	10	10	10	7.4	7.9	7.6	8.1	25.1		2010
	5	10	10	10	10	7.0	8.0	7.4	8.3	25.3		2120
	6	10	10	10	10	7.2	8.2	7.4	8.2	25.1		2020
	7	10	10	10	10	7.4		7.4		24.9		
2.0 g/L	0	10	10	10	10		7.9		8.0	Post: 25.4		3840
	1	10	10	10	10	6.9	7.7	7.7	8.2	25.4		3920
	2	10	10	10	10	7.1	8.0	7.6	8.4	25.3		3800
	3	10	*10 8	*10 9	10	6.9	7.8	7.5	8.3	25.6		3870
	4	*10 10	*8 8	*9 9	*10 9	7.5	8.0	7.6	8.2	25.2		3710
	5	8	7	8	6	6.9	8.0	7.5	8.3	25.4		3810
	6	6	6	8	5	7.3	8.1	7.5	8.2	24.9		3810
	7	5	6	8	4	7.6		7.5		24.8		
4.0 g/L	0	10	10	10	10		7.9		8.1	Post: 25.3		7200
	1	10	10	9	10	7.0	7.7	7.7	8.1	25.3		7260
	2	9	10	9	10	7.2	8.0	7.6	8.3	25.3		7090
	3	9	10	7	10	6.8	7.7	7.5	8.2	25.2		7210
	4	5	6	6	7	7.4	7.9	7.6	8.2	25.2		7050
	5	2	3	2	5	6.9	8.0	7.5	8.2	25.3		7040
	6	0	1	2	2	7.3	8.2	7.5	8.1	24.9		7030
	7	5	1	1	1	7.6		7.5		25.1		
8.0 g/L	0	10	10	10	10		7.9		8.0	Post: 25.2		13250
	1	9	9	10	9	7.0	7.7	7.8	8.0	25.3		13550
	2	4	2	0	0	7.2	8.0	7.5	8.3	25.3		13160
	3	1	0			6.7	7.7	7.5	8.1	24.8		13650
	4	1				7.4	8.1	7.4	8.2	25.4		12640
	5	1				7.0	8.1	7.5	8.1	25.2		13150
	6	0				7.4	8.2	7.4	8.0	25.1		13340
	7	5										

✓ Indicates one organism inadvertently poured off during solution renewal, replaced into container. Day 0 Temperatures = Post-renewals  
 "M" = organism missing, start count reduced. "Inj" = organism injured, remove from stats. Therm ID# = Thermometer ID used for all measurements that day.  
 "F" = fungus noted on dead organisms. Pre = Pre-renewal solutions. Post = Post-renewal solutions. 23.8 = Temp. out of recommended range

**Endpoint IC25 Cusum Chart Limits**  
 Survival 1.6 1.4 to 4.1  
 Growth 1.4\* 1.6 to 3.2

Task Manager [Signature]  
 Project Manager [Signature]  
 QA Officer [Signature]

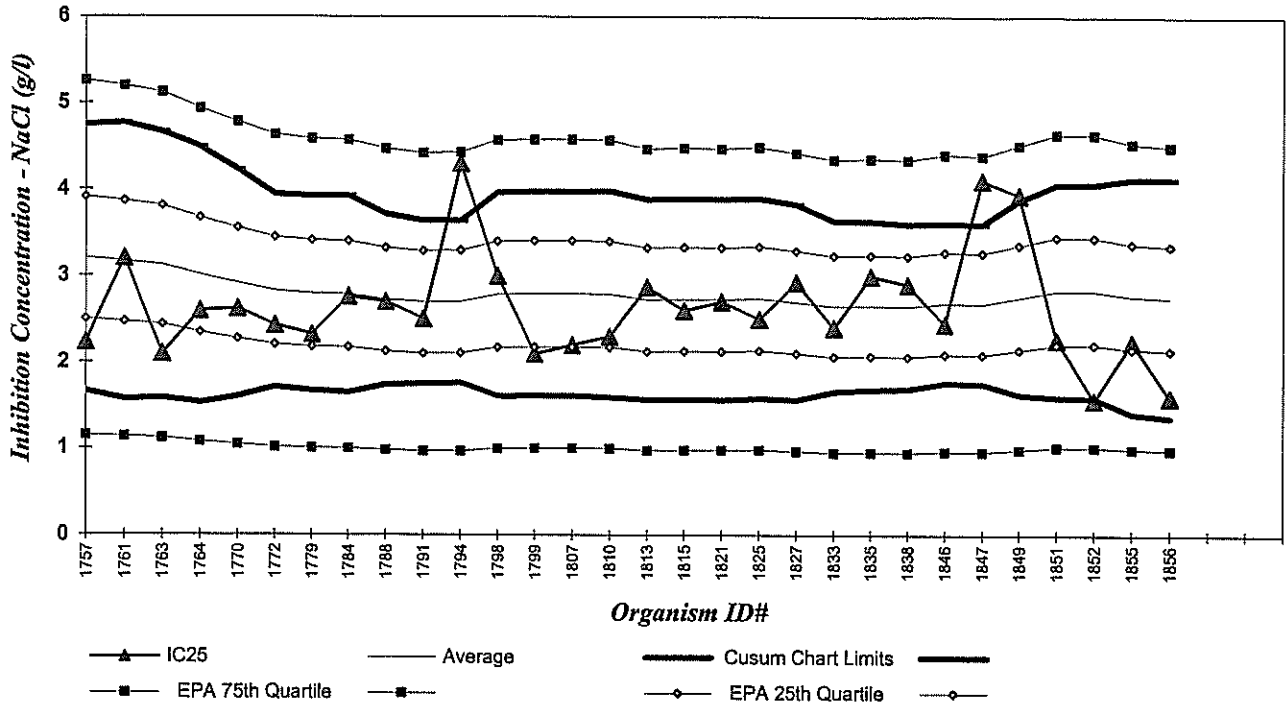
# BIOASSAY REFTOX AND CUSUM CHART EVALUATION FORM

Test Type: <input type="checkbox"/> Acute -OR- <input checked="" type="checkbox"/> Chronic Surv./ Growth Test Endpoint: <input type="checkbox"/> Survival -OR- <input checked="" type="checkbox"/> Growth REFTOX test start date: 2-2-2016	Analyst doing review: <i>Doug Winn</i> Peer Reviewer: <i>B. [Signature]</i> Bioassay Section Lead: <i>B. [Signature]</i> QA Officer:	Date: 2-11-2016 Date: 2-11-2016 Date: 2-11-2016 Date:
--	---	--

No USEPA Quantiles apply	ASL limits within USEPA 25th Quartile	ASL limits between USEPA 25th Quartile and USEPA 75th Quartile	ASL limits outside of the USEPA 75th Quartile
<p><input type="checkbox"/> Cultured inhouse -OR-  <input type="checkbox"/> Reflux results from supplier available  <input type="checkbox"/> Test is Routine (10+ per year)  <input type="checkbox"/> Previous 19+ reflux tests in limits  <input type="checkbox"/> NOT subject to WDOE regulation</p> <p>IF ALL APPLY, reflux test need NOT be immediately repeated                      Otherwise, REPEAT as soon as Practicable (preferably within same calendar month)</p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p><input type="checkbox"/> Cultured inhouse -OR-  <input type="checkbox"/> Reflux results from supplier available  <input type="checkbox"/> Test is Routine (10+ per year)  <input type="checkbox"/> Previous 9+ reflux tests in limits  <input type="checkbox"/> Cusum chart shows long term 95%+ of reflux tests being in range  <input type="checkbox"/> NOT subject to WDOE regulation</p> <p>IF ALL APPLY, reflux test need NOT be immediately repeated                      Otherwise, REPEAT as soon as Practicable (preferably within same calendar month)</p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p><input type="checkbox"/> Cultured inhouse -OR-  <input type="checkbox"/> Reflux results from supplier available  <input checked="" type="checkbox"/> Test is Routine (10+ per year)  <input type="checkbox"/> Previous 19+ reflux tests in limits  <input type="checkbox"/> NOT subject to WDOE regulation</p> <p>IF ALL APPLY, reflux test need NOT be immediately repeated                      Otherwise, REPEAT as soon as Practicable (preferably within same calendar month)</p> <p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p>REPEAT as soon as Practicable (preferably within same calendar month)</p> <p>Does the reflux test need to be repeated?  <input checked="" type="checkbox"/> Yes</p>
<p>Root Cause Analysis:                      Correct Toxicant used?: <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No                      Correct Water used?: <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No                      Correct Temperature used?: <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No                      DO, pH, Cond./Salinity OK?: <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p> <p>If in disagreement with Analyst assessment, forward to Section Leader QA officer</p>	<p>Other Comments / Observations:                      Last 4 tests (of 6) have been out of Cusum chart range!</p> <p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p> <p>If in disagreement with Analyst assessment, forward to Section Leader QA officer</p>	<p>FHM Chronic Ref Tox test rescheduled to begin on Feb. 16, 2016! D.W.                      2 scheduled: tradit. supplier &amp; new organ-ism supplier!</p> <p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p> <p>If in disagreement with Analyst assessment, forward to Section Leader QA officer</p>	<p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes</p>
<p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p>Does the reflux test need to be repeated? (check one)  <input type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>	<p>Does the reflux test need to be repeated? (check one)  <input checked="" type="checkbox"/> Yes -OR- <input type="checkbox"/> No</p>

Analyst: \_\_\_\_\_  
 Peer Reviewer: \_\_\_\_\_  
 Sect. Lead: \_\_\_\_\_

**REFERENCE TOXICANT CUMLATIVE SUMMARY (CUSUM) CHART**  
*Pimphales promelas* Chronic Survival - IC25 Values



***Pimphales promelas* - Chronic (EPA Test Method 1000.0)**

**SODIUM CHLORIDE (g/L)**

From EPA 833-R-00-003:

Endpoint: Chronic Survival

10th Quartile CV (control limit) = 0.03

Stats Method: Linear Interpolation

25th Quartile CV (warning limit) = 0.11

Test Conditions: Recon MH, 25 oC

75th Quartile CV (warning limit) = 0.32

90th Quartile CV (control limit) = 0.52

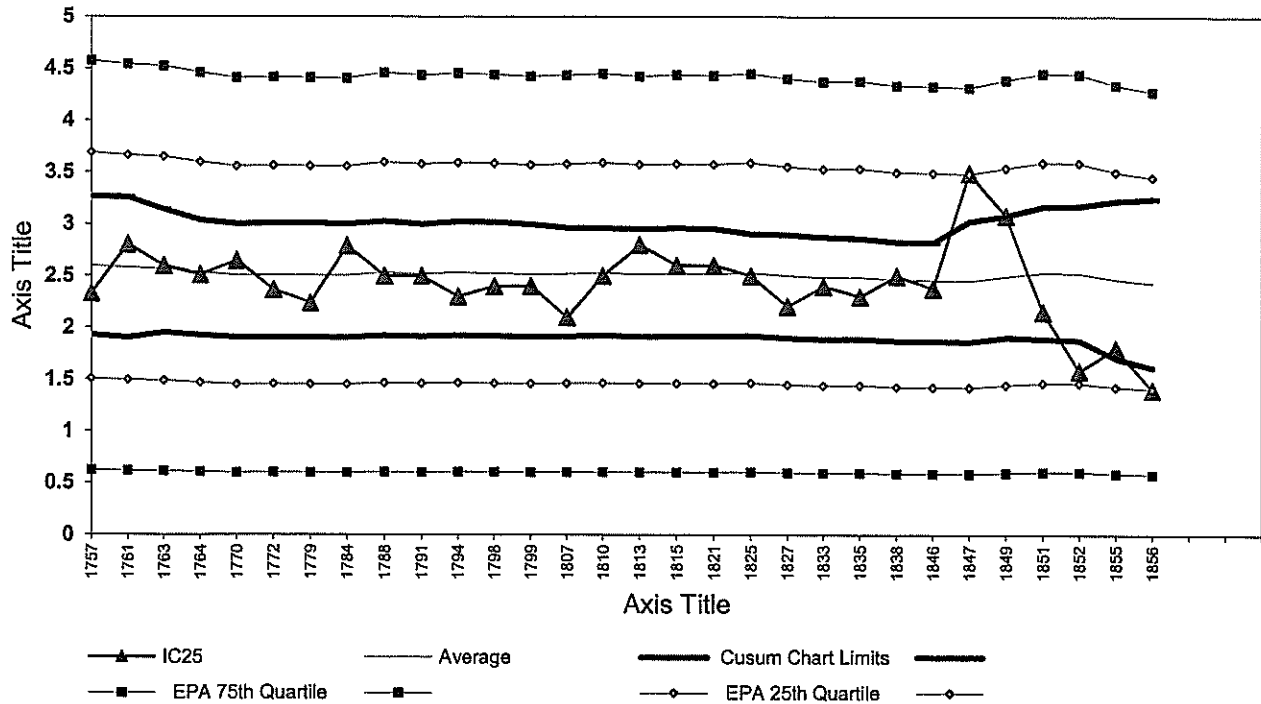
*Intralab CV is compared to EPA Warning limits (25th and 75th CV's) and Control limits (10th and 90th CV's),*

*If lab CV is outside EPA Control limits, the EPA Control limits are used to set Cusum chart limits.*

Event #	FHM ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
321	1827	07/07/15	2.9	2.7	0.57	1.6	3.8	0.19
322	1833	08/18/15	2.4	2.6	0.49	1.7	3.6	0.18
323	1835	09/15/15	3.0	2.7	0.49	1.7	3.6	0.18
324	1838	10/06/15	2.9	2.6	0.48	1.7	3.6	0.17
325	1846	11/17/15	2.4	2.7	0.46	1.8	3.6	0.17
326	1847	12/01/15	4.1	2.7	0.46	1.8	3.6	0.20
327	1849	12/15/15	3.937	2.8	0.56	1.63	3.88	0.22
328	1851	12/29/16	2.3	2.8	0.62	1.6	4.1	0.22
329	1852	01/12/16	1.57	2.8	0.62	1.59	4.06	0.25
330	1855	01/26/16	2.2	2.8	0.68	1.4	4.1	0.25
331	1856	02/02/16	1.6	2.7	0.69	1.4	4.1	0.27
332								
333								



**REFERENCE TOXICANT CUMULATIVE SUMMARY (CUSUM) CHART**  
*Pimephales promelas* Chronic Biomass - IC25 Values



***Pimephales promelas* - Chronic (EPA Test Method 1000.0)**

**SODIUM CHLORIDE (g/L)**

Endpoint: Chronic Growth (Biomass)

Stats Method: Linear Interpolation

Test Conditions: Recon MH, 25 oC

From EPA 833-R-00-003:

10th Quartile CV (control limit) = 0.12

25th Quartile CV (warning limit) = 0.21

75th Quartile CV (warning limit) = 0.38

90th Quartile CV (control limit) = 0.45

*Intralab CV is compared to EPA Warning limits (25th and 75th CV's) and Control limits (10th and 90th CV's).*

*If lab CV is outside EPA Control limits, the EPA Control limits are used to set Cusum chart limits.*

Event #	FHM ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
321	1827	7/7/2015	2.2	2.5	0.19	1.9	2.9	0.08
322	1833	8/18/2015	2.4	2.5	0.20	1.9	2.9	0.08
323	1835	9/15/2015	2.3	2.5	0.19	1.9	2.9	0.07
324	1838	10/6/2015	2.5	2.5	0.18	1.9	2.8	0.07
325	1846	11/17/2015	2.4	2.5	0.18	1.9	2.8	0.07
326	1847	12/1/2015	3.5	2.5	0.18	1.9	3.0	0.12
327	1849	12/15/2015	3.081	2.5	0.29	1.907	3.080	0.13
328	1851	12/29/2015	2.2	2.5	0.32	1.9	3.2	0.13
329	1852	1/12/2016	1.6	2.5	0.32	1.9	3.2	0.15
330	1855	1/26/2016	1.8	2.5	0.38	1.7	3.2	0.17
331	1856	2/2/2016	1.4	2.4	0.41	1.6	3.2	0.20
332								
333								

**APPENDIX C**  
**CHAIN OF CUSTODY**

# CH2MHILL

## CHAIN OF CUSTODY RECORD FOR NPDES COMPLIANCE BIOMONITORING

Client City of Molalla WWTF  
Address 12424 S Taliver Road

NPDES# \_\_\_\_\_

Ship Samples to:  
CH2M HILL - Applied Sciences Laboratory  
Attention: Bioassay Lab  
1100 NE Circle Blvd, Suite 300  
Corvallis, OR 97330  
Lab Phone: (541) 768-3160  
Customer Service: (541) 768-3120

Contact Person: Jason Clifford  
Phone: 503-793-5283  
E-mail: jclifford@cityofmolalla.com

CH2M HILL Project # / Purchase Order # WASTWATER

### Composite Sample Information:

Initiated: Date 1-31-16 Time 0600  
Ended: Date 2-1-16 Time 0600  
Chilled During Collection? Yes  No   
Dechlorinated prior to shipping? Yes  No

### Analysis Required / Comments

Sample ID	Date	Time	Sample Type		# of Containers	Lab ID#	Fathead Acute	Fathead Chronic	Cerio Acute	Cerio Chronic	Green Algae	Trout Acute	Sheepshead Acute	Sheepshead Chronic	Menidia Acute	Menidia Chronic	Mysid Acute	Mysid Chronic	Haz Waste	Concentration and/or Comments		
			Comp.	Grab																		
Final Effluent (Dms)	2-1-16	0820		✓	1		✓															

Sampled By: <i>[Signature]</i> <u>Jon Randall</u> (Please sign and print name)	Date/Time <u>2/1/16 / 0830</u>	Relinquished By: <i>[Signature]</i> <u>Jon Randall</u> (Please sign and print name)	Date/Time <u>2/1/16 / 1103</u>
Received By	Date/Time	Relinquished By	Date/Time
Received By	Date/Time	Relinquished By	Date/Time
Received By: <i>[Signature]</i> (Please sign and print name)	Date/Time <u>02/01/16/1103</u>	Shipped Via	Shipping #
Work Authorized By: <i>[Signature]</i> (Please sign and print name)	Remarks: <u>B3474A</u>	UPS <input type="checkbox"/> Bus <input type="checkbox"/> Fed-Ex <input type="checkbox"/> Hand <input type="checkbox"/> Other <input type="checkbox"/>	

HARD Delivery 7.5°C



Batch Number: B 3474A  
Client/Project: City of Waiia

Date Received: 02/02/16  
Received By: JP

- Were custody seals intact?  Yes  No  N/A
- Packing Material:  Ice  Blue Ice  Box
- Temp OK? (<6C) Therm ID: TH173 Exp. 04/16 HAND Delivery 7.5 °C  Yes  No  N/A
- Was a Chain of Custody (CoC) Provided?  Yes  No  N/A
- Was the CoC correctly filled out (If No, document below)  Yes  No  N/A
- Were the sample containers in good condition (not broken or leaking)?  Yes  No  N/A
- Are all samples within 36 hours of collection?  Yes  No  N/A
- Method of Shipment:  Hand Delivered  FedEx  UPS  Greyhound  Other: \_\_\_\_\_  N/A

**Sample Exception Report** (The following exceptions were noted)

Sample Chilled in transit; going ahead with testing.  
MC  
2-1-16

Client was notified on: \_\_\_\_\_ Client contact: \_\_\_\_\_

Resolution to Exception:

# CH2MHILL

## CHAIN OF CUSTODY RECORD FOR NPDES COMPLIANCE BIOMONITORING

Client City of Malilla WWTP  
 Address 12424 S. Toliver Road

NPDES# 101514

Ship Samples to:  
 CH2M HILL - Applied Sciences Laboratory  
 Attention: Bioassay Lab  
 1100 NE Circle Blvd. Suite 300  
 Corvallis, OR 97330  
 Lab Phone: (541) 768-3160  
 Customer Service: (541) 768-3120

### Composite Sample Information:



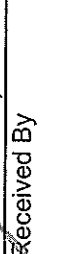
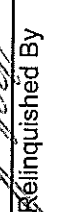



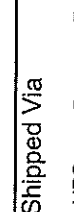
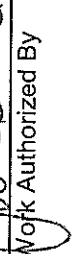
Initiated: Date 2-2-16 Time 0600  
 Ended: Date 2-3-16 Time 0600  
 Chilled During Collection? Yes  No   
 Dechlorinated prior to shipping? Yes  No

CH2M HILL Project # / Purchase Order # wastewater

Contact Person: Jason Clifford  
 Phone: 503-793-5283  
 E-mail: jclifford@cityofmalilla.com

### Analysis Required / Comments

Sample ID	Date	Time	Comp.	Grab	# of Containers	Lab ID#	Analysis Required / Comments										Concentration and/or Comments								
							Fathead Acute	Fathead Chronic	Cerio Acute	Cerio Chronic	Green Algae	Trout Acute	Sheepshead Acute	Sheepshead Chronic	Menidia Acute	Menidia Chronic		Mysid Acute	Mysid Chronic	Haz Waste					
Final Effluent (Dms)	2-3-16	0900	<input checked="" type="checkbox"/>		1	B3474B	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>																	

Sampled By & Title 	(Please sign and print name) Jon Randall	Date/Time 2/3/16	Date/Time 0850	Relinquished By 	(Please sign and print name) Jon Randall	Date/Time 2/3/16	Date/Time 1105
Received By 	(Please sign and print name)	Date/Time	Date/Time	Relinquished By 	(Please sign and print name)	Date/Time	Date/Time
Received By 	(Please sign and print name)	Date/Time	Date/Time	Relinquished By 	(Please sign and print name)	Date/Time	Date/Time
Received By 	(Please sign and print name)	Date/Time 2/3/16	Date/Time 1105	Relinquished By 	(Please sign and print name)	Date/Time	Date/Time
Work Authorized By 	(Please sign and print name)	Shipped Via UPS <input type="checkbox"/> Bus <input type="checkbox"/> Fed-Ex <input type="checkbox"/> Hand <input type="checkbox"/>	Shipping #	Remarks B3474B HAND DELIVERY, ice 4.6°C			



Batch Number: B3474B  
Client/Project: City of Lowell A

Date Received: 02/03/16 @ 1105  
Received By: JR

- Were custody seals intact? *HAND Delivery*  Yes  No  N/A
- Packing Material:  Ice  Blue Ice  Box
- Temp OK? (<6C) Therm ID: TH173 Exp. 04/16 4.6°C  Yes  No  N/A
- Was a Chain of Custody (CoC) Provided?  Yes  No  N/A
- Was the CoC correctly filled out (If No, document below)  Yes  No  N/A
- Were the sample containers in good condition (not broken or leaking)?  Yes  No  N/A
- Are all samples within 36 hours of collection?  Yes  No  N/A
- Method of Shipment:  Hand Delivered  FedEx  UPS  Greyhound  Other: \_\_\_\_\_  N/A

**Sample Exception Report** (The following exceptions were noted)

Client was notified on: \_\_\_\_\_ Client contact: \_\_\_\_\_

Resolution to Exception:

# CH2MHILL

## CHAIN OF CUSTODY RECORD FOR NPDES COMPLIANCE BIOMONITORING

Client City of Molalla WWT  
 Address 12424 Stalvey Road

NPDES# 101514

Ship Samples to:  
 CH2M HILL - Applied Sciences Laboratory  
 Attention: Bioassay Lab  
 1100 NE Circle Blvd. Suite 300  
 Corvallis, OR 97330  
 Lab Phone: (541) 768-3160  
 Customer Service: (541) 768-3120

**Composite Sample Information:**

Initiated: Date 2-4-16 Time 0600  
 Ended: Date 2-5-16 Time 0600  
 Chilled During Collection? Yes  No   
 Dechlorinated prior to shipping? Yes  No

CH2M HILL Project # / Purchase Order # wastewater

Contact Person: Jason Clifford  
 Phone: 503-793-5283  
 E-mail: jclifford@cityofmolalla.com

**Analysis Required / Comments**

Sample ID	Date	Time	Sample Type		# of Containers	Lab ID#	Fathead Acute	Fathead Chronic	Cerio Acute	Cerio Chronic	Green Algae	Trout Acute	Sheepshead Acute	Sheepshead Chronic	Menidia Acute	Menidia Chronic	Mysid Acute	Mysid Chronic	Haz Waste	Concentration and/or Comments		
			Comp.	Grab																		
Final Effluent (Dms)	2-5-16	0810	<input checked="" type="checkbox"/>		1	B347403	<input checked="" type="checkbox"/>															

Sampled By & Title: J. Randall Jon Randall (Please sign and print name) Date/Time: 2/5/16 0810 Date/Time: 2/5/16 0953

Received By: [Signature] (Please sign and print name) Date/Time: 2/5/16 0953 Date/Time: 2/5/16 0953

Relinquished By: [Signature] (Please sign and print name) Date/Time: 2/5/16 0953 Date/Time: 2/5/16 0953

Relinquished By: [Signature] (Please sign and print name) Date/Time: 2/5/16 0953 Date/Time: 2/5/16 0953

Relinquished By: [Signature] (Please sign and print name) Date/Time: 2/5/16 0953 Date/Time: 2/5/16 0953

Shipped Via: Hand Delivery Shipping # 242

Remarks: B347403

Work Authorized By: [Signature] (Please sign and print name)



Batch Number: B3474C  
Client/Project: City of MO/ALIA

Date Received: 02/05/16  
Received By: JRP

- Were custody seals intact? *HAND Delivery*  Yes  No  N/A
- Packing Material:  Ice  Blue Ice  Box
- Temp OK? (<6C) Therm ID: TH173 Exp. 04/16 2.4 °C  Yes  No  N/A
- Was a Chain of Custody (CoC) Provided?  Yes  No  N/A
- Was the CoC correctly filled out (If No, document below)  Yes  No  N/A
- Were the sample containers in good condition (not broken or leaking)?  Yes  No  N/A
- Are all samples within 36 hours of collection?  Yes  No  N/A
- Method of Shipment:  Hand Delivered  FedEx  UPS  Greyhound  Other: \_\_\_\_\_  N/A

**Sample Exception Report** (The following exceptions were noted)

Client was notified on: \_\_\_\_\_ Client contact: \_\_\_\_\_

Resolution to Exception: