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Comments: Bioassay toxicity analysis was subcontracted to Nautilus Environmental Ltd. located in Burnaby, BC. Refer to their report appended for detail.

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ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample ID Description Sampled Date Sampled Time Client ID					
Grouping	Analyte				

Reference Information

Test Method References:

ALS Test Code	Matrix	Test Description	Method Reference**
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** ALS test methods may incorporate modifications from specified reference methods to improve performance.

The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location
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Chain of Custody Numbers:

1

GLOSSARY OF REPORT TERMS

Surrogate - A compound that is similar in behaviour to target analyte(s), but that does not occur naturally in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery.

mg/kg - milligrams per kilogram based on dry weight of sample.

mg/kg wwt - milligrams per kilogram based on wet weight of sample.

mg/kg lwt - milligrams per kilogram based on lipid-adjusted weight of sample.

mg/L - milligrams per litre.

< - Less than.

D.L. - The reported Detection Limit, also known as the Limit of Reporting (LOR).

N/A - Result not available. Refer to qualifier code and definition for explanation.

Test results reported relate only to the samples as received by the laboratory.

UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.

Analytical results in unsigned test reports with the DRAFT watermark are subject to change, pending final QC review.



**Toxicity testing of L1426336-1 (R10), L1426336-2 (NF1),
L1426336-3 (NF2), L1426336-4 (X1), L1426336-5 (X14),
L1426336-6 (X3A) and L1426336-7 (R3)**

Samples collected February 25, 2014

Final Report

Report date:
April 29, 2013

Submitted to:

ALS Environmental
Burnaby, BC

8664 Commerce Court
Burnaby, BC
V5A 4N7

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SIGNATURE PAGE



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This report has been prepared by Nautilus Environmental Company Inc. based on data and/or samples provided by our client and the results of this study are for their sole benefit. Any reliance on the data by a third party is at the sole and exclusive risk of that party. The results presented here relate only to the samples tested.

1.0 INTRODUCTION

Nautilus Environmental conducted acute and sub-lethal toxicity tests for ALS Environmental on seven samples identified as L1426336-1 (R10), L1426336-2 (NF1), L1426336-3 (NF2), L1426336-4 (X1), L1426336-5 (X14), L1426336-6 (X3A) and L1426336-7 (R3). All samples were collected on February 25, 2014 and delivered to the laboratory in Burnaby, BC on February 27, 2014. Samples were transported in two 20-L plastic containers per sample. The samples were received at temperatures ranging from 2.8 - 4.3°C and were stored in the dark at $4 \pm 2^\circ\text{C}$ prior to testing. The following toxicity tests were performed on all samples:

- *Ceriodaphnia dubia* survival and reproduction
- 7-d *Lemna minor* growth inhibition
- 72-h *Pseudokirchneriella subcapitata* growth inhibition
- 96-h rainbow trout (*Oncorhynchus mykiss*) LC50

This report describes the results of these toxicity tests. Copies of laboratory data sheets and printouts of statistical analyses for each test are provided in Appendices A to D. The chain-of-custody form is provided in Appendix E.

2.0 METHODS

Methods for the toxicity tests are summarized in Tables 1 to 4. Testing was conducted according to procedures described by the Environment Canada protocols (2000, 2007a, 2007b and 2007c). Statistical analyses for the tests were performed using CETIS (Tidepool Scientific Software, 2013).

Table 1. Summary of test conditions: *Ceriodaphnia dubia* survival and reproduction test.

Test organism	<i>Ceriodaphnia dubia</i>
Test organism source	In-house culture
Test organism age	<24 h old neonates produced within 12 h
Test type	Static-renewal
Test duration	7 ± 1 day
Test vessel	20 mL test tube
Test volume	15 mL
Test replicates	10 test replicates per treatment
No. of organisms	1 per replicate
Control water	20% Perrier water
Test solution renewal	Daily
Test temperature	25 ± 1°C
Feeding	<i>Pseudokirchneriella subcapitata</i> and YCT
Light intensity	100 to 600 lux at water surface
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	Environment Canada (2007a), EPS 1/RM/21
Statistical software	CETIS (2013)
Test endpoint	Survival and reproduction
Test acceptability criteria for controls	≥80% survival; ≥15 young per surviving control producing three broods; ≥60% of controls producing three or more broods
Reference toxicant	Sodium chloride

Table 2. Summary of test conditions: *Lemna minor* growth inhibition test.

Test organism	<i>Lemna minor</i> , CPCC#490
Test organism source	In-house culture, obtained from Canadian Phycological Culture Centre, and originally isolated from Wainfleet, Stinking Barn, Niagra Peninsula, Ontario, Canada.
Test organism age	7 to 10 day old
Test type	Static
Test duration	7 days
Test vessel	250-mL glass containers
Test volume	100 mL
Test replicates	4 per treatment
No. of organisms	Two 3-frond plants per replicate
Control water	Deionized water with supplemented nutrients
Test solution renewal	None
Test temperature	25 ± 2°C
Feeding	None
Light intensity	4000 to 5600 lux full spectrum light
Photoperiod	24 h light
Aeration	None
Test protocol	Environment Canada (2007b), EPS 1/RM/37
Statistical software	CETIS (2013)
Test endpoint	Number of fronds and dry weight
Test acceptability criteria for controls	≥ 8-fold increase in number of fronds
Reference toxicant	Potassium chloride

Table 3. Summary of test conditions: *Pseudokirchneriella subcapitata* growth inhibition test.

Test organism	<i>Pseudokirchneriella subcapitata</i> , strain UTCC #37
Test organism source	In-house culture, obtained from Canadian Phycological Culture Centre, and originally isolated from Nitelva River, Norway.
Test organism age	3-to 7-day old culture in logarithmic growth phase
Test type	Static
Test duration	72 hours
Test vessel	Microplate
Test volume	220 µL
Test replicates	4 replicates per treatment; 8 replicates for control
No. of organisms	10, 000 cells/mL
Control water	Deionized water with supplemented nutrients
Test solution renewal	None
Test temperature	24 ± 2°C
Feeding	None
Light intensity	3600 to 4400 lux
Photoperiod	24 hours light
Aeration	None
Test protocol	Environment Canada (2007c), EPS 1/RM/25
Statistical software	CETIS (2013)
Test endpoint	Algal cell growth inhibition
Test acceptability criteria for controls	≥ 16-fold increase in number of algal cells; CV ≤20%; no trend when analyzed using Mann-Kendall test
Reference toxicant	Zinc

Table 4. Summary of test conditions: 96-h rainbow trout (*Oncorhynchus mykiss*) LC50 test.

Test organism	<i>Oncorhynchus mykiss</i>
Test organism source	Commercial hatchery
Test organism age	Juvenile
Test type	Static
Test duration	96 hours
Test vessel	20 L glass aquarium
Test volume	10 to 20 L (dependent on size of fish)
Test replicates	1 test replicate per treatment
No. of organisms	10 per replicate
Control water	Municipal dechlorinated water
Test solution renewal	None
Test temperature	15 ± 1°C
Feeding	None
Light intensity	100 to 500 lux
Photoperiod	16 hours light/8 hours dark
Aeration	6.5 ± 1 mL/min/L
Test protocol	Environment Canada (2000), EPS 1/RM/13
Statistical software	CETIS (2013)
Test endpoint	LC50
Test acceptability criteria for controls	Survival ≥ 90%
Reference toxicant	Sodium nitrite

3.0 RESULTS

The results of toxicity tests conducted on samples R10, NF1, NF2, X1, X14, X3A and R3 are provided in Tables 5 to 26. For samples R10 and NF1, no adverse effects were observed in any species tested. In addition, stimulation was observed in the *P. subcapitata* tests in most concentrations (Tables 19 and 20). For samples NF2, X1, X14, X3A and R3 all species tested were adversely effected, with the exception of *L. minor* dry weight and rainbow trout survival. The LC50 value for rainbow trout was >100% in all samples tested.

Table 5. Results: *Ceriodaphnia dubia* survival and reproduction test with sample R10.

Concentration (% v/v)	Survival (%)	Reproduction (mean ± SD)
Control	100	16.7 ± 2.4
5	100	16.5 ± 3.1
10	100	15.0 ± 4.1
20	100	17.5 ± 3.0
40	90	13.5 ± 6.0
60	90	15.2 ± 5.5
80	90	13.7 ± 6.8
100	100	16.8 ± 4.3
Test endpoint (% v/v)		
LC50	>100	-
IC25	-	>100
IC50	-	>100

SD = Standard Deviation, LC= Lethal Concentration, IC= Inhibition Concentration.

Table 6. Results: *Ceriodaphnia dubia* survival and reproduction test with sample NF1.

Concentration (% v/v)	Survival (%)	Reproduction (mean ± SD)	Stimulation (%)
Control	100	14.4 ± 5.1*	-
5	100	16.8 ± 2.6	16.7
10	100	15.3 ± 4.5	6.2
20	100	17.1 ± 4.3	18.8
40	100	17.2 ± 3.2	19.4
60	90	15.5 ± 2.9	7.6
80	100	15.7 ± 5.7	9.0
100	100	17.8 ± 2.1	23.6
Test endpoint (% v/v)			
LC50	>100	-	-
IC25	-	>100	-
IC50	-	>100	-

SD = Standard Deviation, LC= Lethal Concentration, IC= Inhibition Concentration.

*This result met the Environment Canada criterion of ≥15 young per adult female based on surviving organisms that had produced three broods, which was 15.8 young per adult.

Table 7. Results: *Ceriodaphnia dubia* survival and reproduction test with sample NF2.

Concentration (% v/v)	Survival (%)	Reproduction (mean ± SD)
Control	90	14.1 ± 5.0*
5	100	15.4 ± 2.0
10	100	14.8 ± 1.9
20	30	2.2 ± 3.3
40	0	0.0 ± 0.0
60	0	0.0 ± 0.0
80	0	0.0 ± 0.0
100	0	0.0 ± 0.0
Test endpoint (% v/v)		
LC50 (95% CL)	17.5 (14.3 - 21.5)	-
IC25 (95% CL)	-	12.3 (11.3 - 12.7)
IC50 (95% CL)	-	15.1 (14.0 - 16.2)

SD = Standard Deviation, LC= Lethal Concentration, IC= Inhibition Concentration, CL= Confidence Limits.

*This result met the Environment Canada criterion of ≥15 young per adult female based on surviving organisms that had produced three broods, which was 15.7 young per adult.

Table 8. Results: *Ceriodaphnia dubia* survival and reproduction test with sample X1.

Concentration (% v/v)	Survival (%)	Reproduction (mean ± SD)
Control	100	15.2 ± 1.5
5	80	15.1 ± 3.2
10	90	14.1 ± 5.5
20	100	14.3 ± 1.8
40	0	0.0 ± 0.0
60	0	0.0 ± 0.0
80	0	0.0 ± 0.0
100	0	0.0 ± 0.0
Test endpoint (% v/v)		
LC50 (95% CL)	27.2 (25.1 – 29.5)	-
IC25 (95% CL)	-	23.0 (20.6 – 23.6)
IC50 (95% CL)	-	27.7 (25.8 – 28.2)

SD = Standard Deviation, LC= Lethal Concentration, IC= Inhibition Concentration, CL= Confidence Limits.

Table 9. Results: *Ceriodaphnia dubia* survival and reproduction test with sample X14.

Concentration (% v/v)	Survival (%)	Reproduction (mean ± SD)
Control	100	26.0 ± 11.4
1.56	100	31.2 ± 5.2 †
3.13	100	32.4 ± 4.7 †
6.25	90	30.7 ± 10.0 †
12.5	100	34.6 ± 3.0 †
25	100	20.5 ± 10.3
50	100	16.9 ± 8.8
100	70	2.7 ± 3.1
Test endpoint (% v/v)		
LC50	>100	-
IC25 (95% CL)	-	30.4 (7.8 – 52.0)
IC50 (95% CL)	-	60.5 (37.1 – 67.6)

SD = Standard Deviation, LC= Lethal Concentration, IC= Inhibition Concentration, CL= Confidence Limits.

† = The hormesis model was conducted but the data did not fit the model; therefore reproduction was adjusted to that of the control value for analysis.

Table 10. Results: *Ceriodaphnia dubia* survival and reproduction test with sample X3A.

Concentration (% v/v)	Survival (%)	Reproduction (mean ± SD)
Control	100	25.0 ± 10.8
1.56	100	25.1 ± 7.6
3.13	100	30.5 ± 3.3 †
6.25	100	28.9 ± 4.0 †
12.5	100	24.2 ± 10.7
25	100	22.6 ± 10.3
50	30	0.0 ± 0.0
100	0	0.0 ± 0.0
Test endpoint (% v/v)		
LC50 (95% CL)	43.5 (35.6 – 53.2)	-
IC25 (95% CL)	-	28.2 (5.4 – 29.8)
IC50 (95% CL)	-	34.1 (27.3 – 35.4)

SD = Standard Deviation, LC= Lethal Concentration, IC= Inhibition Concentration, CL= Confidence Limits.

† = The hormesis model was conducted but the data did not fit the model; therefore reproduction was adjusted to that of the control value for analysis.

Table 11. Results: *Ceriodaphnia dubia* survival and reproduction test with sample R3.

Concentration (% v/v)	Survival (%)	Reproduction (mean ± SD)
Control	100	28.9 ± 9.0
1.56	100	19.8 ± 8.3
3.13	100	19.2 ± 10.3
6.25	90	24.6 ± 13.0
12.5	100	29.9 ± 4.5
25	100	25.6 ± 7.7
50	90	22.6 ± 10.8
100	80	11.8 ± 7.9
Test endpoint (% v/v)		
LC50	>100	-
IC25 (95% CL)	-	70.7 (n/a - 100)
IC50 (95% CL)	-	97.4 (71.9 - 100)

SD = Standard Deviation, LC= Lethal Concentration, IC= Inhibition Concentration, CL= Confidence Limits.

Table 12. Results: *Lemna minor* growth inhibition test with sample R10.

Concentration (% v/v)	FronD Growth (No. of Fronds) (mean ± SD)	Stimulation (%)	Dry Weight (mg) (mean ± SD)	Stimulation (%)
Control	70.5 ± 6.5	-	5.7 ± 0.6	-
1.5	85.5 ± 22.6	21.3	7.3 ± 2.2	27.7
3.0	68.0 ± 17.7	-	5.4 ± 1.5	-
6.1	61.0 ± 16.2	-	5.1 ± 1.4	-
12.1	88.0 ± 14.0	24.8	7.2 ± 1.0	26.8
24.2	86.5 ± 5.8	22.7	7.7 ± 0.6	35.3
48.5	91.2 ± 21.4	29.4	8.6 ± 2.0*	51.0
97	69.5 ± 21.6	-	6.3 ± 1.8	10.7
Test endpoint (% v/v)				
IC25	>97	-	>97	-
IC50	>97	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration.

*Indicates concentrations that are significantly greater than the control.

Table 13. Results: *Lemna minor* growth inhibition test with sample NF1.

Concentration (% v/v)	FronD Growth (No. of Fronds) (mean ± SD)	Stimulation (%)	Dry Weight (mg) (mean ± SD)	Stimulation (%)
Control	71.2 ± 14.6	-	6.9 ± 1.1	-
1.5	85.0 ± 11.2	19.3	7.7 ± 0.9	11.0
3.0	68.5 ± 5.8	-	6.9 ± 0.8	-
6.1	85.0 ± 23.6	19.3	7.8 ± 1.5	12.5
12.1	80.2 ± 11.1	12.6	7.6 ± 1.2	9.9
24.2	80.0 ± 21.3	12.3	7.7 ± 2.0	11.2
48.5	71.8 ± 10.3	0.7	7.2 ± 1.0	3.4
97	66.8 ± 14.0	-	6.8 ± 1.4	-
Test endpoint (% v/v)				
IC25	>97	-	>97	-
IC50	>97	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration.

Table 14. Results: *Lemna minor* growth inhibition test with sample NF2.

Concentration (% v/v)	FronD Growth (No. of Fronds) (mean ± SD)	Stimulation (%)	Dry Weight (mg) (mean ± SD)	Stimulation (%)
Control	67.8 ± 11.9	-	6.0 ± 0.9	-
1.5	66.5 ± 4.5	-	6.1 ± 0.9	1.8
3.0	64.2 ± 11.4	-	5.9 ± 0.8	-
6.1	83.0 ± 20.6 [†]	22.5	7.7 ± 1.4 [†]	28.1
12.1	77.5 ± 15.4 [†]	14.4	7.3 ± 0.9 [†]	22.3
24.2	54.0 ± 1.2	-	5.5 ± 0.3	-
48.5	50.2 ± 3.3	-	5.3 ± 0.7	-
97	45.5 ± 2.6	-	4.8 ± 0.4	-
Test endpoint (% v/v)				
IC25 (95% CL)	43.7 (7.6 - 87.6)	-	>97	-
IC50	>97	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

[†] The hormesis model was conducted but the data did not fit the model; therefore the number of fronds was adjusted to that of the control value for analysis.

Table 15. Results: *Lemna minor* growth inhibition test with sample X1.

Concentration (% v/v)	FronD Growth (No. of Fronds) (mean ± SD)	Stimulation (%)	Dry Weight (mg) (mean ± SD)	Stimulation (%)
Control	71.2 ± 20.4	-	5.7 ± 1.3	-
1.5	79.5 ± 9.0	11.6	6.7 ± 1.0	18.0
3.0	70.2 ± 10.5	-	6.2 ± 0.9	8.9
6.1	63.8 ± 5.9	-	5.5 ± 0.7	-
12.1	69.2 ± 9.5	-	6.2 ± 0.8	9.7
24.2	63.8 ± 17.8	-	5.9 ± 1.0	5.1
48.5	52.8 ± 7.8	-	5.4 ± 0.4	-
97	48.8 ± 4.6	-	5.2 ± 0.6	-
Test endpoint (% v/v)				
IC25 (95% CL)	48.5 (24.5 - 85.6)	-	>97	-
IC50	>97	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

Table 16. Results: *Lemna minor* growth inhibition test with sample X14.

Concentration (% v/v)	FronD Growth (No. of Fronds) (mean ± SD)	Stimulation (%)	Dry Weight (mg) (mean ± SD)	Stimulation (%)
Control	91.8 ± 9.6	-	8.2 ± 0.9	-
1.5	91.0 ± 19.8	-	9.1 ± 1.8	10.9
3.0	101.3 ± 27.4	10.4	9.6 ± 2.4	16.2
6.1	87.2 ± 13.9	-	8.7 ± 0.9	5.3
12.1	91.0 ± 15.9	-	8.8 ± 1.1	6.7
24.2	91.8 ± 3.6	-	9.2 ± 0.2	12.3
48.5	59.8 ± 9.4	-	8.3 ± 0.9	0.5
97	45.0 ± 6.6	-	7.7 ± 1.3	-
Test endpoint (% v/v)				
IC25 (95% CL)	37.5 (25.8 – 53.2)	-	>97	-
IC50 (95% CL)	87.0 (48.0 – 97)	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

Table 17. Results: *Lemna minor* growth inhibition test with sample X3A.

Concentration (% v/v)	FronD Growth (No. of Fronds) (mean ± SD)	Stimulation (%)	Dry Weight (mg) (mean ± SD)	Stimulation (%)
Control	65.2 ± 9.8	-	5.5 ± 0.7	-
1.5	57.0 ± 8.3	-	5.6 ± 0.9	0.5
3.0	64.0 ± 7.8	-	6.2 ± 0.6	12.5
6.1	61.5 ± 15.4	-	6.1 ± 1.8	10.0
12.1	60.2 ± 13.1	-	6.1 ± 1.3	10.9
24.2	58.8 ± 17.5	-	6.0 ± 1.7	8.8
48.5	47.8 ± 2.5	-	5.8 ± 0.4	4.4
97	48.2 ± 10.0	-	5.7 ± 0.9	3.9
Test endpoint (% v/v)				
IC25 (95% CL)	90.0 (25.5 – 97)	-	>97	-
IC50	>97	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

Table 18. Results: *Lemna minor* growth inhibition test with sample R3.

Concentration (% v/v)	FronD Growth (No. of Fronds) (mean ± SD)	Stimulation (%)	Dry Weight (mg) (mean ± SD)	Stimulation (%)
Control	66.8 ± 10.6	-	5.9 ± 0.7	-
1.5	78.0 ± 18.4	16.8	6.5 ± 1.4	11.2
3.0	62.8 ± 2.6	-	5.4 ± 0.4	-
6.1	63.8 ± 7.4	-	5.7 ± 0.6	-
12.1	63.5 ± 5.4	-	5.8 ± 0.6	-
24.2	51.5 ± 7.9	-	5.2 ± 1.0	-
48.5	50.0 ± 5.0	-	5.4 ± 0.4	-
97	33.8 ± 2.5	-	4.6 ± 0.2	-
Test endpoint (% v/v)				
IC25 (95% CL)	36.0 (26.5 – 47.0)	-	>97	-
IC50	>97	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

Table 19. Results: *Pseudokirchneriella subcapitata* growth inhibition test with sample R10.

Concentration (% v/v)	Cell Density (x 10 ⁴ cells/mL) (mean ± SD)	Stimulation (%)
Control	46.8 ± 7.2	-
1.5	49.8 ± 6.8	6.4
3.0	55.0 ± 7.1	17.6
6.0	68.5 ± 2.4*	46.5
11.9	86.2 ± 10.9*	84.5
23.8	155.8 ± 9.9*	233.2
47.6	162.3 ± 18.5*	247.1
95.2	148.8 ± 8.5*	218.2
Test endpoint (% v/v)		
IC25	>95.2	-
IC50	>95.2	-

SD = Standard Deviation, IC = Inhibition Concentration.

*Indicates concentrations that are significantly greater than the control.

Table 20. Results: *Pseudokirchneriella subcapitata* growth inhibition test with sample NF1.

Concentration (% v/v)	Cell Density ($\times 10^4$ cells/mL) (mean \pm SD)	Stimulation (%)
Control	49.1 \pm 6.8	-
1.5	49.0 \pm 4.1	-
3.0	62.2 \pm 6.5*	26.7
6.0	79.5 \pm 7.0*	61.8
11.9	67.0 \pm 8.9*	36.4
23.8	84.0 \pm 6.3*	71.0
47.6	112.5 \pm 14.6*	129.0
95.2	132.3 \pm 11.0*	169.2
Test endpoint (% v/v)		
IC25	>95.2	-
IC50	>95.2	-

SD = Standard Deviation, IC = Inhibition Concentration.

*Indicates concentrations that are significantly greater than the control.

Table 21. Results: *Pseudokirchneriella subcapitata* growth inhibition test with sample NF2.

Concentration (% v/v)	Cell Density ($\times 10^4$ cells/mL) (mean \pm SD)	Stimulation (%)
Control	42.5 \pm 2.6	-
1.5	50.0 \pm 9.0†	17.6
3.0	55.8 \pm 4.8†	31.2
6.0	44.5 \pm 5.7†	4.7
11.9	21.5 \pm 5.4	-
23.8	0.8 \pm 1.0	-
47.6	0.0 \pm 0.0	-
95.2	0.0 \pm 0.0	-
Test endpoint (% v/v)		
IC25 (95% CL)	8.5 (7.5 - 9.7)	-
IC50 (95% CL)	12.0 (9.5 - 14.5)	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

† The hormesis model was conducted but the data did not fit the model; therefore the cell yield was adjusted to that of the control value for analysis.

Table 22. Results: *Pseudokirchneriella subcapitata* growth inhibition test with sample X1.

Concentration (% v/v)	Cell Density ($\times 10^4$ cells/mL) (mean \pm SD)	Stimulation (%)
Control	48.1 \pm 5.7	-
1.5	47.0 \pm 8.8	-
3.0	53.8 \pm 2.9 [†]	11.7
6.0	51.0 \pm 6.5 [†]	6.0
11.9	32.8 \pm 2.6	-
23.8	9.2 \pm 1.7	-
47.6	0.2 \pm 0.5	-
95.2	1.0 \pm 1.4	-
Test endpoint (% v/v)		
IC25 (95% CL)	10.2 (7.9 – 11.8)	-
IC50 (95% CL)	15.4 (13.2 – 16.6)	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

[†] The hormesis model was conducted but the data did not fit the model; therefore the cell yield was adjusted to that of the control value for analysis.

Table 23. Results: *Pseudokirchneriella subcapitata* growth inhibition test with sample X14.

Concentration (% v/v)	Cell Density ($\times 10^4$ cells/mL) (mean \pm SD)	Stimulation (%)
Control	50.0 \pm 4.8	-
1.5	54.8 \pm 6.1 [†]	9.5
3.0	59.8 \pm 5.0 [†]	19.5
6.0	84.0 \pm 14.4 [†]	68.0
11.9	55.5 \pm 3.7 [†]	11.0
23.8	32.0 \pm 3.8	-
47.6	11.8 \pm 3.4	-
95.2	0.0 \pm 0.0	-
Test endpoint (% v/v)		
IC25 (95% CL)	19.3 (16.8 – 21.8)	-
IC50 (95% CL)	30.3 (26.4 – 33.6)	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

[†] The hormesis model was conducted but the data did not fit the model; therefore the cell yield was adjusted to that of the control value for analysis.

Table 24. Results: *Pseudokirchneriella subcapitata* growth inhibition test with sample X3A.

Concentration (% v/v)	Cell Density (x 10 ⁴ cells/mL) (mean ± SD)	Stimulation (%)
Control	54.1 ± 4.4	-
1.5	51.5 ± 4.5	-
3.0	56.5 ± 6.1	4.4
6.0	45.0 ± 12.2	-
11.9	33.5 ± 4.2	-
23.8	23.2 ± 1.9	-
47.6	0.8 ± 1.5	-
95.2	0.0 ± 0.0	-
Test endpoint (% v/v)		
IC25 (95% CL)	7.8 (3.6 – 11.1)	-
IC50 (95% CL)	18.4 (13.4 – 22.2)	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

Table 25. Results: *Pseudokirchneriella subcapitata* growth inhibition test with sample R3.

Concentration (% v/v)	Cell Density (x 10 ⁴ cells/mL) (mean ± SD)	Stimulation (%)
Control	44.6 ± 4.2	-
1.5	50.0 ± 6.4 [†]	12.0
3.0	86.5 ± 10.0 [†]	93.8
6.0	105.0 ± 5.5 [†]	135.3
11.9	85.0 ± 5.1 [†]	90.5
23.8	59.8 ± 6.4 [†]	33.9
47.6	25.8 ± 7.0	-
95.2	4.8 ± 2.2	-
Test endpoint (% v/v)		
IC25 (95% CL)	35.8 (30.1 – 44.9)	-
IC50 (95% CL)	53.1 (38.5 – 63.4)	-

SD = Standard Deviation, IC = Inhibition Concentration, CL= Confidence Limits.

[†] The hormesis model was conducted but the data did not fit the model; therefore the cell yield was adjusted to that of the control value for analysis.

Table 26. Results: 96-h rainbow trout (*Oncorhynchus mykiss*) LC50 test.

Survival (%)							
Concentration (% v/v)	R10	NF1	NF2	X1	X14	X3A	R3
Control	100	100	100	100	100	90	100
6.25	100	100	100	100	100	100	100
12.5	100	100	100	100	100	100	100
25.0	100	100	90	100	100	100	100
50	100	100	80	90	100	100	100
100	100	100	60	100	100	60	100
Test endpoint (% v/v)							
LC50	>100	>100	>100	>100	>100	>100	>100

LC= Lethal Concentration.

4.0 QA/QC

The health histories of the test organisms used in the exposures were acceptable and met the requirements of the Environment Canada protocols. The tests met all control acceptability criteria and water quality parameters remained within ranges specified in the protocols throughout the tests. There were no deviations from the test methodologies. Uncertainty associated with these tests is best described by the standard deviation around the mean and/or the confidence intervals around the point estimates.

In the rainbow trout test, the 100% (v/v) treatment of sample X14 had 11 fish instead of ten. The addition of an extra fish did not impact results since there were no mortalities observed in the test.

Results of the reference toxicant tests conducted during the testing program are summarized in Table 27. Results for these tests fell within the range for organism performance of mean and range, based on historical results obtained by the laboratory with these tests. Thus, the sensitivities of the organisms evaluated in the reference toxicant tests were appropriate.

Table 27. Reference toxicant results.

Test Species	Endpoint	Historical Mean (2 SD Range)	CV (%)	Test Date
<i>C. dubia</i> (Nautilus)	Survival (LC50): 1.4 g/L NaCl	1.7 (1.2 - 2.5)	21	February 21, 2014
	Reproduction (IC50): 1.3 g/L NaCl	1.2 (0.8 - 1.6)	18	
<i>C. dubia</i> (IRC)	Survival (LC50): 3.3 g/L NaCl	3.3 (3.1 - 3.4)	5	February 19, 2014
	Reproduction (IC50): 3.1 g/L NaCl	3.1 (2.9 - 3.3)	6	
<i>L. minor</i>	No. Fronds (IC25): 3.6 g/L KCl	4.4 (3.5 - 5.5)	12	February 20, 2014
<i>P. subcapitata</i>	Growth (IC50): 24.7 µg/L Zn	22.7 (15.2 - 33.8)	22	February 21, 2014
<i>O. mykiss</i>	Survival (LC50): 4.7 mg/L NaNO ₂	5.4 (2.2 - 13.4)	57	February 19, 2014

SD = Standard Deviation, CV = Coefficient of Variation, IC = Inhibition Concentration, LC = Lethal Concentration.

5.0 REFERENCES

- Environment Canada. 2000. Biological test method: reference method for determining acute lethality of effluents to rainbow trout. Environmental Protection Series. Report EPS 1/RM/13, Second Edition, December 2000, including May 2007 amendments. Environment Canada, Method Development and Application Section, Environmental Technology Centre, Ottawa, ON. 23 pp.
- Environment Canada. 2007a. Biological test method: test of reproduction and survival using the cladoceran *Ceriodaphnia dubia*. Environmental Protection Series. Report EPS 1/RM/21, Second Edition, February 2007. Environment Canada, Method Development and Application Section, Environmental Science and Technology Centre, Science and Technology Branch, Ottawa, ON. 74 pp.
- Environment Canada. 2007b. Biological test method: tests for measuring the inhibition of growth using the freshwater macrophyte, *Lemna minor*. Environmental Protection Series, Report EPS 1/RM/37. Second Edition. January 2007. Environment Canada, Method Development and Application Section, Environmental Technology Centre, Ottawa, ON. 112 pp.
- Environment Canada. 2007c. Biological test method: growth inhibition test using the freshwater alga. Environmental Protection Series, Report EPS 1/RM/25. Second Edition, March 2007. Environment Canada, Method Development and Application Section, Environmental Science and Technology Centre, Science and Technology Branch, Ottawa, ON. 53 pp.
- Tidepool Scientific Software. 2013. CETIS comprehensive environmental toxicity information system, version 1.8.4.29 Tidepool Scientific Software, McKinleyville, CA. 222 pp.

APPENDIX A - *Ceriodaphnia dubia* Toxicity Test Data

Ceriodaphnia dubia Summary Sheet

Client: ALS Environmental
 Work Order No.: 14076

Start Date/Time: Feb 27/14 @ 1300
 Set up by: Erin Lyle

Sample Information:

Sample ID: L1426336-1 (R10)
 Sample Date: Feb 25/14
 Date Received: Feb 27/14
 Sample Volume: 2x20L

Test Validity Criteria:

- 1) Mean survival of first generation controls is $\geq 80\%$
- 2) At least 60% of controls have produced three broods within 8 days
- 3) An average of ≥ 15 live young produced per surviving female in the control solutions during the first three broods.
- 4) Invalid if ephippia observed in any control solution at any time.

WQ Ranges:

T (°C) = 25 ± 1 , DO (mg/L) = 3.3 to 8.4, pH = 6.0 to 8.5

Test Organism Information:

Broodstock No.: _____
 Age of young (Day 0): _____
 Avg No. young in first 3 broods of previous 7 d: _____
 Mortality (%) in previous 7 d: _____
 Individual female # used ≥ 8 young on test day

Q21914
<24-h (within 12-h)
17
0
1, 4, 5, 6, 8, 9, 16, 17, 18, 21, 22, 23
25, 28, 31, 32, 33, 34, 36, 37, 40.

NaCl Reference Toxicant Results:

Reference Toxicant ID: Ca108
 Stock Solution ID: 13 Na 03
 Date Initiated: Feb 21/14

7-d LC50 (95% CL): 1.4 (1.1 - 1.8) g/L NaCl
 7-d IC50 (95% CL): 1.3 (1.0 - 1.8) g/L NaCl

7-d LC50 Reference Toxicant Mean and Historical Range: 1.7 (1.2 - 2.5) g/L NaCl CV (%): 21
 7-d IC50 Reference Toxicant Mean and Historical Range: 1.2 (0.8 - 1.6) g/L NaCl CV (%): 16

Test Results:

	Survival	Reproduction
LC50 % (v/v) (95% CL)	>100	
IC25 % (v/v) (95% CL)		>105
IC50 % (v/v) (95% CL)		>100

Reviewed by: JOU

Date reviewed: March 26/14

Chronic Freshwater Toxicity Test Initial and Final Water Quality Measurements

Client: ALS
 Sample ID: R10 (14726336-1)
 Work Order #: 14076

Start Date & Time: Feb 27/14 @ 1300
 Stop Date & Time: March 7/14 @ 130h.
 Test Species: Ceriodaphnia dubia

Concentration	Days														Final		
	0		1		2		3		4		5		6			7	8
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final		new	
Control																	
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	24.5	24.0	24.5	24.0	25.0	24.0	25.0	24.0	25.0	
DO (mg/L)	8.2	7.8	8.0	7.9	7.9	7.4	8.1	7.4	8.1	7.7	7.9	7.4	8.0	6.9	8.2	7.2	
pH	8.0	7.7	7.9	7.8	7.9	7.9	8.0	7.7	7.9	7.7	7.9	7.7	8.0	7.5	7.9	7.5	
Cond. (µS/cm)	210	210		211				211				212				212	
Initials	EMM	EMM						EMM		EMM		EMM		JW	EMM	JW	

Concentration	Days														Final		
	0		1		2		3		4		5		6			7	8
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final		new	
(VIV) 5%																	
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	24.5	24.0	24.5	24.0	25.0	24.0	25.0	24.0	25.0	
DO (mg/L)	7.8	7.8	8.0	7.5	7.9	7.4	8.0	7.6	8.0	7.6	8.0	7.5	8.0	7.0	7.8	7.3	
pH	7.7	7.8	7.8	7.8	7.9	7.8	7.9	7.6	7.9	7.7	7.9	7.7	7.8	7.6	7.7	7.6	
Cond. (µS/cm)	213	214		216		218		217		217		216		226		211	
Initials	EMM	EMM						EMM		EMM		EMM		JW	EMM	JW	

Concentration	Days														Final		
	0		1		2		3		4		5		6			7	8
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final		new	
(CIV) 40%																	
Temperature (°C)	24.0	25.0	24.5	25.0	24.0	25.0	24.0	24.5	24.5	24.5	24.0	25.0	24.5	25.0	24.0	25.0	
DO (mg/L)	7.6	7.8	7.9	7.7	7.9	7.8	8.0	7.6	8.0	7.6	7.8	7.4	8.1	6.9	7.8	7.2	
pH	7.7	7.8	7.8	7.9	7.9	7.8	7.9	7.7	7.8	7.8	7.8	7.7	7.8	7.6	7.6	7.6	
Cond. (µS/cm)	245	246		251		248		248		250		247		260		244	
Initials	EMM	EMM						EMM		EMM		EMM		JW	EMM	JW	

Concentration	Days														Final		
	0		1		2		3		4		5		6			7	8
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final		new	
(CIV) 100%																	
Temperature (°C)	25.5	25.0	26.0	25.0	24.0	25.0	24.0	24.5	24.5	24.5	24.0	25.0	24.5	25.0	24.0	25.0	
DO (mg/L)	8.1	7.9	7.8	7.8	7.8	7.5	7.8	7.5	8.1	7.3	8.2	7.6	8.1	6.9	7.7	7.2	
pH	7.3	7.9	7.6	8.0	7.8	7.9	7.8	7.7	7.7	7.7	7.5	7.6	7.5	7.7	7.6	7.6	
Cond. (µS/cm)	304	306		301		299		308		308		306		309		290	
Initials	EMM	EMM						EMM		EMM		EMM		JW	EMM	JW	

	Control	100% (CIV)
Hardness*	100	148
Alkalinity*	89	126

Analysts: AWD, EMM, JW

Reviewed by: JGW
 Date reviewed: March 26/14

* mg/L as CaCO3

WQ Ranges: T (°C) = 25 ± 1; DO (mg/L) = 3.3 to 8.4 (mg/L); pH = 6 to 8.5

Sample Description: clear

Comments: Broadboard Used: 021914

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: ALS
 Sample ID: P16 (114763361)
 Work Order: 14076
 Start Date & Time: Feb 27/14 @ 1300
 Stop Date & Time: March 7/14 @ 1300
 Set up by: EMM/VVL

Days	Concentration: 5										Concentration: 10															
	A	B	C	D	E	F	G	H	I	J	Init	JW	A	B	C	D	E	F	G	H	I	J	Init	JW		
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
7	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Total	14	15	18	16	13	17	17	17	17	17	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22

Days	Concentration: 40										Concentration: 60															
	A	B	C	D	E	F	G	H	I	J	Init	JW	A	B	C	D	E	F	G	H	I	J	Init	JW		
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
7	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Total	19	17	12	18	14	19	18	18	18	18	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17

Days	Concentration: 80										Concentration: 100															
	A	B	C	D	E	F	G	H	I	J	Init	JW	A	B	C	D	E	F	G	H	I	J	Init	JW		
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
7	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Total	7	15	16	11	19	16	18	18	18	18	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17

Notes: X = mortality.
 Sample Description: clear
 Comments: Total # Young only based on the first 3 broods. Fourth and subsequent broods not included in total count.
 Reviewed by: JGH
 Date reviewed: March 26/14

CETIS Analytical Report

Report Date: 25 Mar-14 08:08 (p 1 of 2)
 Test Code: 14076b | 15-0790-2832

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 06-2151-9487	Endpoint: 7d ^{8d} Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 25 Mar-14 8:07	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 06-2588-9811	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 27 Feb-14 13:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 07 Mar-14 13:10	Species: Ceriodaphnia dubia	Brine:
Duration: 8d 0h	Source: In-House Culture	Age: <24h
Sample ID: 00-4133-3542	Code: 276B326	Client: ALS
Sample Date: 25 Feb-14 15:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 46h (3.6 °C)	Station: L1426336-1(R10)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1827371	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	31.8	25.25	N/A	3.144	NA	3.961
EC10	>100	N/A	N/A	<1	NA	NA
EC15	>100	N/A	N/A	<1	NA	NA
EC20	>100	N/A	N/A	<1	NA	NA
EC25	>100	N/A	N/A	<1	NA	NA
EC40	>100	N/A	N/A	<1	NA	NA
EC50	>100	N/A	N/A	<1	NA	NA

7d Survival Rate Summary

Calculated Variate(A/B)

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10
5		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
20		10	1	1	1	0	0	0.0%	0.0%	10	10
40		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10
60		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10
80		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10
100		10	1	1	1	0	0	0.0%	0.0%	10	10

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	Negative Control	1	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
20		1	1	1	1	1	1	1	1	1	1
40		1	1	1	1	0	1	1	1	1	1
60		1	1	1	1	1	1	1	1	1	0
80		1	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: 25 Mar-14 08:08 (p 2 of 2)

Test Code: 14076b | 15-0790-2632

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 06-2151-9487
 Analyzed: 25 Mar-14 8:07

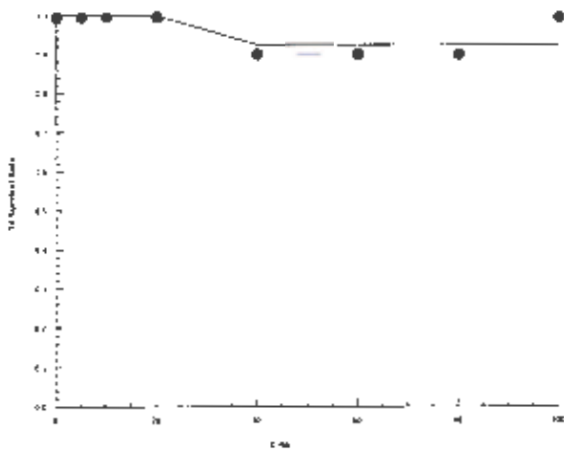
Endpoint: 7d Survival Rate
 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1 8.7
 Official Results: Yes

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	Negative Control	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
10		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
20		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
40		1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
60		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
80		1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1
100		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

Graphics



CETIS Analytical Report

Report Date: 25 Mar-14 08:08 (p 1 of 2)
 Test Code: 14076b | 15-0790-2632

Ceriodaphnia 7-d Survival and Reproduction Test

Nautlius Environmental

Analysis ID: 10-1090-1897	Endpoint: Reproduction	CETIS Version: CETISv1.8.7
Analyzed: 25 Mar-14 8:08	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 06-2588-9811	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 27 Feb-14 13:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 07 Mar-14 13:10	Species: Ceriodaphnia dubia	Brine:
Duration: 8d 0h	Source: In-House Culture	Age: <24h
Sample ID: 00-4133-3542	Code: 276B326	Client: ALS
Sample Date: 25 Feb-14 15:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 46h (3.6 °C)	Station: L1426336-1(R10)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	74627	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	24.08	0.8414	N/A	4.152	NA	118.8
IC10	35.87	2.391	N/A	2.788	NA	41.83
IC15	>100	N/A	N/A	<1	NA	NA
IC20	>100	N/A	N/A	<1	NA	NA
IC25	>100	N/A	N/A	<1	NA	NA
IC40	>100	N/A	N/A	<1	NA	NA
IC50	>100	N/A	N/A	<1	NA	NA

Reproduction Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	16.7	13	22	0.7608	2.406	14.41%	0.0%
5		10	16.5	11	20	0.969	3.064	18.57%	1.2%
10		10	15	5	19	1.291	4.082	27.22%	10.18%
20		10	17.5	12	23	0.9339	2.953	16.88%	-4.79%
40		10	13.5	0	21	1.91	6.042	44.75%	19.16%
60		10	15.2	0	19	1.744	5.514	36.27%	8.98%
80		10	13.7	0	24	2.14	6.767	49.39%	17.96%
100		10	16.8	10	24	1.348	4.264	25.38%	-0.6%

Reproduction 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	Negative Control	14	16	18	16	13	17	17	17	17	22
5		17	12	20	19	11	19	16	19	15	17
10		19	15	18	16	5	16	17	12	14	18
20		19	17	12	18	14	19	18	23	18	17
40		14	15	16	21	0	17	18	16	8	10
60		16	17	19	18	18	17	17	14	16	0
80		7	15	16	11	19	16	0	24	18	11
100		19	15	19	18	17	17	10	19	10	24

CETIS Analytical Report

Report Date: 25 Mar-14 08:08 (p 2 of 2)
Test Code: 14076b | 15-0790-2632

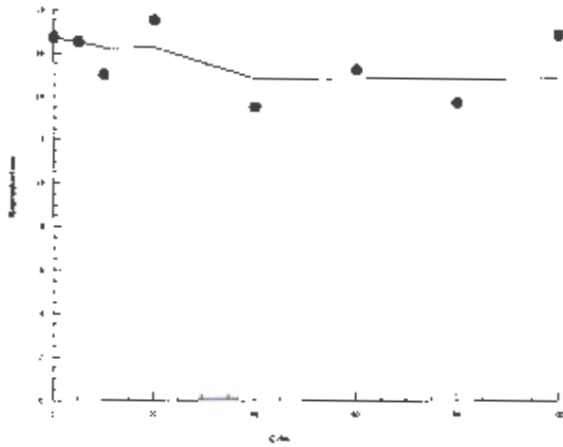
Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 10-1090-1897 Endpoint: Reproduction
Analyzed: 25 Mar-14 8:08 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



Ceriodaphnia dubia Summary Sheet

Client: ALS Environmental
 Work Order No.: 14076

Start Date/Time: Feb 27/14 @ 1300
 Set up by: EMM

Sample Information:

Sample ID: L1426336-2 (NF1)
 Sample Date: Feb 25/14
 Date Received: Feb 27/14
 Sample Volume: 2x 20L

Test Validity Criteria:

- 1) Mean survival of first generation controls is $\geq 80\%$
- 2) At least 60% of controls have produced three broods within 8 days
- 3) An average of ≥ 15 live young produced per surviving female in the control solutions during the first three broods.
- 4) Invalid if ephippia observed in any control solution at any time.

WQ Ranges:

T ($^{\circ}$ C) = 25 ± 1 ; DO (mg/L) = 3.3 to 8.4; pH = 6.0 to 8.5

Test Organism Information:

Broodstock No.: 021914
 Age of young (Day 0): <24-h (within 12-h)
 Avg No. young in first 3 broods of previous 7 d: 17
 Mortality (%) in previous 7 d: 0
 Individual female # used ≥ 8 young on test day: 14, 5, 6, 8, 7, 16, 17, 18, 21, 22, 23, 25, 28, 31, 32, 33, 34, 36, 37, 40

NaCl Reference Toxicant Results:

Reference Toxicant ID: cd 4E
 Stock Solution ID: 13 N203
 Date Initiated: Feb 21/14

7-d LC50 (95% CL): 1.4 (1.1 - 1.8) g/L NaCl
 7-d IC50 (95% CL): 1.3 (1.0 - 1.8) g/L NaCl

7-d LC50 Reference Toxicant Mean and Historical Range: 1.7 (1.2 - 2.5) g/L NaCl CV (%): 21
 7-d IC50 Reference Toxicant Mean and Historical Range: 1.2 (0.7 - 1.6) g/L NaCl CV (%): 18

Test Results:

	Survival	Reproduction
LC50 % (v/v) (95% CL)	>100	>100
IC25 % (v/v) (95% CL)	>100	>100
IC50 % (v/v) (95% CL)	>100	>100

Reviewed by: Joh

Date reviewed: March 27/14

Chronic Freshwater Toxicity Test Initial and Final Water Quality Measurements

Client: ALS
 Sample ID: NFI (L1426336-2)
 Work Order #: 14076

Start Date & Time: Feb 27/14 @ 1300
 Stop Date & Time: March 7/14 @ 1215h
 Test Species: Ceriodaphnia dubia

Concentration	Days														Final	New	Final		
	0		1		2		3		4		5		6					7	
	init.	old	new	old	new	old	new	old	new	old	new	old	new	old				new	final
control																			
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	24.5	24.0	24.5	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	
DO (mg/L)	8.2	7.8	8.0	7.8	7.9	7.4	8.1	7.4	8.1	7.7	7.9	7.6	8.0	7.3	8.2	7.1			
pH	8.0	7.9	7.9	7.8	7.9	7.8	8.0	7.9	7.9	7.7	7.9	7.7	8.0	7.8	7.9	7.5			
Cond. (µS/cm)	210	210		216		211		211		211		212				211	211		
Initials	EMM	EMM						EMM		EMM		EMM				EMM	JW		

Concentration	Days														Final	New	Final		
	0		1		2		3		4		5		6					7	
	init.	old	new	old	new	old	new	old	new	old	new	old	new	old				new	final
5%																			
Temperature (°C)	24.5	25.0	24.5	25.0	24.0	25.0	24.0	24.5	24.0	24.5	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	
DO (mg/L)	8.2	7.7	7.9	7.7	7.9	7.4	8.1	7.8	8.0	7.6	8.0	7.5	8.0	7.4	7.9	7.0			
pH	7.9	7.9	7.9	7.9	7.9	7.8	8.0	7.8	7.9	7.8	7.9	7.7	7.8	7.8	7.9	7.6			
Cond. (µS/cm)	212	214		218		216		217		214		216				220	217		
Initials	EMM	EMM						EMM		EMM		EMM				EMM	JW		

Concentration	Days														Final	New	Final		
	0		1		2		3		4		5		6					7	
	init.	old	new	old	new	old	new	old	new	old	new	old	new	old				new	final
40%																			
Temperature (°C)	25.0	25.0	25.0	25.0	24.0	25.0	24.0	24.5	24.0	24.5	25.0	25.0	24.5	25.0	24.0	25.0	24.0	25.0	
DO (mg/L)	8.1	7.6	7.8	7.8	7.9	7.5	8.0	7.7	8.1	7.6	7.9	7.6	8.1	7.4	7.9	7.0			
pH	7.8	8.0	7.7	7.9	7.8	7.8	7.9	7.6	7.9	7.7	7.7	7.7	7.6	7.8	7.6	7.6			
Cond. (µS/cm)	248	252		248		250		256		249		251				261	249		
Initials	EMM	EMM						EMM		EMM		EMM				EMM	JW		

Concentration	Days														Final	New	Final		
	0		1		2		3		4		5		6					7	
	init.	old	new	old	new	old	new	old	new	old	new	old	new	old				new	final
100%																			
Temperature (°C)	25.0	25.0	25.5	25.0	24.0	25.0	24.0	24.5	24.0	24.5	25.0	25.0	24.5	25.0	24.0	25.0	24.0	25.0	
DO (mg/L)	8.2	7.6	7.8	7.9	8.0	7.4	8.0	7.2	8.1	7.6	7.9	7.4	8.1	7.5	7.9	7.0			
pH	7.3	7.4	7.6	7.8	7.7	7.7	7.8	7.6	7.8	7.7	7.5	7.8	7.5	7.8	7.4	7.8			
Cond. (µS/cm)	315	315		306		310		316		317		318				321	303		
Initials	EMM	EMM						EMM		EMM		EMM				EMM	JW		

	Control	100% (v/v)
Hardness*	100	156
Alkalinity*	84	132

Analysts: AWD, EMM, JW, MK

Reviewed by: JW
 Date reviewed: March 26/14

* mg/L as CaCO3

WQ Ranges: T (°C) = 25 ± 1; DO (mg/L) = 3.3 to 8.4 (mg/L); pH = 6 to 8.5

Sample Description: Slightly yellow, clear

Comments: Broodboard Used: 021914

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: ALS NFI (L49263862) Start Date & Time: Feb 27 7:40 300
 Sample ID: 10076 10076 Stop Date & Time: MARCH 7 14 @ 12:5h
 Work Order: 10076 10076 Set up by: EMM

Days	Concentration: 5 % (v/v)										Concentration: 10											
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init
1	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM
2	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM
3	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM
4	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM
5	/	/	3	3	2	3	2	2	2	2	YML	2	3	2	3	2	3	2	2	2	2	YML
6	/	/	3	3	3	3	3	3	3	3	YML	4	5	4	5	3	7	5	7	5	5	YML
7	/	/	3	3	5	5	5	5	5	5	YML	7	6	7	6	4	5	10	7	10	5	YML
8	/	/	3	3	5	5	5	5	5	5	YML	11	10	10	10	10	10	11	11	11	10	YML
Total	2	18	14	19	10	14	17	14	14	14	EMM	18	16	17	14	14	14	14	19	17	17	EMM

Days	Concentration: 20										Concentration: 40										Concentration: 60												
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init
1	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM
2	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	EMM	
3	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	EMM	
4	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	EMM	
5	/	/	2	2	3	3	3	3	3	3	YML	2	2	2	2	2	3	2	3	3	2	YML	2	3	2	2	2	2	3	2	2	YML	
6	/	/	2	2	4	4	4	4	4	4	YML	5	6	5	5	7	6	7	5	6	5	YML	5	7	5	4	5	4	5	4	5	YML	
7	/	/	2	2	5	5	5	5	5	5	YML	7	8	6	6	8	10	10	7	8	7	YML	10	5	10	5	5	6	6	4	4	YML	
8	/	/	2	2	5	5	5	5	5	5	YML	11	10	11	10	10	10	11	11	11	10	YML	10	10	10	8	8	8	8	8	8	YML	
Total	11	14	16	20	17	27	16	16	19	15	EMM	18	10	19	17	20	15	18	21	19	15	EMM	17	17	15	14	10	16	14	21	14	EMM	

Days	Concentration: 80										Concentration: 100																					
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init										
1	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	EMM
2	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	EMM
3	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	EMM
4	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	/	EMM	/	/	/	/	/	/	/	/	/	EMM
5	/	/	4	3	2	2	2	2	2	2	YML	3	2	2	2	3	2	2	3	3	2	YML	2	3	2	2	2	2	3	2	2	YML
6	/	/	3	3	5	5	5	5	5	5	YML	6	5	5	5	7	6	7	5	6	5	YML	6	7	5	4	5	4	5	4	5	YML
7	/	/	3	3	6	6	6	6	6	6	YML	8	7	6	6	8	10	10	6	7	6	YML	8	7	6	5	6	5	6	5	6	YML
8	/	/	3	3	6	6	6	6	6	6	YML	11	10	11	10	10	10	11	11	11	10	YML	10	10	10	8	8	8	8	8	8	YML
Total	23	11	26	12	16	12	20	10	10	17	EMM	15	21	15	17	17	19	16	20	19	19	EMM	17	17	15	14	10	16	14	21	14	EMM

Notes: X = mortality.

Sample Description: Slightly yellow, clear

Comments: Total # Young only based on the first 3 Broods. Fourth and subsequent broods not included in total count.

Reviewed by: Joh Date reviewed: March 26/14

CETIS Analytical Report

Report Date: 25 Mar-14 07:55 (p 1 of 2)
 Test Code: 14076a | 07-6558-8075

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 15-1663-2841	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 25 Mar-14 7:50	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 20-9586-2233	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 27 Feb-14 13:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 07 Mar-14 12:15	Species: Ceriodaphnia dubia	Brine:
Duration: 7d 23h	Source: In-House Culture	Age: <24h
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 46h (4.3 °C)	Station: L1426336-2(NF1)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	51729	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	>100	N/A	N/A	<1	NA	NA
EC10	>100	N/A	N/A	<1	NA	NA
EC15	>100	N/A	N/A	<1	NA	NA
EC20	>100	N/A	N/A	<1	NA	NA
EC25	>100	N/A	N/A	<1	NA	NA
EC40	>100	N/A	N/A	<1	NA	NA
EC50	>100	N/A	N/A	<1	NA	NA

7d Survival Rate Summary

C-%	Control Type	Count	Calculated Variate(A/B)									
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B	
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10	
5		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	
20		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	
60		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10	
80		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	

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	Negative Control	1	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
20		1	1	1	1	1	1	1	1	1	1
40		1	1	1	1	1	1	1	1	1	1
60		1	1	1	1	1	1	1	0	1	1
80		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: 25 Mar-14 07:55 (p 2 of 2)
 Test Code: 14078a | 07-6558-8075

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 15-1663-2841
 Analyzed: 25 Mar-14 7:50

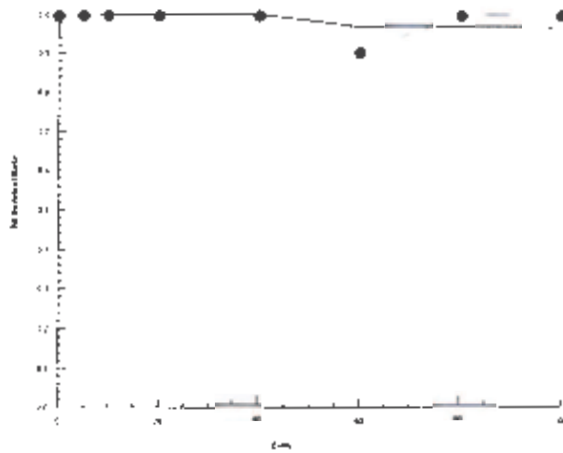
Endpoint: ^{afternoon} 7d Survival Rate
 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1 8.7
 Official Results: Yes

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	Negative Control	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
10		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
20		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
40		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
60		1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1
80		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
100		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

Graphics



CETIS Analytical Report

Report Date: 25 Mar-14 07:55 (p 1 of 2)
 Test Code: 14076a | 07-6558-8075

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 18-5388-6492	Endpoint: Reproduction	CETIS Version: CETISv1.8.7
Analyzed: 25 Mar-14 7:51	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 20-9586-2233	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 27 Feb-14 13:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 07 Mar-14 12:15	Species: Ceriodaphnia dubia	Brine:
Duration: 7d 23h	Source: In-House Culture	Age: <24h
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 46h (4.3 °C)	Station: L1426336-2(NF1)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2119951	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>100	N/A	N/A	<1	NA	NA
IC10	>100	N/A	N/A	<1	NA	NA
IC15	>100	N/A	N/A	<1	NA	NA
IC20	>100	N/A	N/A	<1	NA	NA
IC25	>100	N/A	N/A	<1	NA	NA
IC40	>100	N/A	N/A	<1	NA	NA
IC50	>100	N/A	N/A	<1	NA	NA

Reproduction Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	14.4	2	19	1.6	5.06	35.14%	0.0%
5		10	16.8	13	21	0.8138	2.573	15.32%	-16.67%
10		10	15.3	10	21	1.438	4.547	29.72%	-6.25%
20		10	17.1	11	27	1.354	4.28	25.03%	-18.75%
40		10	17.2	10	21	1.009	3.19	18.55%	-19.44%
60		10	15.5	10	21	0.9098	2.877	18.56%	-7.64%
80		10	15.7	10	26	1.808	5.716	36.41%	-9.03%
100		10	17.8	15	21	0.6633	2.098	11.78%	-23.61%

Reproduction 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	Negative Control	2	18	14	19	10	14	17	16	17	17
5		18	16	17	14	14	19	21	13	19	17
10		21	10	12	11	13	10	21	19	19	17
20		11	14	16	20	17	27	16	16	19	15
40		18	10	19	17	20	15	18	21	19	15
60		17	17	17	15	14	10	16	14	21	14
80		23	11	26	12	16	12	20	10	10	17
100		15	21	15	17	17	19	16	20	19	19

CETIS Analytical Report

Report Date: 25 Mar-14 07:55 (p 2 of 2)
Test Code: 14076a | 07-6558-8075

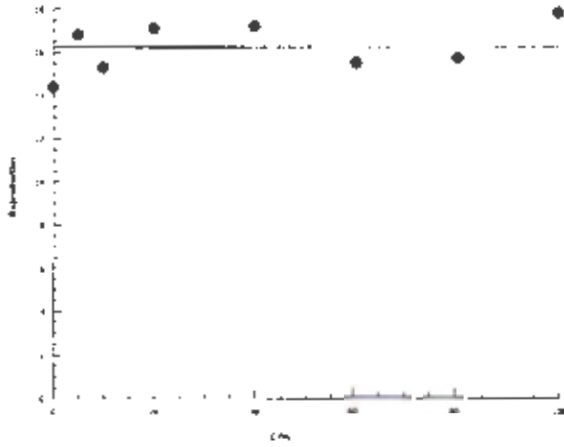
Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 18-5388-6492 Endpoint: Reproduction
Analyzed: 25 Mar-14 7:51 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 31 Mar-14 12:55 (p 1 of 2)
 Test Code: 14076a | 07-6558-8075

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 07-0267-9219	Endpoint: Reproduction	CETIS Version: CETISv1 8.7
Analyzed: 25 Mar-14 7:57	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 20-9586-2233	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 27 Feb-14 13:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 07 Mar-14 12:15	Species: Ceriodaphnia dubia	Brine:
Duration: 7d 23h	Source: In-House Culture	Age: <24h
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 46h (4.3 °C)	Station: L1426336-2(NF1)	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	29.5%	100	>100	NA	1

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		5	1.348	2.386	4.248	18	0.3199	CDF	Non-Significant Effect
		10	0.5056	2.386	4.248	18	0.7041	CDF	Non-Significant Effect
		20	1.517	2.386	4.248	18	0.2533	CDF	Non-Significant Effect
		40	1.573	2.386	4.248	18	0.2329	CDF	Non-Significant Effect
		60	0.6179	2.386	4.248	18	0.6557	CDF	Non-Significant Effect
		80	0.7303	2.386	4.248	18	0.6047	CDF	Non-Significant Effect
		100	1.91	2.386	4.248	18	0.1325	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	95.15	13.59286	7	0.8579	0.5439	Non-Significant Effect
Error	1140.8	15.84444	72			
Total	1235.95		79			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	14.09	18.48	0.0497	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9845	0.9579	0.4477	Normal Distribution

Reproduction Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	10	14.4	10.78	18.02	16.5	2	19	1.6	35.14%	0.0%
5		10	16.8	14.96	18.64	17	13	21	0.8138	15.32%	-16.67%
10		10	15.3	12.05	18.55	15	10	21	1.438	29.72%	-6.25%
20		10	17.1	14.04	20.16	16	11	27	1.354	25.03%	-18.75%
40		10	17.2	14.92	19.48	18	10	21	1.009	18.55%	-19.44%
60		10	15.5	13.44	17.56	15.5	10	21	0.9098	18.56%	-7.64%
80		10	15.7	11.61	19.79	14	10	26	1.808	36.41%	-9.03%
100		10	17.8	16.3	19.3	18	15	21	0.6633	11.78%	-23.61%

Reproduction 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	Negative Control	2	18	14	19	10	14	17	16	17	17
5		18	16	17	14	14	19	21	13	19	17
10		21	10	12	11	13	10	21	19	19	17
20		11	14	16	20	17	27	16	16	19	15
40		18	10	19	17	20	15	18	21	19	15
60		17	17	17	15	14	10	16	14	21	14
80		23	11	26	12	16	12	20	10	10	17
100		15	21	15	17	17	19	16	20	19	19

CETIS Analytical Report

Report Date: 31 Mar-14 12:55 (p 2 of 2)
Test Code: 14076a | 07-6558-8075

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 07-0267-5219

Endpoint: Reproduction

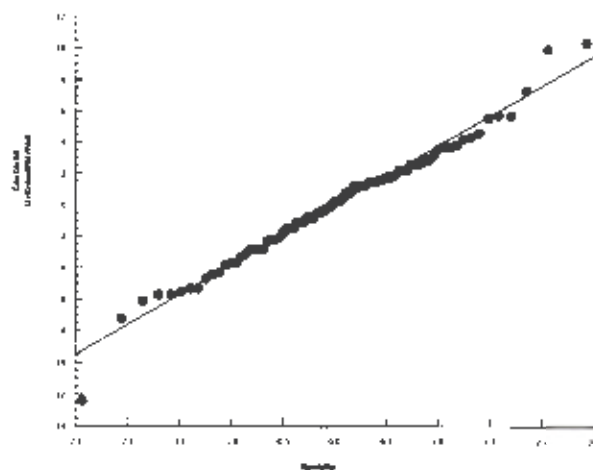
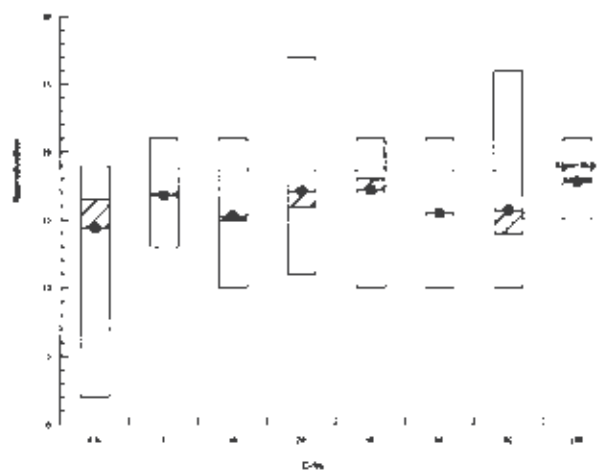
CETIS Version: CETISv1 8.7

Analyzed: 25 Mar-14 7:57

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Graphics



Ceriodaphnia dubia Summary Sheet

Client: ALS Environmental
 Work Order No.: 14076

Start Date/Time: Feb 28/14 @ 1100h
 Set up by: EMM

Sample Information:

Sample ID: L1426336-3 (NF2)
 Sample Date: Feb 25/14
 Date Received: Feb 27/14
 Sample Volume: 2x20L

Test Validity Criteria:

- 1) Mean survival of first generation controls is $\geq 80\%$
- 2) At least 60% of controls have produced three broods within 8 days
- 3) An average of >15 live young produced per surviving female in the control solutions during the first three broods
- 4) Invalid if ephippia observed in any control solution at any time

WQ Ranges:

T (°C) = 25 ± 1 ; DO (mg/L) = 3.3 to 8.4; pH = 6.0 to 8.5

Test Organism Information:

Broodstock No.: 021914
 Age of young (Day 0): <24-h (within 12-h)
 Avg No. young in first 3 broods of previous 7 d: 17
 Mortality (%) in previous 7 d: 0
 Individual female # used ≥ 8 young on test day: 2, 4, 5, 6, 7, 8, 9, 11, 14, 16, 17, 18, 19, 22, 23, 25, 26, 28, 32, 33, 36, 37

NaCl Reference Toxicant Results:

Reference Toxicant ID: cd 108
 Stock Solution ID: 1311203
 Date Initiated: Feb 21/14

7-d LC50 (95% CL): 1.4 (1.1 - 1.8) g/L NaCl
 7-d IC50 (95% CL): 1.3 (1.0 - 1.8) g/L NaCl

7-d LC50 Reference Toxicant Mean and Historical Range: 1.7 (1.2 - 1.5) g/L NaCl CV (%): 21
 7-d IC50 Reference Toxicant Mean and Historical Range: 1.2 (0.9 - 1.6) g/L NaCl CV (%): 18

Test Results:

	Survival	Reproduction
LC50 % (v/v) (95% CL)	17.5 (14.3 - 21.5)	
IC25 % (v/v) (95% CL)		12.3 (11.2 - 12.6) ^{emp} (11.3 - 12.7)
IC50 % (v/v) (95% CL)		15.1 (14.0 - 16.0) _{emp} 16.2

Reviewed by: Joh

Date reviewed: March 27/14

Chronic Freshwater Toxicity Test Initial and Final Water Quality Measurements

Client: AIS
 Sample ID: NE2 (L1426336-3)
 Work Order #: 14076

Start Date & Time: Feb 28/14 @ 1100h
 Stop Date & Time: March 8/14 @ 1325h
 Test Species: Ceriodaphnia dubia

Concentration <i>control</i>	Days															8		
	0	1		2		3		4		5		6		7 ^{old}	8			
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final			new	Final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	24.5	24.0	24.5	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.0	7.8	7.9	7.4	8.1	7.6	8.1	7.7	7.9	7.3	8.0	7.7	8.2	7.4	8.1	8.1	7.6	
pH	7.9	7.8	7.9	7.8	8.1	7.7	7.9	7.6	7.9	7.7	8.0	7.7	7.9	7.8	8.1	7.9		
Cond. (µS/cm)	210		210		211		211		211		213		216		213	208		
Initials	EMM		A		A		EMM		EMM		EMM		EMM		EMM	A		

Concentration <i>(V/V) 5%</i>	Days															8		
	0	1		2		3		4		5		6		7 ^{old}	8			
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final			new	Final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	24.5	24.0	24.5	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.0	7.5	7.9	7.5	8.0	7.6	8.1	7.7	7.9	7.2	8.0	7.3	8.0	7.4	8.0	7.5	7.5	
pH	7.9	7.8	7.8	7.9	7.8	7.6	7.9	7.7	7.8	7.7	7.8	7.6	7.7	7.8	7.8	8.1		
Cond. (µS/cm)	215		220		218		219		216		214		221		217	213		
Initials	EMM		A		A		EMM		EMM		EMM		EMM		EMM	A		

Concentration <i>(V/V) 40%</i>	Days														
	0	1		2		3		4		5		6		7	
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final	
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	24.5									
DO (mg/L)	8.1	7.5	7.9	7.6	8.0	7.4									
pH	7.7	7.9	7.8	7.9	7.8	7.6									
Cond. (µS/cm)	257		255		259	269									
Initials	EMM		A		A	EMM									

Concentration <i>(V/V) 100%</i>	Days														
	0	1		2		3		4		5		6		7	
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final	
Temperature (°C)	24.5														
DO (mg/L)	8.1														
pH	7.5														
Cond. (µS/cm)	327														
Initials	EMM														

	Control	100% (V/V)
Hardness*	100	160
Alkalinity*	84	124

Analysts: AWD, EMM

Reviewed by: JBL

Date reviewed: March 26/14

* mg/L as CaCO₃

WQ Ranges: T (°C) = 25 ± 1; DO (mg/L) = 3.3 to 8.4 (mg/L); pH = 6 to 8.5

Sample Description: clear

Comments: Broodboard Used: 021914

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: ALS Start Date & Time: Feb 28/14 @ 11:06
 Sample ID: NF2 (14716336-3) Stop Date & Time: March 8/14 @ 13:25h
 Work Order: 14676 Set up by: EMM

Days	Concentration: 5										Concentration: 10																					
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init										
1																																
2	X																															
3																																
4																																
5																																
6																																
7																																
8																																
Total																																

Days	Concentration: 20										Concentration: 40										Concentration: 60																						
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init										
1																																											
2																																											
3	X																																										
4																																											
5																																											
6																																											
7																																											
8																																											
Total																																											

Days	Concentration: 80										Concentration: 100																					
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init										
1	X																															
2																																
3																																
4																																
5																																
6																																
7																																
8																																
Total																																

Notes: X = mortality.

Sample Description: clear

Comments: Total # Young only based on the first 3 broods. Fourth and subsequent broods not included in total count.

Reviewed by: JGH Date reviewed: March 26/14

CETIS Analytical Report

Report Date: 25 Mar-14 08:20 (p 1 of 2)
 Test Code: 14076c | 11-8877-8198

Ceriodaphnia 7-d Survival and Reproduction Test *9.1.2014* Nautilus Environmental

Analysis ID: 00-5603-8265	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 25 Mar-14 8:17	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes
Batch ID: 14-3157-7400	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 28 Feb-14 11:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 08 Mar-14 13:25	Species: Ceriodaphnia dubia	Brine:
Duration: 8d 2h	Source: In-House Culture	Age: <24h
Sample ID: 07-0729-5812	Code: 2A287A44	Client: ALS
Sample Date: 25 Feb-14 13:05	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 70h (3.3 °C)	Station: L1426336-3(NF2)	

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.1	0.00%	1.244	0.04404	17.54	14.32	21.48

7d Survival Rate Summary

C-%	Control Type	Count	Calculated Variate(A/B)								
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	0.9	0	1	0.1	0.3162	35.14%	0.0%	9	10
5		10	1	1	1	0	0	0.0%	-11.11%	10	10
10		10	1	1	1	0	0	0.0%	-11.11%	10	10
20		10	0.3	0	1	0.1528	0.483	161.0%	66.67%	3	10
40		10	0	0	0	0	0		100.0%	0	10
60		10	0	0	0	0	0		100.0%	0	10
80		10	0	0	0	0	0		100.0%	0	10
100		10	0	0	0	0	0		100.0%	0	10

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	Negative Control	0	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
20		0	0	0	0	0	0	1	0	1	1
40		0	0	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

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	Negative Control	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
10		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
20		0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	1/1	1/1
40		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
60		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
80		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
100		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

CETIS Analytical Report

Report Date: 25 Mar-14 08:20 (p 2 of 2)
Test Code: 14076c | 11-8877-819B

Ceriodaphnia 7-d Survival and Reproduction Test *P.L. 2012*

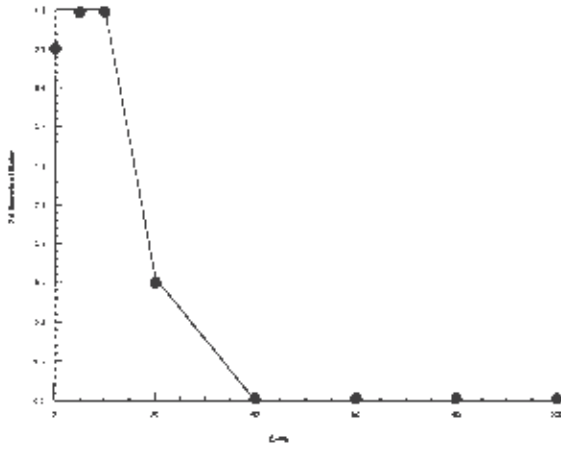
Nautilus Environmental

Analysis ID: 00-5603-8265
Analyzed: 25 Mar-14 8:17

Endpoint: 7d Survival Rate
Analysis: Untrimmed Spearman-Kärber

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 25 Mar-14 09:00 (p 1 of 2)
 Test Code: 14076c | 11-8877-8198

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 08-3838-3862	Endpoint: Reproduction	CETIS Version: CETISv1.8.7
Analyzed: 25 Mar-14 8:59	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 14-3157-7400	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 28 Feb-14 11:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 08 Mar-14 13:25	Species: Ceriodaphnia dubia	Brine:
Duration: 8d 2h	Source: In-House Culture	Age: <24h
Sample ID: 07-0729-5812	Code: 2A287A44	Client: ALS
Sample Date: 25 Feb-14 13:05	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 70h (3.3 °C)	Station: L1426336-3(NF2)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1683215	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	10.43	2.711	10.5	9.591	9.527	36.89
IC10	10.87	8.221	11.02	9.201	9.077	12.16
IC15	11.33	10.32	11.56	8.828	8.651	9.694
IC20	11.81	10.8	12.13	8.471	8.246	9.259
IC25	12.3	11.26	12.72	8.129	7.862	8.883
IC40	13.91	12.77	14.68	7.191	6.81	7.832
IC50	15.08	14.01	16.19	6.63	6.178	7.139

Reproduction Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	14.1	0	17	1.588	5.021	35.61%	0.0%
5		10	15.4	12	18	0.6182	1.955	12.7%	-9.22%
10		10	14.8	12	18	0.611	1.932	13.06%	-4.97%
20		10	2.2	0	9	1.052	3.327	151.2%	84.4%
40		10	0	0	0	0	0		100.0%
60		10	0	0	0	0	0		100.0%
80		10	0	0	0	0	0		100.0%
100		10	0	0	0	0	0		100.0%

Reproduction 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	Negative Control	0	15	17	16	16	16	16	14	16	15
5		16	16	12	12	17	16	16	18	15	16
10		12	16	14	18	13	14	16	17	13	15
20		0	3	0	0	0	0	9	0	3	7
40		0	0	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

CETIS Analytical Report

Report Date: 25 Mar-14 09:00 (p 2 of 2)

Test Code: 14076c | 11-8877-8198

Ceriodaphnia 7-d Survival and Reproduction Test

Nauticus Environmental

Analysis ID: 08-3838-3862

Endpoint: Reproduction

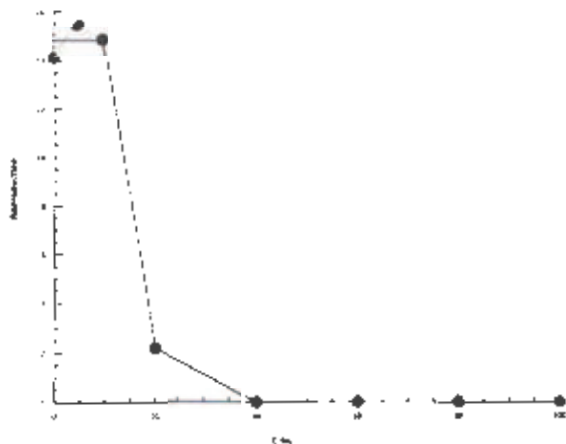
CETIS Version: CETISv1.8.7

Analyzed: 25 Mar-14 8:59

Analysis: Linear Interpolation (ICPIN)

Official Results: Yes

Graphics



Ceriodaphnia dubia Summary Sheet

Client: AUS Environmental
 Work Order No.: 14076

Start Date/Time: FEB 28/14 @ 1100h
 Set up by: ENJM

Sample Information:

Sample ID: L1426331-4 (X1)
 Sample Date: FEB 25/14
 Date Received: FEB 27/14
 Sample Volume: 2x 20L

Test Validity Criteria:

- 1) Mean survival of first generation controls is $\geq 80\%$
- 2) At least 60% of controls have produced three broods within 8 days
- 3) An average of ≥ 15 live young produced per surviving female in the control solutions during the first three broods
- 4) Invalid if ephippia observed in any control solution at any time

WQ Ranges:

T (°C) = 25 ± 1 ; DO (mg/L) = 3.3 to 8.4; pH = 6.0 to 8.5

Test Organism Information:

Broodstock No.: 022014
 Age of young (Day 0): <24-h (within 12-h)
 Avg No. young in first 3 broods of previous 7 d. 17
 Mortality (%) in previous 7 d: 0
 Individual female # used >8 young on test day 3, 4, 5, 6, 7, 10, 11, 12, 17, 18

NaCl Reference Toxicant Results:

Reference Toxicant ID: CA102
 Stock Solution ID: 131203
 Date Initiated: FEB 26/14

7-d LC50 (95% CL): 1.4 (1.1-1.8) g/L NaCl
 7-d IC50 (95% CL): 1.3 (1.0-1.8) g/L NaCl

7-d LC50 Reference Toxicant Mean and Historical Range: 1.7 (1.2-2.5) g/L NaCl CV (%): 21
 7-d IC50 Reference Toxicant Mean and Historical Range: 1.2 (0.9-1.6) g/L NaCl CV (%): 18

Test Results:

	Survival	Reproduction
LC50 % (v/v) (95% CL)	27.2 (25.1-29.5)	
IC25 % (v/v) (95% CL)		23.0 (20.6-23.6)
IC50 % (v/v) (95% CL)		27.7 (25.9-28.2)

Reviewed by: John

Date reviewed: March 27/14

Chronic Freshwater Toxicity Test Initial and Final Water Quality Measurements

Client: ALS
 Sample ID: X1 (1426336-4)
 Work Order #: 18076

Start Date & Time: Feb 28/14 @ 1100h
 Stop Date & Time: March 8/14 @ 1400h
 Test Species: Ceriodaphnia dubia

Concentration <i>control</i>	Days															
	0	1		2		3		4		5		6		7 ^{exam}	8	
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final	new	Final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	24.5	24.0	24.5	24.0	25.0	24.0	24.0	24.0	25.0
DO (mg/L)	8.0	7.9	7.9	7.4	8.1	7.6	8.1	7.8	7.9	8.0	8.0	7.3	8.2	7.9	8.1	7.2
pH	7.9	7.8	7.9	7.8	8.0	7.8	7.9	7.6	7.9	7.8	8.0	7.8	7.9	7.8	8.1	7.9
Cond. (µS/cm)	210	216		211		211		211		212		216		213		208
Initials	Emm	A		A		Emm		Emm		Emm		Emm		Emm		A

Concentration <i>5</i>	Days															
	0	1		2		3		4		5		6		7 ^{exam}	8	
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final	new	Final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	24.5	24.0	24.5	24.0	25.6	24.0	24.0	24.0	25.0
DO (mg/L)	8.2	7.5	7.9	7.5	8.1	7.5	8.2	7.8	7.9	8.6	8.0	7.3	7.9	8.0	7.9	7.4
pH	7.9	7.9	7.8	7.8	7.8	7.8	7.9	7.8	7.9	7.8	7.8	7.7	7.7	7.9	7.9	7.9
Cond. (µS/cm)	217	218		220		218		213		214		220		219		212
Initials	Emm	A		A		Emm		Emm		Emm		Emm		Emm		A

Concentration <i>40%</i>	Days															
	0	1		2		3		4		5		6		7		
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final	new	Final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0										
DO (mg/L)	8.1	7.4	7.8	7.7	7.8	7.4										
pH	7.6	7.9	7.7	7.9	7.6	7.6										
Cond. (µS/cm)	262	251		248		252										
Initials	Emm	A		A		Emm										

Concentration <i>100%</i>	Days															
	0	1		2		3		4		5		6		7		
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final	new	Final
Temperature (°C)	24.5															
DO (mg/L)	8.1															
pH	7.4															
Cond. (µS/cm)	346															
Initials	Emm															

	Control	100% (VIV)
Hardness*	100	186
Alkalinity*	89	130

Analysts: AWD, Emm

Reviewed by: Jou

Date reviewed: March 27/14

* mg/L as CaCO₃

WQ Ranges: T (°C) = 25 ± 1; DO (mg/L) = 3.3 to 8.4 (mg/L); pH = 6 to 8.5

Sample Description: clear

Comments: Broodboard Used: 022014

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: ALS Sample ID: *1 (L1426354) Start Date & Time: Feb 28/14 02:10:00
 Work Order: 14076 Set up by: EMM Stop Date & Time: March 8/14 14:00

Days	Concentration: 5										Concentration: 10											
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init
1	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
2	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
5	4	2	3	3	3	3	2	4	3	2	EMM	2	3	3	3	2	3	X	3	2	3	EMM
6	7	4	6	7	7	7	7	4	6	7	JW	7	7	8	8	7	8	1	5	6	7	JW
7	6	7	6	6	6	6	5	7	6	6	JW	6	7	10	7	6	7	1	5	6	7	JW
8	6	7	6	6	6	6	6	6	6	6	EMM	6	7	7	6	6	7	1	5	6	7	EMM
Total	17	13	15	14	15	18	14	16	15	15	EMM	16	19	15	18	16	15	17	10	15	17	EMM

Days	Concentration: 40										Concentration: 60											
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init
1	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
2	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
5	3	2	3	3	3	2	4	3	2	3	EMM	3	3	3	3	2	3	X	3	2	3	EMM
6	8	6	6	6	6	6	6	6	6	6	JW	6	7	10	7	6	7	1	5	6	7	JW
7	6	7	6	6	6	6	6	6	6	6	JW	6	7	7	6	6	7	1	5	6	7	JW
8	6	7	6	6	6	6	6	6	6	6	EMM	6	7	7	6	6	7	1	5	6	7	EMM
Total	17	15	11	15	15	13	14	12	16	15	EMM	16	19	15	18	16	15	17	10	15	17	EMM

Days	Concentration: 80										Concentration: 100											
	A	B	C	D	E	F	G	H	I	J	Init	A	B	C	D	E	F	G	H	I	J	Init
1	X	X	X	X	X	X	X	X	X	X	/	X	X	X	X	X	X	X	X	X	X	/
2	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
5	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
6	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
7	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
8	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Total	0	0	0	0	0	0	0	0	0	0	EMM	0	0	0	0	0	0	0	0	0	0	EMM

Notes: X = mortality.
 Sample Description: clear
 Comments: Total # Young only based on the first 3 Broods. Fourth and subsequent broods not included in total count.
 Reviewed by: JBL Date reviewed: March 28/14

CETIS Analytical Report

Report Date: 25 Mar-14 08:30 (p 1 of 2)
 Test Code: 14076d | 03-3685-9732

Ceriodaphnia 7-d Survival and Reproduction Test Nautilus Environmental

Analysis ID: 00-8120-2686	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 25 Mar-14 8:28	Analysis: Trimmed Spearman-Kärber	Official Results: Yes
Batch ID: 07-6970-9125	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 28 Feb-14 11:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 08 Mar-14 14:00	Species: Ceriodaphnia dubia	Brine:
Duration: 8d 3h	Source: In-House Culture	Age: <24h
Sample ID: 19-2140-4869	Code: 72864FC5	Client: ALS
Sample Date: 25 Feb-14 11:10	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 72h (4 °C)	Station: L1426336-4(X1)	

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	10.00%	1.435	0.01763	27.22	25.09	29.52

7d Survival Rate Summary

Calculated Variate(A/B)

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10
5		10	0.8	0	1	0.1333	0.4216	52.7%	20.0%	8	10
10		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10
20		10	1	1	1	0	0	0.0%	0.0%	10	10
40		10	0	0	0	0	0		100.0%	0	10
60		10	0	0	0	0	0		100.0%	0	10
80		10	0	0	0	0	0		100.0%	0	10
100		10	0	0	0	0	0		100.0%	0	10

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	Negative Control	1	1	1	1	1	1	1	1	1	1
5		1	1	1	0	1	1	1	1	1	0
10		1	1	1	1	1	1	1	0	1	1
20		1	1	1	1	1	1	1	1	1	1
40		0	0	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

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	Negative Control	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
5		1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	0/1
10		1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1
20		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
40		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
60		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
80		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
100		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

CETIS Analytical Report

Report Date: 25 Mar-14 08:30 (p 2 of 2)
Test Code: 14076d | 03-3685-9732

Ceriodaphnia 7-d Survival and Reproduction Test

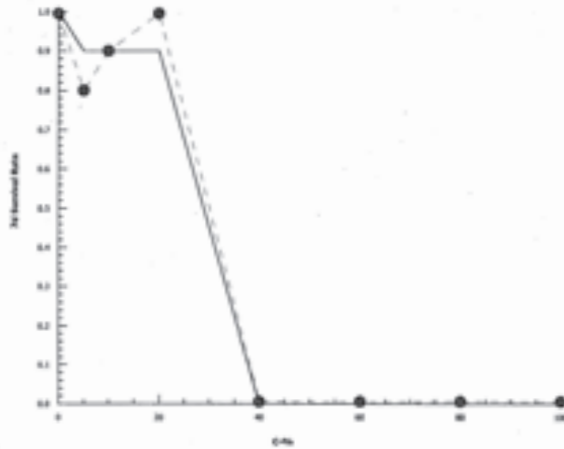
Nautilus Environmental

Analysis ID: 00-8120-2686
Analyzed: 25 Mar-14 8:28

Endpoint: ~~7d~~ Survival Rate
Analysis: Trimmed Spearman-Kärber

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 25 Mar-14 08:31 (p 1 of 2)
 Test Code: 14076d | 03-3685-9732

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 01-0863-1339	Endpoint: Reproduction	CETIS Version: CETISv1.8.7
Analyzed: 25 Mar-14 8:30	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 07-6970-9125	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 28 Feb-14 11:00	Protocol: EC/EPS 1/RM/21	Diluent: 20% Perrier Water
Ending Date: 08 Mar-14 14:00	Species: Ceriodaphnia dubia	Brine:
Duration: 8d 3h	Source: In-House Culture	Age: <24h
Sample ID: 19-2140-4869	Code: 72864FC5	Client: ALS
Sample Date: 25 Feb-14 11:10	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 72h (4 °C)	Station: L1426336-4(X1)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1024824	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	8.358	1.278	20.51	11.96	4.877	78.28
IC10	20.52	4.187	21.25	4.873	4.706	23.88
IC15	21.31	7.245	22.02	4.694	4.542	13.8
IC20	22.12	9.471	22.81	4.521	4.384	10.56
IC25	22.96	20.63	23.63	4.355	4.231	4.847
IC40	25.68	23.58	26.28	3.894	3.806	4.24
IC50	27.66	25.77	28.19	3.615	3.547	3.88

Reproduction Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	15.2	13	18	0.4667	1.476	9.71%	0.0%
5		10	15.1	8	20	1.027	3.247	21.5%	0.66%
10		10	14.1	0	19	1.741	5.507	39.05%	7.24%
20		10	14.3	11	17	0.5783	1.829	12.79%	5.92%
40		10	0	0	0	0	0		100.0%
60		10	0	0	0	0	0		100.0%
80		10	0	0	0	0	0		100.0%
100		10	0	0	0	0	0		100.0%

Reproduction 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	Negative Control	17	13	15	14	15	18	14	16	15	15
5		20	17	16	12	17	14	16	16	15	8
10		16	19	15	18	16	15	17	0	10	15
20		17	15	11	15	15	13	14	12	16	15
40		0	0	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

CETIS Analytical Report

Report Date: 25 Mar-14 08:31 (p 2 of 2)
Test Code: 14076d | 03-3685-9732

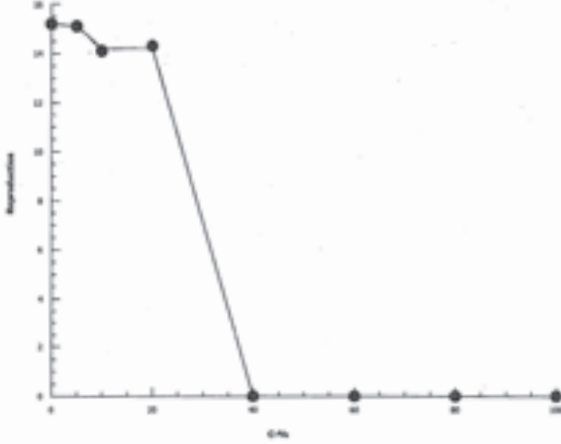
Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 01-0863-1339 Endpoint: Reproduction
Analyzed: 25 Mar-14 8:30 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



Client: ALS Environmental

W.O.#: 14076

Hardness and Alkalinity Datasheet

Sample ID	Sample Date	Sample Volume (mL)	Alkalinity				Hardness			Technician
			(mL) 0.02N HCL/H ₂ SO ₄ used to pH 4.5	(mL) of 0.02N HCL/H ₂ SO ₄ used to pH 4.2	Total Alkalinity (mg/L CaCO ₃)	Sample Volume (mL)	Volume of 0.01M EDTA Used (mL)	Total Hardness (mg/L CaCO ₃)		
L1426326-4 (X1)	Feb 27/14	50	6.6	6.7	130	50	9.3	136	KLP	
L1426326-1 (R10)	↓	↓	6.4	6.5	126	↓	7.4	148	KLP	
L1426326-2 (NF1)	↓	↓	6.7	6.8	132	↓	7.8	156	KLP	
L1426326-3 (NF2)	↓	↓	6.3	6.4	124	↓	8.0	160	KLP	
286.5 mixer control	↓	↓	4.3	4.4	84	↓	5.0	100	KLP	
							5.0			

Notes:

Reviewed by: JGA

Date Reviewed: March 26/14

CERIODAPHNIA DUBIA TOXICITY TESTING
February 2014

Prepared for:

NAUTILUS ENVIRONMENTAL
8664 Commerce Court
Burnaby, BC
V5A 4N7

Prepared by:

IRC INTEGRATED RESOURCE CONSULTANTS INC.
160 - 14480 River Road
Richmond, B.C.
V6V 1L4
Tel: 604-278-7714
Fax: 604-278-7741

21 March 2014

Nautilus Environmental
8664 Commerce Court
Burnaby, BC
V5A 4N7

Attention: Krysta Pearcy

Reference: *Ceriodaphnia dubia* bioassay on the X14 sample received on 27 February 2014.


Dear Ms. Pearcy,

Enclosed please find the final report for the *Ceriodaphnia dubia* toxicity testing results, for Nautilus Environmental X14 sample dated 25 February 2014. This report includes *Ceriodaphnia dubia* test reproduction and survival data as well as daily water quality readings and reference toxicant results.

The result of the *Ceriodaphnia dubia* bioassay indicated that a concentration that would cause 50% mortality (LC50) was greater than 100%. The survival No Observed Effect Level (NOEL) was 100% and the Lowest Observed Effect Level (LOEL) was greater than 100%. The concentration that would cause a 50% inhibition in reproduction (IC50) in the culture tested was 53.58% with a 95% confidence interval between 24.92% and 62.32%, while the 25% inhibition value (IC25) was 20.91% with a 95% confidence interval between 17.52% and 32.90%. The reproduction No Observed Effect Level (NOEL) was 50% and the Lowest Observed Effect Level (LOEL) was 100%.

Should you have any questions regarding these results, please do not hesitate to call me at 604-278-7714.

Sincerely,



Ditty Chacko Kakkassery
Laboratory Biologist
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Encl.

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1.0 CERIODAPHNIA DUBIA BIOASSAY METHOD AND RESULTS

1.1 SAMPLE DESCRIPTION

IRC Sample ID No.:	1402154
Sample Name:	X14
Effluent type:	Effluent
Date collected:	25 February 2014
Date, time received:	27 February 2014; 1425 hrs
Collection Method:	Grab
Amount, Container:	6 x 1 L glass containers
Date, time test initiated:	27 February 2014; 1620 hrs.
Date, time test completed:	05 March 2014; 1230 hrs.
Physical description:	Translucent slightly yellow liquid

1.2 METHOD

The method used for this test was as per the IRC laboratory "Standard Operating Procedure for *Ceriodaphnia dubia* Testing and Culturing" CDver3. This procedure follows the "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*" Report EPS 1/RMS/21 Second Edition - February 2007. The NOEL and LOEL were calculated by Fisher Exact/Bonferroni-Holm method, IC50 and IC25 using Linear Interpolation method and LC50 by Spearman-Kärber or Linear Interpolation method with the CETIS, ver 1.6.6E (2008) software.

Ceriodaphnia dubia are cultured on site from an original culture obtained from Carolina Biological Supply Ltd. Organisms are maintained in 1 L mass cultures and in a series of 23 mL glass test tubes containing a single *Ceriodaphnid* from which test organisms are obtained. Culture, control and test dilution water was a mixture of 80% distilled water and 20% Perrier.

Tests were conducted in 23mL glass test tubes containing 20mL of test solution, at a depth of 12 cm. Ten replicates of each concentration (100%, 50%, 25%, 12.5%, 6.25%, 3.13% and 1.56%) and control were tested. Test temperature was maintained throughout the test period at 25±1°C, with a photoperiod of 16 hours light and 8 hours dark.

Initiation of the bioassay was carried out by placing a single neonate *Ceriodaphnid* of less than 24 hours, into each test vessel. New test solutions were prepared daily into which organisms were transferred. Daily measurements of the mortality and number of young produced in each replicate were recorded. Records were also maintained for daily readings of dissolved oxygen, pH, temperature and conductivity for each test concentration and control solution. The test was completed when ≥ 60% of control organisms had 3 broods.

On the day of test initiation, adult *Ceriodaphnia* were placed in test tubes at 0600 hours; young used in testing were pulled directly from these test tubes at 1600 hours, ranging in age from 0 to 1000 hours. There was no unusual appearance or behaviour noted in the test organisms prior to their use in the test. No ephippia were observed in brood cultures and mass cultures in the seven day period preceding the test.

Sample used for testing was collected on 25 February 2014. Sample containers were marked with the sample ID: 7 Day Chronic *Ceriodaphnia*. The sample arrival temperature was 10.5°C; nothing unusual was noted regarding the sample appearance. Sample in the 6 x 1 litre glass jugs received were stored in the dark at 4±1°C until used for testing. The required volume of sample was poured out into a labeled beaker on each day of testing. The sample was not pH adjusted or filtered prior to being used in testing. The test was complete at day 6 as ≥60% of control organisms had produced 3 broods at this time.

1.3 RESULTS

	Results	95% Confidence Interval
<i>Ceriodaphnia dubia</i> LC ₅₀	> 100%	-
NOEL (Survival)	100%	-
LOEL (Survival)	> 100%	-
<i>Ceriodaphnia dubia</i> IC ₂₅	20.91%	17.52% - 32.90%
<i>Ceriodaphnia dubia</i> IC ₅₀	53.58%	24.92% - 62.32%
NOEL (Reproduction)	50%	-
LOEL (Reproduction)	100%	-

LC₅₀ = Concentration which would cause a 50% mortality

IC₂₅ = Concentration which would cause a 25% inhibition in reproduction or growth.

IC₅₀ = Concentration which would cause a 50% inhibition in reproduction or growth.

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

1.4 WATER QUALITY READINGS AND TEST DATA

Test set up technician was DB. Daily reading technicians were DB, DC, CW and MH. The initial dissolved oxygen level of the sample was 11.5 mg/L at 11.0°C, the initial conductivity was 795 µS/cm and the initial pH was 7.4. The sample was not pH adjusted or filtered prior to testing. For daily water quality readings, please see appendices.

Daily Initial Readings of Undiluted sample (after warming)

	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (µS/cm)	PRE-AERATION*
DAY 0	11.3	25.0	7.3	795	20 minutes
DAY 1	10.9	25.0	7.4	787	20 minutes
DAY 2	11.4	25.5	7.3	789	20 minutes
DAY 3	11.2	24.0	7.4	793	20 minutes
DAY 4	11.3	25.5	7.3	791	20 minutes
DAY 5	11.5	25.0	7.4	790	20 minutes

*Pre-aeration of the sample is carried out if the dissolved oxygen level is either less than 40% saturation or greater than 100% saturation. Pre-aeration is for a maximum of 20 minutes.

Daily 0 Hour Refresh Solutions

Concentration	Dissolved Oxygen Range (mg/L)	pH Range	Conductivity Range (µS/cm)	Hardness (mg/L)
100%	9.5-10.0	7.4-7.6	773-791	410-424
50%	8.7-8.9	7.5-7.7	495-507	
25%	8.4-8.6	7.7-7.8	246-346	
12.5%	8.2-8.5	7.7-7.9	256-263	
6.25%	8.1-8.4	7.7-7.9	211-219	
3.13%	8.1-8.4	7.7-7.9	188-196	
1.56%	8.1-8.4	7.7-7.9	175-185	
Control	8.1-8.4	7.7-8.0	164-176	74-86

Daily 24 Hour Old Solutions

Concentration	Dissolved Oxygen Range (mg/L)	pH Range	Conductivity Range (µS/cm)
100%	7.1-7.4	7.9-8.1	776-791
50%	6.9-7.4	7.9-8.1	500-509
25%	6.8-7.3	7.7-8.0	342-353
12.5%	6.7-7.2	7.6-7.9	260-269
6.25%	6.7-7.2	7.6-7.9	215-221
3.13%	6.7-7.4	7.6-7.9	192-200
1.56%	6.7-7.3	7.6-7.9	180-188
Control	6.9-7.4	7.6-7.8	171-180

REPRODUCTION AND SURVIVAL RESULTS:

Summary of Total Young Produced Per *Ceriodaphnia*

Concentration	Total Young Produced per <i>Ceriodaphnid</i> in its First 3 Broods										Mean Young in First 3 Broods	Standard Deviation
	1	2	3	4	5	6	7	8	9	10		
100%	5	D	8	1	7	0	D	D	3	3	2.7	3.1
50%	6	14	16	2	17	23	27	29	23	12	16.9	8.8
25%	25	27	2	30	8	36	13	25	20	17	20.5	10.3
12.5%	30	36	32	37	34	37	34	32	40	34	34.6	3.0
6.25%	35	38	33	38	29	39	D12	36	13	34	30.7	10.0
3.13%	34	38	38	32	27	34	22	35	35	36	32.4	4.7
1.56%	31	22	34	25	31	35	41	33	30	30	31.2	5.2
Control	32	38	14	35	12	11	36	32	15	35	26.0	11.4

'D' – Dead

① 31 emm

'X' – Cerio lost due to technician error

Summary of *Ceriodaphnia* Survival

Concentration	Percent Survival					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
100%	100	80	70	70	70	70
50%	100	100	100	100	100	100
25%	100	100	100	100	100	100
12.5%	100	100	100	100	100	100
6.25%	100	100	100	100	100	90
3.13%	100	100	100	100	100	100
1.56%	100	100	100	100	100	100
Control	100	100	100	100	100	100

Percent survival in each concentration is based on a single individual in each of ten replicates.

1.5 QUALITY CONTROL

Test controls conducted concurrently with the test and reference toxicant bioassays affirmed the validity of the *Ceriodaphnia dubia* test. Testing of the reference toxicant was performed as per protocol requirements with no deviations and conditions were within testing limits for measured parameters as specified by the bioassay protocol.

The brood organisms used to supply neonates in the *Ceriodaphnia dubia* survival and reproductive bioassay maintained the requirements of mortality rates less than or equal to 20% prior to testing; with a minimum of 9 young produced in the previous brood and an average of 32.2 young produced per adult in its first 3 broods. The brood stock was challenged with a reference toxicant (reagent grade sodium chloride) within fourteen days of sample testing. The value obtained in this test was within warning limits (± 2 standard deviations) of the laboratory mean, established through repetitive testing with the reference toxicant and brood culture. Dilution water controls run concurrent with the test produced three broods per test organism in at least 60% of the control replicates with an average of greater than 15 live young per adult. Control mortalities were less than 20%.

Test Brood Stock Health Summary

	Actual	Required
Age of Neonates	0-1000 hours	≤ 24 hours
Age of brood adults	6 days	≤ 14 days
Mean % mortality in 7 days prior to testing	0%	$\leq 20\%$
Average of number of young produced per adult in its first 3 broods	32.2	≥ 15
Minimum number of young produced in previous brood	9	≥ 8
Ehippia observations	None	None

Reference Toxicant Results

Chemical Used:	Sodium Chloride
Date Tested:	19 February 2014
7 day IC_{50} (Log Value):	3.147 mg/L, with a 95% confidence interval between 3.097 mg/L and 3.198 mg/L
Lab Geometric Mean (Log Value):	3.079 mg/L \pm 0.189 mg/L (two standard deviations) N = 31
Warning Limits (Log Values):	2.890 mg/L to 3.268 mg/L
7 day LC_{50} (Log Value):	3.327 mg/L, with a 95% confidence interval between 3.231 mg/L and 3.422 mg/L
Lab Geometric Mean (Log Value):	3.253 mg/L \pm 0.166 mg/L (two standard deviations) N = 31
Warning Limits (Log Values):	3.087 mg/L to 3.419 mg/L

***Ceriodaphnia dubia* Brood Stock Health Record**

CERIODAPHNIA BROOD STOCK HEALTH RECORD

Client:	Nautilus Environmental
IRC ID#:	1402154
Sample Name:	X14
Sample Date:	25-Feb-14
Date Tested:	27-Feb-14
STOCK BIRTH DAY/DATES:	19-Feb-14

Mortality rate in 7 day period preceding test:	
# Dead:	0
Total organisms:	30
% Mortality:	0.0%
Required:	≤20%

EPHIPPIA OBSERVATIONS (yes/no): No

Young Produced 19-Feb-14

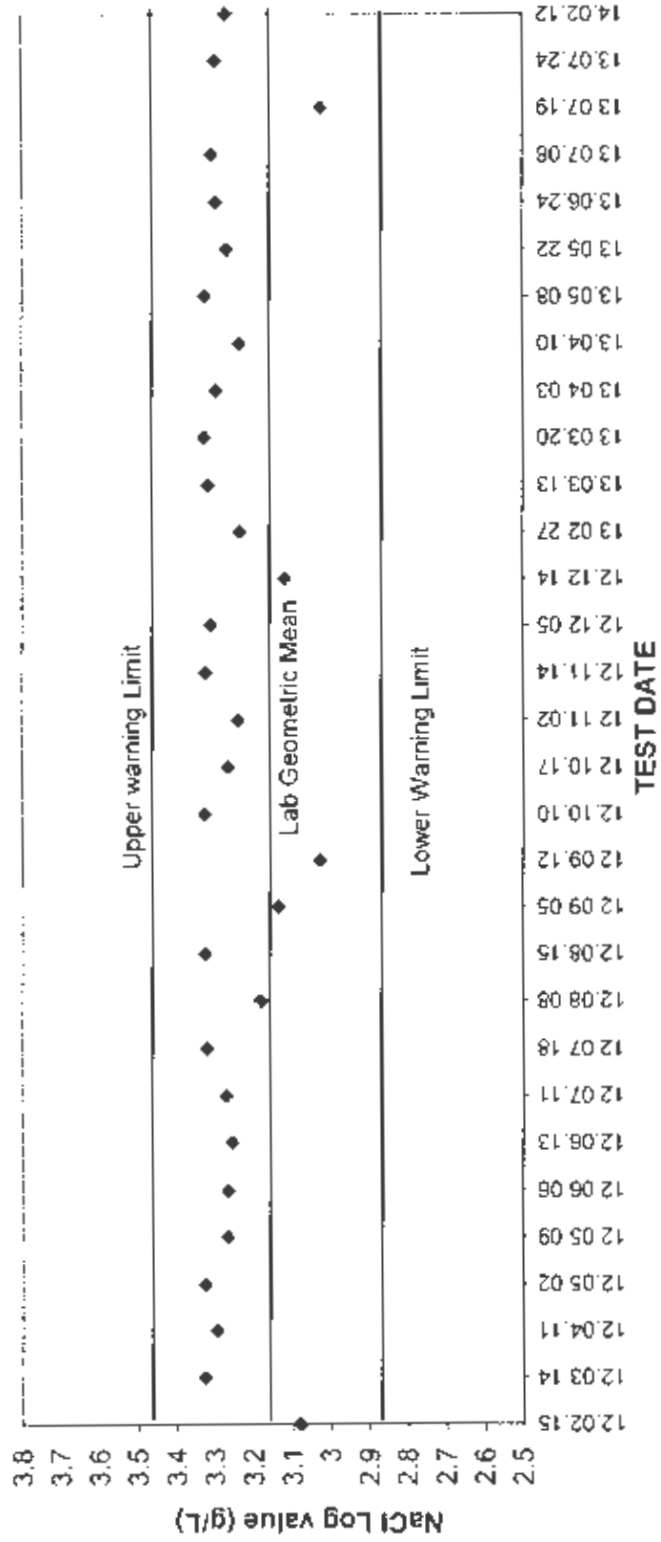
Date	Age (Days)	Organism #											
		1	2	3	4	5	6	7	8	9	10		
20-Feb-14	1	0	0	0	0	0	0	0	0	0	0	0	0
21-Feb-14	2	0	0	0	0	0	0	0	0	0	0	0	0
22-Feb-14	3	3	4	4	3	3	0	4	4	5	5		
23-Feb-14	4	0	0	0	0	0	6	0	0	0	0		
24-Feb-14	5	9	12	12	12	12	9	12	11	8	11		
25-Feb-14	6	20	17	19	21	17	0	17	9	16	20		
26-Feb-14	7	0	15	0	0	0	0	0	19	0	0		
27-Feb-14	8	20	22	19	17	22	19	24	0	20	20		
28-Feb-14	9	20	20	21	24	23	25	25	21	27	20		
1-Mar-14	10	23	0	17	27	0	21	24	19	22	20		
2-Mar-14	11	0	25	0	0	26	0	0	20	0	0		
Total in first 3 broods:		32	33	35	36	32	15	33	24	29	36		

Average in first 3 broods: 32.2 Required: ≥15
 Minimum brood size on day of testing: 9 Required: ≥8

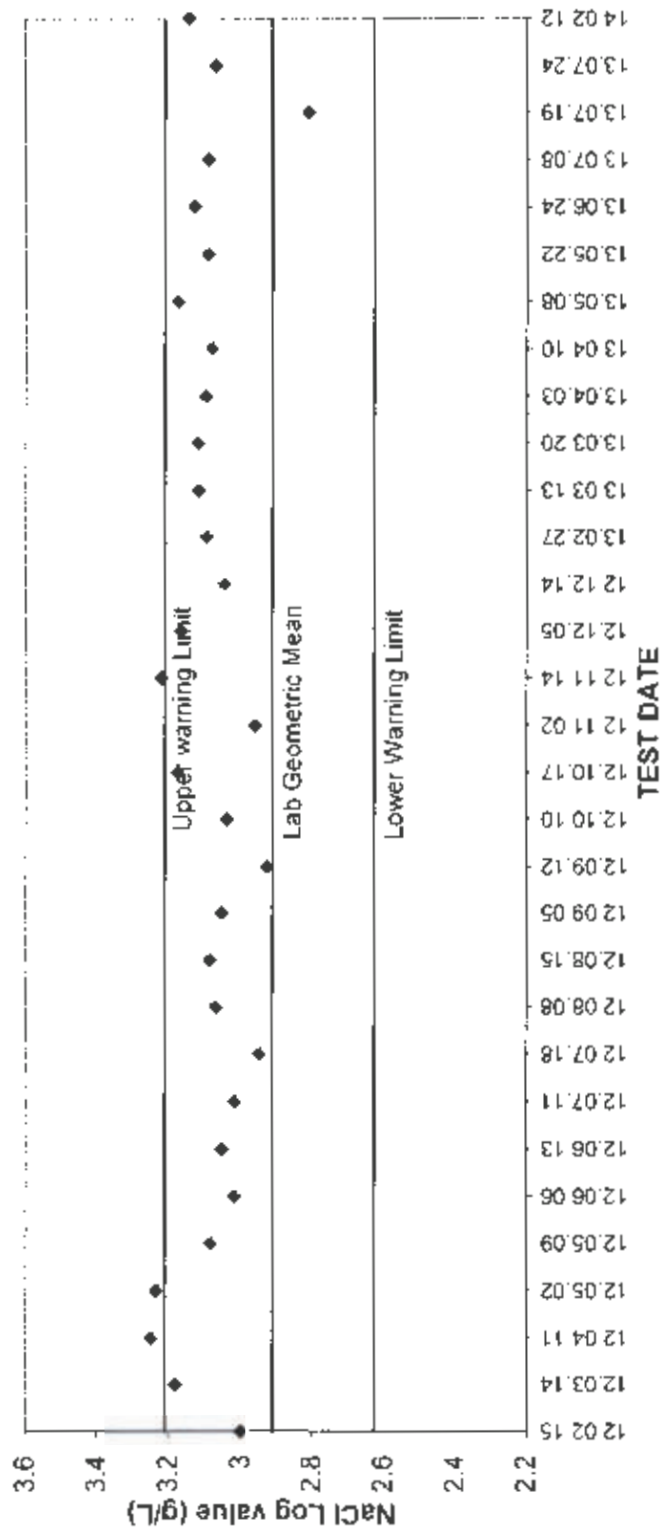
"d" - dead; "X" - Tech Error

Ceriodaphnia dubia Bioassay Reference Toxicant
Warning Charts

CERIODAPHNIA REFERENCE TOXICANT LC50 WARNING CHART - LOG VALUES



CERIODAPHNIA REFERENCE TOXICANT IC50 WARNING CHART - LOG VALUES



Test Brood Count and Solution Readings

CERIODAPHNIA BROOD COUNT SUMMARY

Client:	Nautilus Environmental
IRC ID#:	1402154
Sample Name:	X14
Sample Date:	25-Feb-14
Date Tested:	27-Feb-14

CONCENTRATION:	100%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	d	0	0	0	0	d	d	0	0
4	0	d	0	0	0	0	d	d	0	0
5	5	d	0	1	0	0	d	d	0	3
6	0	d	8	0	7	0	d	d	3	0
TOTAL:	5	0	8	1	7	0	0	0	3	3

CONCENTRATION:	50%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	0	0	0	0	0	0	0	0	0
4	0	5	6	2	0	5	3	5	4	4
5	5	9	10	0	6	8	9	10	6	8
6	1	0	0	0	11	10	15	14	13	0
TOTAL:	6	14	16	2	17	23	27	29	23	12

CONCENTRATION:	25%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	5	0	0	5	0	5	0	3	0	0
4	0	0	0	0	2	0	7	1	0	5
5	6	10	0	9	6	12	8	10	5	12
6	14	17	2	16	0	19	0	11	15	0
TOTAL:	25	27	2	30	8	36	15	25	20	17

CONCENTRATION:	12.50%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	0	0	4	4	5	0	5	0	5
4	4	4	6	0	0	0	5	0	7	0
5	11	13	12	14	13	14	11	11	10	11
6	15	19	14	19	17	18	18	16	23	18
TOTAL:	30	36	32	37	34	37	34	32	40	34

CONCENTRATION:	6.25%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	3	5	6	6	0	5	0	0	0	7
4	1	0	0	0	1	0	0	5	4	0
5	15	15	11	14	11	12	12	12	9	13
6	16	18	16	18	17	22	d	19	0	14
TOTAL:	35	38	33	38	29	39	12	36	13	34

CONCENTRATION:	3.13%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	5	6	4	0	0	5	5	6	5
4	6	0	0	0	4	4	0	0	0	0
5	13	11	12	10	12	12	9	13	12	12
6	15	15	20	18	11	18	8	17	17	19
TOTAL:	34	31	38	32	27	34	22	35	35	36

CONCENTRATION:	1.56%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	0	0	0	6	4	6	5	4	3
4	5	3	5	3	0	0	6	0	0	0
5	11	8	13	11	11	11	13	10	8	9
6	15	11	16	11	14	20	16	18	18	18
TOTAL:	31	22	34	25	31	35	41	33	30	30

CONCENTRATION:	Control									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	4	0	0	0	0	0	0	5	0	0
4	0	5	5	4	4	3	5	0	6	6
5	10	14	9	14	8	8	11	9	9	12
6	18	19	0	17	0	0	20	18	0	17
TOTAL:	32	38	14	35	12	11	36	32	15	35

"d" - dead; "X" - Tech Error

CONCENTRATION	100%	50%	25%	12.5%	6.25%	3.13%	1.56%	Control
BROOD COUNT MEANS	2.7	16.9	20.5	34.6	30.7	32.4	31.2	26.0
SD	3.1	8.8	10.3	3.0	10.0	4.7	5.2	11.4

Client:	Nautilus Environmental																		
IRC ID#:	1402154																		
Sample Name:	X14																		
Sample Date:	25-Feb-14																		
Date Tested:	27-Feb-14																		
FRESH SOLUTIONS										OLD SOLUTIONS									
DISSOLVED OXYGEN										DISSOLVED OXYGEN									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	9.9	9.8	9.8	10.0	9.6	9.5		9.5	10.0	100	7.3	7.4	7.3	7.4	7.3	7.1		7.1	7.4
50	8.9	8.7	8.9	8.9	8.7	8.8		8.7	8.9	50	7.4	7.3	7.1	7.3	7.2	6.9		6.9	7.4
25	8.6	8.4	8.4	8.4	8.4	8.5		8.4	8.6	25	7.2	7.2	7.1	7.3	7.0	6.8		6.8	7.3
12.5	8.5	8.3	8.2	8.2	8.3	8.4		8.2	8.5	12.5	7.1	7.0	7.0	7.2	6.9	6.7		6.7	7.2
6.25	8.4	8.3	8.1	8.2	8.2	8.4		8.1	8.4	6.25	7.2	7.0	7.1	7.1	6.9	6.7		6.7	7.2
3.13	8.4	8.3	8.1	8.1	8.2	8.3		8.1	8.4	3.13	7.3	7.4	7.1	7.2	7.0	6.7		6.7	7.4
1.56	8.4	8.2	8.1	8.2	8.2	8.3		8.1	8.4	1.56	7.2	7.3	7.1	7.2	7.1	6.7		6.7	7.3
CONTROL	8.4	8.1	8.2	8.4	8.3	8.4		8.1	8.4	CONTROL	7.1	7.1	7.2	7.4	7.2	6.9		6.9	7.4
pH										pH									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	7.4	7.5	7.4	7.5	7.4	7.6		7.4	7.6	100	7.9	8.1	8.1	8.0	8.0	8.0		7.9	8.1
50	7.5	7.7	7.6	7.6	7.6	7.7		7.5	7.7	50	7.9	8.1	8.0	7.9	7.9	7.9		7.9	8.1
25	7.7	7.7	7.7	7.8	7.7	7.8		7.7	7.8	25	7.8	8.0	7.9	7.8	7.8	7.7		7.7	8.0
12.5	7.7	7.8	7.8	7.8	7.8	7.9		7.7	7.9	12.5	7.7	7.9	7.8	7.7	7.7	7.6		7.6	7.9
6.25	7.7	7.8	7.9	7.9	7.8	7.9		7.7	7.9	6.25	7.7	7.9	7.8	7.7	7.7	7.6		7.6	7.9
3.13	7.7	7.8	7.9	7.9	7.8	7.9		7.7	7.9	3.13	7.7	7.9	7.8	7.7	7.7	7.6		7.6	7.9
1.56	7.7	7.9	7.9	7.9	7.9	7.9		7.7	7.9	1.56	7.7	7.9	7.8	7.7	7.7	7.6		7.6	7.9
CONTROL	7.7	7.9	8.0	7.9	7.9	7.9		7.7	8.0	CONTROL	7.7	7.8	7.8	7.7	7.7	7.6		7.6	7.8
CONDUCTIVITY										CONDUCTIVITY									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	775	773	791	773	785	786		773	791	100	776	780	791	782	789	786		776	791
50	499	502	507	495	504	503		495	507	50	500	509	506	505	509	506		500	509
25	343	343	346	246	346	345		246	346	25	342	348	345	353	350	349		342	353
12.5	258	256	258	263	262	259		256	263	12.5	260	261	261	269	265	263		260	269
6.25	214	213	211	219	218	215		211	219	6.25	216	216	215	221	221	218		215	221
3.13	192	190	188	195	196	192		188	196	3.13	194	192	193	198	200	195		192	200
1.56	180	175	176	181	185	181		175	185	1.56	182	180	181	184	188	183		180	188
CONTROL	171	168	164	174	176	168		164	176	CONTROL	175	171	173	176	180	175		171	180
AFTER WARMING:																			
DAY	0	1	2	3	4	5	6	MIN	MAX										
Dissolved Oxygen	11.3	10.9	11.4	11.2	11.3	11.5		10.9	11.5										
Temperature	25.0	25.0	25.5	24.0	25.5	25.0		24	25.5										
pH	7.3	7.4	7.3	7.4	7.3	7.4		7.3	7.4										
Conductivity	795	787	789	793	791	790		787	795										
Aeration:	20 min	20 min	20 min	20 min	20 min	20 min													
HARDNESS:																			
Sample	410	410	412	421	424	422		410	424										
Dilution Water	86	74	80	76	80	76		74	86										

***Ceriodaphnia dubia* Bioassay Calculation Printouts**
7 Day Chronic *Ceriodaphnia* IC₂₅ and IC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:11 (p 1 of 2)
Test Code: 04-6579-5047/1402154

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 17-4870-5124	Endpoint: Reproduction	CETIS Version: CETISv1.6.6
Analyzed: 18 Mar-14 16:09	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Test Run No: 08-0175-3567	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 0h	Source: In-House Culture	Age:
Sample No: 02-1487-4161	Code: 214874161	Client: Nautilus
Sample Date: 18 Mar-14 16:05 ①	Material: Unknown	Project: Special Studies
Receive Date: 18 Mar-14 16:05 ②	Source: X14	
Sample Age: N/A	Station:	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X + 1)	Linear	57951	200	Yes	Two-Point Interpolation

Point Estimates

Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	13.87	5.387	15.6	7.208	6.41	18.56
IC10	15.39	13.79	19.42	6.499	5.15	7.252
IC15	17.05	15.16	24.11	5.864	4.148	6.597
IC20	18.89	16.38	29.3	5.294	3.413	6.106
IC25	20.91	17.52	32.9	4.782	3.04	5.707
IC40	36.19	21.75	55.54	2.764	1.801	4.598
IC50	53.58	24.92	62.32	1.866	1.605	4.013

Reproduction Summary

Conc-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	10	26	11	38	2.076	11.37	43.74%	0.0%
1.56		10	31.2	22	41	0.9576	5.245	16.81%	-20.0%
3.13		10	32.4	22	38	0.8658	4.742	14.64%	-24.62%
6.25		10	30.7	12	39	1.83	10.02	32.65%	-18.08%
12.5		10	34.6	30	40	0.5389	2.951	8.53%	-33.08%
25		10	20.5	2	36	1.88	10.3	50.24%	21.15%
50		10	16.9	2	29	1.607	8.8	52.07%	35.0%
100		10	2.7	0	8	0.5581	3.057	113.2%	89.62%

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	32	38	14	35	12	11	36	32	15	35
1.56		31	22	34	25	31	35	41	33	30	30
3.13		34	31	38	32	27	34	22	35	35	36
6.25		35	38	33	38	29	39	12	36	13	34
12.5		30	36	32	37	34	37	34	32	40	34
25		25	27	2	30	8	36	15	25	20	17
50		6	14	16	2	17	23	27	29	23	12
100		5	0	8	1	7	0	0	0	3	3

① 25 Feb-14 09:00
 ② 27 Feb-14 10:30

CETIS Analytical Report

Report Date: 18 Mar-14 16:11 (p 2 of 2)
Test Code: 04-6579-5047/1402154

Ceriodaphnia 7-d Survival and Reproduction Test

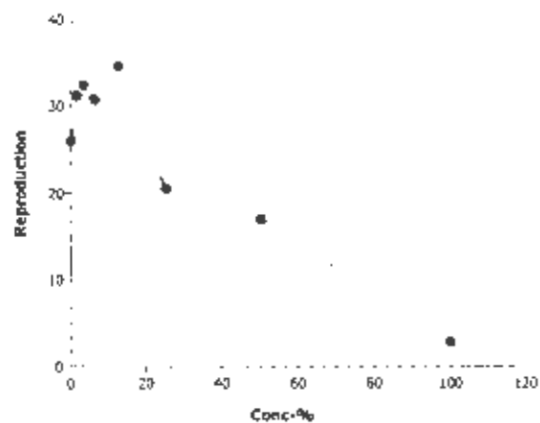
IRC Integrated Research Consultants

Analysis No: 17-4870-5124
Analyzed: 18 Mar-14 16:09

Endpoint: Reproduction
Analysis: Linear Interpolation (ICP₁₀)

CETIS Version: CETISv1.6.6
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 16 Apr-14 09:08 (p 1 of 2)

Test Code: 04-6579-5047/14 | 16-7175-0758

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 10-4675-8167	Endpoint: Reproduction	CETIS Version: CETISv1.8.7
Analyzed: 16 Apr-14 9:08	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 19-7346-0589	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine:
Duration: 6d 0h	Source: In-House Culture	Age:
Sample ID: 18-2798-0256	Code: 6CF4C3E0	Client: ALS
Sample Date: 25 Feb-14 09:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 39h (2.8 °C)	Station: L1426336-5(X14)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1385258	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	14.76	0.2584	28.51	6.774	3.772	387
IC10	17.4	0.5836	30.01	5.746	3.332	171.3
IC15	20.49	0.9929	34.2	4.881	2.924	100.7
IC20	24.09	1.508	44.39	4.152	2.253	66.32
IC25	30.35	7.799	52	3.295	1.923	12.82
IC40	53.29	20.53	60.79	1.876	1.645	4.87
IC50	60.53	37.09	67.63	1.652	1.479	2.696

Reproduction Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	26	11	38	3.596	11.37	43.74%	0.0%
1.56		10	26	11	38	3.596	11.37	43.74%	0.0%
3.13		10	26	11	38	3.596	11.37	43.74%	0.0%
6.25		10	26	11	38	3.596	11.37	43.74%	0.0%
12.5		10	26	11	38	3.596	11.37	43.74%	0.0%
25		10	20.5	2	36	3.257	10.3	50.24%	21.15%
50		10	16.9	2	29	2.783	8.8	52.07%	35.0%
100		10	2.7	0	8	0.9667	3.057	113.2%	89.62%

Reproduction 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	Negative Control	32	38	14	35	12	11	36	32	15	35
1.56		32	38	14	35	12	11	36	32	15	35
3.13		32	38	14	35	12	11	36	32	15	35
6.25		32	38	14	35	12	11	36	32	15	35
12.5		32	38	14	35	12	11	36	32	15	35
25		25	27	2	30	8	36	15	25	20	17
50		6	14	16	2	17	23	27	29	23	12
100		5	0	8	1	7	0	0	0	3	3

CETIS Analytical Report

Report Date: 16 Apr-14 09:08 (p 2 of 2)
Test Code: 04-6579-5047/14 | 16-7175-0758

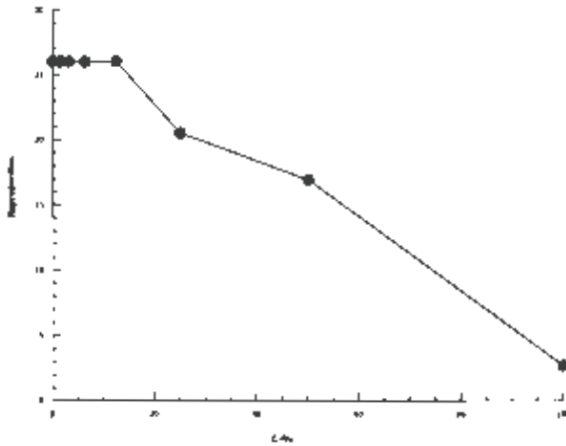
Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 10-4675-8167 Endpoint: Reproduction
Analyzed: 16 Apr-14 9:08 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 18 Mar-14 16:10 (p 1 of 2)
 Test Code: 04-6579-5047/1402154

Ceriodaphnia 7-d Survival and Reproduction Test			IRC Integrated Research Consultants		
Analysis No: 12-9097-9978	Endpoint: Reproduction	CETIS Version: CETISv1.6.6			
Analyzed: 18 Mar-14 16:09	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes			
Test Run No: 08-0175-3567	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko			
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water			
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable			
Duration: 6d 0h	Source: In-House Culture	Age:			
Sample No: 02-1487-4161	Code: 214874161	Client: Nautilus			
Sample Date: 18 Mar-14 16:05	Material: Unknown	Project: Special Studies			
Receive Date: 18 Mar-14 16:05	Source: X14				
Sample Age: N/A	Station:				

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	50	100	70.71	2	31.84%

Steel Many-One Rank Test

Control	vs	Conc-%	Test Stat	Critical	Ties	P-Value	Decision(5%)
Dilution Water		1.56	108	74	1	0.9234	Non-Significant Effect
		3.13	116	74	4	0.9851	Non-Significant Effect
		6.25	119	74	4	0.9929	Non-Significant Effect
		12.5	123.5	74	2	0.9980	Non-Significant Effect
		25	91	74	2	0.4477	Non-Significant Effect
		50	83	74	2	0.1973	Non-Significant Effect
		100*	55	74	0	0.0005	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	7988.75	1141.25	7	18.97	0.0000	Significant Effect
Error	4332	60.16667	72			
Total	12320.75	1201.41666793823	79			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Bartlett Equality of Variance	29.93	18.48	0.0001	Unequal Variances
Distribution	Shapiro-Wilk Normality	0.9568		0.0086	Non-normal Distribution

Reproduction Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	10	26	21.67	30.33	11	38	2.112	11.37	43.74%	0.0%
1.56		10	31.2	29.2	33.2	22	41	0.974	5.245	16.81%	-20.0%
3.13		10	32.4	30.6	34.2	22	38	0.8806	4.742	14.64%	-24.62%
6.25		10	30.7	26.89	34.51	12	39	1.861	10.02	32.65%	-18.08%
12.5		10	34.6	33.48	35.72	30	40	0.5481	2.951	8.53%	-33.08%
25		10	20.5	16.58	24.42	2	36	1.912	10.3	50.24%	21.15%
50		10	16.9	13.55	20.25	2	29	1.634	8.8	52.07%	35.0%
100		10	2.7	1.537	3.863	0	8	0.5676	3.057	113.2%	89.62%

CETIS Analytical Report

Report Date: 18 Mar-14 16:10 (p 2 of 2)
 Test Code: 04-6579-5047/1402154

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 12-9097-9978

Endpoint: Reproduction

CETIS Version: CETISv1.6.6

Analyzed: 18 Mar-14 16:09

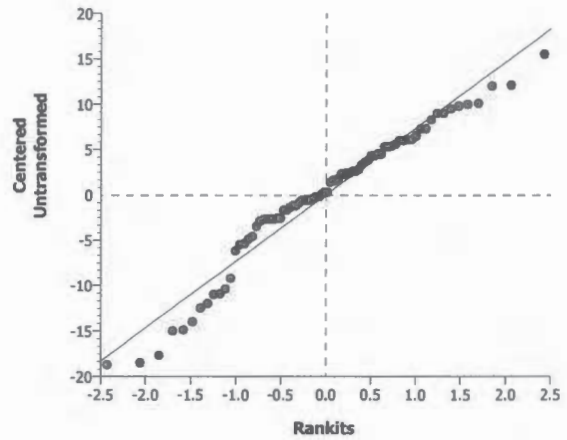
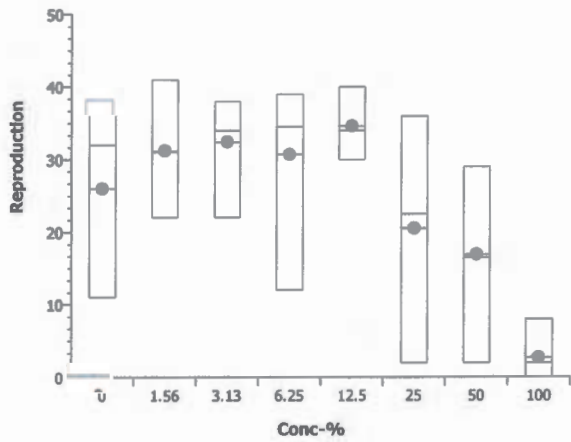
Analysis: Nonparametric-Control vs Treatments

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	32	38	14	35	12	11	36	32	15	35
1.56		31	22	34	25	31	35	41	33	30	30
3.13		34	31	38	32	27	34	22	35	35	36
6.25		35	38	33	38	29	39	12	36	13	34
12.5		30	36	32	37	34	37	34	32	40	34
25		25	27	2	30	8	36	15	25	20	17
50		6	14	16	2	17	23	27	29	23	12
100		5	0	8	1	7	0	0	0	3	3

Graphics



***Ceriodaphnia dubia* Bioassay Calculation Printouts**
7 Day Chronic *Ceriodaphnia* LC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:10 (p 1 of 2)
Test Code: 04-6579-5047/1402154

Ceriodaphnia 7-d Survival and Reproduction Test			IRC Integrated Research Consultants		
Analysis No: 19-9176-3377	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.6.6			
Analyzed: 18 Mar-14 16:08	Analysis: Linear Regression (MLE)	Official Results: Yes			
Test Run No: 08-0175-3567	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko			
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water			
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable			
Duration: 6d 0h	Source: In-House Culture	Age:			
Sample No: 02-1487-4161	Code: 214874161	Client: Nautilus			
Sample Date: 18 Mar-14 16:05	Material: Unknown	Project: Special Studies			
Receive Date: 18 Mar-14 16:05	Source: X14				
Sample Age: N/A	Station:				

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0	Yes	Yes	No	Yes

Regression Summary

Iters	LL	AICc	Mu	Sigma	G Stat	Chi-Sq	Critical	P-Value	Decision(5%)
6	-13.07	33.14	2.085	1.007	1.239	7.853	11.07	0.1645	Non-Significant Heterogeneity

Point Estimates

Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC10	45.79	N/A	N/A	2.184	N/A	N/A
EC15	80.85	N/A	N/A	1.237	N/A	N/A
EC20	127	N/A	N/A	0.7872	N/A	N/A
EC25	187.2	N/A	N/A	0.5342	N/A	N/A
EC40	497.2	N/A	N/A	0.2011	N/A	N/A
EC50	894.7	N/A	N/A	0.1118	N/A	N/A

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(5%)
Slope	0.9927	0.5638	-0.1123	2.098	1.761	0.1386	Non-Significant Parameter
Intercept	2.07	0.9006	0.3046	3.835	2.298	0.0699	Non-Significant Parameter

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(5%)
Extreme Value	Grubbs Extreme Value	1.838	2.02	0.1895	No Outliers Detected
Distribution	Shapiro-Wilk Normality	0.8731		0.1977	Normal Distribution

6d Survival Rate Summary

Conc-%	Control Type	Count	Calculated Variate(A/B)								
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%	A	B
0	Dilution Water	10	1	1	1	0	0	0.0%	0.0%	10	10
1.56		10	1	1	1	0	0	0.0%	0.0%	10	10
3.13		10	1	1	1	0	0	0.0%	0.0%	10	10
6.25		10	0.9	0	1	0.05774	0.3162	35.14%	10.0%	9	10
12.5		10	1	1	1	0	0	0.0%	0.0%	10	10
25		10	1	1	1	0	0	0.0%	0.0%	10	10
50		10	1	1	1	0	0	0.0%	0.0%	10	10
100		10	0.7	0	1	0.08819	0.483	69.01%	30.0%	7	10

CETIS Analytical Report

Report Date: 18 Mar-14 16:10 (p 2 of 2)
 Test Code: 04-6579-5047/1402154

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 19-9176-3377
 Analyzed: 18 Mar-14 16:08

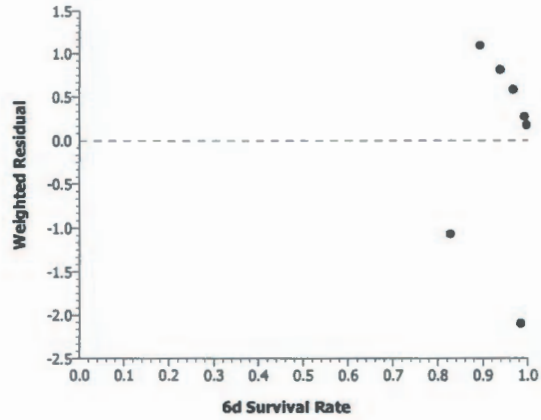
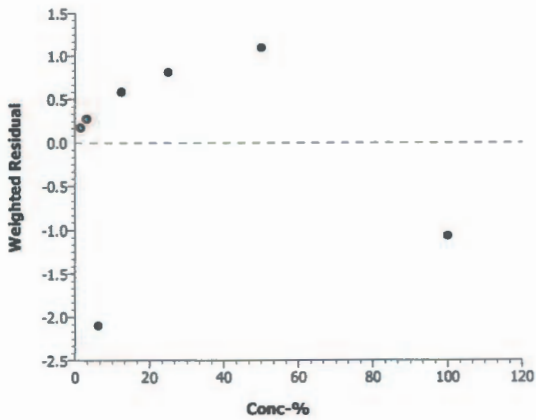
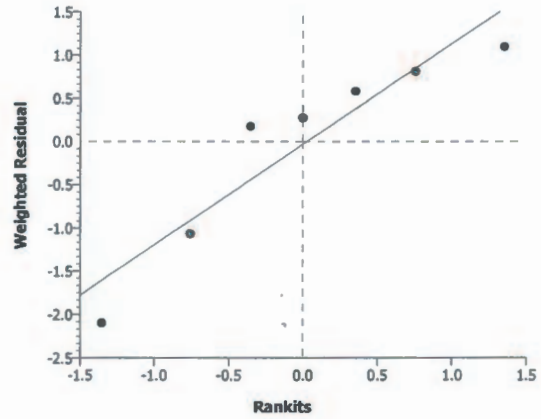
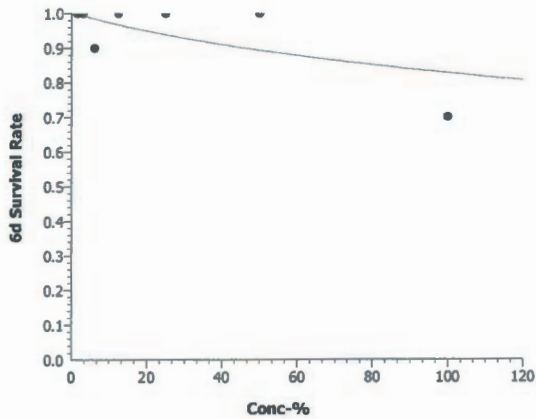
Endpoint: 6d Survival Rate
 Analysis: Linear Regression (MLE)

CETIS Version: CETISv1.6.6
 Official Results: Yes

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
1.56		1	1	1	1	1	1	1	1	1	1
3.13		1	1	1	1	1	1	1	1	1	1
6.25		1	1	1	1	1	1	0	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		1	1	1	1	1	1	1	1	1	1
100		1	0	1	1	1	1	0	0	1	1

Graphics



CETIS Analytical Report

Report Date: 18 Mar-14 16:09 (p 1 of 2)
Test Code: 04-6579-5047/1402154

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 18-5844-3650	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.6.6
Analyzed: 18 Mar-14 16:08	Analysis: STP 2x2 Contingency Tables	Official Results: Yes
Test Run No: 08-0175-3567	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 0h	Source: In-House Culture	Age:
Sample No: 02-1487-4161	Code: 214874161	Client: Nautilus
Sample Date: 18 Mar-14 16:05	Material: Unknown	Project: Special Studies
Receive Date: 18 Mar-14 16:05	Source: X14	
Sample Age: N/A	Station:	

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

Fisher Exact/Bonferroni-Holm Test

Control	vs	Conc-%	Test Stat	P-Value	Decision(0.05)
Dilution Water		1.56	1	1	Non-Significant Effect
		3.13	1	1	Non-Significant Effect
		6.25	0.5	1	Non-Significant Effect
		12.5	1	1	Non-Significant Effect
		25	1	1	Non-Significant Effect
		50	1	1	Non-Significant Effect
		100	0.1053	0.7368	Non-Significant Effect

Data Summary

Conc-%	Control Type	No-Resp	Resp	Total
0	Dilution Water	10	0	10
1.56		10	0	10
3.13		10	0	10
6.25		9	1	10
12.5		10	0	10
25		10	0	10
50		10	0	10
100		7	3	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
1.56		1	1	1	1	1	1	1	1	1	1
3.13		1	1	1	1	1	1	1	1	1	1
6.25		1	1	1	1	1	1	0	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		1	1	1	1	1	1	1	1	1	1
100		1	0	1	1	1	1	0	0	1	1

CETIS Analytical Report

Report Date: 18 Mar-14 16:09 (p 2 of 2)
Test Code: 04-6579-5047/1402154

Ceriodaphnia 7-d Survival and Reproduction Test

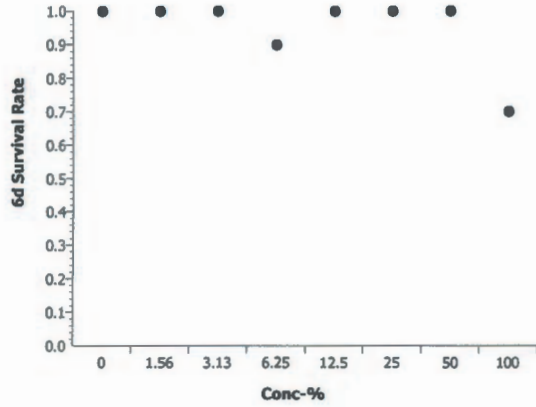
IRC Integrated Research Consultants

Analysis No: 18-5844-3650
Analyzed: 18 Mar-14 16:08

Endpoint: 6d Survival Rate
Analysis: STP 2x2 Contingency Tables

CETIS Version: CETISv1.6.6
Official Results: Yes

Graphics



Ceriodaphnia dubia Bioassay Calculation Printouts
Reftox IC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:28 (p 1 of 2)
 Test Code: 12-0152-3261

Ceriodaphnia 7-d Survival and Reproduction Test			IRC Integrated Research Consultants		
Analysis No: 09-4477-0338	Endpoint: Reproduction	CETIS Version: CETISv1.6.6	Analyzed: 05 Mar-14 10:15	Analysis: Nonlinear Regression	Official Results: Yes
Test Run No: 20-2489-0876	Test Type: Reproduction-Survival (7d)	Analyst:	Start Date: 19 Feb-14 13:27	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 25 Feb-14 14:55	Species: Ceriodaphnia dubia	Brine: Not Applicable	Duration: 6d 1h	Source: In-House Culture	Age:
Sample No: 13-0089-8086	Code: 1300898086	Client: Internal Lab	Sample Date: 27 Feb-14 11:50	Material: Sodium chloride	Project: Special Studies
Receive Date: 27 Feb-14 11:50	Source: Reference Toxicant		Sample Age: N/A	Station:	

Non-Linear Regression Options				
Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1-Phi(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary								
Iters	Log LL	AICc	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(5%)
6	-87.09	180.9	0.6237	Yes	0.4101	4.113	0.5260	Non-Significant Lack of Fit

Point Estimates			
Level	Conc-gm/	95% LCL	95% UCL
IC10	0.8703	N/A	1.124
IC15	0.9536	N/A	1.215
IC20	1.026	N/A	1.285
IC25	1.091	0.7556	1.342
IC40	1.277	1.089	1.474
IC50	1.404	1.25	1.576

Regression Parameters							
Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(5%)
A	29.8	1.253	27.26	32.34	23.78	0.0000	Significant Parameter
C	0.3731	0.1463	0.07675	0.6695	2.551	0.0150	Significant Parameter
D	1.404	0.08842	1.225	1.583	15.88	0.0000	Significant Parameter

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Lack of Fit	12.89863	12.89863	1	0.4101	0.5260	Non-Significant
Model	2063.101	1031.551	2	33.33	0.0000	Significant
Pure Error	1132.4	31.45555	36			
Residual	1145.299	30.95402	37			

Residual Analysis					
Attribute	Method	Test Stat	Critical	P-Value	Decision(5%)
Variances	Bartlett Equality of Variance	3.027	7.815	0.3876	Equal Variances
	Mod Levene Equality of Variance	0.1617	2.866	0.9214	Equal Variances
Distribution	Shapiro-Wilk Normality	0.9514		0.0844	Normal Distribution

Reproduction Summary			Calculated Variate						
Conc-gm/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	10	29	14	35	1.171	6.412	22.11%	0.0%
0.375		10	30.6	18	37	1.202	6.586	21.52%	-5.52%
0.75		10	28.4	22	33	0.6896	3.777	13.3%	2.07%
1.5		10	12.8	7	23	0.9499	5.203	40.65%	55.86%

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 09-4477-0338
 Analyzed: 05 Mar-14 10:15

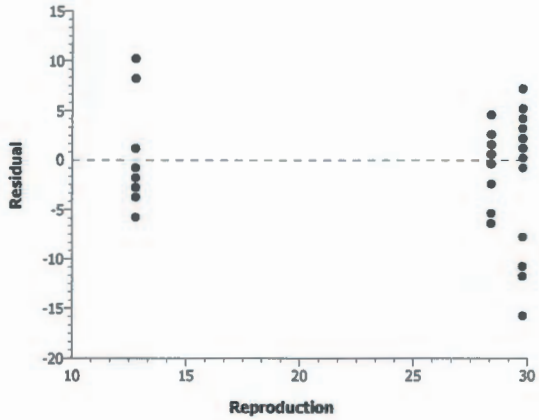
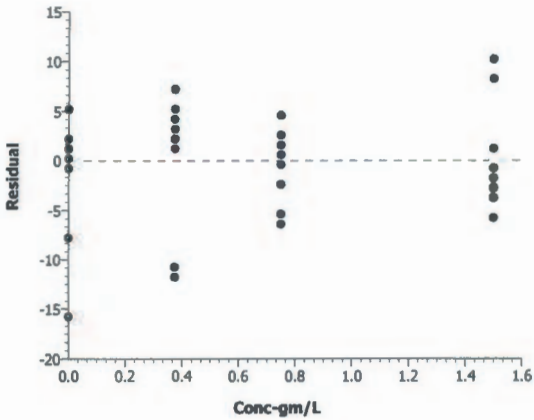
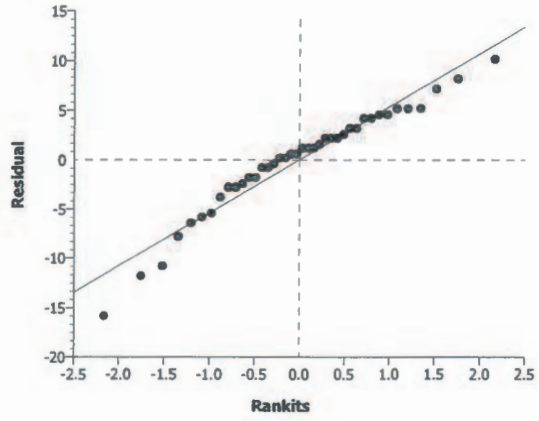
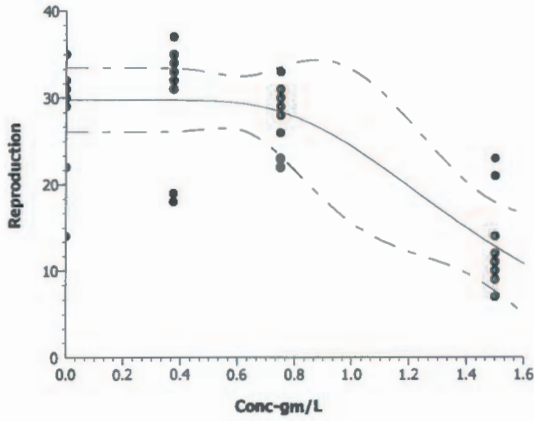
Endpoint: Reproduction
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.6.6
 Official Results: Yes

Reproduction Detail

Conc-gm/L	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	32	14	32	22	30	35	31	29	35	30
0.375		37	31	32	33	34	34	19	35	33	18
0.75		33	28	29	31	30	33	22	29	23	26
1.5		9	10	11	10	7	11	23	12	21	14

Graphics



Ceriodaphnia dubia Bioassay Calculation Printouts
Reftox LC₅₀

CERIODAPHNIA DUBIA TOXICITY TESTING
February 2014

Prepared for:

NAUTILUS ENVIRONMENTAL
8664 Commerce Court
Burnaby, BC
V5A 4N7

Prepared by:

IRC INTEGRATED RESOURCE CONSULTANTS INC.
160 - 14480 River Road
Richmond, B.C.
V6V 1L4
Tel: 604-278-7714
Fax: 604-278-7741

22 March 2014

Nautilus Environmental
8664 Commerce Court
Burnaby, BC
V5A 4N7

Attention: Krysta Pearcy

Reference: *Ceriodaphnia dubia* bioassay on the X3A sample received on 27 February 2014.

Dear Ms. Pearcy,

Enclosed please find the final report for the *Ceriodaphnia dubia* toxicity testing results, for Nautilus Environmental X3A sample dated 25 February 2014. This report includes *Ceriodaphnia dubia* test reproduction and survival data as well as daily water quality readings and reference toxicant results.

The result of the *Ceriodaphnia dubia* bioassay indicated that a concentration that would cause 50% mortality (LC50) was 46.65% with 95% confidence interval between 37.64% and 57.83%. The survival No Observed Effect Level (NOEL) was 25% and the Lowest Observed Effect Level (LOEL) was 50%. The concentration that would cause a 50% inhibition in reproduction (IC50) in the culture tested was 32.91% with a 95% confidence interval between 27.14% and 35.41%, while the 25% inhibition value (IC25) was 26.65% with a 95% confidence interval between 9.852% and 29.77%. The reproduction No Observed Effect Level (NOEL) was 25% and the Lowest Observed Effect Level (LOEL) was greater than 25%.

Should you have any questions regarding these results, please do not hesitate to call me at 604-278-7714.

Sincerely,



Ditty Chacko Kakkassery
Laboratory Biologist
IRC Integrated Resource Consultants Inc.
Encl.

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1.0 CERIODAPHNIA DUBIA BIOASSAY METHOD AND RESULTS

1.1 SAMPLE DESCRIPTION

IRC Sample ID No.:	1402155
Sample Name:	X3A
Effluent type:	Effluent
Date collected:	25 February 2014
Date, time received:	27 February 2014; 1425 hrs
Collection Method:	Grab
Amount, Container:	7 x 1 L glass containers
Date, time test initiated:	27 February 2014; 1640 hrs.
Date, time test completed:	05 March 2014; 1115 hrs.
Physical description:	Translucent slightly yellow liquid

1.2 METHOD

The method used for this test was as per the IRC laboratory "Standard Operating Procedure for *Ceriodaphnia dubia* Testing and Culturing" CDver3. This procedure follows the "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*" Report EPS 1/RMS/21 Second Edition - February 2007. The NOEL and LOEL were calculated by Fisher Exact/Bonferroni-Holm method, IC50 and IC25 using Linear Interpolation method and LC50 by Spearman-Kärber or Linear Interpolation method with the CETIS, ver 1.6.6E (2008) software.

Ceriodaphnia dubia are cultured on site from an original culture obtained from Carolina Biological Supply Ltd. Organisms are maintained in 1 L mass cultures and in a series of 23 mL glass test tubes containing a single *Ceriodaphnid* from which test organisms are obtained. Culture, control and test dilution water was a mixture of 80% distilled water and 20% Perrier.

Tests were conducted in 23mL glass test tubes containing 20mL of test solution, at a depth of 12 cm. Ten replicates of each concentration (100%, 50%, 25%, 12.5%, 6.25%, 3.13% and 1.56%) and control were tested. Test temperature was maintained throughout the test period at 25 \pm 1 $^{\circ}$ C, with a photoperiod of 16 hours light and 8 hours dark.

Initiation of the bioassay was carried out by placing a single neonate *Ceriodaphnid* of less than 24 hours, into each test vessel. New test solutions were prepared daily into which organisms were transferred. Daily measurements of the mortality and number of young produced in each replicate were recorded. Records were also maintained for daily readings of dissolved oxygen, pH, temperature and conductivity for each test concentration and control solution. The test was completed when \geq 60% of control organisms had 3 broods.

On the day of test initiation, adult *Ceriodaphnia* were placed in test tubes at 0600 hours; young used in testing were pulled directly from these test tubes at 1600 hours, ranging in age from 0 to 1000 hours. There was no unusual appearance or behaviour noted in the test organisms prior to their use in the test. No ephippia were observed in brood cultures and mass cultures in the seven day period preceding the test.

Sample used for testing was collected on 25 February 2014. Sample containers were marked with the sample ID: 7 Day Chronic *Ceriodaphnia*. The sample arrival temperature was 9.8 $^{\circ}$ C; nothing unusual was noted regarding the sample appearance. Sample in the 7 x 1 litre glass jugs received were stored in the dark at 4 \pm 1 $^{\circ}$ C until used for testing. The required volume of sample was poured out into a labeled beaker on each day of testing. The sample was not pH adjusted or filtered prior to being used in testing. The test was complete at day 6 as \geq 60% of control organisms had produced 3 broods at this time.

1.3 RESULTS

	Results	95% Confidence Interval
<i>Ceriodaphnia dubia</i> LC ₅₀	46.65%	37.64% - 57.83%
NOEL (Survival)	25%	-
LOEL (Survival)	50%	-
<i>Ceriodaphnia dubia</i> IC ₂₅	26.65%	9.852% - 29.77%
<i>Ceriodaphnia dubia</i> IC ₅₀	32.91%	27.14% - 35.41%
NOEL (Reproduction)	50%	-
LOEL (Reproduction)	> 25%	-

LC₅₀= Concentration which would cause a 50% mortality

IC₂₅= Concentration which would cause a 25% inhibition in reproduction or growth.

IC₅₀= Concentration which would cause a 50% inhibition in reproduction or growth.

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

1.4 WATER QUALITY READINGS AND TEST DATA

Test set up technician was DB. Daily reading technicians were DB, DC, CW and MH. The initial dissolved oxygen level of the sample was 11.7 mg/L at 12.0°C, the initial conductivity was 327 µS/cm and the initial pH was 7.4. The sample was not pH adjusted or filtered prior to testing. For daily water quality readings, please see appendices.

Daily Initial Readings of Undiluted sample (after warming)

	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (µS/cm)	PRE-AERATION*
DAY 0	11.6	25.0	7.4	330	20 minutes
DAY 1	11.0	25.0	7.4	329	20 minutes
DAY 2	11.7	25.0	7.4	334	20 minutes
DAY 3	11.1	24.0	7.5	332	20 minutes
DAY 4	11.2	24.5	7.5	331	20 minutes
DAY 5	11.5	24.5	7.5	331	20 minutes

*Pre-aeration of the sample is carried out if the dissolved oxygen level is either less than 40% saturation or greater than 100% saturation. Pre-aeration is for a maximum of 20 minutes.

Daily 0 Hour Refresh Solutions

Concentration	Dissolved Oxygen Range (mg/L)	pH Range	Conductivity Range (µS/cm)	Hardness (mg/L)
100%	9.6-10.1	7.5-7.6	324-335	164-190
50%	8.7-9.0	7.6-7.7	244-253	
25%	8.4-8.6	7.6-7.9	205-213	
12.5%	8.2-8.4	7.7-7.9	187-193	
6.25%	8.1-8.4	7.7-8.0	176-183	
3.13%	8.0-8.3	7.7-8.0	170-178	
1.56%	8.0-8.4	7.7-8.0	167-176	
Control	8.0-8.4	7.8-8.0	164-174	74-86

Daily 24 Hour Old Solutions

Concentration	Dissolved Oxygen Range (mg/L)	pH Range	Conductivity Range (µS/cm)
100%	7.4	7.9	327
50%	7.1-7.6	7.8-8.0	249-255
25%	6.7-7.3	7.7-7.9	209-216
12.5%	6.6-7.2	7.6-7.9	190-196
6.25%	6.5-7.3	7.6-7.9	179-186
3.13%	6.5-7.4	7.6-7.9	174-181
1.56%	6.7-7.6	7.6-7.9	170-178
Control	6.7-7.3	7.6-7.8	170-176

REPRODUCTION AND SURVIVAL RESULTS:

Summary of Total Young Produced Per *Ceriodaphnia*

Concentration	Total Young Produced per <i>Ceriodaphnid</i> in its First 3 Broods										Mean Young in First 3 Broods	Standard Deviation
	1	2	3	4	5	6	7	8	9	10		
100%	D	D	D	D	D	D	D	D	D	D	0.0	0.0
50%	D	D	0	D	0	D	D	D	0	D	0.0	0.0
25%	19	34	25	27	31	0	14	18	25	33	22.6	10.3
12.5%	13	33	27	2	36	28	X	31	22	26	24.2	12.6
6.25%	31	29	21	26	31	31	25	31	29	35	28.9	4.0
3.13%	24	33	30	28	35	32	30	28	31	34	30.5	3.3
1.56%	27	32	25	30	34	15	25	33	16	14	25.1	7.6
Control	33	37	6	28	34	13	30	18	35	16	25.0	10.8

'D' – Dead

'X' – Cerio lost due to technician error

Summary of *Ceriodaphnia* Survival

Concentration	Percent Survival					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
100%	0	0	0	0	0	0
50%	50	50	30	30	30	30
25%	100	100	100	100	100	100
12.5%	100	100	100	100	100	100
6.25%	100	100	100	100	100	100
3.13%	100	100	100	100	100	100
1.56%	100	100	100	100	100	100
Control	100	100	100	100	100	100

Percent survival in each concentration is based on a single individual in each of ten replicates.

1.5 QUALITY CONTROL

Test controls conducted concurrently with the test and reference toxicant bioassays affirmed the validity of the *Ceriodaphnia dubia* test. Testing of the reference toxicant was performed as per protocol requirements with no deviations and conditions were within testing limits for measured parameters as specified by the bioassay protocol.

The brood organisms used to supply neonates in the *Ceriodaphnia dubia* survival and reproductive bioassay maintained the requirements of mortality rates less than or equal to 20% prior to testing; with a minimum of 9 young produced in the previous brood and an average of 32.2 young produced per adult in its first 3 broods. The brood stock was challenged with a reference toxicant (reagent grade sodium chloride) within fourteen days of sample testing. The value obtained in this test was within warning limits (± 2 standard deviations) of the laboratory mean, established through repetitive testing with the reference toxicant and brood culture. Dilution water controls run concurrent with the test produced three broods per test organism in at least 60% of the control replicates with an average of greater than 15 live young per adult. Control mortalities were less than 20%.

Test Brood Stock Health Summary

	Actual	Required
Age of Neonates	0-1000 hours	≤ 24 hours
Age of brood adults	6 days	≤ 14 days
Mean % mortality in 7 days prior to testing	0%	$\leq 20\%$
Average of number of young produced per adult in its first 3 broods	32.2	≥ 15
Minimum number of young produced in previous brood	9	≥ 8
Ehippia observations	None	None

Reference Toxicant Results

Chemical Used:	Sodium Chloride
Date Tested:	19 February 2014
7 day IC ₅₀ (Log Value):	3.147 mg/L, with a 95% confidence interval between 3.097 mg/L and 3.198 mg/L
Lab Geometric Mean (Log Value):	3.079 mg/L \pm 0.189 mg/L (two standard deviations) N = 31
Warning Limits (Log Values):	2.890 mg/L to 3.268 mg/L
7 day LC50 (Log Value):	3.327 mg/L, with a 95% confidence interval between 3.231 mg/L and 3.422 mg/L
Lab Geometric Mean (Log Value):	3.253 mg/L \pm 0.166 mg/L (two standard deviations) N = 31
Warning Limits (Log Values):	3.087 mg/L to 3.419 mg/L

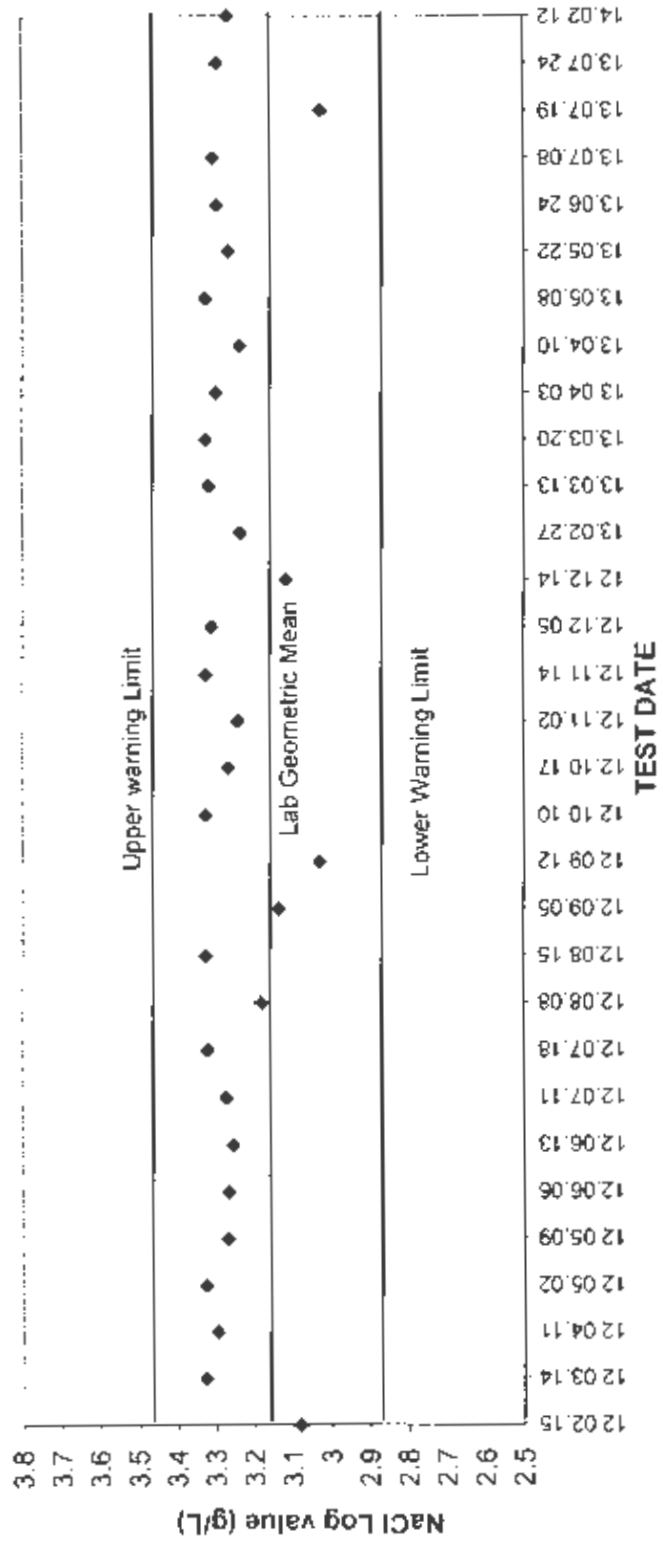
Ceriodaphnia dubia Brood Stock Health Record

CERIODAPHNIA BROOD STOCK HEALTH RECORD

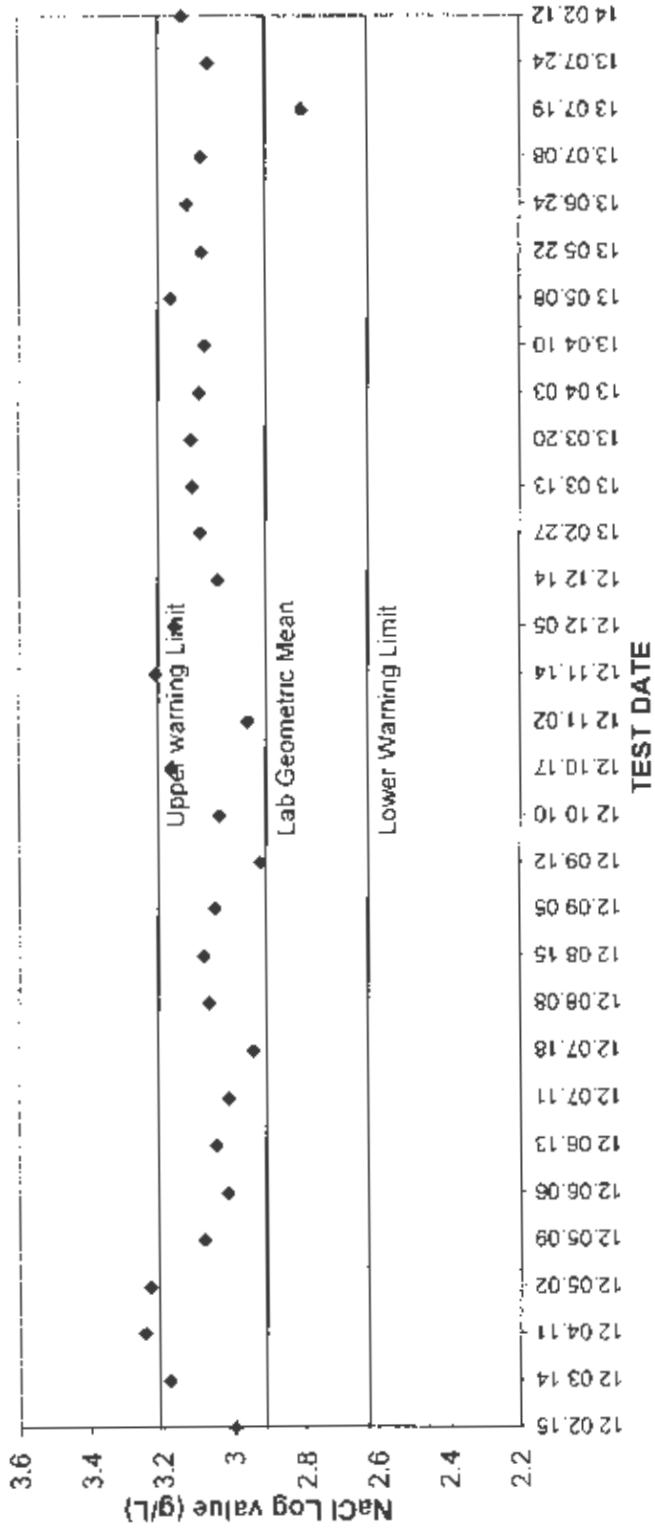
Client: Nautilus Environmental											
IRC ID#: 1402155											
Sample Name: X3A											
Sample Date: 25-Feb-14											
Date Tested: 27-Feb-14											
STOCK BIRTH DAY/DATES: 19-Feb-14											
EPHIPPIA OBSERVATIONS (yes/no): No											
Young Produced 19-Feb-14											
		Organism #									
		1	2	3	4	5	6	7	8	9	10
Date	Age (Days)										
20-Feb-14	1	0	0	0	0	0	0	0	0	0	0
21-Feb-14	2	0	0	0	0	0	0	0	0	0	0
22-Feb-14	3	3	4	4	3	3	0	4	4	5	5
23-Feb-14	4	0	0	0	0	0	6	0	0	0	0
24-Feb-14	5	9	12	12	12	12	9	12	11	8	11
25-Feb-14	6	20	17	19	21	17	0	17	9	16	20
26-Feb-14	7	0	15	0	0	0	0	0	19	0	0
27-Feb-14	8	20	22	19	17	22	19	24	0	20	20
28-Feb-14	9	20	20	21	24	23	25	25	21	27	20
1-Mar-14	10	23	0	17	27	0	21	24	19	22	20
2-Mar-14	11	0	25	0	0	26	0	0	20	0	0
Total in first 3 broods:		32	33	35	36	32	15	33	24	29	36
Average in first 3 broods:		32.2 Required: ≥15									
Minimum brood size on day of testing:		9 Required: ≥8									
"d" - dead; "X" - Tech Error											

Ceriodaphnia dubia Bioassay Reference Toxicant
Warning Charts

CERIODAPHNIA REFERENCE TOXICANT LC50 WARNING CHART - LOG VALUES



CERIODAPHNIA REFERENCE TOXICANT IC50 WARNING CHART - LOG VALUES



Test Brood Count and Solution Readings

CERIODAPHNIA BROOD COUNT SUMMARY

Client:	Nautilus Environmental
IRC ID#:	1402155
Sample Name:	X3A
Sample Date:	25-Feb-14
Date Tested:	27-Feb-14

CONCENTRATION:		100%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10	
3	d	d	d	d	d	d	d	d	d	d	
4	d	d	d	d	d	d	d	d	d	d	
5	d	d	d	d	d	d	d	d	d	d	
6	d	d	d	d	d	d	d	d	d	d	
TOTAL:	0	0	0	0	0	0	0	0	0	0	

CONCENTRATION:		50%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10	
3	d	d	0	d	0	d	d	d	0	d	
4	d	d	0	d	0	d	d	d	0	d	
5	d	d	0	d	0	d	d	d	0	d	
6	d	d	0	d	0	d	d	d	0	d	
TOTAL:	0	0	0	0	0	0	0	0	0	0	

CONCENTRATION:		25%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10	
3	0	0	2	5	0	0	0	3	0	0	
4	4	5	0	0	4	0	5	0	4	5	
5	8	11	7	6	9	0	7	8	8	12	
6	7	18	16	16	18	0	2	7	13	16	
TOTAL:	19	34	25	27	31	0	14	18	25	33	

CONCENTRATION:		12.50%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10	
3	0	5	0	0	0	0	X	0	0	0	
4	4	0	0	0	4	3	X	4	0	0	
5	9	11	13	2	14	10	X	13	9	12	
6	0	17	14	0	18	15	X	14	13	14	
TOTAL:	13	33	27	2	36	28	0	31	22	26	

CONCENTRATION:		6.25%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10	
3	5	0	4	4	0	6	0	5	2	4	
4	3	0	0	0	4	0	0	1	0	0	
5	10	12	7	8	11	10	11	10	11	12	
6	13	17	10	14	16	15	14	15	16	19	
TOTAL:	31	29	21	26	31	31	25	31	29	35	

CONCENTRATION:		3.13%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10	
3	3	4	0	5	6	4	6	0	5	5	
4	0	0	0	0	0	0	0	3	0	0	
5	9	13	11	9	11	10	10	7	12	11	
6	12	16	19	14	18	18	14	18	14	18	
TOTAL:	24	33	30	28	35	32	30	28	31	34	

CONCENTRATION:		1.56%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10	
3	5	6	0	2	0	0	4	0	0	0	
4	0	0	3	0	6	4	0	5	4	3	
5	8	10	11	12	11	0	8	10	11	11	
6	14	16	11	16	17	11	13	18	1	0	
TOTAL:	27	32	25	30	34	15	25	33	16	14	

CONCENTRATION:		Control									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10	
3	0	0	0	3	6	0	0	0	6	0	
4	5	7	0	0	0	4	6	6	0	5	
5	10	12	6	11	12	9	9	12	11	11	
6	18	18	0	14	16	0	15	0	18	0	
TOTAL:	33	37	6	28	34	13	30	18	35	16	

"d" - dead; "X" - Tech Error

CONCENTRATION	100%	50%	25%	12.5%	6.25%	3.13%	1.56%	Control
BROOD COUNT MEANS	0.0	0.0	22.6	24.2	28.9	30.5	25.1	25.0
SD	0.0	0.0	10.3	12.6	4.0	3.3	7.6	10.8

Client:	Nautilus Environmental																		
IRC ID#:	1402155																		
Sample Name:	X3A																		
Sample Date:	25-Feb-14																		
Date Tested:	27-Feb-14																		
FRESH SOLUTIONS										OLD SOLUTIONS									
DISSOLVED OXYGEN										DISSOLVED OXYGEN									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	10.0	9.9	10.0	10.1	9.7	9.6		9.6	10.1	100	7.4							7.4	7.4
50	9.0	8.8	8.9	8.8	8.7	8.8		8.7	9.0	50	7.2	7.6	7.5	7.4	7.5	7.1		7.1	7.6
25	8.6	8.5	8.4	8.4	8.4	8.5		8.4	8.6	25	7.2	7.3	7.0	7.2	7.3	6.7		6.7	7.3
12.5	8.3	8.3	8.2	8.3	8.3	8.4		8.2	8.4	12.5	7.2	7.1	6.9	7.1	7.2	6.6		6.6	7.2
6.25	8.1	8.2	8.1	8.2	8.1	8.4		8.1	8.4	6.25	7.3	7.1	7.0	7.2	7.3	6.5		6.5	7.3
3.13	8.1	8.2	8.0	8.2	8.1	8.3		8.0	8.3	3.13	7.4	7.4	7.1	7.2	7.2	6.5		6.5	7.4
1.56	8.2	8.2	8.0	8.2	8.1	8.4		8.0	8.4	1.56	7.3	7.6	6.9	7.3	7.3	6.7		6.7	7.6
CONTROL	8.3	8.3	8.0	8.4	8.2	8.4		8.0	8.4	CONTROL	7.3	7.2	7.1	7.3	7.3	6.7		6.7	7.3
pH										pH									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	7.5	7.6	7.5	7.5	7.6	7.6		7.5	7.6	100	7.9							7.9	7.9
50	7.6	7.7	7.7	7.7	7.7	7.7		7.6	7.7	50	7.8	8.0	7.9	7.9	7.9	7.8		7.8	8.0
25	7.6	7.8	7.8	7.9	7.7	7.7		7.6	7.9	25	7.7	7.9	7.9	7.8	7.8	7.7		7.7	7.9
12.5	7.7	7.8	7.8	7.9	7.8	7.8		7.7	7.9	12.5	7.7	7.9	7.8	7.8	7.7	7.6		7.6	7.9
6.25	7.7	7.8	7.9	8.0	7.8	7.8		7.7	8.0	6.25	7.7	7.9	7.8	7.8	7.7	7.6		7.6	7.9
3.13	7.7	7.8	7.9	8.0	7.8	7.8		7.7	8.0	3.13	7.7	7.9	7.8	7.7	7.7	7.6		7.6	7.9
1.56	7.7	7.8	8.0	8.0	7.8	7.9		7.7	8.0	1.56	7.7	7.9	7.8	7.7	7.7	7.6		7.6	7.9
CONTROL	7.8	7.8	8.0	7.9	7.8	7.9		7.8	8.0	CONTROL	7.7	7.8	7.8	7.7	7.7	7.6		7.6	7.8
CONDUCTIVITY										CONDUCTIVITY									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	326	324	335	324	328	328		324	335	100	327							327	327
50	248	244	250	249	253	249		244	253	50	249	252	251	255	254	251		249	255
25	209	205	208	209	213	209		205	213	25	211	210	209	214	216	212		209	216
12.5	189	187	187	191	193	188		187	193	12.5	190	191	190	194	196	191		190	196
6.25	179	177	176	181	183	178		176	183	6.25	180	179	180	183	186	181		179	186
3.13	173	171	170	175	178	172		170	178	3.13	175	174	174	179	181	176		174	181
1.56	171	168	167	172	176	170		167	176	1.56	173	170	173	177	178	174		170	178
CONTROL	169	169	164	171	174	166		164	174	CONTROL	175	170	172	176	176	172		170	176
AFTER WARMING:																			
DAY	0	1	2	3	4	5	6	MIN	MAX										
Dissolved Oxygen	11.6	11.0	11.7	11.1	11.2	11.5		11	11.7										
Temperature	25.0	25.0	25.0	24.0	24.5	24.5		24	25										
pH	7.4	7.4	7.4	7.5	7.5	7.5		7.4	7.5										
Conductivity	330	329	334	332	331	331		329	334										
Aeration:	20 min	20 min	20 min	20 min	20 min	20 min													
HARDNESS:																			
Sample	164	164	164	190	172	166		164	190										
Dilution Water	86	74	80	76	80	76		74	86										

***Ceriodaphnia dubia* Bioassay Calculation Printouts**
7 Day Chronic *Ceriodaphnia* IC₂₅ and IC₅₀

CETIS Analytical Report

Report Date: 22 Mar-14 15:28 (p 1 of 2)
 Test Code: 06-5160-2182/1402155

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 08-2456-1812	Endpoint: Reproduction	CETIS Version: CETISv1.6.6
Analyzed: 22 Mar-14 15:27	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Test Run No: 13-5600-6431	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 0h	Source: In-House Culture	Age:
Sample No: 17-6399-0549	Code: 1763990549	Client: Nautilus
Sample Date: 18 Mar-14 16:42 ①	Material: Unknown	Project: Special Studies
Receive Date: 18 Mar-14 16:42 ②	Source: X3A	
Sample Age: N/A <i>emm</i>	Station:	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X + 1)	Linear	57951	200	Yes	Two-Point Interpolation

Point Estimates

Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	8.496	0.5744	25.89	11.77	3.862	174.1
IC10	11.44	1.479	26.81	8.742	3.73	67.63
IC15	18.84	6.793	27.76	5.307	3.602	14.72
IC20	25.55	8.46	28.75	3.914	3.478	11.82
IC25	26.65	9.852	29.77	3.752	3.359	10.15
IC40	30.26	23.08	33.04	3.305	3.026	4.332
IC50	32.91	27.14	35.41	3.038	2.824	3.685

Reproduction Summary

Calculated Variate

Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	10	25	6	37	1.98	10.84	43.37%	0.0%
1.56		10	25.1	14	34	1.394	7.637	30.43%	-0.4%
3.13		10	30.5	24	35	0.5978	3.274	10.74%	-22.0%
6.25		10	28.9	21	35	0.7224	3.957	13.69%	-15.6%
12.5		9	24.22	2	36	1.949	10.67	44.07%	3.11%
25		10	22.6	0	34	1.884	10.32	45.66%	9.6%
50		10	0	0	0	0	0		100.0%
100		10	0	0	0	0	0		100.0%

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	33	37	6	28	34	13	30	18	35	16
1.56		27	32	25	30	34	15	25	33	16	14
3.13		24	33	30	28	35	32	30	28	31	34
6.25		31	29	21	26	31	31	25	31	29	35
12.5		13	33	27	2	36	28	31	22	26	
25		19	34	25	27	31	0	14	18	25	33
50		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

① 25-Feb-14 11:35
 ② 27-Feb-14 10:30

CETIS Analytical Report

Report Date: 16 Apr-14 09:30 (p 1 of 2)
 Test Code: 06-5160-2182/14 | 01-4554-2479

Ceriodaphnia 7-d Survival and Reproduction Test

Nautillus Environmental

Analysis ID: 11-8058-7376	Endpoint: Reproduction	CETIS Version: CETISv1.8.7
Analyzed: 16 Apr-14 9:30	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 10-6454-5487	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine:
Duration: 6d 0h	Source: In-House Culture	Age:
Sample ID: 05-2872-4258	Code: 1F83B120	Client: ALS
Sample Date: 25 Feb-14 11:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 36h (3.9 °C)	Station: L1426336-6(X3A)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	20290	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	15.34	0.327	25.89	6.52	3.862	305.8
IC10	25.08	0.7608	26.81	3.988	3.73	131.4
IC15	26.07	1.337	27.76	3.836	3.602	74.82
IC20	27.1	2.885	28.75	3.691	3.478	34.67
IC25	28.16	5.439	29.77	3.551	3.359	18.39
IC40	31.61	23.67	33.04	3.163	3.026	4.224
IC50	34.13	27.33	35.41	2.93	2.824	3.659

Reproduction Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	25	6	37	3.429	10.84	43.37%	0.0%
1.56		10	25	6	37	3.429	10.84	43.37%	0.0%
3.13		10	25	6	37	3.429	10.84	43.37%	0.0%
6.25		10	25	6	37	3.429	10.84	43.37%	0.0%
12.5		9	24.22	2	36	3.558	10.67	44.07%	3.11%
25		10	22.6	0	34	3.263	10.32	45.66%	9.6%
50		10	0	0	0	0	0		100.0%
100		10	0	0	0	0	0		100.0%

Reproduction 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	Negative Control	33	37	6	28	34	13	30	18	35	16
1.56		33	37	6	28	34	13	30	18	35	16
3.13		33	37	6	28	34	13	30	18	35	16
6.25		33	37	6	28	34	13	30	18	35	16
12.5		13	33	27	2	36	28	31	22	26	
25		19	34	25	27	31	0	14	18	25	33
50		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

CETIS Analytical Report

Report Date: 16 Apr-14 09:30 (p 2 of 2)
Test Code: 06-5160-2182/14 | 01-4554-2479

Cariodaphnia 7-d Survival and Reproduction Test

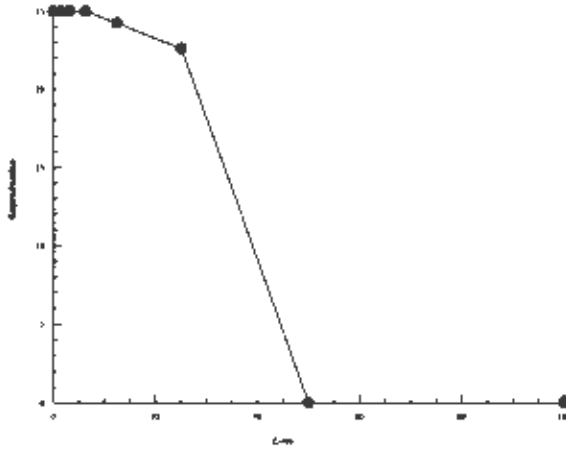
Nautilus Environmental

Analysis ID: 11-8058-7376
Analyzed: 16 Apr-14 9:30

Endpoint: Reproduction
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 18 Mar-14 16:25 (p 1 of 2)
Test Code: 06-5160-2182/1402155

Ceriodaphnia 7-d Survival and Reproduction Test			IRC Integrated Research Consultants		
Analysis No: 18-8739-9187	Endpoint: Reproduction	CETIS Version: CETISv1.6.6			
Analyzed: 18 Mar-14 16:23	Analysis: Nonparametric-Multiple Comparison	Official Results: Yes			
Test Run No: 13-5600-6431	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko			
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water			
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable			
Duration: 6d 0h	Source: In-House Culture	Age:			
Sample No: 17-6399-0549	Code: 1763990549	Client: Nautilus			
Sample Date: 18 Mar-14 16:12	Material: Unknown	Project: Special Studies			
Receive Date: 18 Mar-14 16:12	Source: X3A				
Sample Age: N/A	Station:				

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	25	>25	N/A	4	35.81%

Wilcoxon/Bonferroni Adj Test

Control	vs	Conc-%	Test Stat	Critical	Ties	P-Value	Decision(5%)
Dilution Water		1.56	99		4	1.0000	Non-Significant Effect
		3.13	113.5		5	1.0000	Non-Significant Effect
		6.25	109.5		1	1.0000	Non-Significant Effect
		12.5	84.5		3	1.0000	Non-Significant Effect
		25	96.5		3	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	448.3207	89.66415	5	1.288	0.2831	Non-Significant Effect
Error	3690.256	69.62746	53			
Total	4138.57632446289	159.291610717773	58			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Bartlett Equality of Variance	18.57	15.09	0.0023	Unequal Variances
Distribution	Shapiro-Wilk Normality	0.9359		0.0039	Non-normal Distribution

Reproduction Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	10	25	20.88	29.12	6	37	2.013	10.84	43.37%	0.0%
1.56		10	25.1	22.2	28	14	34	1.418	7.637	30.43%	-0.4%
3.13		10	30.5	29.25	31.75	24	35	0.6081	3.274	10.74%	-22.0%
6.25		10	28.9	27.39	30.41	21	35	0.7347	3.957	13.69%	-15.6%
12.5		9	24.22	20.16	28.28	2	36	1.982	10.67	44.07%	3.11%
25		10	22.6	18.67	26.53	0	34	1.916	10.32	45.66%	9.6%

CETIS Analytical Report

Report Date: 18 Mar-14 16:25 (p 2 of 2)
 Test Code: 06-5160-2182/1402155

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 18-8739-9187

Endpoint: Reproduction

CETIS Version: CETISv1.6.6

Analyzed: 18 Mar-14 16:23

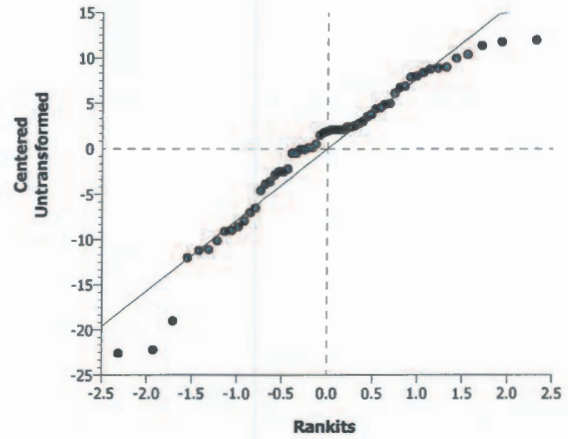
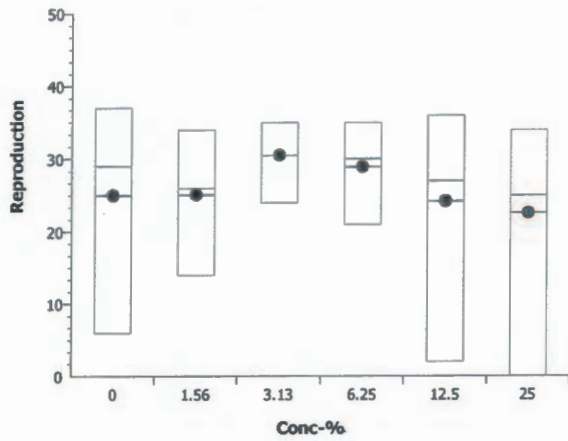
Analysis: Nonparametric-Multiple Comparison

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	33	37	6	28	34	13	30	18	35	16
1.56		27	32	25	30	34	15	25	33	16	14
3.13		24	33	30	28	35	32	30	28	31	34
6.25		31	29	21	26	31	31	25	31	29	35
12.5		13	33	27	2	36	28	31	22	26	
25		19	34	25	27	31	0	14	18	25	33

Graphics



***Ceriodaphnia dubia* Bioassay Calculation Printouts**
7 Day Chronic *Ceriodaphnia* LC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:24 (p 1 of 1)
 Test Code: 06-5160-2182/1402155

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 08-9346-4144	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.6.6
Analyzed: 18 Mar-14 16:15	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes
Test Run No: 13-5600-6431	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 0h	Source: In-House Culture	Age:
Sample No: 17-6399-0549	Code: 1763990549	Client: Nautilus
Sample Date: 18 Mar-14 16:12	Material: Unknown	Project: Special Studies
Receive Date: 18 Mar-14 16:12	Source: X3A	
Sample Age: N/A	Station:	

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC/LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.669	0.04664	46.65	37.64	57.83

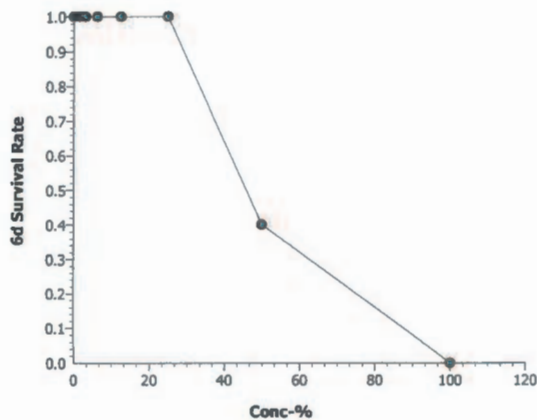
6d Survival Rate Summary

Conc-%	Control Type	Count	Calculated Variate(A/B)								
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%	A	B
0	Dilution Water	10	1	1	1	0	0	0.0%	0.0%	10	10
1.56		10	1	1	1	0	0	0.0%	0.0%	10	10
3.13		10	1	1	1	0	0	0.0%	0.0%	10	10
6.25		10	1	1	1	0	0	0.0%	0.0%	10	10
12.5		9	1	1	1	0	0	0.0%	0.0%	9	9
25		10	1	1	1	0	0	0.0%	0.0%	10	10
50		10	0.4	0	1	0.09428	0.5164	129.1%	60.0%	4	10
100		10	0	0	0	0	0		100.0%	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
1.56		1	1	1	1	1	1	1	1	1	1
3.13		1	1	1	1	1	1	1	1	1	1
6.25		1	1	1	1	1	1	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		0	0	1	0	1	0	0	0	1	1 0 emm
100		0	0	0	0	0	0	0	0	0	0

Graphics



CETIS Analytical Report

Report Date: 18 Mar-14 16:24 (p 1 of 2)
Test Code: 06-5160-2182/1402155

Ceriodaphnia 7-d Survival and Reproduction Test **IRC Integrated Research Consultants**

Analysis No: 13-8864-6600	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.6.6
Analyzed: 18 Mar-14 16:15	Analysis: STP 2x2 Contingency Tables	Official Results: Yes
Test Run No: 13-5600-6431	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 0h	Source: In-House Culture	Age:
Sample No: 17-6399-0549	Code: 1763990549	Client: Nautilus
Sample Date: 18 Mar-14 16:12	Material: Unknown	Project: Special Studies
Receive Date: 18 Mar-14 16:12	Source: X3A	
Sample Age: N/A	Station:	

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	25	50	35.36	4	N/A

Fisher Exact/Bonferroni-Holm Test

Control	vs	Conc-%	Test Stat	P-Value	Decision(0.05)
Dilution Water		1.56	1	1	Non-Significant Effect
		3.13	1	1	Non-Significant Effect
		6.25	1	1	Non-Significant Effect
		12.5	1	1	Non-Significant Effect
		25	1	1	Non-Significant Effect
		50	0.005418	0.03251	Significant Effect
		100	5.413E-06	3.789E-05	Significant Effect

Data Summary

Conc-%	Control Type	No-Resp	Resp	Total
0	Dilution Water	10	0	10
1.56		10	0	10
3.13		10	0	10
6.25		10	0	10
12.5		9	0	9
25		10	0	10
50		4	6	10
100		0	10	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
1.56		1	1	1	1	1	1	1	1	1	1
3.13		1	1	1	1	1	1	1	1	1	1
6.25		1	1	1	1	1	1	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		0	0	1	0	1	0	0	0	1	1
100		0	0	0	0	0	0	0	0	0	0

CETIS Analytical Report

Report Date: 18 Mar-14 16:24 (p 2 of 2)
Test Code: 06-5160-2182/1402155

Ceriodaphnia 7-d Survival and Reproduction Test

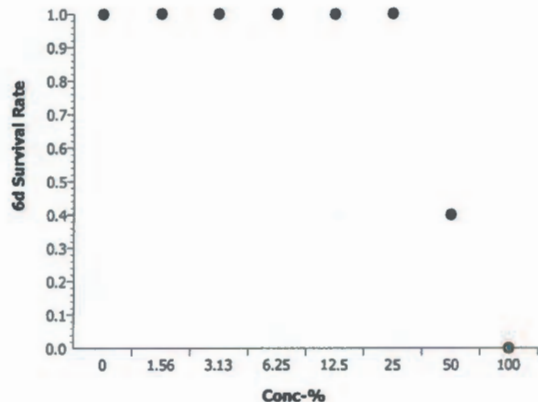
IRC Integrated Research Consultants

Analysis No: 13-8864-6600
Analyzed: 18 Mar-14 16:15

Endpoint: 6d Survival Rate
Analysis: STP 2x2 Contingency Tables

CETIS Version: CETISv1.6.6
Official Results: Yes

Graphics



Ceriodaphnia dubia Bioassay Calculation Printouts
Reflux IC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:28 (p 1 of 2)
 Test Code: 12-0152-3261

Ceriodaphnia 7-d Survival and Reproduction Test			IRC Integrated Research Consultants		
Analysis No: 09-4477-0338	Endpoint: Reproduction	CETIS Version: CETISv1.6.6			
Analyzed: 05 Mar-14 10:15	Analysis: Nonlinear Regression	Official Results: Yes			
Test Run No: 20-2489-0876	Test Type: Reproduction-Survival (7d)	Analyst:			
Start Date: 19 Feb-14 13:27	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water			
Ending Date: 25 Feb-14 14:55	Species: Ceriodaphnia dubia	Brine: Not Applicable			
Duration: 6d 1h	Source: In-House Culture	Age:			
Sample No: 13-0089-8086	Code: 1300898086	Client: Internal Lab			
Sample Date: 27 Feb-14 11:50	Material: Sodium chloride	Project: Special Studies			
Receive Date: 27 Feb-14 11:50	Source: Reference Toxicant				
Sample Age: N/A	Station:				

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(5%)
6	-87.09	180.9	0.6237	Yes	0.4101	4.113	0.5260	Non-Significant Lack of Fit

Point Estimates

Level	Conc-gm/l	95% LCL	95% UCL
IC10	0.8703	N/A	1.124
IC15	0.9536	N/A	1.215
IC20	1.026	N/A	1.285
IC25	1.091	0.7556	1.342
IC40	1.277	1.089	1.474
IC50	1.404	1.25	1.576

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(5%)
A	29.8	1.253	27.26	32.34	23.78	0.0000	Significant Parameter
C	0.3731	0.1463	0.07675	0.6695	2.551	0.0150	Significant Parameter
D	1.404	0.08842	1.225	1.583	15.88	0.0000	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Lack of Fit	12.89863	12.89863	1	0.4101	0.5260	Non-Significant
Model	2063.101	1031.551	2	33.33	0.0000	Significant
Pure Error	1132.4	31.45555	36			
Residual	1145.299	30.95402	37			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(5%)
Variances	Bartlett Equality of Variance	3.027	7.815	0.3876	Equal Variances
	Mod Levene Equality of Variance	0.1617	2.866	0.9214	Equal Variances
Distribution	Shapiro-Wilk Normality	0.9514		0.0844	Normal Distribution

Reproduction Summary

Conc-gm/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	10	29	14	35	1.171	6.412	22.11%	0.0%
0.375		10	30.6	18	37	1.202	6.586	21.52%	-5.52%
0.75		10	28.4	22	33	0.6896	3.777	13.3%	2.07%
1.5		10	12.8	7	23	0.9499	5.203	40.65%	55.86%

CETIS Analytical Report

Report Date: 18 Mar-14 16:28 (p 2 of 2)
 Test Code: 12-0152-3261

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

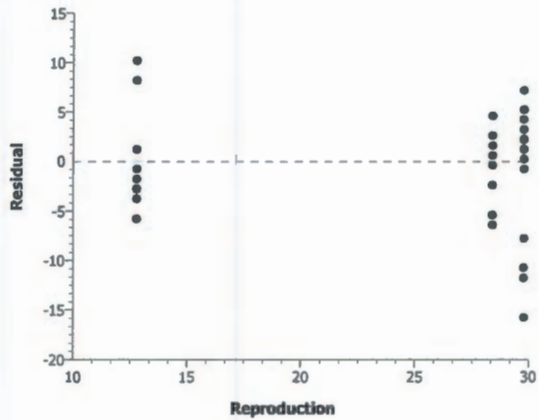
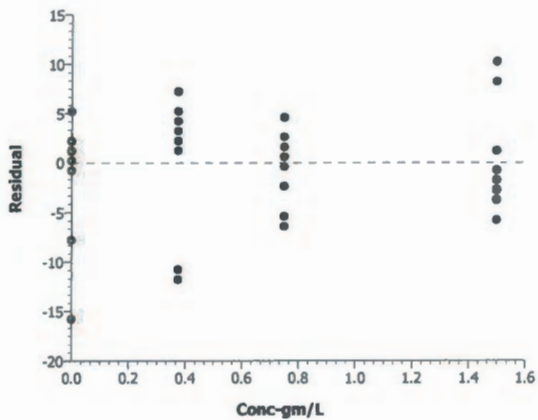
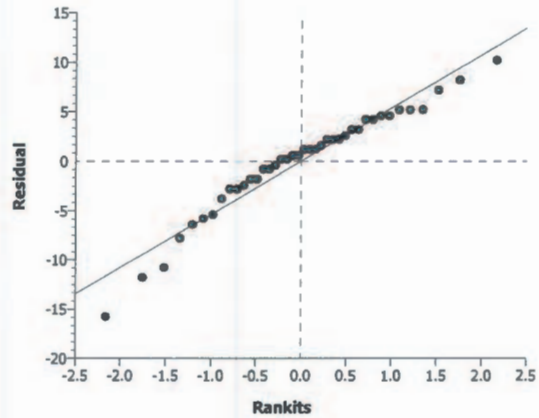
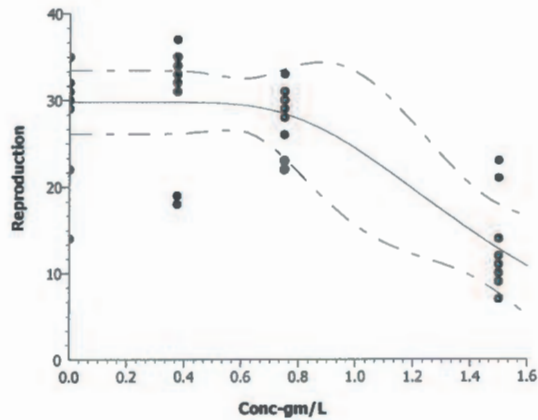
Analysis No: 09-4477-0338 Endpoint: Reproduction
 Analyzed: 05 Mar-14 10:15 Analysis: Nonlinear Regression

CETIS Version: CETISv1.6.6
 Official Results: Yes

Reproduction Detail

Conc-gm/L	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	32	14	32	22	30	35	31	29	35	30
0.375		37	31	32	33	34	34	19	35	33	18
0.75		33	28	29	31	30	33	22	29	23	26
1.5		9	10	11	10	7	11	23	12	21	14

Graphics



Ceriodaphnia dubia Bioassay Calculation Printouts
Reflux LC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:27 (p 1 of 1)
 Test Code: 12-0152-3261

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 00-3619-3653	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.6.6
Analyzed: 05 Mar-14 10:14	Analysis: Binomial Method	Official Results: Yes
Test Run No: 20-2489-0876	Test Type: Reproduction-Survival (7d)	Analyst:
Start Date: 19 Feb-14 13:27	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 25 Feb-14 14:55	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 1h	Source: In-House Culture	Age:
Sample No: 13-0089-8086	Code: 1300898086	Client: Internal Lab
Sample Date: 27 Feb-14 11:50	Material: Sodium chloride	Project: Special Studies
Receive Date: 27 Feb-14 11:50	Source: Reference Toxicant	
Sample Age: N/A	Station:	

Binomial/Graphical Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC/LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	0.3266	0	2.121	1.704	2.641

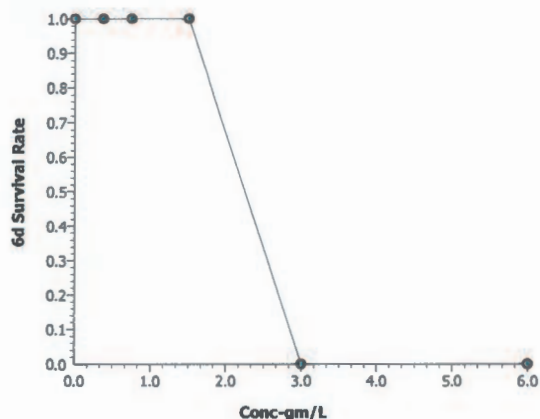
6d Survival Rate Summary

Conc-gm/L	Control Type	Count	Calculated Variate(A/B)								
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%	A	B
0	Dilution Water	7	1	1	1	0	0	0.0%	0.0%	7	7
0.375		10	1	1	1	0	0	0.0%	0.0%	10	10
0.75		10	1	1	1	0	0	0.0%	0.0%	10	10
1.5		10	1	1	1	0	0	0.0%	0.0%	10	10
3		10	0	0	0	0	0		100.0%	0	10
6		10	0	0	0	0	0		100.0%	0	10

6d Survival Rate Detail

Conc-gm/L	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			
0.375		1	1	1	1	1	1	1	1	1	1
0.75		1	1	1	1	1	1	1	1	1	1
1.5		1	1	1	1	1	1	1	1	1	1
3		0	0	0	0	0	0	0	0	0	0
6		0	0	0	0	0	0	0	0	0	0

Graphics



CERIODAPHNIA DUBIA TOXICITY TESTING
February 2014

Prepared for:

NAUTILUS ENVIRONMENTAL
8664 Commerce Court
Burnaby, BC
V5A 4N7

Prepared by:

IRC INTEGRATED RESOURCE CONSULTANTS INC.
160 - 14480 River Road
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24 March 2014

Nautilus Environmental
8664 Commerce Court
Burnaby, BC
V5A 4N7

Attention: Krysta Percy

Reference: *Ceriodaphnia dubia* bioassay on the R3 sample received on 27 February 2014.

Dear Ms. Percy,

Enclosed please find the final report for the *Ceriodaphnia dubia* toxicity testing results, for Nautilus Environmental R3 sample dated 25 February 2014. This report includes *Ceriodaphnia dubia* test reproduction and survival data as well as daily water quality readings and reference toxicant results.

The result of the *Ceriodaphnia dubia* bioassay indicated that a concentration that would cause 50% mortality (LC50) was greater than 100%. The survival No Observed Effect Level (NOEL) was 100% and the Lowest Observed Effect Level (LOEL) was greater than 100%. The concentration that would cause a 50% inhibition in reproduction (IC50) in the culture tested was 97.39% with a 95% confidence interval between 71.93% and 131.9%, while the 25% inhibition value (IC25) was 70.72% with a 95% confidence interval between N/A and 107.5%. The reproduction No Observed Effect Level (NOEL) was 50% and the Lowest Observed Effect Level (LOEL) was 100%.

Should you have any questions regarding these results, please do not hesitate to call me at 604-278-7714.

Sincerely,

Dity Chacko Kakkassery
Laboratory Biologist
IRC Integrated Resource Consultants Inc.
Encl.

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1.0 CERIODAPHNIA DUBIA BIOASSAY METHOD AND RESULTS

1.1 SAMPLE DESCRIPTION

IRC Sample ID No.:	1402156
Sample Name:	R3
Effluent type:	Effluent
Date collected:	25 February 2014; 1330 hrs
Date, time received:	27 February 2014; 1530 hrs
Collection Method:	Grab
Amount, Container:	3 x 2L & 1 x 1L plastic containers
Date, time test initiated:	27 February 2014; 1650 hrs.
Date, time test completed:	05 March 2014; 1055 hrs.
Physical description:	Translucent slightly yellow liquid

1.2 METHOD

The method used for this test was as per the IRC laboratory "Standard Operating Procedure for *Ceriodaphnia dubia* Testing and Culturing" CDver3. This procedure follows the "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*" Report EPS 1/RMS/21 Second Edition - February 2007. The NOEL and LOEL were calculated by Fisher Exact/Bonferroni-Holm method, IC50 and IC25 using Linear Interpolation method and LC50 by Spearman-Kärber or Linear Interpolation method with the CETIS, ver 1.6.6E (2008) software.

Ceriodaphnia dubia are cultured on site from an original culture obtained from Carolina Biological Supply Ltd. Organisms are maintained in 1 L mass cultures and in a series of 23 mL glass test tubes containing a single *Ceriodaphnid* from which test organisms are obtained. Culture, control and test dilution water was a mixture of 80% distilled water and 20% Perrier.

Tests were conducted in 23mL glass test tubes containing 20mL of test solution, at a depth of 12 cm. Ten replicates of each concentration (100%, 50%, 25%, 12.5%, 6.25%, 3.13% and 1.56%) and control were tested. Test temperature was maintained throughout the test period at 25± 1°C, with a photoperiod of 16 hours light and 8 hours dark.

Initiation of the bioassay was carried out by placing a single neonate *Ceriodaphnid* of less than 24 hours, into each test vessel. New test solutions were prepared daily into which organisms were transferred. Daily measurements of the mortality and number of young produced in each replicate were recorded. Records were also maintained for daily readings of dissolved oxygen, pH, temperature and conductivity for each test concentration and control solution. The test was completed when ≥ 60% of control organisms had 3 broods.

On the day of test initiation, adult *Ceriodaphnia* were placed in test tubes at 0600 hours; young used in testing were pulled directly from these test tubes at 1600 hours, ranging in age from 0 to 1000 hours. There was no unusual appearance or behaviour noted in the test organisms prior to their use in the test. No ephippia were observed in brood cultures and mass cultures in the seven day period preceding the test.

Sample used for testing was collected on 25 February 2014. Sample containers were marked with the sample ID: 7 Day Chronic *Ceriodaphnia*. The sample arrival temperature was 9.3°C; nothing unusual was noted regarding the sample appearance. Sample in the 3 x 2 litre and 1 x 1 litre plastic jugs received were stored in the dark at 4±1°C until used for testing. The required volume of sample was poured out into a labeled beaker on each day of testing. The sample was not pH adjusted or filtered prior to being used in testing. The test was complete at day 6 as ≥60% of control organisms had produced 3 broods at this time.

1.3 RESULTS

	Results	95% Confidence Interval
<i>Ceriodaphnia dubia</i> LC ₅₀	> 100%	-
NOEL (Survival)	100%	-
LOEL (Survival)	> 100%	-
<i>Ceriodaphnia dubia</i> IC ₂₅	70.72%	N/A – 101.5%
<i>Ceriodaphnia dubia</i> IC ₅₀	97.39%	71.93% - 131.9%
NOEL (Reproduction)	50%	-
LOEL (Reproduction)	100%	-

LC₅₀= Concentration which would cause a 50% mortality

IC₂₅= Concentration which would cause a 25% inhibition in reproduction or growth.

IC₅₀= Concentration which would cause a 50% inhibition in reproduction or growth.

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

1.4 WATER QUALITY READINGS AND TEST DATA

Test set up technician was DB. Daily reading technicians were DB, CW and MH. The initial dissolved oxygen level of the sample was 11.7 mg/L at 9.5°C, the initial conductivity was 635 µS/cm and the initial pH was 7.5. The sample was not pH adjusted or filtered prior to testing. For daily water quality readings, please see appendices.

Daily Initial Readings of Undiluted sample (after warming)

	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (µS/cm)	PRE-AERATION*
DAY 0	11.4	24.0	7.5	638	20 minutes
DAY 1	11.0	25.5	7.5	618	20 minutes
DAY 2	11.2	24.5	7.6	641	20 minutes
DAY 3	11.2	24.5	7.5	639	20 minutes
DAY 4	11.0	26.0	7.6	638	20 minutes
DAY 5	11.6	24.0	7.5	639	20 minutes

*Pre-aeration of the sample is carried out if the dissolved oxygen level is either less than 40% saturation or greater than 100% saturation. Pre-aeration is for a maximum of 20 minutes.

Daily 0 Hour Refresh Solutions

Concentration	Dissolved Oxygen Range (mg/L)	pH Range	Conductivity Range (µS/cm)	Hardness (mg/L)
100%	9.2-10.1	7.6-7.7	624-639	330-362
50%	8.6-9.0	7.7-7.8	414-419	
25%	8.2-8.6	7.7-7.9	296-301	
12.5%	8.1-8.6	7.7-7.9	229-238	
6.25%	8.1-8.5	7.8-8.0	198-206	
3.13%	8.1-8.5	7.8-8.0	181-190	
1.56%	8.1-8.4	7.8-8.0	172-183	
Control	8.1-8.5	7.8-8.0	165-176	74-86

Daily 24 Hour Old Solutions

Concentration	Dissolved Oxygen Range (mg/L)	pH Range	Conductivity Range (µS/cm)
100%	7.0-7.5	8.0-8.1	628-641
50%	6.8-7.4	7.8-8.0	419-426
25%	6.8-7.3	7.8-8.0	301-305
12.5%	6.7-7.3	7.8-7.9	234-242
6.25%	6.8-7.4	7.7-7.9	201-209
3.13%	6.7-7.4	7.7-7.9	185-193
1.56%	6.7-7.4	7.6-7.9	176-184
Control	6.9-7.4	7.6-7.8	171-180

REPRODUCTION AND SURVIVAL RESULTS:

Summary of Total Young Produced Per *Ceriodaphnia*

Concentration	Total Young Produced per <i>Ceriodaphnid</i> in its First 3 Broods										Mean Young in First 3 Broods	Standard Deviation
	1	2	3	4	5	6	7	8	9	10		
100%	25	10	D	9	6	7	21	D(6)	18	16	11.8	7.9
50%	24	33	20	27	26	30	D(1)	30	29	6	22.6	10.8
25%	28	33	32	16	19	28	17	33	34	16	25.6	7.7
12.5%	18	32	30	29	33	30	32	32	29	34	29.9	4.5
6.25%	12	32	30	34	31	D	35	35	30	7	24.6	13.0
3.13%	9	32	30	6	30	23	9	12	28	13	19.2	10.3
1.56%	15	33	18	32	18	17	28	13	9	15	19.8	8.3
Control	10	41	33	17	32	29	28	32	36	31	28.9	9.0

'D' – Dead

'X' – Cerio lost due to technician error

Summary of *Ceriodaphnia* Survival

Concentration	Percent Survival					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
100%	100	100	90	90	90	80
50%	100	100	100	90	90	90
25%	100	100	100	100	100	100
12.5%	100	100	100	100	100	100
6.25%	100	100	100	90	90	90
3.13%	100	100	100	100	100	100
1.56%	100	100	100	100	100	100
Control	100	100	100	100	100	100

Percent survival in each concentration is based on a single individual in each of ten replicates.

1.5 QUALITY CONTROL

Test controls conducted concurrently with the test and reference toxicant bioassays affirmed the validity of the *Ceriodaphnia dubia* test. Testing of the reference toxicant was performed as per protocol requirements with no deviations and conditions were within testing limits for measured parameters as specified by the bioassay protocol.

The brood organisms used to supply neonates in the *Ceriodaphnia dubia* survival and reproductive bioassay maintained the requirements of mortality rates less than or equal to 20% prior to testing; with a minimum of 9 young produced in the previous brood and an average of 32.2 young produced per adult in its first 3 broods. The brood stock was challenged with a reference toxicant (reagent grade sodium chloride) within fourteen days of sample testing. The value obtained in this test was within warning limits (± 2 standard deviations) of the laboratory mean, established through repetitive testing with the reference toxicant and brood culture. Dilution water controls run concurrent with the test produced three broods per test organism in at least 60% of the control replicates with an average of greater than 15 live young per adult. Control mortalities were less than 20%.

Test Brood Stock Health Summary

	Actual	Required
Age of Neonates	0-1000 hours	≤ 24 hours
Age of brood adults	6 days	≤ 14 days
Mean % mortality in 7 days prior to testing	0%	$\leq 20\%$
Average of number of young produced per adult in its first 3 broods	32.2	≥ 15
Minimum number of young produced in previous brood	9	≥ 8
Ehippia observations	None	None

Reference Toxicant Results

Chemical Used:	Sodium Chloride
Date Tested:	19 February 2014
7 day IC_{50} (Log Value):	3.147 mg/L, with a 95% confidence interval between 3.097 mg/L and 3.198 mg/L
Lab Geometric Mean (Log Value):	3.079 mg/L \pm 0.189 mg/L (two standard deviations) N = 31
Warning Limits (Log Values):	2.890 mg/L to 3.268 mg/L
7 day LC_{50} (Log Value):	3.327 mg/L, with a 95% confidence interval between 3.231 mg/L and 3.422 mg/L
Lab Geometric Mean (Log Value):	3.253 mg/L \pm 0.166 mg/L (two standard deviations) N = 31
Warning Limits (Log Values):	3.087 mg/L to 3.419 mg/L

Ceriodaphnia dubia Brood Stock Health Record

CERIODAPHNIA BROOD STOCK HEALTH RECORD

Client:	Nautilus Environmental
IRC ID#:	1402156
Sample Name:	R3
Sample Date:	25-Feb-14
Date Tested:	27-Feb-14
STOCK BIRTH DAY/DATES:	19-Feb-14

Mortality rate in 7 day period preceding test:	
# Dead:	0
Total organisms:	30
% Mortality:	0.0%
Required:	≤20%

EPHIPPIA OBSERVATIONS (yes/no): No

Young Produced 19-Feb-14

Date	Age (Days)	Organism #	1	2	3	4	5	6	7	8	9	10
20-Feb-14	1	0	0	0	0	0	0	0	0	0	0	0
21-Feb-14	2	0	0	0	0	0	0	0	0	0	0	0
22-Feb-14	3	3	4	4	3	3	0	4	4	5	5	5
23-Feb-14	4	0	0	0	0	0	6	0	0	0	0	0
24-Feb-14	5	9	12	12	12	12	9	12	11	8	11	11
25-Feb-14	6	20	17	19	21	17	0	17	9	16	20	20
26-Feb-14	7	0	15	0	0	0	0	0	19	0	0	0
27-Feb-14	8	20	22	19	17	22	19	24	0	20	20	20
28-Feb-14	9	20	20	21	24	23	25	25	21	27	20	20
1-Mar-14	10	23	0	17	27	0	21	24	19	22	20	20
2-Mar-14	11	0	25	0	0	26	0	0	20	0	0	0
Total in first 3 broods:		32	33	35	36	32	15	33	24	29	36	36

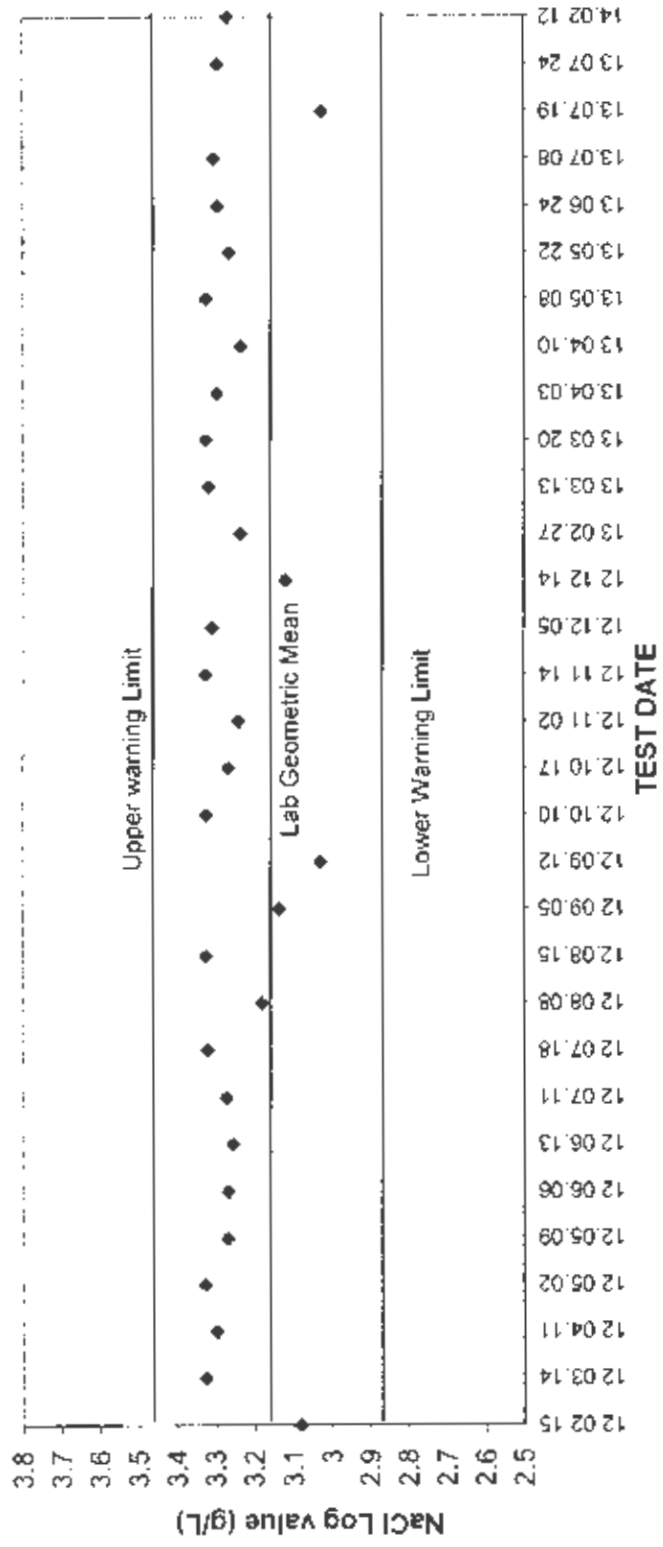
Average in first 3 broods: 32.2 Required: ≥15

Minimum brood size on day of testing: 9 Required: ≥8

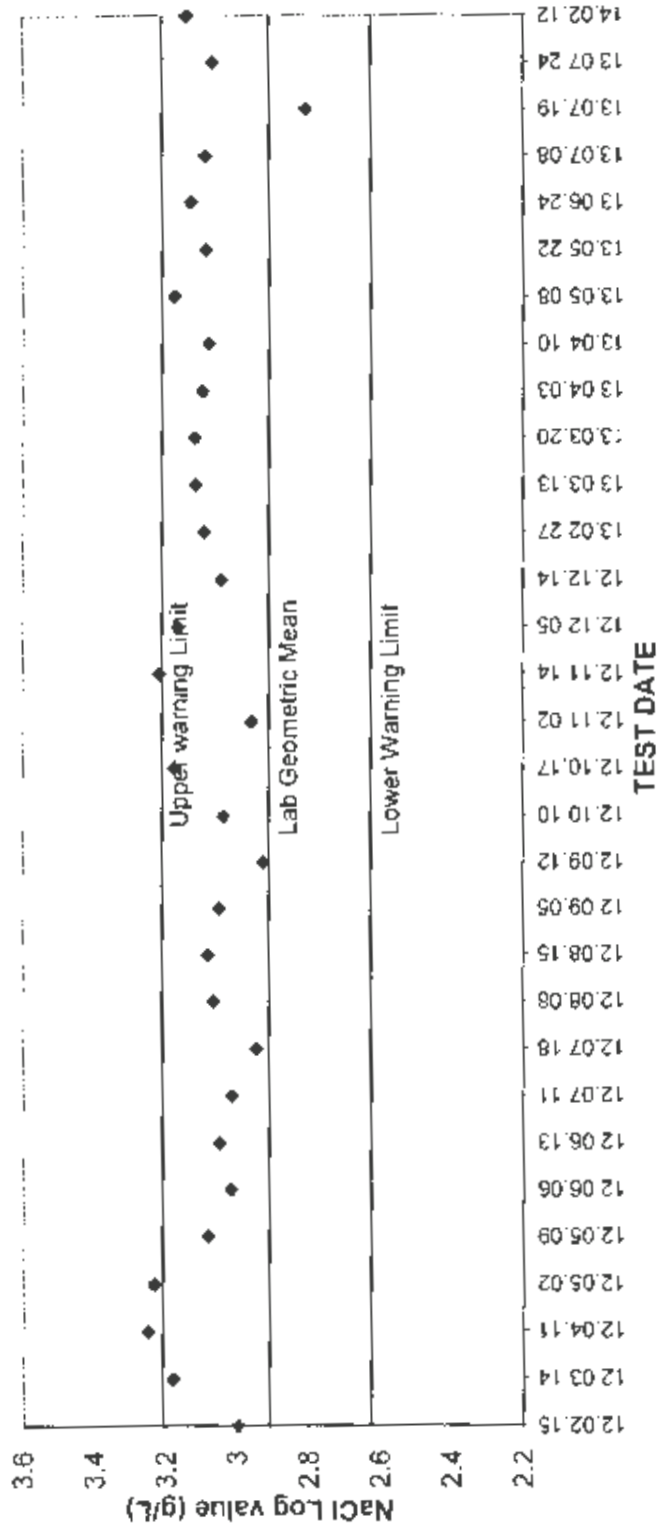
"d" - dead; "X" - Tech Error

***Ceriodaphnia dubia* Bioassay Reference Toxicant
Warning Charts**

CERIODAPHNIA REFERENCE TOXICANT LC50 WARNING CHART - LOG VALUES



CERIODAPHNIA REFERENCE TOXICANT IC50 WARNING CHART - LOG VALUES



Test Brood Count and Solution Readings

CERIODAPHNIA BROOD COUNT SUMMARY

Client:	Nautilus Environmental
IRC ID#:	1402156
Sample Name:	R3
Sample Date:	25-Feb-14
Date Tested:	27-Feb-14

CONCENTRATION:	100%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	0	d	0	0	0	0	0	0	0
4	4	5	d	3	1	2	3	0	5	3
5	7	5	d	6	5	5	6	6	8	7
6	14	0	d	0	0	0	12	d	5	6
TOTAL:	25	10	0	9	6	7	21	6	18	16

CONCENTRATION:	50%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	5	0	0	6	5	0	0	0	0
4	4	0	3	5	0	0	d(1)	5	5	2
5	8	12	7	10	8	11	d	12	11	4
6	12	16	10	12	12	14	d	13	13	0
TOTAL:	24	33	20	27	26	30	1	30	29	6

CONCENTRATION:	25%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	5	0	0	0	0	4	0	5	5	0
4	0	6	6	6	0	0	6	0	0	5
5	9	12	12	10	7	10	11	12	14	11
6	14	15	14	0	12	14	0	16	15	0
TOTAL:	28	33	32	16	19	28	17	33	34	16

CONCENTRATION:	12.50%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	6	4	0	5	5	0	0	0	0
4	7	0	0	5	0	0	5	6	4	5
5	11	10	11	11	12	10	10	10	11	11
6	0	16	15	13	16	15	17	16	14	18
TOTAL:	18	32	30	29	33	30	32	32	29	34

CONCENTRATION:	6.25%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	0	0	0	0	0	0	0	5	0
4	5	6	6	6	5	d	6	6	0	1
5	7	11	10	12	10	d	10	12	10	6
6	0	15	14	16	16	d	19	17	15	0
TOTAL:	12	32	30	34	31	0	35	35	30	7

CONCENTRATION:	3.13%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	0	0	0	0	0	0	0	0	0
4	1	3	6	5	6	5	3	0	4	5
5	0	12	10	1	10	9	6	4	14	8
6	8	17	14	0	14	9	0	8	10	0
TOTAL:	9	32	30	6	30	23	9	12	28	13

CONCENTRATION:	1.56%									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	0	0	0	0	0	0	0	0	0
4	4	5	5	4	5	7	3	4	3	3
5	11	11	13	12	13	0	11	9	6	0
6	0	17	0	16	0	10	14	0	0	12
TOTAL:	15	33	18	32	18	17	28	13	9	15

CONCENTRATION:	Control									
Day# / Adult #:	1	2	3	4	5	6	7	8	9	10
3	0	5	4	0	0	0	0	4	5	0
4	3	0	0	4	0	5	6	0	0	5
5	7	12	12	13	11	8	9	10	12	9
6	0	24	17	0	21	16	13	18	19	17
TOTAL:	10	41	33	17	32	29	28	32	36	31

"d" - dead; "X" - Tech Error

CONCENTRATION	100%	50%	25%	12.5%	6.25%	3.13%	1.56%	Control
BROOD COUNT MEANS	11.8	22.6	25.6	29.9	24.6	19.2	19.8	28.9
SD	7.9	10.8	7.7	4.5	13.0	10.3	8.3	9.0

Client:	Nautilus Environmental																		
IRC ID#:	1402156																		
Sample Name:	R3																		
Sample Date:	25-Feb-14																		
Date Tested:	27-Feb-14																		
FRESH SOLUTIONS										OLD SOLUTIONS									
DISSOLVED OXYGEN										DISSOLVED OXYGEN									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	10.1	10.1	9.9	10.0	9.2	9.7		9.2	10.1	100	7.2	7.5	7.3	7.5	7.5	7.0		7.0	7.5
50	8.8	9.0	8.9	8.8	8.6	8.8		8.6	9.0	50	7.2	7.4	7.2	7.3	7.2	6.8		6.8	7.4
25	8.4	8.6	8.5	8.2	8.3	8.6		8.2	8.6	25	7.3	7.2	7.2	7.1	7.2	6.8		6.8	7.3
12.5	8.2	8.6	8.4	8.1	8.2	8.4		8.1	8.6	12.5	7.3	7.0	7.2	7.0	7.2	6.7		6.7	7.3
6.25	8.2	8.5	8.2	8.1	8.1	8.3		8.1	8.5	6.25	7.4	7.2	7.2	7.2	7.3	6.8		6.8	7.4
3.13	8.1	8.5	8.2	8.1	8.1	8.3		8.1	8.5	3.13	7.4	7.2	7.2	7.1	7.2	6.7		6.7	7.4
1.56	8.2	8.4	8.1	8.1	8.2	8.4		8.1	8.4	1.56	7.4	7.4	7.3	7.2	7.2	6.7		6.7	7.4
CONTROL	8.3	8.4	8.1	8.3	8.3	8.5		8.1	8.5	CONTROL	7.4	7.1	7.3	7.4	7.2	6.9		6.9	7.4
pH										pH									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	7.6	7.7	7.7	7.6	7.7	7.6		7.6	7.7	100	8.0	8.1	8.1	8.0	8.1	8.0		8.0	8.1
50	7.7	7.8	7.8	7.8	7.8	7.8		7.7	7.8	50	7.8	8.0	8.0	7.9	7.9	7.9		7.8	8.0
25	7.7	7.8	7.9	7.8	7.8	7.8		7.7	7.9	25	7.8	8.0	7.9	7.8	7.8	7.8		7.8	8.0
12.5	7.7	7.9	7.9	7.9	7.9	7.9		7.7	7.9	12.5	7.7	7.9	7.8	7.7	7.8	7.7		7.7	7.9
6.25	7.8	7.9	8.0	7.9	7.9	7.9		7.8	8.0	6.25	7.7	7.9	7.8	7.7	7.8	7.7		7.7	7.9
3.13	7.8	7.9	8.0	7.9	7.9	7.9		7.8	8.0	3.13	7.7	7.9	7.8	7.7	7.7	7.7		7.7	7.9
1.56	7.8	7.9	8.0	7.9	7.9	7.9		7.8	8.0	1.56	7.7	7.9	7.8	7.6	7.7	7.7		7.6	7.9
CONTROL	7.8	7.8	8.0	7.8	7.9	7.9		7.8	8.0	CONTROL	7.7	7.8	7.8	7.6	7.7	7.7		7.6	7.8
CONDUCTIVITY										CONDUCTIVITY									
DAY	0	1	2	3	4	5	6	MIN	MAX	DAY	1	2	3	4	5	6	7	MIN	MAX
100	629	624	639	625	635	631		624	639	100	630	629	640	628	641	636		628	641
50	416	417	419	414	419	417		414	419	50	419	424	426	421	425	425		419	426
25	299	296	299	298	301	301		296	301	25	301	302	302	303	305	305		301	305
12.5	234	229	233	235	238	236		229	238	12.5	236	234	236	239	242	240		234	242
6.25	202	198	200	205	206	202		198	206	6.25	205	201	203	207	209	205		201	209
3.13	185	181	183	187	190	186		181	190	3.13	188	185	186	191	193	189		185	193
1.56	177	172	174	178	183	178		172	183	1.56	179	176	178	182	184	181		176	184
CONTROL	169	168	165	174	176	168		165	176	CONTROL	175	171	173	180	177	176		171	180
AFTER WARMING:																			
DAY	0	1	2	3	4	5	6	MIN	MAX										
Dissolved Oxygen	11.4	11.0	11.2	11.2	11.0	11.6		11	11.6										
Temperature	24.0	25.5	24.5	24.5	26.0	24.0		24	26										
pH	7.5	7.5	7.6	7.5	7.6	7.5		7.5	7.6										
Conductivity	638	618	641	639	638	639		618	641										
Aeration:	20 min	20 min	20 min	20 min	20 min	20 min													
HARDNESS:																			
Sample	330	330	330	362	332	338		330	362										
Dilution Water	86	74	80	76	80	76		74	86										

***Ceriodaphnia dubia* Bioassay Calculation Printouts**
7 Day Chronic *Ceriodaphnia* IC₂₅ and IC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:04 (p 1 of 2)
Test Code: 15-6796-1810/1402156

Ceriodaphnia 7-d Survival and Reproduction Test **IRC Integrated Research Consultants**

Analysis No: 12-0946-5570	Endpoint: Reproduction	CETIS Version: CETISv1.6.6
Analyzed: 18 Mar-14 16:01	Analysis: Nonlinear Regression	Official Results: Yes
Test Run No: 13-1595-1917	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 0h	Source: In-House Culture	Age:
Sample No: 09-0880-0885	Code: 908800885	Client: Nautilus
Sample Date: 18 Mar-14 15:54	Material: Unknown	Project: Special Studies
Receive Date: 18 Mar-14 15:54	Source: R3	
Sample Age: N/A	Station:	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ((log(X/D)/C)))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(5%)
23	-219.7	445.8	0.1437	Yes	2.335	2.342	0.0506	Non-Significant Lack of Fit

Point Estimates

Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC10	53.03	N/A	82.79	1.886	1.208	N/A
IC15	59.57	N/A	90.95	1.679	1.099	N/A
IC20	65.33	N/A	96.89	1.531	1.032	N/A
IC25	70.72	N/A	101.5	1.414	0.9853	N/A
IC40	86.36	61.5	114.5	1.158	0.8731	1.626
IC50	97.39	71.93	131.9	1.027	0.7584	1.39

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(5%)
A	24.66	1.261	22.15	27.17	19.56	0.0000	Significant Parameter
C	0.4744	0.3017	-0.1264	1.075	1.572	0.1200	Non-Significant Parameter
D	97.39	14.24	69.03	125.7	6.838	0.0000	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Model	1418.375	709.1877	2	7.631	0.0009	Significant
Lack of Fit	998.6246	199.7249	5	2.335	0.0506	Non-Significant
Pure Error	6157.8	85.525	72			
Residual	7156.425	92.94058	77			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(5%)
Variances	Mod Levene Equality of Variance	0.9523	2.14	0.4725	Equal Variances
Distribution	Shapiro-Wilk Normality	0.9068		0.0000	Non-normal Distribution

Reproduction Summary

Conc-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	10	28.9	10	41	1.652	9.049	31.31%	0.0%
1.56		10	19.8	9	33	1.508	8.257	41.7%	31.49%
3.13		10	19.2	6	32	1.887	10.34	53.84%	33.56%
6.25		10	24.6	0	35	2.382	13.05	53.04%	14.88%
12.5		10	29.9	18	34	0.823	4.508	15.08%	-3.46%
25		10	25.6	16	34	1.407	7.706	30.1%	11.42%
50		10	22.6	1	33	1.963	10.75	47.57%	21.8%
100		10	11.8	0	25	1.434	7.857	66.59%	59.17%

CETIS Analytical Report

Report Date: 18 Mar-14 16:04 (p 2 of 2)
 Test Code: 15-6796-1810/1402156

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

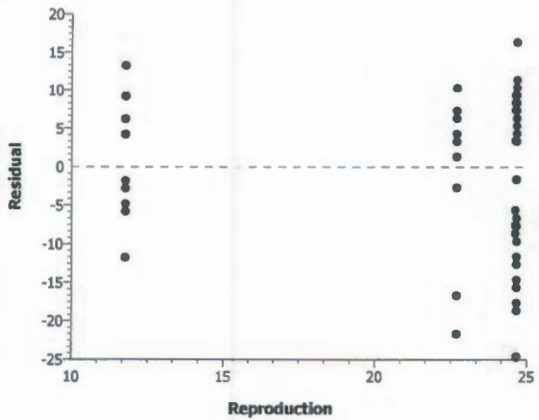
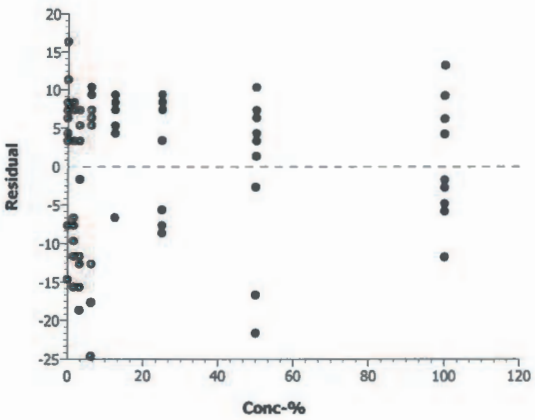
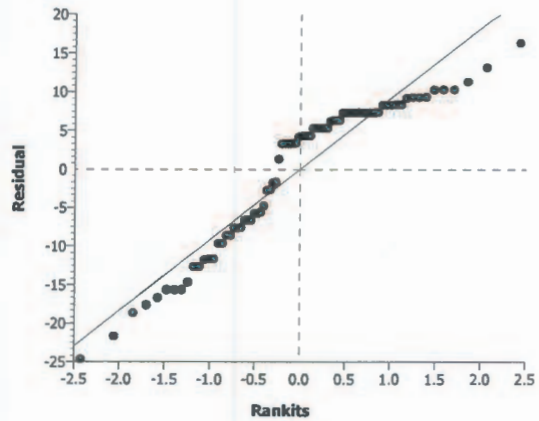
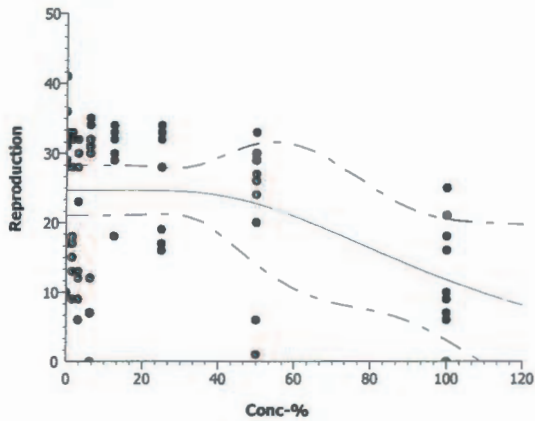
Analysis No: 12-0946-5570 Endpoint: Reproduction
 Analyzed: 18 Mar-14 16:01 Analysis: Nonlinear Regression

CETIS Version: CETISv1.6.6
 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	10	41	33	17	32	29	28	32	36	31
1.56		15	33	18	32	18	17	28	13	9	15
3.13		9	32	30	6	30	23	9	12	28	13
6.25		12	32	30	34	31	0	35	35	30	7
12.5		18	32	30	29	33	30	32	32	29	34
25		28	33	32	16	19	28	17	33	34	16
50		24	33	20	27	26	30	1	30	29	6
100		25	10	0	9	6	7	21	6	18	16

Graphics



CETIS Analytical Report

Report Date: 18 Mar-14 16:04 (p 2 of 2)
Test Code: 15-6796-1810/1402156

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

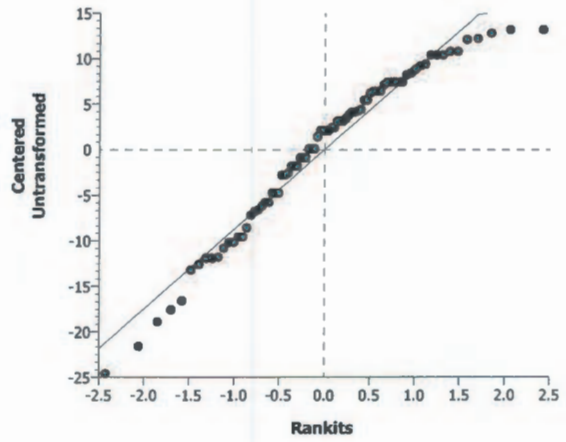
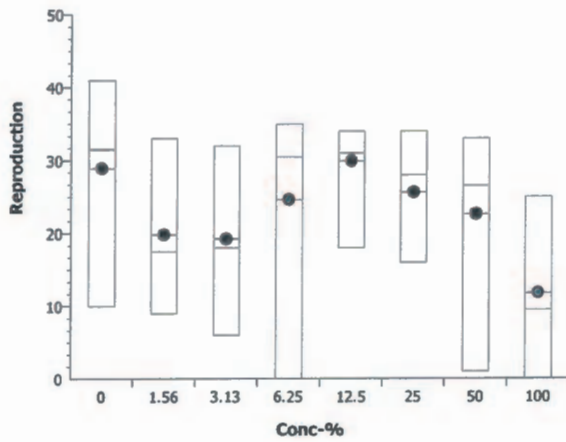
Analysis No: 19-0206-1856 **Endpoint:** Reproduction
Analyzed: 18 Mar-14 16:02 **Analysis:** Nonparametric-Control vs Treatments

CETIS Version: CETISv1.6.6
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	10	41	33	17	32	29	28	32	36	31
1.56		15	33	18	32	18	17	28	13	9	15
3.13		9	32	30	6	30	23	9	12	28	13
6.25		12	32	30	34	31	0	35	35	30	7
12.5		18	32	30	29	33	30	32	32	29	34
25		28	33	32	16	19	28	17	33	34	16
50		24	33	20	27	26	30	1	30	29	6
100		25	10	0	9	6	7	21	6	18	16

Graphics



***Ceriodaphnia dubia* Bioassay Calculation Printouts**
7 Day Chronic *Ceriodaphnia* LC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:03 (p 1 of 2)
 Test Code: 15-6796-1810/1402156

Ceriodaphnia 7-d Survival and Reproduction Test **IRC Integrated Research Consultants**

Analysis No: 10-2491-4294	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.6.6
Analyzed: 18 Mar-14 16:01	Analysis: Linear Regression (MLE)	Official Results: Yes
Test Run No: 13-1595-1917	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 0h	Source: In-House Culture	Age:
Sample No: 09-0880-0885	Code: 908800885	Client: Nautilus
Sample Date: 18 Mar-14 15:54	Material: Unknown	Project: Special Studies
Receive Date: 18 Mar-14 15:54	Source: R3	
Sample Age: N/A	Station:	

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0	Yes	Yes	No	Yes

Regression Summary

Iters	LL	AICc	Mu	Sigma	G Stat	Chi-Sq	Critical	P-Value	Decision(5%)
5	-13.59	34.18	2.692	1.175	1.427	4.406	11.07	0.4926	Non-Significant Heterogeneity

Point Estimates

Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC10	47.58	N/A	N/A	2.102	N/A	N/A
EC15	92.35	N/A	N/A	1.083	N/A	N/A
EC20	156.5	N/A	N/A	0.6392	N/A	N/A
EC25	245.9	N/A	N/A	0.4067	N/A	N/A
EC40	768.5	N/A	N/A	0.1301	N/A	N/A
EC50	1525	N/A	N/A	0.06556	N/A	N/A

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(5%)
Slope	0.851	0.5187	-0.1656	1.868	1.641	0.1618	Non-Significant Parameter
Intercept	2.291	0.8074	0.7085	3.874	2.838	0.0364	Significant Parameter

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(5%)
Extreme Value	Grubbs Extreme Value	2.027	2.02	0.0468	Outlier Detected
Distribution	Shapiro-Wilk Normality	0.8586		0.1471	Normal Distribution

6d Survival Rate Summary

Conc-%	Control Type	Count	Calculated Variate(A/B)							A	B
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%		
0	Dilution Water	10	1	1	1	0	0	0.0%	0.0%	10	10
1.56		10	1	1	1	0	0	0.0%	0.0%	10	10
3.13		10	1	1	1	0	0	0.0%	0.0%	10	10
6.25		10	0.9	0	1	0.05774	0.3162	35.14%	10.0%	9	10
12.5		10	1	1	1	0	0	0.0%	0.0%	10	10
25		10	1	1	1	0	0	0.0%	0.0%	10	10
50		10	0.9	0	1	0.05774	0.3162	35.14%	10.0%	9	10
100		10	0.8	0	1	0.07698	0.4216	52.7%	20.0%	8	10

CETIS Analytical Report

Report Date: 18 Mar-14 16:03 (p 2 of 2)
 Test Code: 15-6796-1810/1402156

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

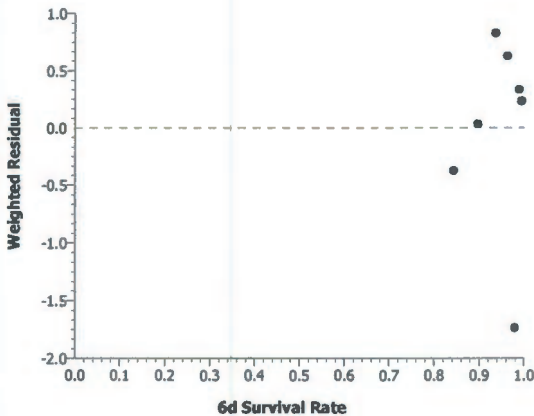
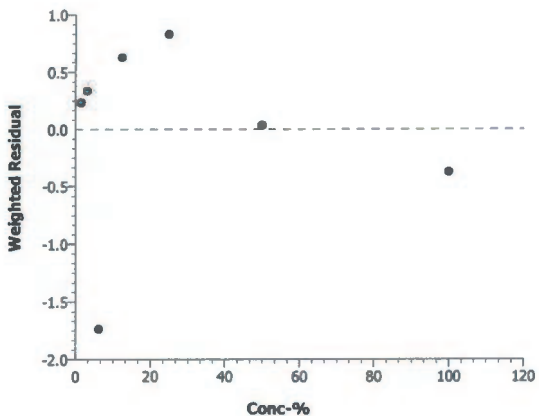
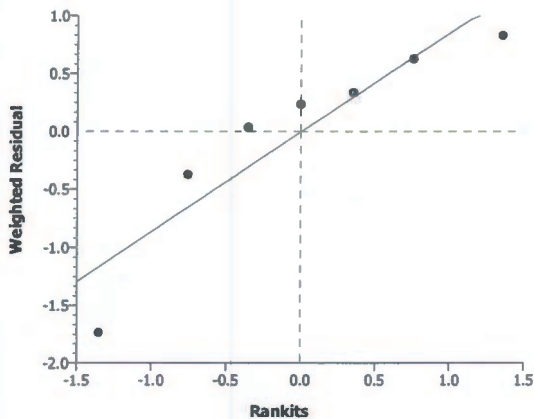
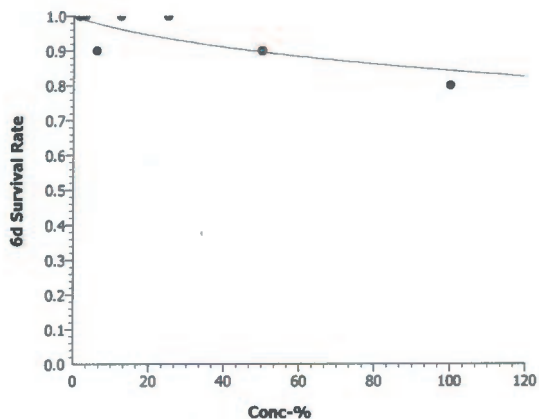
Analysis No: 10-2491-4294 Endpoint: 6d Survival Rate
 Analyzed: 18 Mar-14 16:01 Analysis: Linear Regression (MLE)

CETIS Version: CETISv1.6.6
 Official Results: Yes

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
1.56		1	1	1	1	1	1	1	1	1	1
3.13		1	1	1	1	1	1	1	1	1	1
6.25		1	1	1	1	1	0	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		1	1	1	1	1	1	0	1	1	1
100		1	1	0	1	1	1	1	0	1	1

Graphics



CETIS Analytical Report

Report Date: 18 Mar-14 16:02 (p 1 of 2)
 Test Code: 15-6796-1810/1402156

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 18-0581-1198	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.6.6
Analyzed: 18 Mar-14 16:01	Analysis: STP 2x2 Contingency Tables	Official Results: Yes
Test Run No: 13-1595-1917	Test Type: Reproduction-Survival (7d)	Analyst: Ditty Chacko
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 0h	Source: In-House Culture	Age:
Sample No: 09-0880-0885	Code: 908800885	Client: Nautilus
Sample Date: 18 Mar-14 15:54	Material: Unknown	Project: Special Studies
Receive Date: 18 Mar-14 15:54	Source: R3	
Sample Age: N/A	Station:	

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

Fisher Exact/Bonferroni-Holm Test

Control	vs	Conc-%	Test Stat	P-Value	Decision(0.05)
Dilution Water		1.56	1	1	Non-Significant Effect
		3.13	1	1	Non-Significant Effect
		6.25	0.5	1	Non-Significant Effect
		12.5	1	1	Non-Significant Effect
		25	1	1	Non-Significant Effect
		50	0.5	1	Non-Significant Effect
		100	0.2368	1	Non-Significant Effect

Data Summary

Conc-%	Control Type	No-Resp	Resp	Total
0	Dilution Water	10	0	10
1.56		10	0	10
3.13		10	0	10
6.25		9	1	10
12.5		10	0	10
25		10	0	10
50		9	1	10
100		8	2	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
1.56		1	1	1	1	1	1	1	1	1	1
3.13		1	1	1	1	1	1	1	1	1	1
6.25		1	1	1	1	1	0	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		1	1	1	1	1	1	0	1	1	1
100		1	1	0	1	1	1	1	0	1	1

CETIS Analytical Report

Report Date: 18 Mar-14 16:02 (p 2 of 2)

Test Code: 15-6796-1810/1402156

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 18-0581-1198

Endpoint: 6d Survival Rate

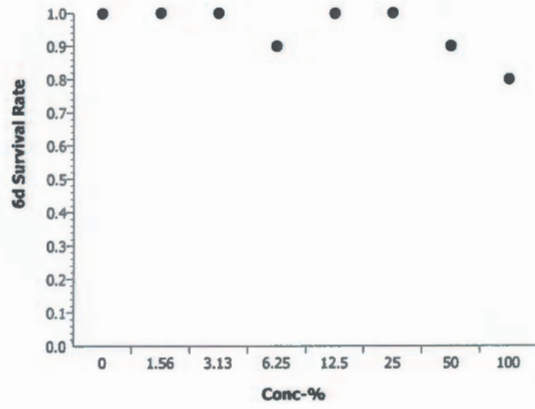
CETIS Version: CETISv1.6.6

Analyzed: 18 Mar-14 16:01

Analysis: STP 2x2 Contingency Tables

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 23 Apr-14 09:32 (p 1 of 2)
 Test Code: 06-5160-2182/14 | 12-2209-7749

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID: 10-2552-1083	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 23 Apr-14 9:31	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes
Batch ID: 18-8869-1174	Test Type: Reproduction-Survival (7d)	Analyst: Emma Marus
Start Date: 27 Feb-14	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 05 Mar-14	Species: Ceriodaphnia dubia	Brine:
Duration: 6d 0h	Source: In-House Culture	Age:
Sample ID: 05-2872-4256	Code: 1F83B120	Client: ALS
Sample Date: 25 Feb-14 11:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 36h (3.9 °C)	Station: L1426336-6(X3A)	

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.639	0.04362	43.53	35.61	53.21

6d Survival Rate Summary

Calculated Variate(A/B)

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10
1.56		10	1	1	1	0	0	0.0%	0.0%	10	10
3.13		10	1	1	1	0	0	0.0%	0.0%	10	10
6.25		10	1	1	1	0	0	0.0%	0.0%	10	10
12.5		9	1	1	1	0	0	0.0%	0.0%	9	9
25		10	1	1	1	0	0	0.0%	0.0%	10	10
50		10	0.3	0	1	0.1528	0.483	161.0%	70.0%	3	10
100		10	0	0	0	0	0	100.0%	0	0	10

6d 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	Negative Control	1	1	1	1	1	1	1	1	1	1
1.56		1	1	1	1	1	1	1	1	1	1
3.13		1	1	1	1	1	1	1	1	1	1
6.25		1	1	1	1	1	1	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		0	0	1	0	1	0	0	0	1	0
100		0	0	0	0	0	0	0	0	0	0

6d 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	Negative Control	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
1.56		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
3.13		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
6.25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
12.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
50		0/1	0/1	1/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1
100		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

CETIS Analytical Report

Report Date: 23 Apr-14 09:32 (p 2 of 2)
Test Code: 06-5160-2182/14 | 12-2209-7749

Ceriodaphnia 7-d Survival and Reproduction Test

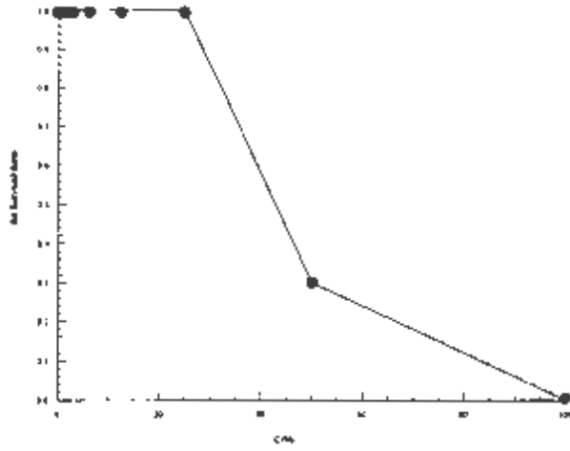
Nautilus Environmental

Analysis ID: 10-2552-1083
Analyzed: 23 Apr-14 9:31

Endpoint: 6d Survival Rate
Analysis: Untrimmed Spearman-Kärber

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



Ceriodaphnia dubia Bioassay Calculation Printouts
Reftox IC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:28 (p 1 of 2)
 Test Code: 12-0152-3261

Ceriodaphnia 7-d Survival and Reproduction Test			IRC Integrated Research Consultants		
Analysis No: 09-4477-0338	Endpoint: Reproduction	CETIS Version: CETISv1.6.6	Analyst:		
Analyzed: 05 Mar-14 10:15	Analysis: Nonlinear Regression	Official Results: Yes	Diluent: Laboratory Water		
Test Run No: 20-2489-0876	Test Type: Reproduction-Survival (7d)		Brine: Not Applicable		
Start Date: 19 Feb-14 13:27	Protocol: EC/EPS 1/RM/21		Age:		
Ending Date: 25 Feb-14 14:55	Species: Ceriodaphnia dubia				
Duration: 6d 1h	Source: In-House Culture				
Sample No: 13-0089-8086	Code: 1300898086	Client: Internal Lab			
Sample Date: 27 Feb-14 11:50	Material: Sodium chloride	Project: Special Studies			
Receive Date: 27 Feb-14 11:50	Source: Reference Toxicant				
Sample Age: N/A	Station:				

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1-Phi(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(5%)
6	-87.09	180.9	0.6237	Yes	0.4101	4.113	0.5260	Non-Significant Lack of Fit

Point Estimates

Level	Conc-gm/l	95% LCL	95% UCL
IC10	0.8703	N/A	1.124
IC15	0.9536	N/A	1.215
IC20	1.026	N/A	1.285
IC25	1.091	0.7556	1.342
IC40	1.277	1.089	1.474
IC50	1.404	1.25	1.576

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(5%)
A	29.8	1.253	27.26	32.34	23.78	0.0000	Significant Parameter
C	0.3731	0.1463	0.07675	0.6695	2.551	0.0150	Significant Parameter
D	1.404	0.08842	1.225	1.583	15.88	0.0000	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Lack of Fit	12.89863	12.89863	1	0.4101	0.5260	Non-Significant
Model	2063.101	1031.551	2	33.33	0.0000	Significant
Pure Error	1132.4	31.45555	36			
Residual	1145.299	30.95402	37			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(5%)
Variances	Bartlett Equality of Variance	3.027	7.815	0.3876	Equal Variances
	Mod Levene Equality of Variance	0.1617	2.866	0.9214	Equal Variances
Distribution	Shapiro-Wilk Normality	0.9514		0.0844	Normal Distribution

Reproduction Summary

Conc-gm/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	10	29	14	35	1.171	6.412	22.11%	0.0%
0.375		10	30.6	18	37	1.202	6.586	21.52%	-5.52%
0.75		10	28.4	22	33	0.6896	3.777	13.3%	2.07%
1.5		10	12.8	7	23	0.9499	5.203	40.65%	55.86%

Ceriodaphnia dubia Bioassay Calculation Printouts
Reflux LC₅₀

CETIS Analytical Report

Report Date: 18 Mar-14 16:27 (p 1 of 1)
 Test Code: 12-0152-3261

Ceriodaphnia 7-d Survival and Reproduction Test

IRC Integrated Research Consultants

Analysis No: 00-3619-3653	Endpoint: 6d Survival Rate	CETIS Version: CETISv1.6.6
Analyzed: 05 Mar-14 10:14	Analysis: Binomial Method	Official Results: Yes
Test Run No: 20-2489-0876	Test Type: Reproduction-Survival (7d)	Analyst:
Start Date: 19 Feb-14 13:27	Protocol: EC/EPS 1/RM/21	Diluent: Laboratory Water
Ending Date: 25 Feb-14 14:55	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 6d 1h	Source: In-House Culture	Age:
Sample No: 13-0089-8086	Code: 1300898086	Client: Internal Lab
Sample Date: 27 Feb-14 11:50	Material: Sodium chloride	Project: Special Studies
Receive Date: 27 Feb-14 11:50	Source: Reference Toxicant	
Sample Age: N/A	Station:	

Binomial/Graphical Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC/LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	0.3266	0	2.121	1.704	2.641

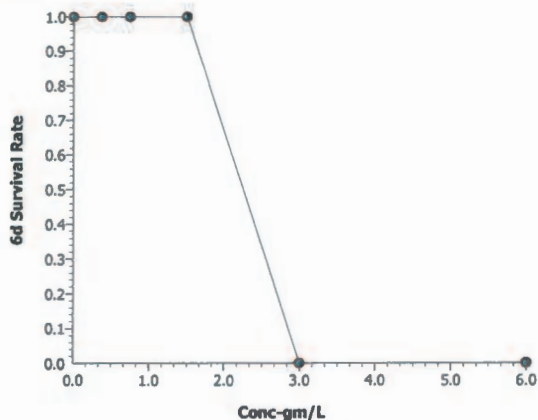
6d Survival Rate Summary

Conc-gm/L	Control Type	Count	Calculated Variate(A/B)								
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%	A	B
0	Dilution Water	7	1	1	1	0	0	0.0%	0.0%	7	7
0.375		10	1	1	1	0	0	0.0%	0.0%	10	10
0.75		10	1	1	1	0	0	0.0%	0.0%	10	10
1.5		10	1	1	1	0	0	0.0%	0.0%	10	10
3		10	0	0	0	0	0		100.0%	0	10
6		10	0	0	0	0	0		100.0%	0	10

6d Survival Rate Detail

Conc-gm/L	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
0.375		1	1	1	1	1	1	1	1	1	1
0.75		1	1	1	1	1	1	1	1	1	1
1.5		1	1	1	1	1	1	1	1	1	1
3		0	0	0	0	0	0	0	0	0	0
6		0	0	0	0	0	0	0	0	0	0

Graphics



APPENDIX B - *Lemna minor* Toxicity Test Data

Lemna minor Summary Sheet

Client: ALS
Work Order No.: 14077

Start Date: Feb 28, 2014
Set up by: JW / JRF

Sample Information:

Sample ID: L 1426330-1 (R10)
Sample Date: Feb 25 / 14 @ 1500h
Date Received: Feb 27 / 14 @ 1030h
Sample Volume: 2x 20L

Test Organism Information:

Culture Date: 02/19/14
Age of culture (Day 0): 9 days
>8X growth in APHA?: Y

KCI Reference Toxicant Results:

Reference Toxicant ID: Lm 100
Date Initiated: Feb 20, 2014

7-d No. of Fronds IC50 (95% CL): 36 (32 - 42)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 44 (35 - 55) CV (%): 12

	Number of Fronds	Dry Weight
Test Results: IC25 % (v/v) (95% CL)	> 97	> 97
IC50 % (v/v) (95% CL)	> 97	> 97

Reviewed by: [Signature]

Date reviewed: March 24/14

Plant Growth Inhibition Toxicity Test Water Quality Measurements

Client : ALS Setup by: JW / JBF
 Sample ID: R10 (L1426336 - 1) (red) Test Date: Feb 28 / 14 @ 1155h
 Work Order No.: 14077 Test Species: Lemna minor
 Culture Source: CRCC # 490
 Test Culture Age: 9 ^{JW} days > 8X Growth? (Y/N): Y
 Light Intensity Range: 4000 - 5600 lux Date Measured: Feb 26 / 14

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	25.0	26.0	26.5	25.5	26.0	26.0	26.0	26.0
Initials	JW	JW	JW	JBF	JW	JW	JW	JW

Sample Characteristics: Initial Water Quality
 Temperature (°C) 24.5 Aeration?: 20 min Adjusted Water Quality 25.0
 DO (mg/L) 9.8 Nutrients added?: Y → 8.8
 pH 7.3 → 7.5
 Conductivity (µS) 310 → 1111

Concentration % (v/v)	Temperature (°C)		pH		Conductivity (µS) 0 h
	Day 0	Day 7	Day 0	Day 7	
Control	24.0	25.0	8.4	8.4	878
1.5	24.0	25.0	8.0	8.4	882
^{JW} 3.0%	24.0	25.0	8.1	8.4	889
6.1	24.0	25.0	8.1	8.5	896
12.1	24.0	25.0	8.0	8.5	910
24.2	24.0	25.0	8.0	8.6	940
48.5	24.0	25.0	7.9	8.6	995
97	25.0	25.0	JW 7.5	8.6	1111
Initials	JW/JBF	KJC	JW/JBF	KJC	JW/JBF

Thermometer: Calibrated Thermometer Cond. Meter: C-2 pH meter: PH-2

Sample Description: clear

Comments: _____

Reviewed: JW Date Reviewed: March 24 / 14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS
 Sample ID: R0 (L1426336 1) (Red)
 Work Order #: 140767
 Start Date: Feb 28 / 14
 Termination Date: March 7 / 14
 Test set up by: JW / JBF

Concentration (% v/v)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
Control	A	6	76										JW
	B	6	72										
	C	6	71										
	D	6	85										
100	A	6	61										JW
	B	6	68										
	C	6	107										
	D	6	110										
200	A	6	96										JW
	B	6	71										
	C	6	76										
	D	6	53										
500	A	6	88										JW
	B	6	49										
	C	6	68										
	D	6	63										
1000	A	6	95										JW
	B	6	102										
	C	6	105										
	D	6	74										
2000	A	6	98										JW
	B	6	97										
	C	6	88										
	D	6	87										

Comments: _____
 Reviewed by: JW
 Date Reviewed: March 24/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS Start Date: Feb 28/14
 Sample ID: R10 (L1426336 - 1) (Red) Termination Date: March 7/14
 Work Order #: 14017 Test set up by: JLV / CRF

Concentration (µg/L)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
45.0	A	6	112										JLV
	B	6	113										
	C	6	97										
	D	6	67										
1.1	A	6	58										JLV
	B	6	106										
	C	6	75										
	D	6	63										
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments

Reviewed by: JLV Date Reviewed: March 24/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: R 10 (L 1426336 - 1)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration % (v/v)	Rep	Pan No. Red	Pan weight (mg)	Pan + plant (mg)	Initials
CONTROL	A	1	1226.70	1232.78	NY/JW
	B	2	1230.15	1235.74	
	C	3	1250.72	1255.59	
	D	4	1232.33	1238.58	
1.5	A	5	1261.77	1266.11	
	B	6	1256.74	1263.74	
	C	7	1229.43	1238.17	
	D	8	1251.41	1260.43	
3.0X JW	A	9	1245.20	1251.97	
	B	10	1292.82	1297.93	
	C	11	1257.63	1264.06	
	D	12	1255.55	1258.96	
6.1	A	13	1258.80	1265.50	
	B	14	1230.66	1234.24	
	C	15	1244.97	1250.71	
	D	16	1267.45	1271.96	
12.1	A	17	1249.89	1256.79	
	B	18	1264.21	1272.27	
	C	19	1244.09	1252.02	
	D	20	1250.86	1256.86	
24.2	A	21	1254.30	1262.26	
	B	22	1231.23	1239.61	
	C	23	1233.61	1240.55	
	D	24	1225.86	1233.42	
48.5	A	25	1237.05	1247.26	
	B	26	1212.28	1222.09	
	C	27	1228.74	1237.26	
	D	28	1225.06	1230.93	

Comments: Reweighed pans 2-1235.83 11-1263.95 24-1233.24

Reviewed by: JGh

Date Reviewed: March 24/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: R10 (L 1426336 - 1)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration % (V/V)	Rep	Pan No. Recd	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1239.67	1244.39	NY/JW ↓ ↓ ↓ ↓
	B	30	1235.43	1244.33	
	C	31	1234.41	1240.60	
	D	32	1264.15	1269.57	
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments: _____

Reviewed by: JW

Date Reviewed: March 24/14

CETIS Analytical Report

Report Date: 26 Mar-14 08:44 (p 1 of 2)
 Test Code: 14077a | 00-2731-5173

Lemna Growth Inhibition Test Nautilus Environmental

Analysis ID: 17-7897-2806	Endpoint: Frond Count	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 8:42	Analysis: Nonlinear Regression	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 00-4133-3542	Code: 276B326	Client: ALS
Sample Date: 25 Feb-14 15:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 57h (3.6 °C)	Station: L1426336-1(R10)	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
2P Exponential EV [Y=A*exp(log(0.5)*X/D)]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
8	-108.7	221.8	224.4		Yes	2.157	2.508	0.0836	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	31670000	N/A	N/A	0.0000031	NA	NA
IC10	65060000	N/A	N/A	0.0000015	NA	NA
IC15	10030000	N/A	N/A	0.0000009	NA	NA
IC20	13780000	N/A	N/A	0.0000007	NA	NA
IC25	17760000	N/A	N/A	0.0000005	NA	NA
IC40	31540000	N/A	N/A	0.0000003	NA	NA
IC50	42800000	N/A	N/A	0.0000002	NA	NA

} > 97% (v/v) JGW

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	77.19	4.166	69.02	85.35	18.53	<0.0001	Significant Parameter
D	4.28E+08	3.60E+14	-7.1E+14	7.06E+14	1.19E-06	1.0000	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	0	0	1	0	1.0000	Non-Significant
Lack of Fit	3682.963	613.8271	6	2.157	0.0836	Non-Significant
Pure Error	6830.75	284.6146	24			
Residual	10513.71	350.4571	30			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	7.67	14.07	0.3626	Equal Variances
	Mod Levene Equality of Variance	0.6982	2.423	0.6732	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9598	0.9338	0.2709	Normal Distribution
	Anderson-Darling A2 Normality	0.4479	2.492	0.2837	Normal Distribution

Frond Count Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	70.5	65	79	3.227	6.455	9.18%	0.0%
1.5		4	85.5	55	104	11.27	22.55	26.37%	-21.28%
3		4	68	47	90	8.841	17.68	26.0%	3.55%
6.1		4	61	43	82	8.073	16.15	26.47%	13.48%
12.1		4	88	68	99	6.988	13.98	15.88%	-24.82%
24.2		4	86.5	81	92	2.901	5.802	6.71%	-22.7%
48.5		4	91.25	61	107	10.73	21.45	23.51%	-29.43%
97		4	69.5	52	100	10.77	21.55	31.0%	1.42%

CETIS Analytical Report

Report Date: 26 Mar-14 08:44 (p 2 of 2)
 Test Code: 14077a | 00-2731-5173

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 17-7897-2806
 Analyzed: 26 Mar-14 8:42

Endpoint: Frond Count
 Analysis: Nonlinear Regression

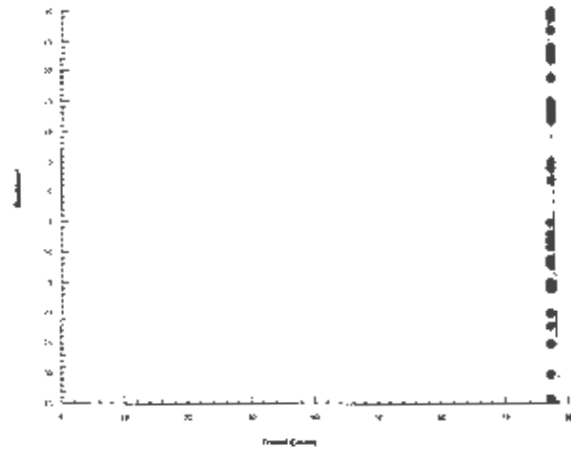
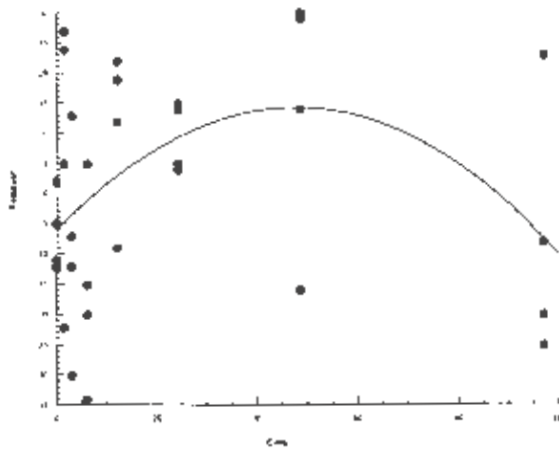
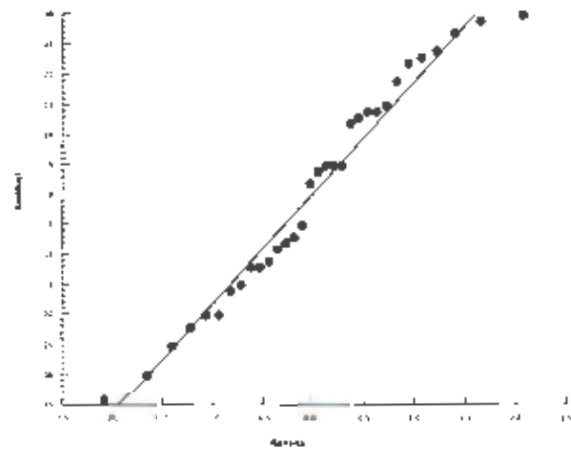
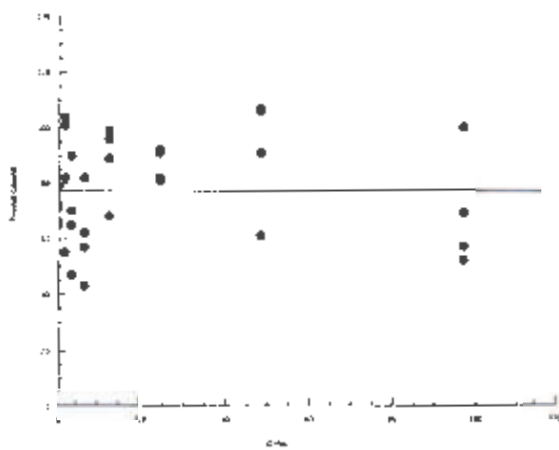
CETIS Version: CETISv1.8.7
 Official Results: Yes

Frond Count Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	72	66	65	79
1.5		55	82	101	104
3		90	65	70	47
6.1		82	43	62	57
12.1		89	96	99	68
24.2		92	91	82	81
48.5		106	107	91	61
97		52	100	69	57

Graphics

2P Exponential EV [Y=A*exp(log(0.5)*X/D)]



CETIS Analytical Report

Report Date: 26 Mar-14 08:44 (p 1 of 2)
 Test Code: 14077a | 00-2731-5173

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 08-0763-0945	Endpoint: Frond Count	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 8:42	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 00-4133-3542	Code: 276B326	Client: ALS
Sample Date: 25 Feb-14 15:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 57h (3.6 °C)	Station: L1426336-1(R10)	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	42.0%	97	>97	NA	1.031

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		1.5	1.257	2.482	29.61	6	0.3649	CDF	Non-Significant Effect
		3	-0.2096	2.482	29.61	6	0.9197	CDF	Non-Significant Effect
		6.1	-0.7964	2.482	29.61	6	0.9821	CDF	Non-Significant Effect
		12.1	1.467	2.482	29.61	6	0.2802	CDF	Non-Significant Effect
		24.2	1.341	2.482	29.61	6	0.3297	CDF	Non-Significant Effect
		48.5	1.739	2.482	29.61	6	0.1890	CDF	Non-Significant Effect
		97	-0.08383	2.482	29.61	6	0.8947	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3679.219	525.6027	7	1.847	0.1241	Non-Significant Effect
Error	6830.75	284.6146	24			
Total	10509.97		31			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.67	18.48	0.3626	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9766	0.9081	0.6962	Normal Distribution

Frond Count Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	4	70.5	60.23	80.77	69	65	79	3.227	9.16%	0.0%
1.5		4	85.5	49.62	121.4	91.5	55	104	11.27	26.37%	-21.28%
3		4	68	39.86	96.14	67.5	47	90	8.841	26.0%	3.55%
6.1		4	61	35.31	86.69	59.5	43	82	8.073	26.47%	13.48%
12.1		4	88	65.76	110.2	92.5	68	99	6.988	15.88%	-24.82%
24.2		4	86.5	77.27	95.73	86.5	81	92	2.901	6.71%	-22.7%
48.5		4	91.25	57.11	125.4	98.5	61	107	10.73	23.51%	-29.43%
97		4	69.5	35.21	103.8	63	52	100	10.77	31.0%	1.42%

Frond Count Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	72	66	65	79
1.5		55	82	101	104
3		90	65	70	47
6.1		82	43	62	57
12.1		89	96	99	68
24.2		92	91	82	81
48.5		106	107	91	61
97		52	100	69	57

CETIS Analytical Report

Report Date: 26 Mar-14 08:44 (p 2 of 2)

Test Code: 14077a | 00-2731-5173

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 08-0763-0945

Endpoint: Frond Count

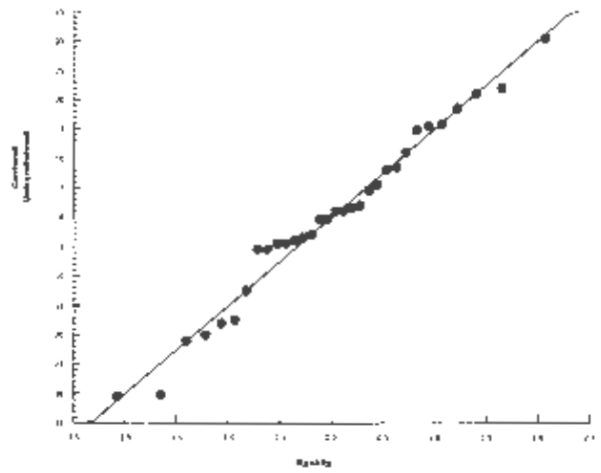
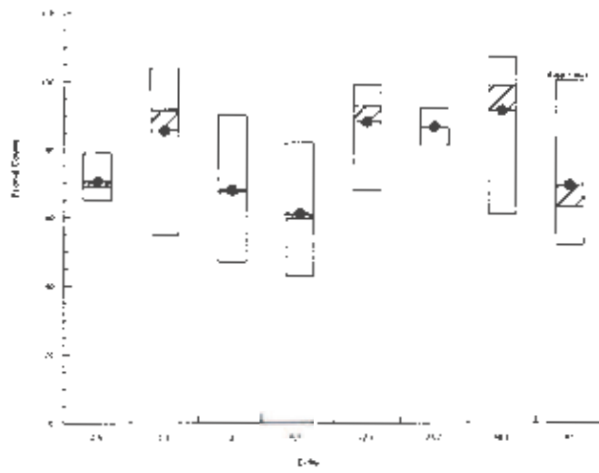
CETIS Version: CETISv1.8.7

Analyzed: 26 Mar-14 8:42

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 26 Mar-14 08:44 (p 1 of 2)
 Test Code: 14077a | 00-2731-5173

Lemna Growth Inhibition Test			Nautilus Environmental
Analysis ID: 10-1135-1669	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7	
Analyzed: 26 Mar-14 8:43	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes	
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya	
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)	
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:	
Duration: 7d 0h	Source: CPCC#490	Age: 9d	
Sample ID: 00-4133-3542	Code: 276B326	Client: ALS	
Sample Date: 25 Feb-14 15:00	Material: Effluent	Project:	
Receive Date: 27 Feb-14 10:30	Source: ALS		
Sample Age: 57h (3.6 °C)	Station: L1426336-1(R10)		

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1391191	200	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	84.87	N/A	N/A	1.178	NA	NA
IC10	>97	N/A	N/A	<1.031	NA	NA
IC15	>97	N/A	N/A	<1.031	NA	NA
IC20	>97	N/A	N/A	<1.031	NA	NA
IC25	>97	N/A	N/A	<1.031	NA	NA
IC40	>97	N/A	N/A	<1.031	NA	NA
IC50	>97	N/A	N/A	<1.031	NA	NA

Total Dry Weight-mg Summary				Calculated Variate					
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	5.698	4.87	6.25	0.3093	0.6186	10.86%	0.0%
1.5		4	7.275	4.34	9.02	1.076	2.151	29.57%	-27.69%
3		4	5.43	3.41	6.77	0.7626	1.525	28.09%	4.7%
6.1		4	5.132	3.58	6.7	0.6846	1.369	26.68%	9.92%
12.1		4	7.223	6	8.06	0.4831	0.9662	13.38%	-26.77%
24.2		4	7.71	6.94	8.38	0.3064	0.6128	7.95%	-35.32%
48.5		4	8.602	5.87	10.21	0.9796	1.959	22.77%	-50.99%
97		4	6.307	4.72	8.9	0.9148	1.83	29.01%	-10.71%

Total Dry Weight-mg Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	6.08	5.59	4.87	6.25
1.5		4.34	7	8.74	9.02
3		6.77	5.11	6.43	3.41
6.1		6.7	3.58	5.74	4.51
12.1		6.9	8.06	7.93	6
24.2		7.96	8.38	6.94	7.56
48.5		10.21	9.81	8.52	5.87
97		4.72	8.9	6.19	5.42

CETIS Analytical Report

Report Date: 26 Mar-14 08:44 (p 2 of 2)
Test Code: 14077a | 00-2731-5173

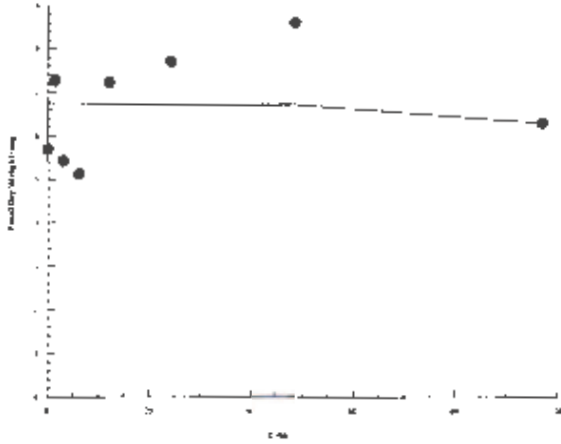
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 10-1135-1669 Endpoint: Total Dry Weight-mg
Analyzed: 26 Mar-14 8:43 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1 8 7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 26 Mar-14 08:44 (p 1 of 2)
 Test Code: 14077a | 00-2731-5173

Lemna Growth Inhibition Test				Nautilus Environmental			
Analysis ID: 04-0148-6424	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7					
Analyzed: 26 Mar-14 8:43	Analysis: Parametric-Control vs Treatments	Official Results: Yes					
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya					
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)					
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:					
Duration: 7d 0h	Source: CPCC#490	Age: 9d					
Sample ID: 00-4133-3542	Code: 276B326	Client: ALS					
Sample Date: 25 Feb-14 15:00	Material: Effluent	Project:					
Receive Date: 27 Feb-14 10:30	Source: ALS						
Sample Age: 57h (3.6 °C)	Station: L1426336-1(R10)						

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	45.8%	24.2	48.5	34.26	4.132

Dunnnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		1.5	1.5	2.482	2.611	6	0.2681	CDF	Non-Significant Effect
		3	-0.2543	2.482	2.611	6	0.9275	CDF	Non-Significant Effect
		6.1	-0.5371	2.482	2.611	6	0.9637	CDF	Non-Significant Effect
		12.1	1.45	2.482	2.611	6	0.2868	CDF	Non-Significant Effect
		24.2	1.913	2.482	2.611	6	0.1429	CDF	Non-Significant Effect
		48.5*	2.762	2.482	2.611	6	0.0280	CDF	Significant Effect
		97	0.5798	2.482	2.611	6	0.6737	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	41.86441	5.980629	7	2.702	0.0325	Significant Effect
Error	53.11691	2.213205	24			
Total	94.98132		31			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.484	18.48	0.3803	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9799	0.9081	0.7959	Normal Distribution

Total Dry Weight-mg Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	4	5.698	4.713	6.682	5.835	4.87	6.25	0.3093	10.86%	0.0%
1.5		4	7.275	3.852	10.7	7.87	4.34	9.02	1.076	29.57%	-27.69%
3		4	5.43	3.003	7.857	5.77	3.41	6.77	0.7626	28.09%	4.7%
6.1		4	5.132	2.954	7.311	5.125	3.58	6.7	0.6846	26.68%	9.92%
12.1		4	7.223	5.685	8.76	7.415	6	8.06	0.4831	13.38%	-26.77%
24.2		4	7.71	6.735	8.685	7.76	6.94	8.38	0.3064	7.95%	-35.32%
48.5		4	8.602	5.485	11.72	9.165	5.87	10.21	0.9796	22.77%	-50.99%
97		4	6.307	3.396	9.219	5.805	4.72	8.9	0.9148	29.01%	-10.71%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	6.08	5.59	4.87	6.25
1.5		4.34	7	8.74	9.02
3		6.77	5.11	6.43	3.41
6.1		6.7	3.58	5.74	4.51
12.1		6.9	8.06	7.93	6
24.2		7.96	8.38	6.94	7.56
48.5		10.21	9.81	8.52	5.87
97		4.72	8.9	6.19	5.42

CETIS Analytical Report

Report Date: 26 Mar-14 08:44 (p 2 of 2)
Test Code: 14077a | 00-2731-5173

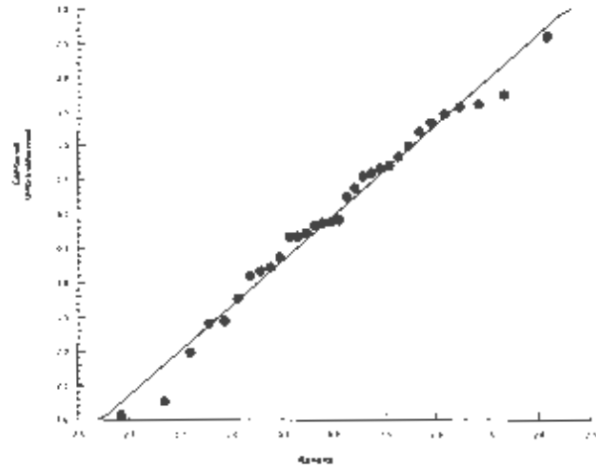
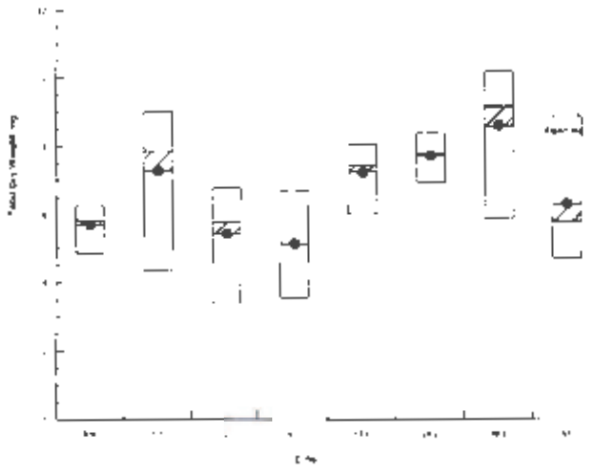
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 04-0148-6424 Endpoint: Total Dry Weight-mg
Analyzed: 26 Mar-14 8:43 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



Lemna minor Summary Sheet

Client: ALS
 Work Order No.: 14077

Start Date: Feb 28, 2014
 Set up by: JW / JBF

Sample Information:

Sample ID: L1426336-2 (NF ¹ ~~2~~)
 Sample Date: Feb 25 / 14 @ 1435h
 Date Received: Feb 27 / 14 @ 1030h
 Sample Volume: 2 x 20L

Test Organism Information:

Culture Date: 02/19/14
 Age of culture (Day 0): 9 days
 >8X growth in APHA?: Y

KCI Reference Toxicant Results:

Reference Toxicant ID: LM 100
 Date Initiated: Feb 20, 2014

7-d No. of Fronds IC50 (95% CL): 3.6 (3.2 - 4.2)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 4.4 (3.9 - 5.5) CV (%): 12

	Number of Fronds	Dry Weight
Test Results. IC25 %(v/v) (95% CL)	> 97	> 97
IC50 %(v/v) (95% CL)	> 97	> 97

Reviewed by: JGh

Date reviewed: March 26/14

Plant Growth Inhibition Toxicity Test Water Quality Measurements

Client: ALS Setup by: JW / JBF
 Sample ID: NF-1 (L1426336-2) (purple) Test Date: Feb 28 / 14 @ 1200h
 Work Order No.: 14077 Test Species: Lemna minor
 Culture Source: CPCC # 490
 Test Culture Age: 9 ^{JW} days > 8X Growth? (Y/N): Y
 Light Intensity Range: 4000 - 5600 lux Date Measured: Feb 26 / 14

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	25.0	26.0	25.5	25.5	26.0	26.0	26.0	26.0
Initials	JW	JW	JW	JBF	JW	JW	JW	JW

Sample Characteristics: Initial Water Quality Adjusted Water Quality

Temperature (°C)	<u>24.5</u>	Aeration?:	<u>20 min</u>	<u>25.0</u>
DO (mg/L)	<u>10.8</u>	Nutrients added?:	<u>Y</u>	<u>8.9</u>
pH	<u>7.2</u>			<u>7.5</u>
Conductivity (µS)	<u>322</u>			<u>1114</u>

Concentration % (v/v)	Temperature (°C)		pH		Conductivity (µS) 0 h
	Day 0	Day 7	Day 0	Day 7	
Control	24.0	25.0	8.4	8.4	878
1.5	24.0	25.0	8.1	8.5	887
3.0 ^{JW}	24.0	25.0	8.1	8.4	889
6.1	25.0	25.0	8.1	8.5	896
12.1	25.0	25.0	8.1	8.3	910
24.2	25.0	25.0	8.0	8.3	939
48.5	25.0	25.0	7.9	8.5	998
97	25.0	25.0	7.5	8.6	1114
Initials	JW / JBF	JW / JBF	JW / JBF	JW / JBF	JW / JBF

Thermometer: Calibrated Thermometer Cond. Meter: C-2 pH meter: PH-2

Sample Description: clear

Comments: _____

Reviewed: JW Date Reviewed: March 25 / 14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALC Start Date: Feb. 28 / 14
 Sample ID: NF-1 (1420336 - 2) (purple) Termination Date: March 7 / 14
 Work Order #: 14017 Test set up by: JW / JEF

Concentration % _{v/v}	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
control	A	6	96			x							JEF
	B	6	69			x							
	C	6	63			x							
	D	6	81										
1.5	A	6	96										
	B	6	79										
	C	6	85										
	D	6	104										
3.0 ¹⁰⁰	A	6	72			x							
	B	6	70			x							
	C	6	83			x							
	D	6	73			x							
6.1	A	6	84		x	x							
	B	6	62		x	x							
	C	6	117										
	D	6	101										
12.1	A	6	102										
	B	6	78										
	C	6	86										
	D	6	79			x							
24.2	A	6	96										
	B	6	97										
	C	6	54			x							
	D	6	97										

Comments: _____

Reviewed by: JEF Date Reviewed: March 25/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS
 Sample ID: NF 1 (L1420326 - 1) (DURC02)
 Work Order #: 14077
 Start Date: Feb 28 / 14
 Termination Date: March 7 / 14
 Test set up by: JW / JBF

Concentration (% v/v)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
4%	A	6	84										JBF
	B	6	74			X							
	C	6	88			X							
	D	6	65			X							
9%	A	6	69										JBF
	B	6	60										
	C	6	87										
	D	6	55										
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments: _____
 Reviewed by: JW Date Reviewed: March 25/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: NF 1 (L 1426336 - 2)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration 9D (V/V)	Rep	Pan No. Purple	Pan weight (mg)	Pan + plant (mg)	Initials
control	A	1	1024.59	1033.10	NYSW
	B	2	1001.77	1007.96	
	C	3	1013.74	1019.81	
	D	4	1019.08	1025.97	
1.5	A	5	1024.77	1032.61 ^{JW}	
	B	6	1005.12	1011.78 ^{JW} 66	
	C	7	1035.36	1042.85	
	D	8	1054.15	1062.98	
3.0% ^{JW}	A	9	1061.60	1068.10	
	B	10	1020.91	1027.38	
	C	11	1000.16	1008.19	
	D	12	1012.44	1018.99	
6.1	A	13	1039.41	1046.76	
	B	14	1043.15	1048.98	
	C	15	1046.25	1055.78 ^{JW} 65	
	D	16	1058.49	1067.02	
12.1	A	17	1041.80	1050.45	
	B	18	1049.52	1056.11	
	C	19	1046.20	1054.79	
	D	20	1051.72	1058.30	
24.2	A	21	1048.60	1057.70	
	B	22	1003.51	1012.08	
	C	23	1016.05	1020.74	
	D	24	1038.92	1047.31	
48.5	A	25	1044.52	1051.74	
	B	26	1000.91	1008.05	
	C	27	991.20	999.60	
	D	28	995.43	1001.27	

Comments: Reweighed pans 4-1025.99 9-1067.66 19-1054.32

Reviewed by: JGU

Date Reviewed: March 25 / 14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: NF1 (L 1426336 - 2)
 Work Order #: 14071

Start Date: Feb 28/14
 Termination Date: March 7 / 14

Concentration <i>% (v/v)</i>	Rep	Pan No. <i>Purple</i>	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1208.97	1215.07	NY/JW ↓ ↓
	B	30	1236.16	1243.74	
	C	31	1226.97	1235.31	
	D	32	1245.36	1250.48	
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments: _____

Reviewed by: JGU

Date Reviewed: March 25/14

CETIS Analytical Report

Report Date: 26 Mar-14 09:01 (p 1 of 2)
 Test Code: 14077b | 00-1835-0189

Lemna Growth Inhibition Test			Nautilus Environmental		
Analysis ID: 16-2678-6901	Endpoint: Frond Count	CETIS Version: CETISv1.8.7			
Analyzed: 26 Mar-14 9:00	Analysis: Nonlinear Regression	Official Results: Yes			
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya			
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)			
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:			
Duration: 7d 0h	Source: CPCC#490	Age: 9d			
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS			
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:			
Receive Date: 27 Feb-14 10:30	Source: ALS				
Sample Age: 57h (4.3 °C)	Station: L1426336-2(NF1)				

Non-Linear Regression Options				
Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
2P Exponential EV [Y=A*exp(log(0.5)*X/D)]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary									
Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
4	-101	206.5	209	0.0297	Yes	0.8056	2.508	0.5755	Non-Significant Lack of Fit

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	32.85	N/A	80.82	3.044	1.237	NA
IC10	67.47	N/A	153.4	1.482	0.6518	NA
IC15	104.1	N/A	248.3	0.9609	0.4027	NA
IC20	142.9	N/A	359.7	0.6998	0.278	NA
IC25	184.2	N/A	490.4	0.5428	0.2039	NA
IC40	327.1	N/A	1109	0.3057	0.09014	NA
IC50	443.9	8.947	2739	0.2253	0.03651	11.18

} > 97% (v/v)

Regression Parameters								
Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)	
A	78.88	3.332	72.35	85.41	23.68	<0.0001	Significant Parameter	
D	443.9	332.8	-208.4	1096	1.334	0.1923	Non-Significant Parameter	

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	422.6493	422.6493	1	1.948	0.1731	Non-Significant
Lack of Fit	1091.226	181.8709	6	0.8056	0.5755	Non-Significant
Pure Error	5418	225.75	24			
Residual	6509.226	216.9742	30			

Residual Analysis						
Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)	
Variances	Bartlett Equality of Variance	6.557	14.07	0.4764	Equal Variances	
	Mod Levene Equality of Variance	0.6925	2.423	0.6776	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9797	0.9338	0.7904	Normal Distribution	
	Anderson-Darling A2 Normality	0.2895	2.492	0.6437	Normal Distribution	

Frond Count Summary			Calculated Variate						
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	71.25	57	90	7.284	14.57	20.45%	0.0%
1.5		4	85	73	98	5.583	11.17	13.14%	-19.3%
3		4	68.5	64	77	2.901	5.802	8.47%	3.86%
6.1		4	85	56	111	11.78	23.57	27.72%	-19.3%
12.1		4	80.25	72	96	5.543	11.09	13.82%	-12.63%
24.2		4	80	48	91	10.67	21.34	26.67%	-12.28%
48.5		4	71.75	59	82	5.17	10.34	14.41%	-0.7%
97		4	66.75	49	81	6.981	13.96	20.92%	6.32%

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 16-2678-6901 Endpoint: Frond Count
 Analyzed: 26 Mar-14 9:00 Analysis: Nonlinear Regression

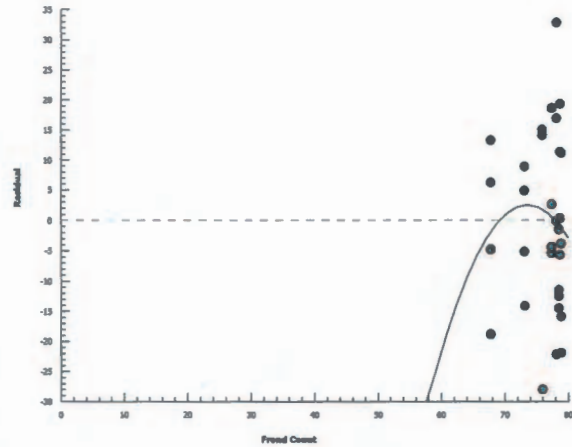
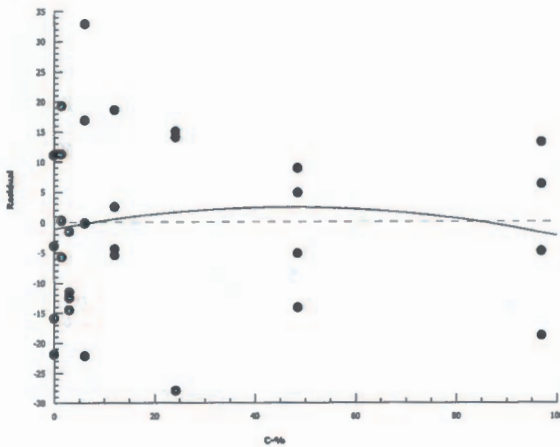
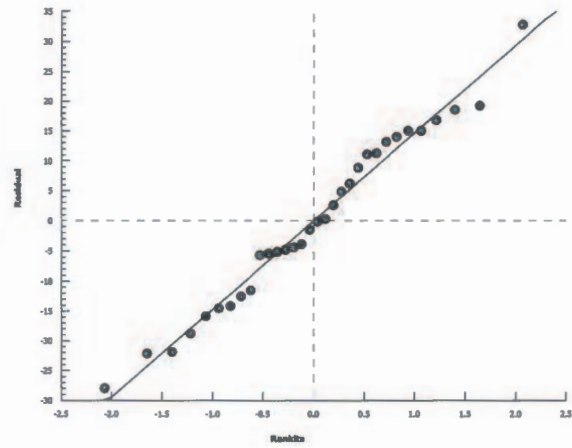
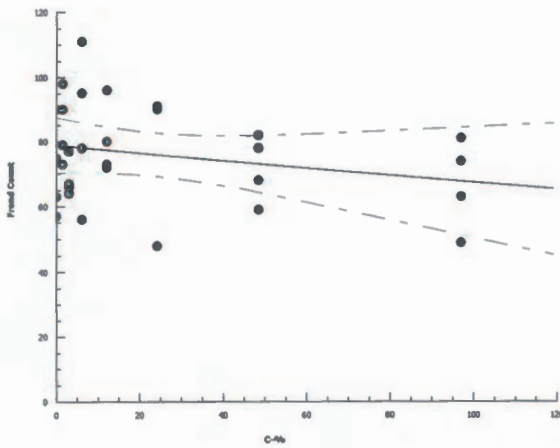
CETIS Version: CETISv1.8.7
 Official Results: Yes

Frond Count Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	90	63	57	75
1.5		90	73	79	98
3		66	64	77	67
6.1		78	56	111	95
12.1		96	72	80	73
24.2		90	91	48	91
48.5		78	68	82	59
97		63	74	81	49

Graphics

2P Exponential EV [Y=A*exp(log(0.5)*X/D)]



CETIS Analytical Report

Report Date: 26 Mar-14 09:01 (p 1 of 2)
 Test Code: 14077b | 00-1835-0189

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 03-7731-0487	Endpoint: Frond Count	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:00	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 57h (4.3 °C)	Station: L1426336-2(NF1)	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	37.0%	97	>97	NA	1.031

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		1.5	1.294	2.482	26.37	6	0.3492	CDF	Non-Significant Effect
		3	-0.2588	2.482	26.37	6	0.9282	CDF	Non-Significant Effect
		6.1	1.294	2.482	26.37	6	0.3492	CDF	Non-Significant Effect
		12.1	0.8471	2.482	26.37	6	0.5524	CDF	Non-Significant Effect
		24.2	0.8236	2.482	26.37	6	0.5634	CDF	Non-Significant Effect
		48.5	0.04706	2.482	26.37	6	0.8629	CDF	Non-Significant Effect
		97	-0.4236	2.482	26.37	6	0.9515	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1513.875	216.2679	7	0.958	0.4828	Non-Significant Effect
Error	5418	225.75	24			
Total	6931.875		31			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.557	18.48	0.4764	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9715	0.9081	0.5410	Normal Distribution

Frond Count Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	4	71.25	48.07	94.43	69	57	90	7.284	20.45%	0.0%
1.5		4	85	67.23	102.8	84.5	73	98	5.583	13.14%	-19.3%
3		4	68.5	59.27	77.73	66.5	64	77	2.901	8.47%	3.86%
6.1		4	85	47.5	122.5	86.5	56	111	11.78	27.72%	-19.3%
12.1		4	80.25	62.61	97.89	76.5	72	96	5.543	13.82%	-12.63%
24.2		4	80	46.05	114	90.5	48	91	10.67	26.67%	-12.28%
48.5		4	71.75	55.3	88.2	73	59	82	5.17	14.41%	-0.7%
97		4	66.75	44.53	88.97	68.5	49	81	6.981	20.92%	6.32%

Frond Count Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	90	63	57	75
1.5		90	73	79	98
3		66	64	77	67
6.1		78	56	111	95
12.1		96	72	80	73
24.2		90	91	48	91
48.5		78	68	82	59
97		63	74	81	49

CETIS Analytical Report

Report Date: 26 Mar-14 09:01 (p 2 of 2)
Test Code: 14077b | 00-1835-0189

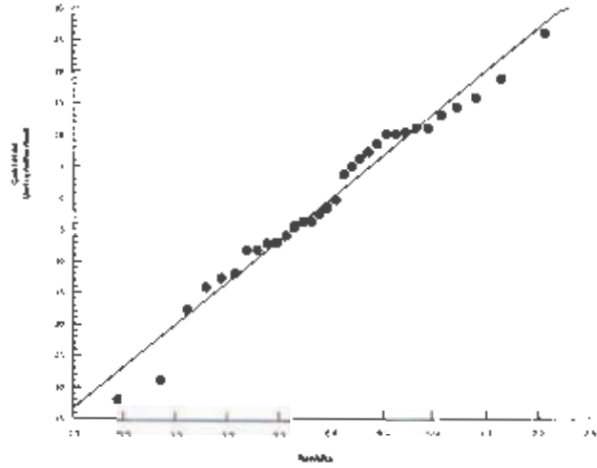
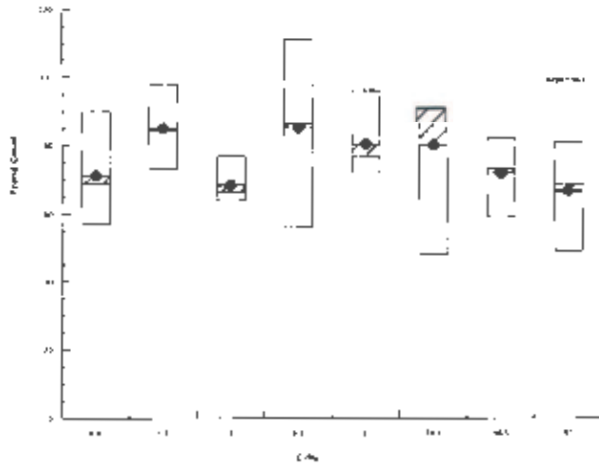
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 03-7731-0487 Endpoint: Frond Count
Analyzed: 26 Mar-14 9:00 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 26 Mar-14 09:01 (p 1 of 2)
 Test Code: 14077b | 00-1835-0189

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 05-6300-6427	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:01	Analysis: Nonlinear Regression	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 57h (4.3 °C)	Station: L1426336-2(NF1)	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
27	-21.39	49.63	53.17		Yes	0.401	2.621	0.8432	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	73.44	N/A	164.3	1.362	0.6086	NA
IC10	104.7	N/A	227	0.9551	0.4406	NA
IC15	133	N/A	546.4	0.7518	0.183	NA
IC20	160.9	N/A	N/A	0.6215	NA	NA
IC25	189.4	N/A	N/A	0.5279	NA	NA
IC40	285.8	N/A	N/A	0.3499	NA	NA
IC50	366	N/A	N/A	0.2732	NA	NA

} > 97% (v/v)
jon

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	7.411	0.27	6.882	7.94	27.45	<0.0001	Significant Parameter
C	0.9765	2.64	-4.197	6.15	0.3699	0.7141	Non-Significant Parameter
D	366	1349	-2278	3010	0.2713	0.7881	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	1.407145	1.407145	1	0.9106	0.3478	Non-Significant
Lack of Fit	3.455154	0.691031	5	0.401	0.8432	Non-Significant
Pure Error	41.35684	1.723202	24			
Residual	44.81199	1.545241	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	3.58	14.07	0.8267	Equal Variances
	Mod Levene Equality of Variance	0.464	2.423	0.8508	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9497	0.9338	0.1416	Normal Distribution
	Anderson-Darling A2 Normality	0.7518	2.492	0.0501	Normal Distribution

CETIS Analytical Report

Report Date: 26 Mar-14 09:01 (p 2 of 2)
 Test Code: 14077b , 00-1835-0189

Lamna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 05-6300-6427 Endpoint: Total Dry Weight-mg CETIS Version: CETISv1.8.7
 Analyzed: 26 Mar-14 9:01 Analysis: Nonlinear Regression Official Results: Yes

Total Dry Weight-mg Summary

Calculated Variate

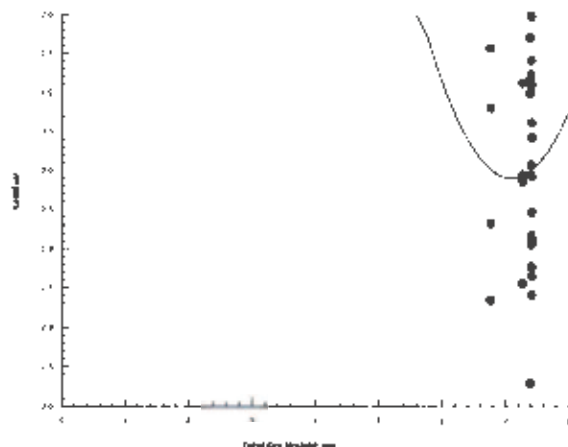
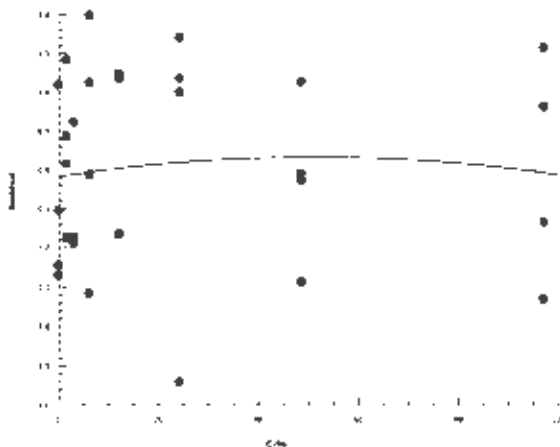
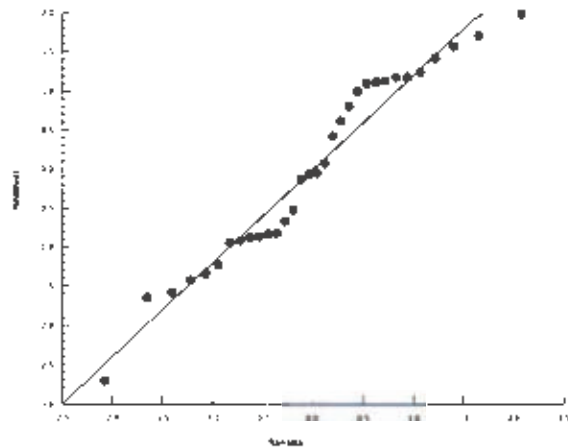
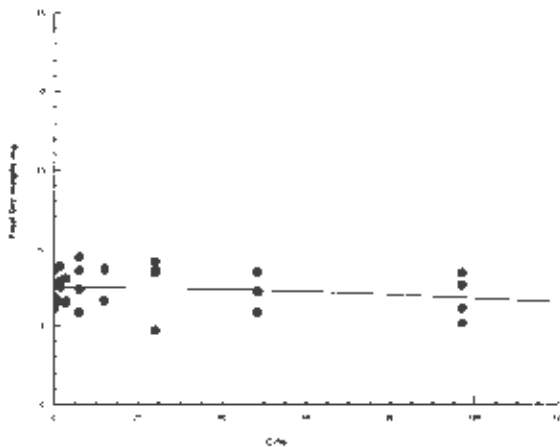
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	6.915	6.07	8.51	0.5616	1.123	16.24%	0.0%
1.5		4	7.675	6.54	8.83	0.4729	0.9458	12.32%	-10.99%
3		4	6.888	6.47	8.03	0.3812	0.7624	11.07%	0.4%
6.1		4	7.777	5.83	9.4	0.7732	1.546	19.88%	-12.47%
12.1		4	7.603	6.58	8.65	0.5876	1.175	15.46%	-9.94%
24.2		4	7.688	4.69	9.1	1.01	2.021	26.29%	-11.17%
48.5		4	7.15	5.84	8.4	0.5231	1.046	14.63%	-3.4%
97		4	6.785	5.12	8.34	0.7241	1.448	21.34%	1.88%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	8.51	6.19	6.07	6.89
1.5		7.84	6.54	7.49	8.83
3		6.5	6.47	8.03	6.55
6.1		7.35	5.83	9.4	8.53
12.1		8.65	6.59	8.59	6.58
24.2		9.1	8.57	4.69	8.39
48.5		7.22	7.14	8.4	5.84
97		6.1	7.58	8.34	5.12

Graphics

3P Cumulative Log-Normal EV (Y=A*(1-Phi(log(X/D)/C)))



CETIS Analytical Report

Report Date: 26 Mar-14 09:01 (p 1 of 2)
 Test Code: 14077b | 00-1835-0189

Lemna Growth Inhibition Test Nautilus Environmental

Analysis ID: 18-9090-8151	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:00	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 57h (4.3 °C)	Station: L1426336-2(NF1)	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	33.3%	97	>97	NA	1.031

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	1.5		0.8187	2.482	2.304	6	0.5656	CDF	Non-Significant Effect
	3		-0.02961	2.482	2.304	6	0.8822	CDF	Non-Significant Effect
	6.1		0.9292	2.482	2.304	6	0.5140	CDF	Non-Significant Effect
	12.1		0.7407	2.482	2.304	6	0.6018	CDF	Non-Significant Effect
	24.2		0.8322	2.482	2.304	6	0.5593	CDF	Non-Significant Effect
	48.5		0.2532	2.482	2.304	6	0.8006	CDF	Non-Significant Effect
	97		-0.14	2.482	2.304	6	0.9065	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.862298	0.6946141	7	0.4031	0.8909	Non-Significant Effect
Error	41.35684	1.723202	24			
Total	46.21914		31			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.58	18.48	0.8267	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9542	0.9081	0.1899	Normal Distribution

Total Dry Weight-mg Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	4	6.915	5.128	8.702	6.54	6.07	8.51	0.5616	16.24%	0.0%
1.5		4	7.675	6.17	9.18	7.665	6.54	8.83	0.4729	12.32%	-10.99%
3		4	6.888	5.674	8.101	6.525	6.47	8.03	0.3812	11.07%	0.4%
6.1		4	7.777	5.317	10.24	7.94	5.83	9.4	0.7732	19.88%	-12.47%
12.1		4	7.603	5.733	9.472	7.59	6.58	8.65	0.5876	15.46%	-9.94%
24.2		4	7.688	4.472	10.9	8.48	4.69	9.1	1.01	26.29%	-11.17%
48.5		4	7.15	5.485	8.815	7.18	5.84	8.4	0.5231	14.63%	-3.4%
97		4	6.785	4.481	9.089	6.84	5.12	8.34	0.7241	21.34%	1.88%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	8.51	6.19	6.07	6.89
1.5		7.84	6.54	7.49	8.83
3		6.5	6.47	8.03	6.55
6.1		7.35	5.83	9.4	8.53
12.1		8.65	6.59	8.59	6.58
24.2		9.1	8.57	4.69	8.39
48.5		7.22	7.14	8.4	5.84
97		6.1	7.58	8.34	5.12

CETIS Analytical Report

Report Date: 26 Mar-14 09:01 (p 2 of 2)
Test Code: 14077b | 00-1835-0189

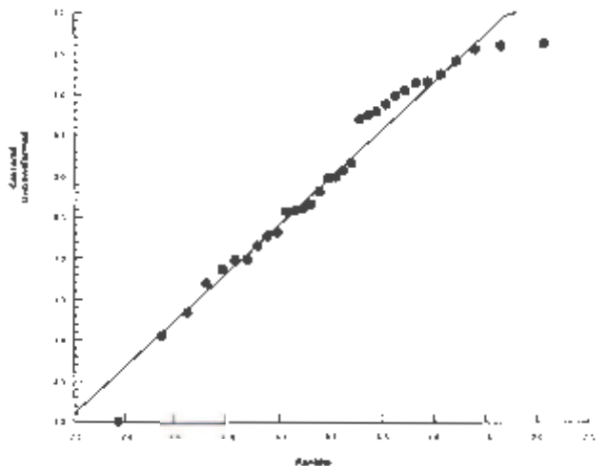
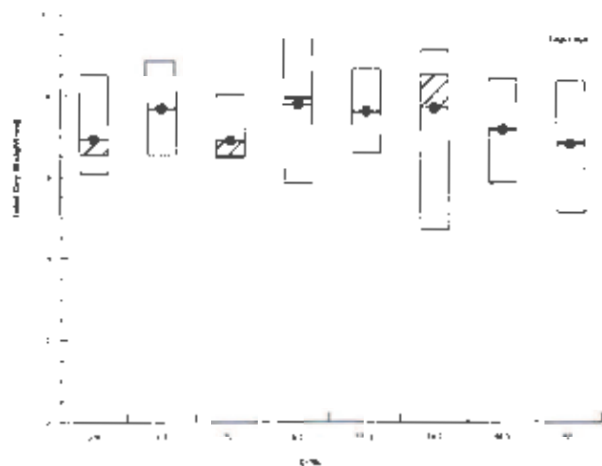
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 18-9090-8151 Endpoint: Total Dry Weight-mg
Analyzed: 26 Mar-14 9:00 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



Lemna minor Summary Sheet

Client: ALG
 Work Order No.: 14097

Start Date: Feb 28 / 14
 Set up by: TW / JBF

Sample Information:

Sample ID: L14205-6 - 3 (Nf 2)
 Sample Date: Feb 25 / 14 @ 1300h
 Date Received: Feb 27 / 14 @ 1030h
 Sample Volume: 2 x 20L

Test Organism Information:

Culture Date: 02/11/14
 Age of culture (Day 0): 9 days
 >8X growth in APHA?: Y

KCl Reference Toxicant Results:

Reference Toxicant ID: LM 100
 Date Initiated: Feb 26 - 2014

7-d No. of Fronds IC50 (95% CL): 5.0 (3.2 - 4.2)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 4.4 (2.5 - 5.5) CV (%): 12

	Number of Fronds	Dry Weight
Test Results: IC25 %(v/v) (95% CL)	43.7 (36 - 87.6)	70.5 - 75.7 - 47.7 >97
IC50 %(v/v) (95% CL)	7.97	>97

Reviewed by: Joh

Date reviewed: March 27 / 14

Plant Growth Inhibition Toxicity Test Water Quality Measurements

Client : ALS Setup by: JW / JBF
 Sample ID: NF - 2 (L1426336 - 3) (Brown) Test Date: Feb 28 / 14
 Work Order No.: 14077 Test Species: Lemna minor
 Culture Source: CPOC # 490
 Test Culture Age: 9 ^{JW} days > 8X Growth? (Y/N): Y
 Light Intensity Range: 4000 - 5600 lux Date Measured: Feb 26 / 14

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	25.0	26.0	25.5	25.5	26.0	26.0	26.0	26.0
Initials	JW	AW	M	JBF	JW	JW	JW	JW

Sample Characteristics: Initial Water Quality Adjusted Water Quality

Temperature (°C)	<u>25.5</u>	Aeration?:	<u>20 min</u>	Temperature (°C)	<u>25.0</u>
DO (mg/L)	<u>10.0</u>	Nutrients added?:	<u>Y</u>	DO (mg/L)	<u>8.9</u>
pH	<u>7.1</u>			pH	<u>7.3</u>
Conductivity (µS)	<u>332</u>			Conductivity (µS)	<u>1127</u>

Concentration % (v/v)	Temperature (°C)		pH		Conductivity (µS) 0 h
	Day 0	Day 7	Day 0	Day 7	
Control	24.0	25.0	8.4	8.2	878
1.5	24.0	25.0	8.1	8.3	885
^{JW} 3.0%	24.0	25.0	8.1	8.3	889
6.1	24.0	25.0	8.1	8.3	895
12.1	24.0	25.0	8.0	8.3	911
24.2	24.0	25.0	7.9	8.2	942
48.5	24.0	25.0	7.8	8.3	1006
97	25.0	25.0	7.3	8.4	1127
Initials	JW / JBF	KSL	JW / JBF	KSL	JW / JBF

Thermometer: Calibrated Thermometer Cond. Meter: C-2 pH meter: PH-2
 Sample Description: clear
 Comments: _____
 Reviewed: JW Date Reviewed: March 25/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS
 Sample ID: N103 (11406336-3) (ST001F)
 Work Order #: 14017 2

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14
 Test set up by: JN / JRF

Concentration (µg/L)	Rep	No. of fronds		Chlorosis ¹	Necrosis	Yellow	Abnormal size	Gleboisity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
Control	A	6	56										JN
	B	6	79										
	C	6	81										
	D	6	79										
10	A	6	66										JN
	B	6	76										
	C	6	75										
	D	6	73										
20	A	6	68										JN
	B	6	64										
	C	6	87										
	D	6	62										
40	A	6	116										JN
	B	6	72										
	C	6	94										
	D	6	74										
80	A	6	84										JN
	B	6	67										
	C	6	104										
	D	6	79										
160	A	6	59										JN
	B	6	61										
	C	6	61										
	D	6	59										

Comments: _____

Reviewed by: JN Date Reviewed: March 26/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALC Start Date: Feb 28 / 14
 Sample ID: AF-R (1496326 3) (R00A) Termination Date: March 7 / 14
 Work Order #: 14071 Test set up by: JW / JEF

Concentration (µg/L)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
42.5	A	6	53			X							JW
	B	6	54			X							
	C	6	58			X							
	D	6	60			X							
44	A	6	52			X							JW
	B	6	50			X							
	C	6	49			X							
	D	6	55			X							
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments: _____
 Reviewed by: John Date Reviewed: March 26/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: NF 2 (14-JW L - 1426336 - 3)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration %D (V/V)	Rep	Pan No. Brown	Pan weight (mg)	Pan + plant (mg)	Initials
control	A	1	991.56	996.26	NY/JW
	B	2	1029.50	1035.81	
	C	3	1044.13	1050.93	
	D	4	1041.15	1047.24	
1.5	A	5	1024.57	1029.40	
	B	6	1037.20	1044.04	
	C	7	1019.92	1026.18	
	D	8	1032.17	1038.57	
3.0% JW	A	9	1018.77	1024.63	
	B	10	1040.24	1045.65	
	C	11	1051.75	1058.84	
	D	12	1005.72	1010.92	
6.1	A	13	1028.86	1038.51	
	B	14	1029.32	1036.10	
	C	15	1015.85	1023.47	
	D	16	1002.84	1009.41	
12.1	A	17	1036.80	1044.63 ^{JW} ₅₃	
	B	18	997.43	1003.70	
	C	19	991.23	999.50	
	D	20	1023.04	1029.99	
24.2	A	21	996.32	1001.94	
	B	22	1005.39	1010.80	
	C	23	1016.18	1021.39	
	D	24	991.94	997.85	
48.5	A	25	1002.93	1007.84	
	B	26	1002.31	1007.37	
	C	27	1012.44	1017.46	
	D	28	992.97	999.28	

Comments: Reweighed pans 4-1047.22 12-1010.80 20-1029.77

Reviewed by: JGU Date Reviewed: March 26/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: NF 2 (L 1426336 - 3)
 Work Order #: 14077

Start Date: Feb 28/14
 Termination Date: March 7/14

Concentration % (v/v)	Rep	Pan No. <small>Brown</small>	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1017.34	1021.95	NJ/JW
	B	30	1019.36	1023.90	↓
	C	31	1019.55	1024.15	↓
	D	32	1033.55	1038.84	↓
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments: _____

Reviewed by: JGu

Date Reviewed: March 26/14

CETIS Analytical Report

Report Date: 26 Mar-14 09:15 (p 1 of 2)
 Test Code: 14077c | 11-2740-7920

Lemna Growth Inhibition Test			Nautilus Environmental		
Analysis ID: 14-2764-6854	Endpoint: Frond Count	CETIS Version: CETISv1.8.7	Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Analyzed: 26 Mar-14 9:10	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes	Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
			Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
			Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 07-0729-5812	Code: 2A287A44	Client: ALS	Sample Date: 25 Feb-14 13:05	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS		Sample Age: 59h (3.3 °C)	Station: L1426336-3(NF2)	

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	930197	200	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	13.95	N/A	14.97	7.17	6.682	NA
IC10	16.06	10.87	18.43	6.228	5.426	9.198
IC15	18.46	13.64	22.61	5.417	4.423	7.33
IC20	21.21	17.01	30.85	4.715	3.241	5.879
IC25	24.89	19.55	72.35	4.018	1.382	5.116
IC40	>97	N/A	N/A	<1.031	NA	NA
IC50	>97	N/A	N/A	<1.031	NA	NA

Frond Count Summary			Calculated Variate						
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	67.75	50	75	5.935	11.87	17.52%	0.0%
1.5		4	66.5	60	70	2.255	4.509	6.78%	1.85%
3		4	64.25	56	81	5.721	11.44	17.81%	5.17%
6.1		4	83	66	110	10.28	20.56	24.77%	-22.51%
12.1		4	77.5	61	98	7.708	15.42	19.89%	-14.39%
24.2		4	54	53	55	0.5774	1.155	2.14%	20.3%
48.5		4	50.25	47	54	1.652	3.304	6.58%	25.83%
97		4	45.5	43	49	1.323	2.646	5.82%	32.84%

Frond Count Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	50	73	75	73
1.5		60	70	69	67
3		62	58	81	56
6.1		110	66	88	68
12.1		78	61	98	73
24.2		53	55	55	53
48.5		47	48	52	54
97		46	44	43	49

CETIS Analytical Report

Report Date: 26 Mar-14 09:15 (p 2 of 2)
Test Code: 14077c | 11-2740-7920

Lemna Growth Inhibition Test

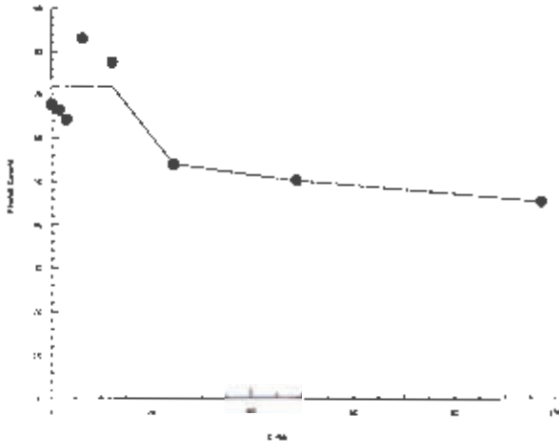
Nautilus Environmental

Analysis ID: 14-2764-6854
Analyzed: 26 Mar-14 9:10

Endpoint: Frond Count
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 27 Mar-14 14:20 (p 1 of 2)
 Test Code: 14077c-1 | 05-9886-5460

Lemna Growth Inhibition Test Nautilus Environmental

Analysis ID: 20-4529-5790	Endpoint: Frond Count	CETIS Version: CETISv1.8.7
Analyzed: 27 Mar-14 14:20	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 07-7012-6724	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age:
Sample ID: 11-0727-3304	Code: 41FFA658	Client: ALS
Sample Date: 25 Feb-14 13:05	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 59h	Station: L1426336-3(NF2)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1457585	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	13.69	N/A	15.75	7.304	6.349	NA
IC10	16.52	N/A	19.91	6.052	5.022	NA
IC15	19.91	N/A	26.24	5.024	3.811	NA
IC20	23.94	8.992	63.24	4.177	1.581	11.12
IC25	43.73	7.605	87.63	2.287	1.141	13.15
IC40	>97	N/A	N/A	<1.031	NA	NA
IC50	>97	N/A	N/A	<1.031	NA	NA

FronD Count Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	67.75	50	75	5.935	11.87	17.52%	0.0%
1.5		4	66.5	60	70	2.255	4.509	6.78%	1.85%
3		4	64.25	56	81	5.721	11.44	17.81%	5.17%
6.1		4	67.75	50	75	5.935	11.87	17.52%	0.0%
12.1		4	67.75	50	75	5.935	11.87	17.52%	0.0%
24.2		4	54	53	55	0.5774	1.155	2.14%	20.3%
48.5		4	50.25	47	54	1.652	3.304	6.58%	25.83%
97		4	45.5	43	49	1.323	2.646	5.82%	32.84%

FronD Count Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	50	73	75	73
1.5		60	70	69	67
3		62	58	81	56
6.1		50	73	75	73
12.1		50	73	75	73
24.2		53	55	55	53
48.5		47	48	52	54
97		46	44	43	49

CETIS Analytical Report

Report Date: 27 Mar-14 14:21 (p 2 of 2)
Test Code: 14077c-1 | 05-9886-5460

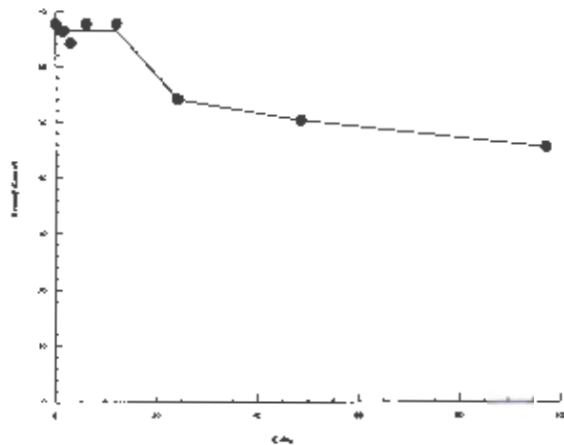
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 20-4529-5790 Endpoint: Frond Count
Analyzed: 27 Mar-14 14:20 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 26 Mar-14 09:15 (p 1 of 2)
 Test Code: 14077c | 11-2740-7920

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 08-7246-2597	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:10	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 07-0729-5812	Code: 2A287A44	Client: ALS
Sample Date: 25 Feb-14 13:05	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 59h (3.3 °C)	Station: L1426336-3(NF2)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1597636	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	15.1	13.49	18.08	6.622	5.532	7.413
IC10	18.79	15.31	26.53	5.323	3.77	6.53
IC15	23.32	17.33	79.74	4.288	1.254	5.771
IC20	52.21	5.336	112.7	1.915	0.8869	18.74
IC25	78.21	15.27	N/A	1.279	NA	6.551
IC40	>97	N/A	N/A	<1.031	NA	NA
IC50	>97	N/A	N/A	<1.031	NA	NA

Total Dry Weight-mg Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	5.975	4.7	6.8	0.4502	0.9003	15.07%	0.0%
1.5		4	6.083	4.83	6.84	0.4354	0.8708	14.32%	-1.8%
3		4	5.89	5.2	7.09	0.423	0.846	14.36%	1.42%
6.1		4	7.655	6.57	9.65	0.7026	1.405	18.36%	-28.12%
12.1		4	7.305	6.27	8.27	0.4387	0.8773	12.01%	-22.26%
24.2		4	5.537	5.21	5.91	0.1497	0.2995	5.41%	7.32%
48.5		4	5.325	4.91	6.31	0.3299	0.6597	12.39%	10.88%
97		4	4.76	4.54	5.29	0.1773	0.3546	7.45%	20.34%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	4.7	6.31	6.8	6.09
1.5		4.83	6.84	6.26	6.4
3		5.86	5.41	7.09	5.2
6.1		9.65	6.78	7.62	6.57
12.1		7.73	6.27	8.27	6.95
24.2		5.62	5.41	5.21	5.91
48.5		4.91	5.06	5.02	6.31
97		4.61	4.54	4.6	5.29

CETIS Analytical Report

Report Date: 26 Mar-14 09:15 (p 2 of 2)
Test Code: 14077c | 11-2740-7920

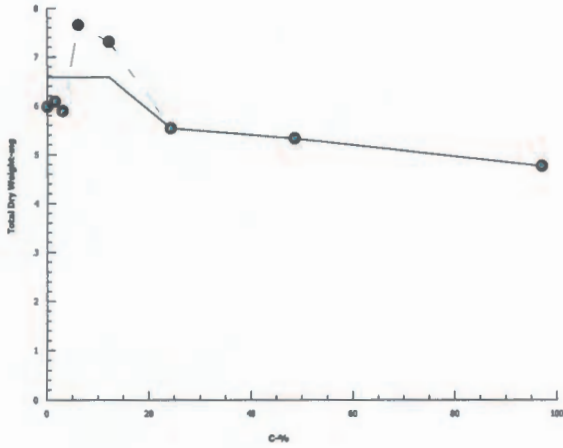
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 08-7246-2597 Endpoint: Total Dry Weight-mg
Analyzed: 26 Mar-14 9:10 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 27 Mar-14 15:22 (p 1 of 2)
 Test Code: 14077c-1 | 05-9886-5460

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 02-8991-5851	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 27 Mar-14 15:21	Analysis: Nonlinear Regression	Official Results: Yes
Batch ID: 07-7012-6724	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age:
Sample ID: 11-0727-3304	Code: 41FFA658	Client: ALS
Sample Date: 25 Feb-14 13:05	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 59h	Station: L1426336-3(NF2)	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
14	-2.612	12.08	15.62	0.2405	Yes	0.06666	2.621	0.9966	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	20.42	N/A	49	4.896	2.041	NA
IC10	40.22	12.49	75.09	2.486	1.332	8.009
IC15	63.54	31.52	104.7	1.574	0.955	3.173
IC20	91.38	40.79	164	1.094	0.6099	2.452
IC25 JW	124.8	40.08	274.2	0.8012	0.3646	2.495
IC40 JW	273.8	22	1508	0.3652	0.06633	4.546
IC50 JW	439.2	13.3	14510	0.2277	0.006894	7.52

} > 97% (V/V)

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	6.014	0.2064	5.609	6.418	29.14	<0.0001	Significant Parameter
C	1.865	1.16	-0.4076	4.138	1.609	0.1185	Non-Significant Parameter
D	439.2	476.3	-494.4	1373	0.922	0.3641	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	5.84683	5.84683	1	11.82	0.0018	Significant
Lack of Fit	0.189846	0.037969	5	0.06666	0.9966	Non-Significant
Pure Error	13.6695	0.569562	24			
Residual	13.85934	0.477908	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	5.479	14.07	0.6017	Equal Variances
	Mod Levene Equality of Variance	0.3557	2.423	0.9188	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9597	0.9338	0.2689	Normal Distribution
	Anderson-Darling A2 Normality	0.3783	2.492	0.4116	Normal Distribution

CETIS Analytical Report

Report Date: 27 Mar-14 15:22 (p 2 of 2)
 Test Code: 14077c-1 | 05-9886-5460

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 02-8991-5851 Endpoint: Total Dry Weight-mg
 Analyzed: 27 Mar-14 15:21 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

Total Dry Weight-mg Summary

Calculated Variate

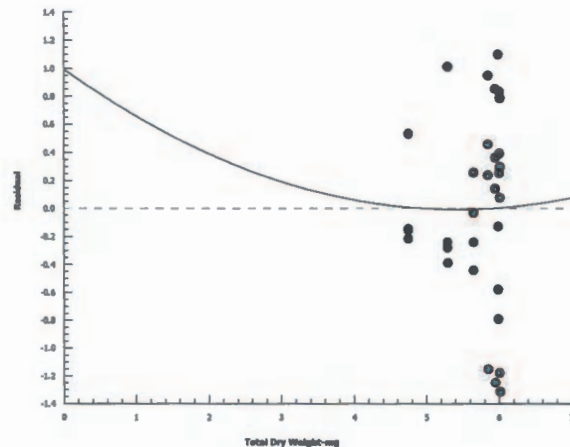
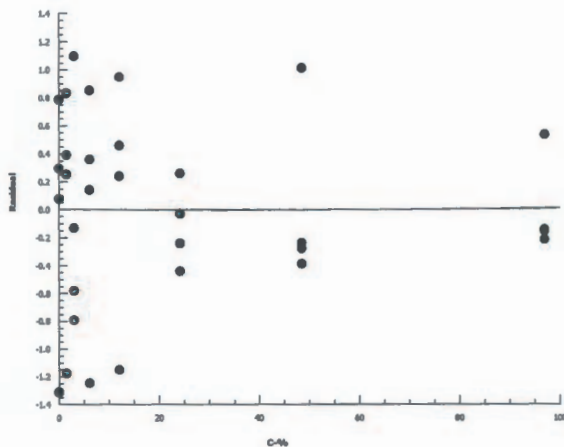
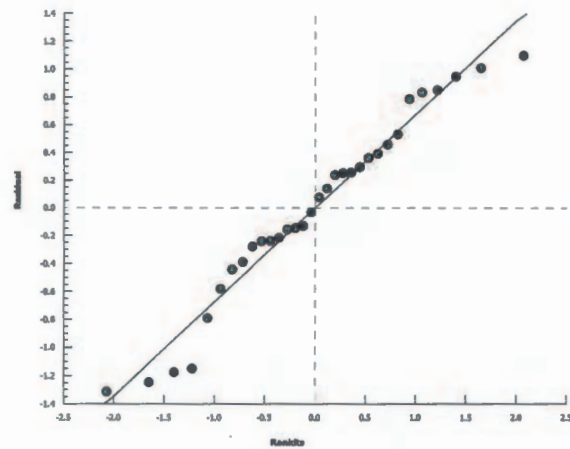
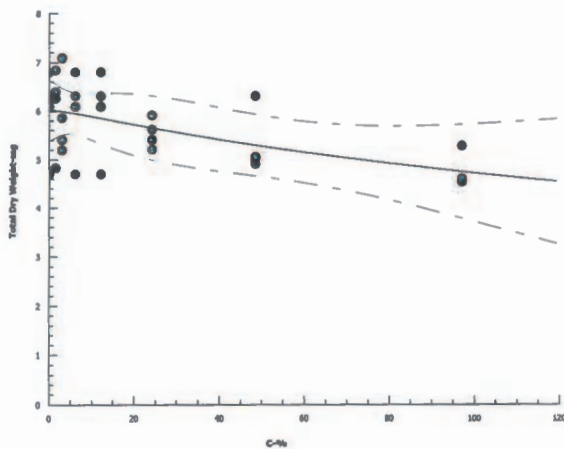
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	5.975	4.7	6.8	0.4502	0.9003	15.07%	0.0%
1.5		4	6.083	4.83	6.84	0.4354	0.8708	14.32%	-1.8%
3		4	5.89	5.2	7.09	0.423	0.846	14.36%	1.42%
6.1		4	5.975	4.7	6.8	0.4502	0.9003	15.07%	0.0%
12.1		4	5.975	4.7	6.8	0.4502	0.9003	15.07%	0.0%
24.2		4	5.537	5.21	5.91	0.1497	0.2995	5.41%	7.32%
48.5		4	5.325	4.91	6.31	0.3299	0.6597	12.39%	10.88%
97		4	4.76	4.54	5.29	0.1773	0.3546	7.45%	20.34%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	4.7	6.31	6.8	6.09
1.5		4.83	6.84	6.26	6.4
3		5.86	5.41	7.09	5.2
6.1		4.7	6.31	6.8	6.09
12.1		4.7	6.31	6.8	6.09
24.2		5.62	5.41	5.21	5.91
48.5		4.91	5.06	5.02	6.31
97		4.61	4.54	4.6	5.29

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



Lemna minor Summary Sheet

Client: ALS
Work Order No.: 14077

Start Date: Feb 28, 2014
Set up by: JW / JBF

Sample Information:

Sample ID: E1420336-4 (X-1)
Sample Date: Feb 26 / 14 @ 110h
Date Received: Feb 27 / 14 @ 1030h
Sample Volume: 2 x 20L

Test Organism Information:

Culture Date: 021914
Age of culture (Day 0): 9 days
>8X growth in APHA?: Y

KCI Reference Toxicant Results:

Reference Toxicant ID: LM 100
Date Initiated: Feb 20, 2014

7-d No. of Fronds IC50 (95% CL): 3.0 (3.2 - 4.2)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 4.4 (3.5 - 5.5) CV (%): 12

	Number of Fronds	Dry Weight
Test Results: IC25 %(v/v) (95% CL)	48.5 (24.5 - 85.6)	>97
IC50 %(v/v) (95% CL)	>97	>97

Reviewed by: J. J. J.

Date reviewed: March 27/14

Plant Growth Inhibition Toxicity Test Water Quality Measurements

Client: ALS Setup by: JW / JBF
 Sample ID: X-1 (L1426336-4) (orange) Test Date: Feb 28/14
 Work Order No.: 14077 Test Species: Lemna minor
 Culture Source: CPCC # 490
 Test Culture Age: 9 th days > 8X Growth? (Y/N): Y
 Light Intensity Range: 4000 - 5600 lux Date Measured: Feb 26/14

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	25.0	26.0	25.5	25.5	26.0	26.0	26.0	26.0
Initials	JW	A	A	JBF	JW	JW	JW	JW

Sample Characteristics: **Initial Water Quality**
 Temperature (°C) 25.5
 DO (mg/L) 10.3
 pH 7.2
 Conductivity (µS) 349

Aeration?: 20 min
 Nutrients added?: Y

Adjusted Water Quality
24.5
8.5
7.5
1141

Concentration % (v/v)	Temperature (°C)		pH		Conductivity (µS) 0 h
	Day 0	Day 7	Day 0	Day 7	
Control	24.0	25.0	8.4	8.3	878
1.5	24.0	25.0	8.1	8.3	898
^{JW} 3.08	24.0	25.0	8.1	8.3	906
6.1	24.0	25.0	8.1	8.4	906
12.1	24.5	25.0	8.1	8.4	919
24.2	25.0	25.0	8.0	8.3	946
48.5	25.0	25.0	7.9	8.4	1014
97	24.5	25.0	7.5	8.4	1141
Initials	JW / JBF	AS	JW / JBF	AS	JW / JBF

Thermometer: Calibrated Thermometer Cond. Meter: C-2 pH meter: pH-2

Sample Description: clear

Comments: _____

Reviewed: JGh Date Reviewed: March 25/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALC Start Date: Feb 28 / 14
 Sample ID: X 1 (L1426336 - 4) (060092) Termination Date: March 7 / 14
 Work Order #: 14077 Test set up by: JW / JEF

Concentration (µg/L)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Roof destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
Control	A	6	66										JW
	B	6	77										
	C	6	60										
	D	6	106										
1.0	A	6	75										
	B	6	97										
	C	6	84										
	D	6	86										
2.0	A	6	66										
	B	6	88										
	C	6	69		x	y							
	D	6	82			x							
4.0	A	6	72			x							
	B	6	64			x							
	C	6	66			x							
	D	6	77			x							
8.0	A	6	75			x							
	B	6	65			y							
	C	6	80			y							
	D	6	73			y							
16.0	A	6	61			y							
	B	6	53			y							
	C	6	94			y							
	D	6	71			y							

Comments: _____

Reviewed by: JW Date Reviewed: March 25/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS Start Date: Feb 28/14
 Sample ID: X1 (L1426136 - 4) : (Orange) Termination Date: March 7 / 14
 Work Order #: 14077 Test set up by: JW / JBF

Concentration (% v/v)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
0.0%	A	60	60			X		X					JW
	B	60	60			X							
	C	60	60			X		X					
	D	60	60			X							
0.1%	A	61	61			X							
	B	60	60			X							
	C	60	60			X							
	D	60	60			X							
0.2%	A	60	60			X							
	B	60	60			X							
	C	60	60			X							
	D	60	60			X							
0.3%	A	60	60			X							
	B	60	60			X							
	C	60	60			X							
	D	60	60			X							

Comments: _____

Reviewed by: JGlu Date Reviewed: March 25/14

7-d Lemna minor Weight Data Sheet

Client: ALS Start Date: Feb 28/14
 Sample ID: X1 (L1426336 - 4) (orange) Termination Date: March 7/14
 Work Order #: 14077

Concentration % (v/v)	Rep	Pan No. <i>Orange</i>	Pan weight (mg)	Pan + plant (mg)	Initials
control	A	1	1222.95	1227.76	NY/JW
	B	2	1244.64	1250.51	
	C	3	1223.28	1227.78	
	D	4	1250.64	1258.09	
1.5	A	5	1218.00	1223.44	
	B	6	1230.65	1238.62	
	C	7	1245.92	1252.55	
	D	8	1246.56	1253.23	
3.0% <i>JW</i>	A	9	1264.17	1269.51	
	B	10	1232.70	1239.84	
	C	11	1234.79	1240.26	
	D	12	1229.47	1236.16	
6.1	A	13	1245.32	1251.62	
	B	14	1247.10	1252.19	
	C	15	1219.59	1224.26	
	D	16	1231.37	1237.25	
12.1	A	17	1229.68	1235.86	
	B	18	1239.35	1245.21 ⁸	
	C	19	1232.49	1239.79	
	D	20	1209.69	1215.20	
24.2	A	21	1224.78	1230.22	
	B	22	1217.25	1222.22	
	C	23	1238.72	1246.03	
	D	24	1245.10	1251.17	
48.5	A	25	1251.74	1257.57	
	B	26	1230.18	1235.85	
	C	27	1254.43	1259.62	
	D	28	1233.55	1238.58	

Comments: Reweighed pans - 8-1253.28 12-1236.13 19-1239.77

Reviewed by: JGu Date Reviewed: March 26/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: X1 (L1426336 - 4)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration % (v/v)	Rep	Pan No. Orange	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1248.17	1254.02	NY/JW ↓ ↓ ↓ ↓
	B	30	1241.45	1247.00	
	C	31	1226.01	1230.94	
	D	32	1231.49	1236.03	
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments: _____

Reviewed by: JBU Date Reviewed: March 26 / 14

CETIS Analytical Report

Report Date: 26 Mar-14 09:22 (p 1 of 2)
 Test Code: 14077d | 11-8240-3329

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 17-4325-8399	Endpoint: Frond Count	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:20	Analysis: Nonlinear Regression	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 19-2140-4869	Code: 72864FC5	Client: ALS
Sample Date: 25 Feb-14 11:10	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 61h (4 °C)	Station: L1426336-4(X1)	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
10	-92.56	192	195.5	0.3445	Yes	0.6124	2.621	0.6914	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	5.115	N/A	15.56	19.55	6.429	NA
IC10	11.88	1.618	28.72	8.42	3.481	61.82
IC15	20.97	7.006	43.22	4.769	2.314	14.27
IC20	32.94	15.11	60.25	3.036	1.66	6.618
IC25	48.53	24.54	85.59	2.06	1.168	4.076
IC40	128.9	42.79	342.8	0.7759	0.2917	2.337
IC50	231.9	49.85	1079	0.4312	0.09268	2.006

} > 97% (v/v)

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	73.89	4.539	65	82.79	16.28	<0.0001	Significant Parameter
C	2.319	1.129	0.1052	4.533	2.053	0.0492	Significant Parameter
D	231.9	156.1	-74.05	537.9	1.486	0.1482	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	2415.18	2415.18	1	18.29	0.0002	Significant
Lack of Fit	433.2885	86.6577	5	0.6124	0.6914	Non-Significant
Pure Error	3396.25	141.5104	24			
Residual	3829.539	132.0531	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	9.301	14.07	0.2317	Equal Variances
	Mod Levene Equality of Variance	1.161	2.423	0.3606	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9585	0.9338	0.2491	Normal Distribution
	Anderson-Darling A2 Normality	0.4328	2.492	0.3079	Normal Distribution

CETIS Analytical Report

Report Date: 26 Mar-14 09:22 (p 2 of 2)
 Test Code: 14077d | 11-8240-3329

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 17-4325-8399 Endpoint: Frond Count CETIS Version: CETISv1.8.7
 Analyzed: 26 Mar-14 9:20 Analysis: Nonlinear Regression Official Results: Yes

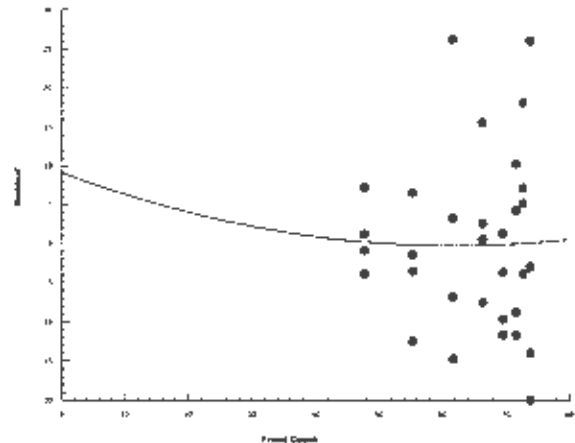
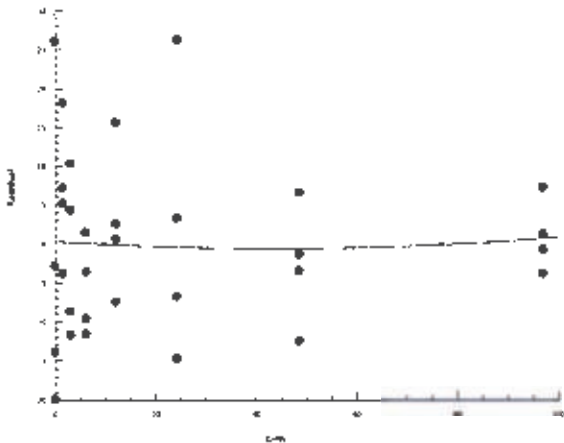
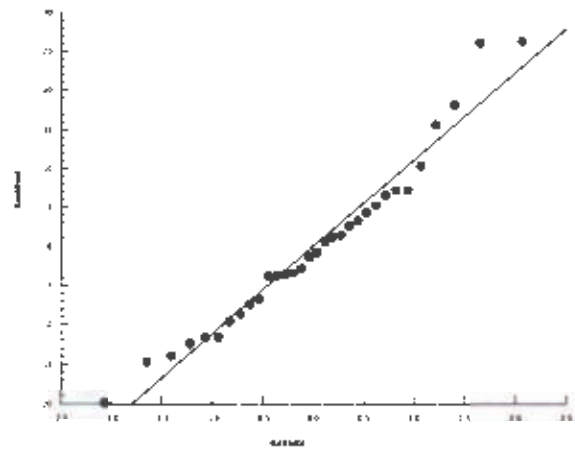
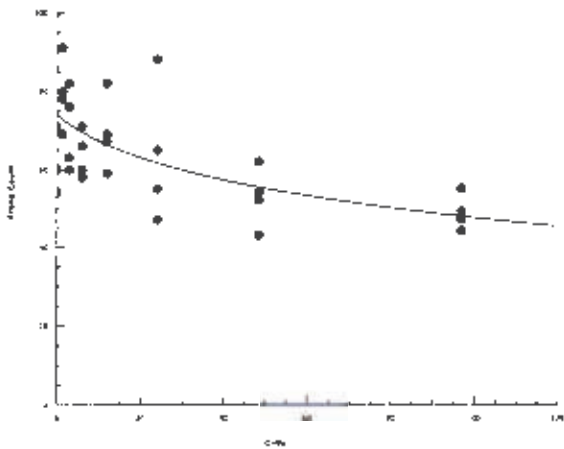
Frond Count Summary			Calculated Variate						
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	71.25	54	100	10.21	20.42	28.66%	0.0%
1.5		4	79.5	69	91	4.518	9.037	11.37%	-11.58%
3		4	70.25	60	82	5.234	10.47	14.9%	1.4%
6.1		4	63.75	58	71	2.955	5.909	9.27%	10.53%
12.1		4	69.25	59	82	4.768	9.535	13.77%	2.81%
24.2		4	63.75	47	88	8.882	17.76	27.87%	10.53%
48.5		4	52.75	43	62	3.902	7.805	14.8%	25.96%
97		4	48.75	44	55	2.323	4.646	9.53%	31.58%

Frond Count Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	60	71	54	100
1.5		69	91	78	80
3		60	82	63	76
6.1		66	58	60	71
12.1		69	59	82	67
24.2		55	47	88	65
48.5		54	62	52	43
97		55	49	47	44

Graphics

3P Cumulative Log-Normal EV (Y=A*(1-Phi(log(X/D)/C)))



CETIS Analytical Report

Report Date: 26 Mar-14 09:22 (p 1 of 2)
 Test Code: 14077d | 11-8240-3329

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 19-3306-0699	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:21	Analysis: Nonlinear Regression	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 19-2140-4869	Code: 72864FC5	Client: ALS
Sample Date: 25 Feb-14 11:10	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 61h (4 °C)	Station: L1426336-4(X1)	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Log-Logistic EV [Y=A/(1+(X/D)^C)]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
32	-10.55	27.96	31.5	0.0494	Yes	0.9671	2.621	0.4574	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	30.78	N/A	92.13	3.249	1.085	NA
IC10	62.41	4.96	128.4	1.602	0.7787	20.16
IC15	96.67	7.895	211.6	1.034	0.4727	12.67
IC20	134.4	N/A	416.5	0.744	0.2401	NA
IC25	176.4	N/A	885	0.5668	0.113	NA
IC40	339.9	N/A	N/A	0.2942	NA	NA
IC50	498.9	N/A	N/A	0.2005	NA	NA

} > 97% (V/V)

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	6.078	0.2748	5.539	6.617	22.12	<0.0001	Significant Parameter
C	1.057	1.298	-1.487	3.601	0.8144	0.4221	Non-Significant Parameter
D	498.9	1062	-1582	2580	0.4698	0.6420	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	2.834797	2.834797	1	3.611	0.0674	Non-Significant
Lack of Fit	3.817487	0.763498	5	0.9671	0.4574	Non-Significant
Pure Error	18.94647	0.789436	24			
Residual	22.76396	0.784964	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	4.666	14.07	0.7007	Equal Variances
	Mod Levene Equality of Variance	0.7234	2.423	0.6536	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9784	0.9338	0.7513	Normal Distribution
	Anderson-Darling A2 Normality	0.2809	2.492	0.6705	Normal Distribution

CETIS Analytical Report

Report Date: 26 Mar-14 09:22 (p 2 of 2)
 Test Code: 14077d | 11-8240-3329

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 19-3306-0699
 Analyzed: 26 Mar-14 9:21

Endpoint: Total Dry Weight-mg
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

Total Dry Weight-mg Summary

Calculated Variate

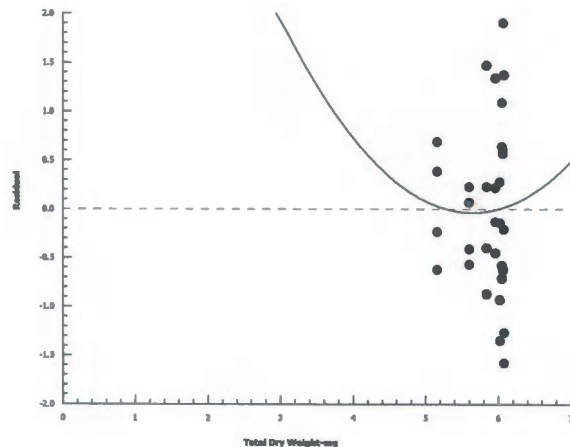
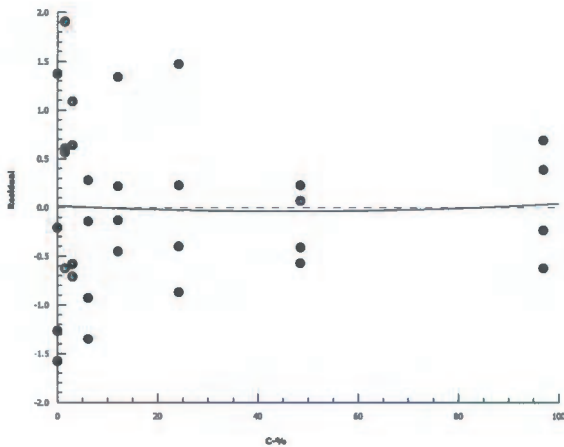
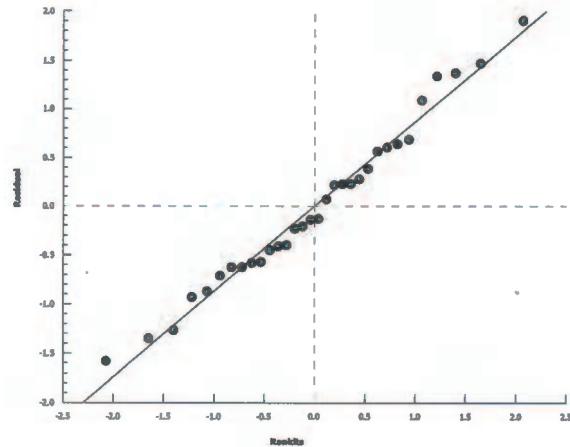
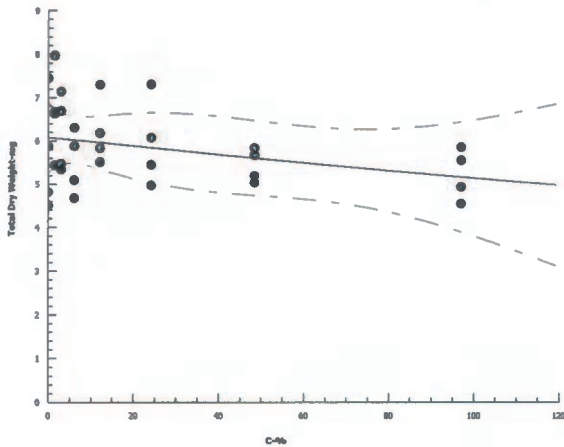
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	5.658	4.5	7.45	0.6656	1.331	23.53%	0.0%
1.5		4	6.677	5.44	7.97	0.5167	1.033	15.48%	-18.03%
3		4	6.16	5.34	7.14	0.4463	0.8926	14.49%	-8.88%
6.1		4	5.485	4.67	6.3	0.3697	0.7395	13.48%	3.05%
12.1		4	6.205	5.51	7.3	0.3898	0.7796	12.56%	-9.68%
24.2		4	5.948	4.97	7.31	0.507	1.014	17.05%	-5.13%
48.5		4	5.43	5.03	5.83	0.1904	0.3809	7.02%	4.02%
97		4	5.217	4.54	5.85	0.2961	0.5922	11.35%	7.78%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	4.81	5.87	4.5	7.45
1.5		5.44	7.97	6.63	6.67
3		5.34	7.14	5.47	6.69
6.1		6.3	5.09	4.67	5.88
12.1		6.18	5.83	7.3	5.51
24.2		5.44	4.97	7.31	6.07
48.5		5.83	5.67	5.19	5.03
97		5.85	5.55	4.93	4.54

Graphics

3P Log-Logistic EV [Y=A/(1+(X/D)^C)]



Lemna minor Summary Sheet

Client: ALS
Work Order No.: 14077

Start Date: Feb 28, 2014
Set up by: JW / JBF

Sample Information:

Sample ID: L 1426335 - 5 (X-14)
Sample Date: Feb 25/14 @ 0900h
Date Received: Feb 27/14 @ 1030h
Sample Volume: 2 X 20L

Test Organism Information:

Culture Date: 021914
Age of culture (Day 0): 9 days
>8X growth in APHA?: Y

KCI Reference Toxicant Results:

Reference Toxicant ID: LM 100
Date Initiated: Feb 20, 2014

7-d No. of Fronds IC50 (95% CL): 3.6 (3.2 - 4.2)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 4.4 (3.5 - 5.5) CV (%): 12

	Number of Fronds	Dry Weight
Test Results: IC25 %(v/v) (95% CL)	37.5 (25.8 - 53.2)	>97
IC50 %(v/v) (95% CL)	87.0 (48.0 - 97)	>97

Reviewed by: JGh

Date reviewed: March 27/14

Plant Growth Inhibition Toxicity Test Water Quality Measurements

Client : ALS Setup by: JW / JBF
 Sample ID: X-14 (L1426336-5) (black) Test Date: Feb 28 / 14
 Work Order No.: 14077 Test Species: Lemna minor
 Culture Source: CPCC # 490
 Test Culture Age: 10 days > 8X Growth? (Y/N): Y
 Light Intensity Range: 4100 - 4600 lux Date Measured: Feb 26 / 14

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	25.0	25.0	25.5	25.5	26.0	26.0	25.0	25.0
Initials	JW	AS	AS	JBF	JW	JW	JW	JW

Sample Characteristics: Initial Water Quality Adjusted Water Quality

Temperature (°C)	<u>25.0</u>	Aeration?: <u>20</u> min	<u>24.0</u>
DO (mg/L)	<u>9.8</u>	Nutrients added?: <u>Y</u>	<u>8.9</u>
pH	<u>7.1</u>		<u>7.4</u>
Conductivity (µS)	<u>831</u>		<u>1552</u>

Concentration % (V/V)	Temperature (°C)		pH		Conductivity (µS) 0 h
	Day 0	Day 7	Day 0	Day 7	
Control	24.0	23.0	8.4	8.2	878
1.5	24.0	23.0	8.1	8.4	894
3.0 ³⁰	24.0	23.0	8.1	8.5	906
6.1	24.0	23.0	8.0	8.6	929
12.1	24.0	23.0	8.0	8.5	974
24.2	24.0	23.0	7.9	8.8 ²⁰	1060
48.5	24.0	23.0	7.7	8.4	1222
97	24.0	23.0	7.4	8.4	1552
Initials	JW / JBF	JW	JW / JBF	JW	JW / JBF

Thermometer: Calibrated Thermometer Cond. Meter: C-2 pH meter: PH-2

Sample Description: clear

Comments: _____

Reviewed: JGh Date Reviewed: March 26 / 14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS
 Sample ID: X.14 (L 426336 - 5) (BIOCK)
 Work Order #: 14077

Start Date: Feb 28/14
 Termination Date: March 7/14
 Test set up by: JW / JBP

Concentration % (v/v)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
control	A	6	96										JW
	B	6	96										
	C	6	88										
	D	6	111										
1.5	A	6	95										JW
	B	6	108			X							
	C	6	115			X							
	D	6	70										
3.0% JW	A	6	130			X							JW
	B	6	84			X							
	C	6	132			X							
	D	6	83			X							
6.1	A	6	93			X							JW
	B	6	84			X							
	C	6	113			X							
	D	6	83			X							
12.1	A	6	119			X							JW
	B	6	81			X							
	C	6	95			X							
	D	6	93			X							
24.2	A	6	101			X							JW
	B	6	93			X							
	C	6	100			X							
	D	6	97			X							

Comments: _____

Reviewed by: JW Date Reviewed: March 26/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS Start Date: Feb 28 / 14
 Sample ID: X 14 (L 1426336 - 5) (BLOCK) Termination Date: MARCh 7 / 14
 Work Order #: 14077 Test set up by: JW / JBF

Concentration % _{v/v}	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
48.5	A	6	59		X	X							JW
	B	6	59		X	X							
	C	6	66		X	X							
	D	6	79		X	X							
97	A	6	46		X	X			X				JW
	B	6	46		X	X			X				
	C	6	52		X	X			X				
	D	6	60		X	X			X				
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments: _____
 Reviewed by: JG Date Reviewed: March 26/14

7-d Lemna minor Weight Data Sheet

Client: ALS Start Date: Feb 28 / 14
 Sample ID: X14 (L1426336 - 5) (black) Termination Date: March 7 / 14
 Work Order #: 14077

Concentration % (v/v)	Rep	Pan No. BLACK	Pan weight (mg)	Pan + plant (mg)	Initials
control	A	1	997.24	1005.42	NY/JW
	B	2	1010.74	1018.13	
	C	3	1003.90	1011.76	
	D	4	984.66	994.13	
1.5	A	5	1009.92	1018.40	
	B	6	995.48	1005.25	
	C	7	999.39	1010.57	
	D	8	1002.88	1009.94	
3.0 ^{3N}	A	9	996.56	1008.58	
	B	10	1008.97	1016.35	
	C	11	1045.11	1056.30	
	D	12	995.21	1002.85	
6.1	A	13	1033.97	1042.44	
	B	14	1041.32	1049.56	
	C	15	1001.57	1011.58	
	D	16	1002.19	1010.10	
12.1	A	17	1005.53	1015.68	
	B	18	994.57	1002.11	
	C	19	1007.01	1015.84	
	D	20	1008.29	1016.88	
24.2	A	21	978.99	988.06	
	B	22	998.46	1007.60	
	C	23	1031.25	1040.77	
	D	24	1021.68	1030.91	
48.5	A	25	1047.87	1056.04	
	B	26	1006.52	1013.80	
	C	27	1015.05	1024.54	
	D	28	1022.77	1030.89	

Comments: Reweighed pans - 5-1018.24 17-1015.57 21-987.66

Reviewed by: JGU Date Reviewed: March 28/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: X 14 (L1426336 - 5)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration % (v/v)	Rep	Pan No. BLACK	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1011.53	1017.86	NY/JW ↓ ↓
	B	30	1020.63	1027.51	
	C	31	1003.88	1012.29	
	D	32	1009.06	1018.16	
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments: _____

Reviewed by: Job

Date Reviewed: March 26 / 14

CETIS Analytical Report

Report Date: 26 Mar-14 09:28 (p 1 of 2)
 Test Code: 14077e | 03-2104-3103

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 00-0534-3718	Endpoint: Frond Count	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:27	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 18-2798-0256	Code: 6CF4C3E0	Client: ALS
Sample Date: 25 Feb-14 09:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 63h (2.8 °C)	Station: L1426336-5(X14)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	806178	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	24.24	N/A	29.15	4.126	3.43	NA
IC10	27.05	N/A	33.64	3.697	2.973	NA
IC15	30.17	N/A	39.1	3.314	2.557	NA
IC20	33.65	22.13	45.84	2.972	2.182	4.519
IC25	37.51	25.77	53.2	2.666	1.88	3.88
IC40	55.75	34.81	84.58	1.794	1.182	2.873
IC50	86.96	48.05	N/A	1.15	NA	2.081

Frond Count Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	91.75	82	105	4.802	9.605	10.47%	0.0%
1.5		4	91	64	109	9.908	19.82	21.78%	0.82%
3		4	101.3	77	126	13.72	27.44	27.1%	-10.35%
6.1		4	87.25	77	107	6.957	13.91	15.95%	4.91%
12.1		4	91	75	113	7.958	15.92	17.49%	0.82%
24.2		4	91.75	87	95	1.797	3.594	3.92%	0.0%
48.5		4	59.75	53	73	4.715	9.43	15.78%	34.88%
97		4	45	40	54	3.317	6.633	14.74%	50.95%

Frond Count Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	90	90	82	105
1.5		89	102	109	64
3		124	78	126	77
6.1		87	78	107	77
12.1		113	75	89	87
24.2		95	87	94	91
48.5		53	53	60	73
97		40	40	46	54

CETIS Analytical Report

Report Date: 26 Mar-14 09:28 (p 2 of 2)
Test Code: 14077e | 03-2104-3103

Lemna Growth Inhibition Test

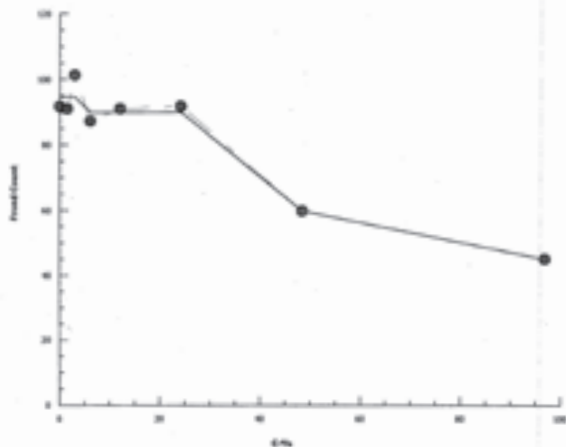
Nautilus Environmental

Analysis ID: 00-0534-3718
Analyzed: 26 Mar-14 9:27

Endpoint: Frond Count
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 26 Mar-14 09:28 (p 1 of 2)
 Test Ccde: 14077e | 03-2104-3103

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 19-1952-5350	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:27	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 18-2798-0256	Code: 6CF4C3E0	Client: ALS
Sample Date: 25 Feb-14 09:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 63h (2.8 °C)	Station: L1426336-5(X14)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	274734	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	36.61	N/A	N/A	2.731	NA	NA
IC10	61.05	N/A	N/A	1.638	NA	NA
IC15	>97	N/A	N/A	<1.031	NA	NA
IC20	>97	N/A	N/A	<1.031	NA	NA
IC25	>97	N/A	N/A	<1.031	NA	NA
IC40	>97	N/A	N/A	<1.031	NA	NA
IC50	>97	N/A	N/A	<1.031	NA	NA

Total Dry Weight-mg Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	8.225	7.39	9.47	0.4456	0.8912	10.83%	0.0%
1.5		4	9.123	7.06	11.18	0.8813	1.763	19.32%	-10.91%
3		4	9.558	7.38	12.02	1.195	2.391	25.01%	-16.2%
6.1		4	8.658	7.91	10.01	0.4652	0.9305	10.75%	-5.26%
12.1		4	8.777	7.54	10.15	0.5364	1.073	12.22%	-6.72%
24.2		4	9.24	9.07	9.52	0.09892	0.1978	2.14%	-12.34%
48.5		4	8.265	7.28	9.49	0.4565	0.9131	11.05%	-0.49%
97		4	7.68	6.33	9.1	0.6463	1.293	16.83%	6.63%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	8.18	7.39	7.86	9.47
1.5		8.48	9.77	11.18	7.06
3		12.02	7.38	11.19	7.64
6.1		8.47	8.24	10.01	7.91
12.1		10.15	7.54	8.83	8.59
24.2		9.07	9.14	9.52	9.23
48.5		8.17	7.28	9.49	8.12
97		6.33	6.88	8.41	9.1

CETIS Analytical Report

Report Date: 26 Mar-14 09:28 (p 2 of 2)
Test Code: 14077e | 03-2104-3103

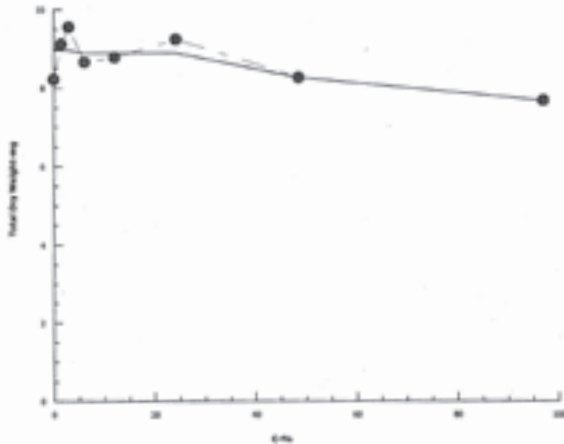
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 19-1952-5350 Endpoint: Total Dry Weight-mg
Analyzed: 26 Mar-14 9:27 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



Lemna minor Summary Sheet

Client: ALS
Work Order No.: IND77

Start Date: Feb 28, 2014
Set up by: JW / JBF

Sample Information:

Sample ID: L 1426336 - 6 (Y3A)
Sample Date: Feb 25 / 14 @ 1135h
Date Received: Feb 27 / 14 @ 1030h
Sample Volume: 2 x 20L

Test Organism Information:

Culture Date: 02/19/14
Age of culture (Day 0): 9 days
>8X growth in APHA?: Y

KCI Reference Toxicant Results:

Reference Toxicant ID: LM 100
Date Initiated: Feb 20, 2014

7-d No. of Fronds IC50 (95% CL): 3.6 (3.2 - 4.2)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 4.4 (3.5 - 5.5) CV (%): 12

	Number of Fronds	Dry Weight
Test Results: IC25 %(v/v) (95% CL)	90.0 (25.5 - 97)	> 97
IC50 %(v/v) (95% CL)	> 97	> 97

Reviewed by: JGh

Date reviewed: March 26 / 14

Plant Growth Inhibition Toxicity Test Water Quality Measurements

Client: ALS Setup by: JW / JBF
 Sample ID: X3A (L1426336 - G) (W40) Test Date: Feb 28 / 14
 Work Order No.: 14077 Test Species: Lemna minor
 Culture Source: CPCC # 490
 Test Culture Age: 9^{3w} days > 8X Growth? (Y/N): Y
 Light Intensity Range: 4000 - 5300 lux Date Measured: Feb 26 / 14

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	25.0	26.0	25.5	25.5	26.0	26.0	26.0	26.0
Initials	JW	JW	JW	JBF	JW	JW	JW	JW

Sample Characteristics: Initial Water Quality Adjusted Water Quality

Temperature (°C)	<u>25.0</u>	Aeration?:	<u>20 min</u>	Adjusted Temperature (°C)	<u>24.0</u>
DO (mg/L)	<u>10.0</u>	Nutrients added?:	<u>Y</u>	Adjusted DO (mg/L)	<u>9.0</u>
pH	<u>7.2</u>			Adjusted pH	<u>7.5</u>
Conductivity (µS)	<u>345</u>			Adjusted Conductivity (µS)	<u>1143</u>

Concentration % (v/v)	Temperature (°C)		pH		Conductivity (µS) 0 h
	Day 0	Day 7	Day 0	Day 7	
Control	24.0	25.0	8.4	8.1	878
1.5	24.0	25.0	8.1	8.3	888
3.0 ^{JW}	24.0	25.0	8.1	8.3	892
6.1	24.0	25.0	8.1	8.2	899
12.1	24.0	25.0	8.1	8.3	917
24.2	24.0	25.0	8.0	8.2	946
48.5	25.0	25.0	7.9	8.3	1028
97	24.0	25.0	7.5	8.5	1143
Initials	JW / JBF		JW / JBF		JW / JBF

Thermometer: Calibrated Thermometer Cond. Meter: C-2 pH meter: PH-2

Sample Description: clear

Comments: _____

Reviewed: JGh Date Reviewed: March 26 / 14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS Start Date: Feb 26 / 14
 Sample ID: X3A (L 1426336 - 6) (Blue) Termination Date: March 7 / 14
 Work Order #: M077 Test set up by: JW / JBF

Concentration % (v/v)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
control	A	6	63										JW
	B	6	64										
	C	6	84										
	D	6	74										
1.5	A	6	60		X	X							JW
	B	6	67										
	C	6	53			X							
	D	6	72										
3.06 ^{JW}	A	6	70			X							JW
	B	6	81			X							
	C	6	65		X	X							
	D	6	64										
6.1	A	6	53			X							JW
	B	6	70			X							
	C	6	88										
	D	6	59			X							
12.1	A	6	82										JW
	B	6	52		X	X							
	C	6	71										
	D	6	60			X							
24.2	A	6	46			X							JW
	B	6	82										
	C	6	77										
	D	6	54			X							

Comments: _____

Reviewed by: JGK Date Reviewed: March 26 / 14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS Start Date: Feb 26 / 14
 Sample ID: X3A (L1426336 - 6) (blue) Termination Date: March 3 / 14
 Work Order #: 14077 Test set up by: JW / JBF

Concentration % (v/v)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
48.5	A	6	57										JW
	B	6	53										
	C	6	51										
	D	6	54		X	X							
97	A	6	67		X	X							JW
	B	6	56		X	X							
	C	6	51		X	X							
	D	6	43		X	X							
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments: _____

Reviewed by: JBF Date Reviewed: March 26/14

7-d Lemna minor Weight Data Sheet

Client: ALC Start Date: Feb 28 / 14
 Sample ID: X3A (L 1426336 - 6) (blue) Termination Date: March 7 / 14
 Work Order #: 14077

Concentration % (v/v)	Rep	Pan No. Blue	Pan weight (mg)	Pan + plant (mg)	Initials
Control	A	1	1240.05	1245.16	NY/JW
	B	2	1250.74	1255.50	
	C	3	1234.78	1241.17	
	D	4	1230.28	1236.12	
1.5	A	5	1274.67	1279.66	
	B	6	1224.28	1230.46	
	C	7	1218.70	1223.35	
	D	8	1211.64	JW 128 1218.04	
3.0 ^{JW} 3.0*	A	9	1243.89	1250.31	
	B	10	1232.83	1239.81	
	C	11	1252.16	1257.95	
	D	12	1248.90	1254.57	
6.1	A	13	1280.14	1284.45	
	B	14	1263.29	1269.66	
	C	15	1242.26	1250.73	
	D	16	1234.35	1239.50	
12.1	A	17	1249.55	1257.28	
	B	18	1274.71	1279.36	
	C	19	1234.75	1240.98	
	D	20	1253.51	1259.40	
24.2	A	21	1239.03	1243.57	
	B	22	1242.78	1250.53	
	C	23	1231.72	1238.88	
	D	24	1232.77	1237.36	
48.5	A	25	1257.99	1264.16	
	B	26	1257.92	1263.20	
	C	27	1240.57	1246.36	
	D	28	1267.71	1273.54	

Comments: Rewighed pans - 8 - 1217.89 25 - 1263.86 31 - 1235.36

Reviewed by: JW Date Reviewed: March 26/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: X3A (L 1426336 - 6)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration % (w/v)	Rep	Pan No. BLUE	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1217.08	1223.42	NY/JW ↓ ↓
	B	30	1224.86	1231.45	
	C	31	1230.27	1235.65	
	D	32	1219.36	1224.02	
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments: _____

Reviewed by: JGh

Date Reviewed: March 26/14

CETIS Analytical Report

Report Date: 26 Mar-14 09:33 (p 1 of 2)
 Test Code: 14077f | 19-5941-2284

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 15-2065-3662	Endpoint: Frond Count	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:32	Analysis: Nonlinear Regression	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 05-2872-4256	Code: 1F83B120	Client: ALS
Sample Date: 25 Feb-14 11:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 60h (3.9 °C)	Station: L1426336-6(X3A)	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1-Phi(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
11	-90.72	188.3	191.8	0.1643	Yes	0.4234	2.621	0.8277	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	10.26	N/A	35.9	9.75	2.785	NA
IC10	23.13	0.1504	61.82	4.323	1.618	664.8
IC15	40.04	11.5	86.57	2.498	1.155	8.694
IC20	61.92	22.15	128.7	1.615	0.7769	4.516
IC25	90.02	25.51	220.7	1.111	0.4531	3.92
IC40	231.1	12.01	1747	0.4328	0.06723	8.327
IC50	407.4	3.797	44770	0.2455	0.002234	26.98

} > 97% (v/v)

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	62.9	3.887	55.29	70.52	16.18	<0.0001	Significant Parameter
C	2.238	1.673	-1.04	5.517	1.338	0.1913	Non-Significant Parameter
D	407.4	530.2	-631.8	1447	0.7684	0.4485	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	952.7588	952.7588	1	8.094	0.0081	Significant
Lack of Fit	276.71	55.34199	5	0.4234	0.8277	Non-Significant
Pure Error	3136.75	130.6979	24			
Residual	3413.46	117.7055	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	8.803	14.07	0.2671	Equal Variances
	Mod Levene Equality of Variance	2.326	2.423	0.0582	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9418	0.9338	0.0843	Normal Distribution
	Anderson-Darling A2 Normality	0.745	2.492	0.0521	Normal Distribution

CETIS Analytical Report

Report Date: 26 Mar-14 09:33 (p 2 of 2)
 Test Code: 140771 | 19-5941-2284

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 15-2065-3662
 Analyzed: 26 Mar-14 9:32

Endpoint: Frond Count
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

Frond Count Summary

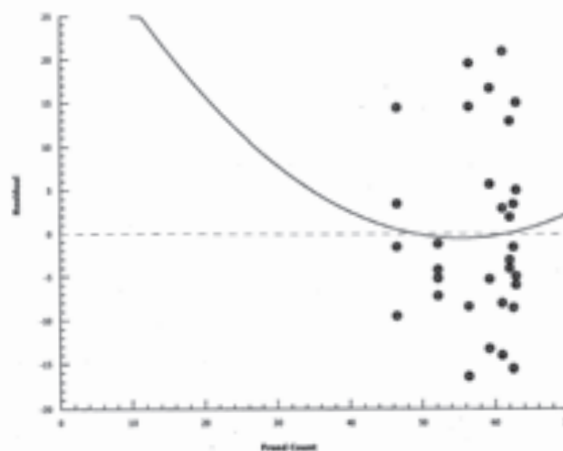
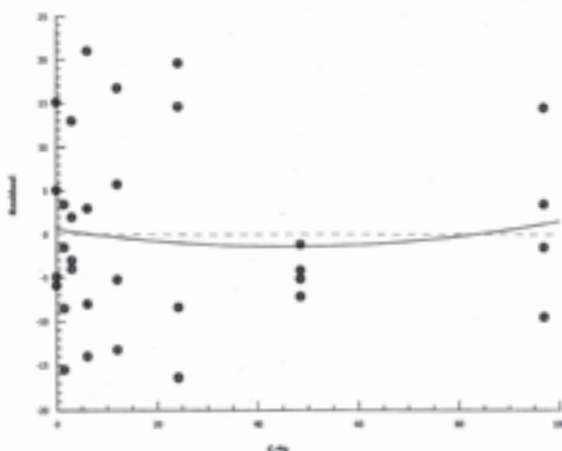
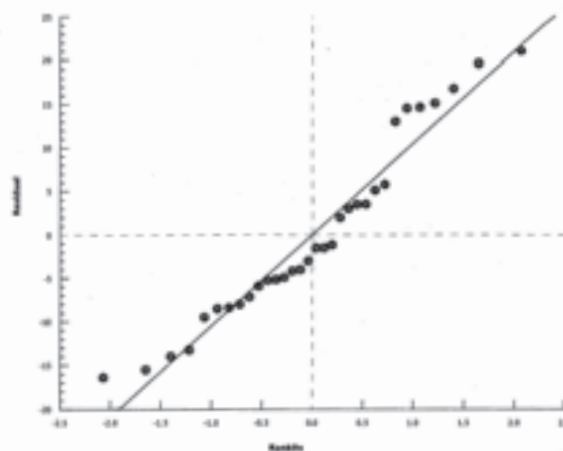
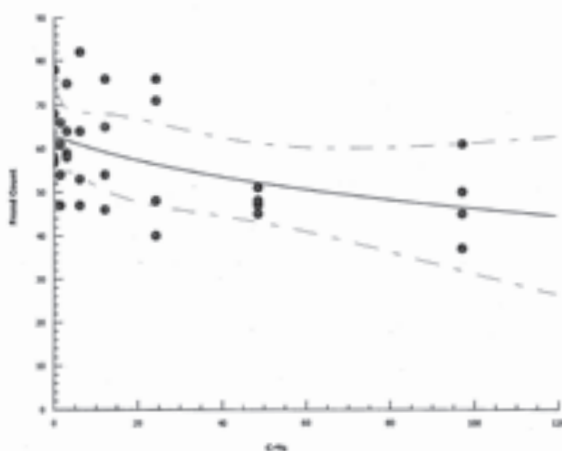
C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	65.25	57	78	4.922	9.845	15.09%	0.0%
1.5		4	57	47	66	4.143	8.287	14.54%	12.64%
3		4	64	58	75	3.894	7.789	12.17%	1.92%
6.1		4	61.5	47	82	7.687	15.37	25.0%	5.75%
12.1		4	60.25	46	76	6.537	13.07	21.7%	7.66%
24.2		4	58.75	40	76	8.731	17.46	29.72%	9.96%
48.5		4	47.75	45	51	1.25	2.5	5.24%	26.82%
97		4	48.25	37	61	5.023	10.05	20.82%	26.05%

Frond Count Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	57	58	78	68
1.5		54	61	47	66
3		64	75	59	58
6.1		47	64	82	53
12.1		76	46	65	54
24.2		40	76	71	48
48.5		51	47	45	48
97		61	50	45	37

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



CETIS Analytical Report

Report Date: 26 Mar-14 09:34 (p 1 of 2)
 Test Code: 140771 | 19-5941-2284

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 13-2665-5955	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:33	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 05-2872-4256	Code: 1F83B120	Client: ALS
Sample Date: 25 Feb-14 11:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 60h (3.9 °C)	Station: L1426336-6(X3A)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1297904	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>97	N/A	N/A	<1.031	NA	NA
IC10	>97	N/A	N/A	<1.031	NA	NA
IC15	>97	N/A	N/A	<1.031	NA	NA
IC20	>97	N/A	N/A	<1.031	NA	NA
IC25	>97	N/A	N/A	<1.031	NA	NA
IC40	>97	N/A	N/A	<1.031	NA	NA
IC50	>97	N/A	N/A	<1.031	NA	NA

Total Dry Weight-mg Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	5.525	4.76	6.39	0.3657	0.7314	13.24%	0.0%
1.5		4	5.555	4.65	6.4	0.4323	0.8646	15.57%	-0.54%
3		4	6.215	5.67	6.98	0.3035	0.607	9.77%	-12.49%
6.1		4	6.075	4.31	8.47	0.9034	1.807	29.74%	-9.96%
12.1		4	6.125	4.65	7.73	0.6336	1.267	20.69%	-10.86%
24.2		4	6.01	4.54	7.75	0.843	1.686	28.05%	-8.78%
48.5		4	5.768	5.28	6.17	0.1835	0.3671	6.36%	-4.39%
97		4	5.743	4.66	6.59	0.4452	0.8904	15.51%	-3.94%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	5.11	4.76	6.39	5.84
1.5		4.99	6.18	4.65	6.4
3		6.42	6.98	5.79	5.67
6.1		4.31	6.37	8.47	5.15
12.1		7.73	4.65	6.23	5.89
24.2		4.54	7.75	7.16	4.59
48.5		6.17	5.28	5.79	5.83
97		6.34	6.59	5.38	4.66

CETIS Analytical Report

Report Date: 26 Mar-14 09:34 (p 2 of 2)
Test Code: 14077f | 19-5941-2284

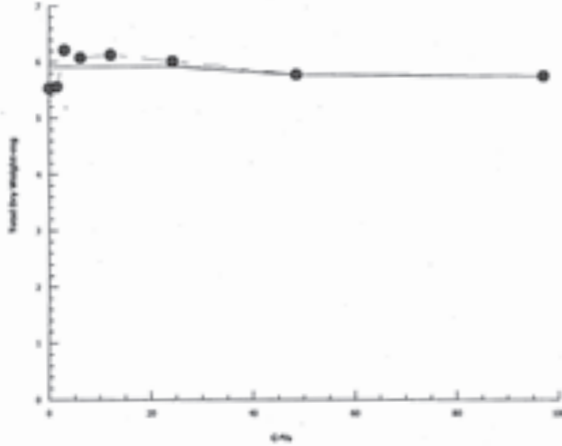
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 13-2665-5955 Endpoint: Total Dry Weight-mg
Analyzed: 26 Mar-14 9:33 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 26 Mar-14 09:33 (p 1 of 2)
 Test Code: 14077f | 19-5941-2284

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 16-7179-8482	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:33	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 05-2872-4256	Code: 1F83B120	Client: ALS
Sample Date: 25 Feb-14 11:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 60h (3.9 °C)	Station: L1426336-6(X3A)	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	36.0%	97	>97	NA	1.031

Dunnett Multiple Comparison Test

Control	vs C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	1.5	0.0374	2.482	1.991	6	0.8654	CDF	Non-Significant Effect
	3	0.8603	2.482	1.991	6	0.5462	CDF	Non-Significant Effect
	6.1	0.6858	2.482	1.991	6	0.6268	CDF	Non-Significant Effect
	12.1	0.7481	2.482	1.991	6	0.5983	CDF	Non-Significant Effect
	24.2	0.6047	2.482	1.991	6	0.6628	CDF	Non-Significant Effect
	48.5	0.3024	2.482	1.991	6	0.7836	CDF	Non-Significant Effect
	97	0.2712	2.482	1.991	6	0.7945	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.961173	0.2801675	7	0.2178	0.9776	Non-Significant Effect
Error	30.87512	1.286463	24			
Total	32.83629		31			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.24	18.48	0.2358	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.982	0.9081	0.8543	Normal Distribution

Total Dry Weight-mg Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	4	5.525	4.361	6.889	5.475	4.76	6.39	0.3657	13.24%	0.0%
1.5		4	5.555	4.179	6.931	5.585	4.65	6.4	0.4323	15.57%	-0.54%
3		4	6.215	5.249	7.181	6.105	5.67	6.98	0.3035	9.77%	-12.49%
6.1		4	6.075	3.2	8.95	5.76	4.31	8.47	0.9034	29.74%	-9.96%
12.1		4	6.125	4.109	8.141	6.06	4.65	7.73	0.6336	20.69%	-10.86%
24.2		4	6.01	3.327	8.693	5.875	4.54	7.75	0.843	28.05%	-8.78%
48.5		4	5.768	5.183	6.352	5.81	5.28	6.17	0.1835	6.36%	-4.39%
97		4	5.743	4.326	7.159	5.86	4.66	6.59	0.4452	15.51%	-3.94%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	5.11	4.76	6.39	5.84
1.5		4.99	6.18	4.65	6.4
3		6.42	6.98	5.79	5.67
6.1		4.31	6.37	8.47	5.15
12.1		7.73	4.65	6.23	5.89
24.2		4.54	7.75	7.16	4.59
48.5		6.17	5.28	5.79	5.83
97		6.34	6.59	5.38	4.86

CETIS Analytical Report

Report Date: 26 Mar-14 09:33 (p 2 of 2)
Test Code: 14077f | 19-5941-2284

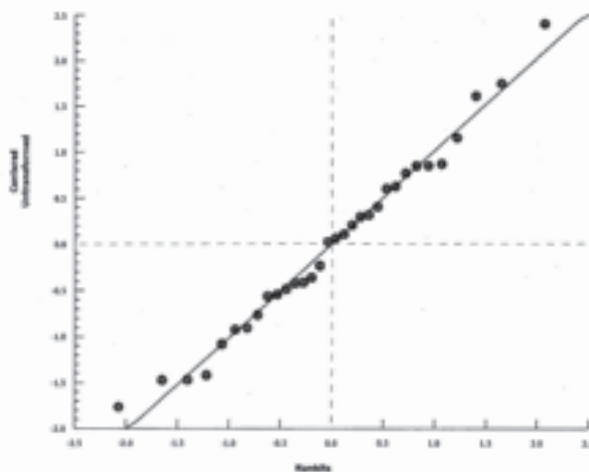
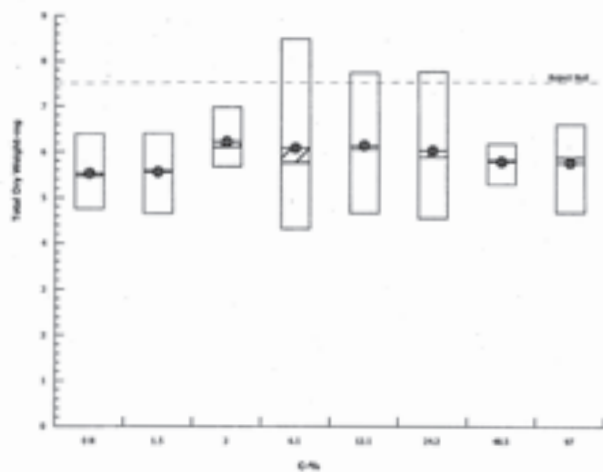
Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 16-7179-8482 Endpoint: Total Dry Weight-mg
Analyzed: 26 Mar-14 9:33 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



Lemna minor Summary Sheet

Client: ALS
 Work Order No.: 14077

Start Date: Feb 28 / 14
 Set up by: JW / JBF

Sample Information:

Sample ID: L1426336-7 (R3)
 Sample Date: Feb 25 / 14 @ 1630h
 Date Received: Feb 27 / 14 @ 1030h
 Sample Volume: 2 x 20L

Test Organism Information:

Culture Date: 021914
 Age of culture (Day 0): 9 days
 >8X growth in APHA?: Y

KCI Reference Toxicant Results:

Reference Toxicant ID: Lm 100
 Date Initiated: Feb 20, 2014

7-d No. of Fronds IC50 (95% CL): 3.6 (3.2 - 4.2)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 4.4 (3.5 - 5.5) CV (%): 12

	Number of Fronds	Dry Weight
Test Results: IC25 %(v/v) (95% CL)	26.0 (26.5 - 47.0)	> 97
IC50 %(v/v) (95% CL)	³⁰ > 97	> 97

Reviewed by: JG

Date reviewed: Apr. 11 / 14

Plant Growth Inhibition Toxicity Test Water Quality Measurements

Client: ALS Setup by: JW / JBF
 Sample ID: R3 (L1426336-7) (green) Test Date: Feb 28 / 14
 Work Order No.: 14077 Test Species: Lemna minor
 Culture Source: CRCC # 490
 Test Culture Age: 9th days > 8X Growth? (Y/N): Y
 Light Intensity Range: 4000 - 5300 lux Date Measured: Feb 26 / 14

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	25.0	26.2	25.5	25.5	26.0	26.0	26.0	26.0
Initials	JW	JW	JW	JBF	JW	JW	JW	JW

Sample Characteristics: Initial Water Quality Adjusted Water Quality

Temperature (°C)	<u>24.0</u>	Aeration?:	<u>20 min</u>	<u>24.0</u>
DO (mg/L)	<u>10.0</u>	Nutrients added?:	<u>Y</u>	<u>9.0</u>
pH	<u>7.3</u>			<u>7.6</u>
Conductivity (µS)	<u>675</u>			<u>1411</u>

Concentration % (v/v)	Temperature (°C)		pH		Conductivity (µS) 0 h
	Day 0	Day 7	Day 0	Day 7	
Control	24.0	24.0 25.0	8.4	8.2	878
1.5	24.0	25.0	8.0	8.3	894
3.09 ^{JW}	24.0	25.0	8.1	8.3	904
6.1	24.0	25.0	8.1	8.3	919
12.1	24.0	25.0	8.0	8.3	954
24.2	24.0	25.0	8.0	8.3	1021
48.5	24.0	25.0	7.9	8.3	1156
97	24.0	25.0	7.6	8.5	1411
Initials	JW / JBF	JW	JW / JBF	JW	JW / JBF

Thermometer: Calibrated Thermometer Cond. Meter: C-2 pH meter: PH-2

Sample Description: clear

Comments: _____

Reviewed: JG Date Reviewed: March 26/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS
 Sample ID: R3 (1426336 - 7) (Green)
 Work Order #: 14077
 Start Date: Feb 28 / 14
 Termination Date: March 7 / 14
 Test set up by: JW / JBF

Concentration % (v/v)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
CONTROL	A	6	72										JW
	B	6	60										
	C	6	73										
	D	6	86										
1.5	A	6	88			X							JW
	B	6	95			X							
	C	6	57			X							
	D	6	96			X							
3.0 ^{JW} 0.8	A	6	65			X							JW
	B	6	71			X							
	C	6	70		X	X							
	D	6	69			X							
6.1	A	6	72			X							JW
	B	6	79			X							
	C	6	66			X							
	D	6	62			X							
12.1	A	6	66			X							JW
	B	6	77			X							
	C	6	69			X							
	D	6	70			X							
24.2	A	6	54			X			X				JW
	B	6	63			X							
	C	6	48			X							
	D	6	65			X							

Comments: _____
 Reviewed by: JW Date Reviewed: March 26/14

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: ALS Start Date: Feb 28/14
 Sample ID: R3 (L1426336 - F) (Green) Termination Date: March 7 / 14
 Work Order #: 14077 Test set up by: JW / JBF

Concentration % (v/v)	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
48.5	A	6	62			X							JW
	B	6	57		X	X							
	C	6	55		X	X							
	D	6	50		X	X							
97	A	6	39		X	X	X						JW
	B	6	37		X	X	X						
	C	6	40		X	X	X						
	D	6	43		X	X							
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments: _____

Reviewed by: JW Date Reviewed: March 26/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: R3 (L1426336 - 7)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration % (v/v)	Rep	Pan No. Green	Pan weight (mg)	Pan + plant (mg)	Initials
control	A	1	1008.54	1014.39	NY/JW
	B	2	1025.89	1031.12	
	C	3	1003.33	1008.85	
	D	4	1051.05	1057.95	
1.5	A	5	1018.53	1025.26	
	B	6	1015.29	1022.47	
	C	7	982.23	986.69	
	D	8	996.48	1004.23	
3.0% <small>JW</small>	A	9	998.41	1003.13	
	B	10	1016.67	1022.40	
	C	11	1003.46	1008.97 <small>JW</small>	
	D	12	993.28	998.76	
6.1	A	13	991.66	997.36	
	B	14	1012.71	1019.11	
	C	15	988.25	994.08	
	D	16	1004.38	1009.31	
12.1	A	17	1000.21	1005.63	
	B	18	1008.93	1015.58	
	C	19	1008.67	1013.99	
	D	20	1001.58	1007.26	
24.2	A	21	991.42	996.97	
	B	22	1009.93	1015.98	
	C	23	1012.53	1016.25	
	D	24	1027.46	1033.03	
48.5	A	25	1032.88	1038.76	
	B	26	1033.72	1039.38	
	C	27	1012.81	1017.79	
	D	28	986.82	992.04	

Comments: Reweighed pans: 6-1022.42 11-1008.30 22-1015.86

Reviewed by: JGh Date Reviewed: March 26/14

7-d Lemna minor Weight Data Sheet

Client: ALS
 Sample ID: R3 (L M26336 - 7)
 Work Order #: 14077

Start Date: Feb 28 / 14
 Termination Date: March 7 / 14

Concentration 90 (v/v)	Rep	Pan No. Green	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1001.37	1005.88	NY/JW ↓ ↓
	B	30	1006.19	1010.74	
	C	31	993.99	988.52	
	D	32	1019.10	1024.06	
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments: _____

Reviewed by: JW Date Reviewed: March 26/14

CETIS Analytical Report

Report Date: 10 Apr-14 16:45 (p 1 of 2)
 Test Code: 14077g | 21-3992-3946

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 18-9997-1101	Endpoint: Frond Count	CETIS Version: CETISv1.8.7
Analyzed: 10 Apr-14 16:45	Analysis: Nonlinear Regression	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 13-8828-3648	Code: 52BF8700	Client: ALS
Sample Date: 25 Feb-14 16:30	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 56h (3.4 °C)	Station: L1426336-7(R3)	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Box-Cox [W=Y^(2Z-2)]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
13	-80.89	168.6	172.2	0.6834	Yes	2.295	2.621	0.0772	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	8.599	N/A	16.01	11.63	6.245	NA
IC10	14.69	6.86	22.97	6.807	4.354	14.58
IC15	21.09	12.59	30.7	4.742	3.258	7.943
IC20	28.1	18.97	38.7	3.558	2.584	5.271
IC25	35.96	26.48	47.01	2.781	2.127	3.776
IC40	66.91	58.21	76.65	1.495	1.305	1.718
IC50	97.21	85.29	110.8	1.029	0.9025	1.172

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	68.3	3.291	61.85	74.75	20.75	<0.0001	Significant Parameter
C	1.474	0.292	0.9022	2.047	5.05	<0.0001	Significant Parameter
D	97.21	10.35	76.93	117.5	9.394	<0.0001	Significant Parameter
Z	-1.24						

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	5.78E-05	5.78E-05	1	68.91	<0.0001	Significant
Lack of Fit	7.87E-06	1.57E-06	5	2.295	0.0772	Non-Significant
Pure Error	1.65E-05	6.86E-07	24			
Residual	2.43E-05	8.39E-07	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	8.702	14.07	0.2748	Equal Variances
	Mod Levene Equality of Variance	0.6783	2.423	0.6887	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9718	0.9338	0.5495	Normal Distribution
	Anderson-Darling A2 Normality	0.3568	2.492	0.4603	Normal Distribution

CETIS Analytical Report

Report Date: 10 Apr-14 16:45 (p 2 of 2)
 Test Code: 14077g | 21-3992-3946

Lemna Growth Inhibition Test

Nautilus Environmental

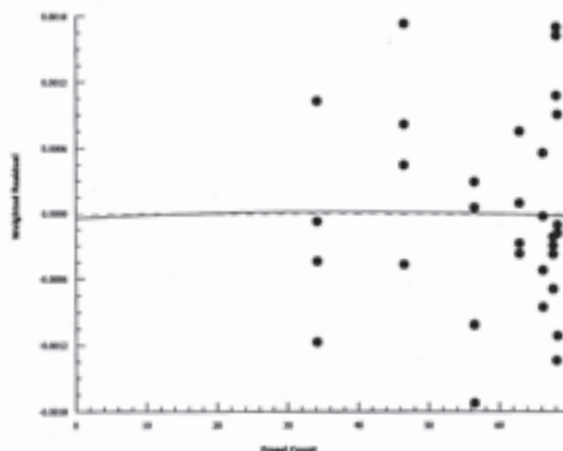
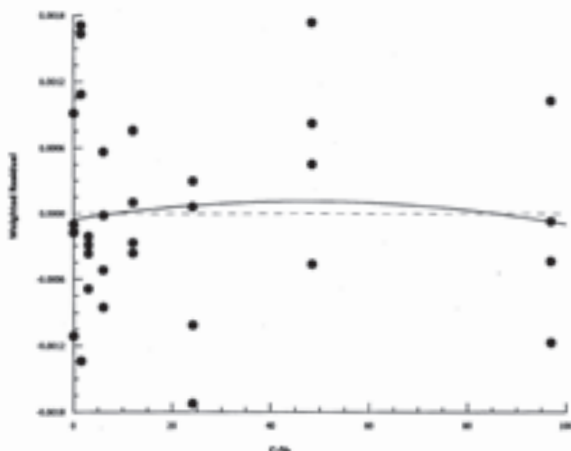
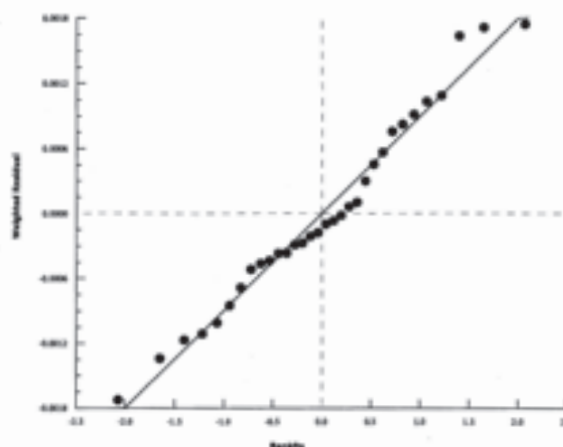
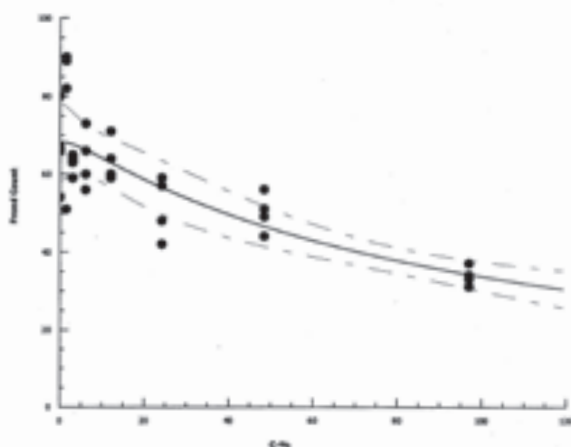
Analysis ID: 18-9997-1101 Endpoint: Frond Count
 Analyzed: 10 Apr-14 16:45 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	66.75	54	80	5.313	10.63	15.92%	0.0%
1.5		4	78	51	90	9.174	18.35	23.52%	-16.85%
3		4	62.75	59	65	1.315	2.63	4.19%	5.99%
6.1		4	63.75	56	73	3.705	7.411	11.62%	4.49%
12.1		4	63.5	59	71	2.723	5.447	8.58%	4.87%
24.2		4	51.5	42	59	3.969	7.937	15.41%	22.85%
48.5		4	50	44	56	2.483	4.967	9.93%	25.09%
97		4	33.75	31	37	1.25	2.5	7.41%	49.44%

Frond Count Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	66	54	67	80
1.5		82	89	51	90
3		59	65	64	63
6.1		66	73	60	56
12.1		60	71	59	64
24.2		48	57	42	59
48.5		56	51	49	44
97		33	31	34	37

Graphics 3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



CETIS Analytical Report

Report Date: 26 Mar-14 09:39 (p 1 of 2)
 Test Code: 14077g | 21-3992-3946

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 15-1033-8830	Endpoint: Total Dry Weight-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 Mar-14 9:39	Analysis: Nonlinear Regression	Official Results: Yes
Batch ID: 18-7938-8836	Test Type: Lemna Growth	Analyst: Jeslin Wijaya
Start Date: 28 Feb-14	Protocol: EC/EPS 1/RM/37	Diluent: APHA (modified)
Ending Date: 07 Mar-14	Species: Lemna minor	Brine:
Duration: 7d 0h	Source: CPCC#490	Age: 9d
Sample ID: 13-8828-3648	Code: 52BF8700	Client: ALS
Sample Date: 25 Feb-14 16:30	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 56h (3.4 °C)	Station: L1426336-7(R3)	

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Log-Gompertz EV [Y=A*exp(log(0.5)(X/D)^C)]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
9	-6.474	19.8	23.34	0.1778	Yes	1.069	2.621	0.4022	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	10.24	N/A	35.33	9.768	2.83	NA
IC10	29.39	4.584	68.26	3.403	1.465	21.81
IC15	55.45	19.88	105.2	1.803	0.9503	5.031
IC20	88.23	27.21	180.4	1.133	0.5542	3.675
IC25	128	20.87	326.2	0.7812	0.3066	4.793
IC40	296.9	N/A	1985	0.3369	0.05038	NA
IC50	464.3	N/A	N/A	0.2154	NA	NA

} > 97% (v/v)

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	5.991	0.3144	5.374	6.607	19.05	<0.0001	Significant Parameter
C	0.6826	0.5376	-0.3711	1.736	1.27	0.2143	Non-Significant Parameter
D	464.3	616.7	-744.5	1673	0.7528	0.4576	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	5.296244	5.296244	1	8.706	0.0062	Significant
Lack of Fit	3.21248	0.642496	5	1.069	0.4022	Non-Significant
Pure Error	14.43046	0.601269	24			
Residual	17.64294	0.608377	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	11.39	14.07	0.1226	Equal Variances
	Mod Levene Equality of Variance	0.7097	2.423	0.6642	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9744	0.9338	0.6283	Normal Distribution
	Anderson-Darling A2 Normality	0.5135	2.492	0.1971	Normal Distribution

CETIS Analytical Report

Report Date: 26 Mar-14 09:39 (p 2 of 2)
 Test Code: 14077g | 21-3992-3946

Lemna Growth Inhibition Test

Nautilus Environmental

Analysis ID: 15-1033-8830
 Analyzed: 26 Mar-14 9:39

Endpoint: Total Dry Weight-mg
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

Total Dry Weight-mg Summary

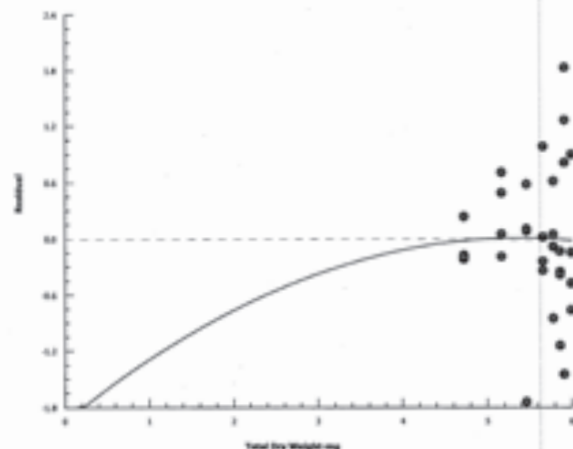
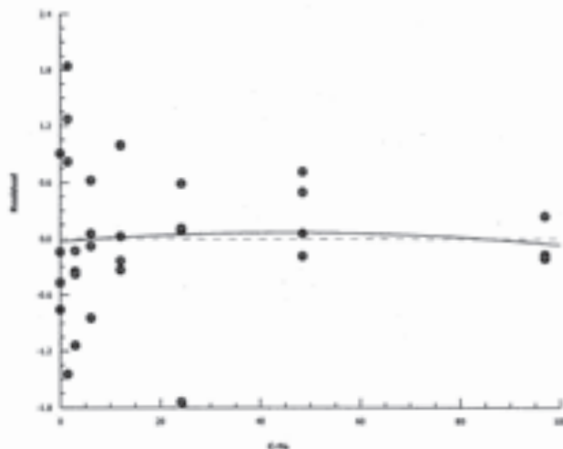
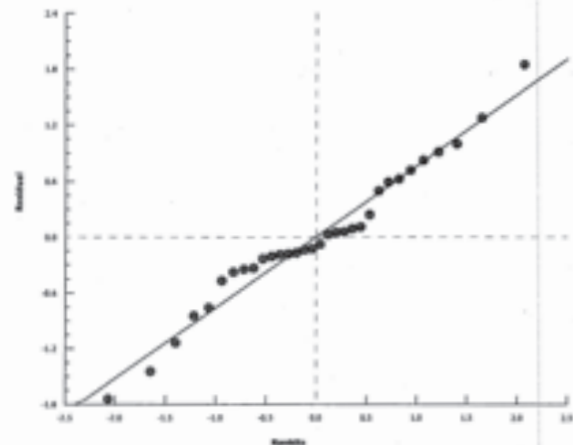
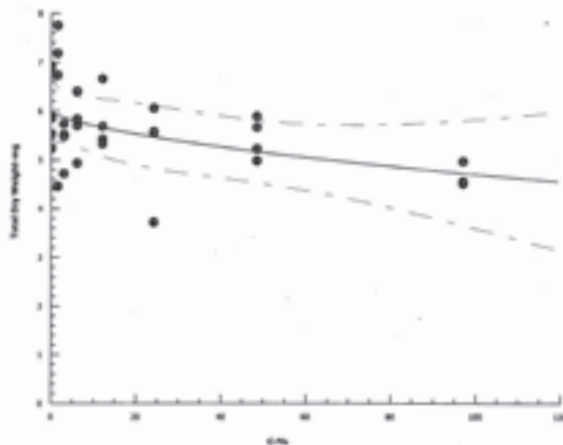
C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	5.875	5.23	6.9	0.3644	0.7287	12.4%	0.0%
1.5		4	6.53	4.46	7.75	0.7209	1.442	22.08%	-11.15%
3		4	5.36	4.72	5.73	0.2205	0.441	8.23%	8.77%
6.1		4	5.715	4.93	6.4	0.3026	0.6052	10.59%	2.72%
12.1		4	5.768	5.32	6.65	0.3038	0.6076	10.53%	1.83%
24.2		4	5.223	3.72	6.05	0.514	1.028	19.68%	11.11%
48.5		4	5.435	4.98	5.88	0.2045	0.409	7.53%	7.49%
97		4	4.638	4.51	4.96	0.1078	0.2157	4.65%	21.06%

Total Dry Weight-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	5.85	5.23	5.52	6.9
1.5		6.73	7.18	4.46	7.75
3		4.72	5.73	5.51	5.48
6.1		5.7	6.4	5.83	4.93
12.1		5.42	6.65	5.32	5.68
24.2		5.55	6.05	3.72	5.57
48.5		5.88	5.66	4.98	5.22
97		4.51	4.55	4.53	4.96

Graphics

3P Log-Gompertz EV [Y=A*exp(log(0.5)(X/D)^C)]



APPENDIX C - *Pseudokirchneriella subcapitata* Toxicity Test Data

***Pseudokirchneriella subcapitata* Summary Sheet**

Client: ALS
Work Order No.: 14078

Start Date: Feb 27/14
Set up by: EMM

Sample Information:

Sample ID: L1426336-1 (R10)
Sample Date: Feb 25/14 @ 1500h
Date Received: Feb 27/14 @ 1030h
Sample Volume: 2x20L

Test Organism Information:

Culture Date: Feb 21/14
Age of culture (Day 0): 6d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC105
Stock Solution ID: 13Zn 01
Date Initiated: Feb 21/14
72-h IC50 (95% CL): 24.7 (16.1 - 31.7) ^{em} µg/L Zn

72-h IC50 Reference Toxicant Mean and Range: 22.7 (15.2 - 33.8) µg/L Zn CV (%): 22

Test Results:	Algal Growth
IC25 %(v/v) (95% CL)	<u>795.2</u>
IC50 %(v/v) (95% CL)	<u>795.2</u>

Reviewed by: JGh

Date reviewed: March 13/14

72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client : ALS Setup by: EMM
 Sample ID: 210 (U426336-1) Test Date/Time: Feb 27/14 @ 1530
 Work Order No.: 14078 Test Species: Pseudokirchneriella subcapitata

Culture Date: Feb 27/14 Age of Culture: 6d Culture Health: Good
 Culture Count: 1562 & 589 Average: 575.5 Culture Cell Density (c1): 575.5 x 10⁴ cells/ml

$$v1 = \frac{220,000 \text{ cells/ml} \times 100 \text{ ml}}{(c1) \quad 575 \times 10^4 \text{ cells/ml}} = 4 \text{ ml}$$

Time Zero Counts: 124 & 20 Average: 22

No. of Cells/mL: 22 x 10⁴ Initial Density: # cells/mL + 220 μL x 10 μL = 10 000 cells/ml

Concentration %(v/v)	Water Quality Measurements					Microplates rotated 2X per day?			
	pH	Temp (°C)				0 h	24 h	48 h	72 h
		0 h	0 h	24 h	48 h				
Control	7.0	24.0	25.0	26.0	26.5	✓	✓	/	/
1.5	7.1	24.5				✓	✓	/	/
3.0	7.2	24.5				✓	✓	/	/
6.0	7.5	24.5				✓	✓	/	/
11.9	7.4	24.5				✓	✓	/	/
23.8	7.5	24.5				✓	✓	/	/
47.6	7.5	24.5				✓	✓	/	/
95.2	7.6	24.5				✓	✓	/	/
						✓	✓	/	/
Initials	EMM	EMM	EMM	EMM	EMM	EMM	EMM	EMM	EMM

Initial control pH: Well 1: 6.8 Well 2: 6.8

Final control pH: Well 1: 6.5 Well 2: 6.5

Light intensity (lux): 3700 Date measured: Feb 27/14

Sample Description: clear

Comments: _____

Reviewed: JGh Date reviewed: March 13/14

***Pseudokirchneriella subcapitata* Toxicity Test Data Sheet**
72-h Algal Cell Counts

Client: ALS Start Date/Time: Feb 27/14 @ 1530
 Work Order #: 14078 Termination Date: March 21/14 @ 1530
 Sample ID: R10 (L1426861) Test set up by: Emm/JW
 % (v/v)

Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	42					Emm
	B	54					
	C	56					
	D	47					
	E	43					
	F	58					
	G	39					
	H	43					
1.5	A	55					↓
	B	57					
	C	42					
	D	49					
3.0	A	47					
	B	60					
	C	63					
	D	54					
6.0	A	67					
	B	71					
	C	68					
	D	72					
11.9	A	85					
	B	78					
	C	83					
	D	105	100				
23.8	A	150					
	B	162					
	C	168					
	D	147					
47.6	A	175					
	B	161					
	C	136	140				
	D	179					
95.2	A	158					
	B	147					
	C	155					
	D	139					

Comments: _____

Reviewed by: Joh Date Reviewed: March 13/14

Pseudokirchneriella subcapitata Algal Counts

Client: ALS
 WO#: 14078
 Sample ID: R10 (L1426336-1)

Start Date/Time: 27-Feb-14 @1530h
 Termination Date: 02-Mar-14 @1530h

Initial Cell Density: 10000 cell/mL
 220000
 0.22
 0.01
 10000

Concentration % v/v	Rep	Count 1 (x 10 ⁴)	Count 2 (x 10 ⁴)	Count 3 (x 10 ⁴)	Count 4 (x 10 ⁴)	Mean (x 10 ⁴)	Cell Yield (x 10 ⁴) cell/mL		
Control	A	42				42	41.0	mean	46.8
	B	54				54	53.0	SD	7.245688
	C	56				56	55.0	CV	15.4988
	D	47				47	46.0		
	E	43				43	42.0		
	F	58				58	57.0		
	G	39				39	38.0		
	H	43				43	42.0		
1.5	A	55				55	54.0		
	B	57				57	56.0		
	C	42				42	41.0		
	D	49				49	48.0		
3	A	47				47	46.0		
	B	60				60	59.0		
	C	63				63	62.0		
	D	54				54	53.0		
6	A	67				67	66.0		
	B	71				71	70.0		
	C	68				68	67.0		
	D	72				72	71.0		
11.9	A	85				85	84.0		
	B	78				78	77.0		
	C	83				83	82.0		
	D	105	100			102.5	101.5		
23.8	A	150				150	149.0		
	B	162				162	161.0		
	C	168				168	167.0		
	D	147				147	146.0		
47.6	A	175				175	174.0		
	B	161				161	160.0		
	C	136	140			138	137.0		
	D	179				179	178.0		
95.2	A	158				158	157.0		
	B	147				147	146.0		
	C	155				155	154.0		
	D	139				139	138.0		

JGL
 March 13/14

CETIS Analytical Report

Report Date: 02 Mar-14 16:20 (p 1 of 2)
 Test Code: 14078a | 14-6494-7294

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 03-1865-4579	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-14 16:20	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 14-3179-1764	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 27 Feb-14 15:30	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 02 Mar-14 15:30	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 6d
Sample ID: 00-4133-3542	Code: 276B326	Client: ALS
Sample Date: 25 Feb-14 15:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 49h (3.6 °C)	Station: L1426336-1(R10)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	977764	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.7195	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>95.2	N/A	N/A	<1.05	NA	NA
IC10	>95.2	N/A	N/A	<1.05	NA	NA
IC15	>95.2	N/A	N/A	<1.05	NA	NA
IC20	>95.2	N/A	N/A	<1.05	NA	NA
IC25	>95.2	N/A	N/A	<1.05	NA	NA
IC40	>95.2	N/A	N/A	<1.05	NA	NA
IC50	>95.2	N/A	N/A	<1.05	NA	NA

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	46.75	38	57	2.562	7.246	15.5%	0.0%
1.5		4	49.75	41	56	3.376	6.752	13.57%	-6.42%
3		4	55	46	62	3.536	7.071	12.86%	-17.65%
6		4	68.5	66	71	1.19	2.38	3.48%	-46.52%
11.9		4	86.25	77	102	5.452	10.9	12.64%	-84.49%
23.8		4	155.8	146	167	4.956	9.912	6.36%	-233.2%
47.6		4	162.3	137	178	9.259	18.52	11.41%	-247.1%
95.2		4	148.8	138	157	4.27	8.539	5.74%	-218.2%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	41	53	55	46	42	57	38	42
1.5		54	56	41	48				
3		46	59	62	53				
6		66	70	67	71				
11.9		84	77	82	102				
23.8		149	161	167	146				
47.6		174	160	137	178				
95.2		157	146	154	138				

QA: JG
 March 13/14

CETIS Analytical Report

Report Date: 02 Mar-14 16:20 (p 2 of 2)
Test Code: 14078a | 14-6494-7294

EC Alga Growth Inhibition Test

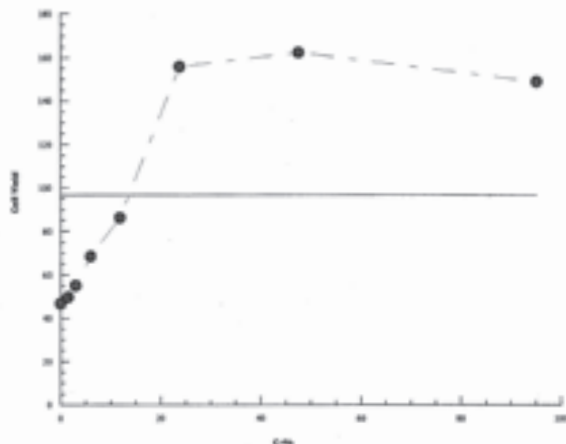
Nautilus Environmental

Analysis ID: 03-1865-4579
Analyzed: 02 Mar-14 16:20

Endpoint: Cell Yield
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 02 Mar-14 16:20 (p 1 of 2)
 Test Code: 14078a | 14-6494-7294

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 15-2090-6552	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-14 16:19	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 14-3179-1764	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 27 Feb-14 15:30	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 02 Mar-14 15:30	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 6d
Sample ID: 00-4133-3542	Code: 276B326	Client: ALS
Sample Date: 25 Feb-14 15:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 49h (3.6 °C)	Station: L1426336-1(R10)	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	31.7%	3	6	4.243	33.33

Dunnett Multiple Comparison Test

Control	vs C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	1.5	0.5113	2.526	14.82	10	0.7787	CDF	Non-Significant Effect
	3	1.406	2.526	14.82	10	0.3520	CDF	Non-Significant Effect
	6*	3.707	2.526	14.82	10	0.0030	CDF	Significant Effect
	11.9*	6.732	2.526	14.82	10	<0.0001	CDF	Significant Effect
	23.8*	18.58	2.526	14.82	10	<0.0001	CDF	Significant Effect
	47.6*	19.69	2.526	14.82	10	<0.0001	CDF	Significant Effect
	95.2*	17.39	2.526	14.82	10	<0.0001	CDF	Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.7195	Non-significant Trend in Controls

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	80186.5	11455.21	7	124.8	<0.0001	Significant Effect
Error	2570.25	91.79464	28			
Total	82756.75		35			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	10.7	18.48	0.1522	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9648	0.9166	0.3001	Normal Distribution

Cell Yield Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	46.75	40.69	52.81	44	38	57	2.562	15.5%	0.0%
1.5		4	49.75	39.01	60.49	51	41	56	3.376	13.57%	-6.42%
3		4	55	43.75	66.25	56	46	62	3.536	12.86%	-17.65%
6		4	68.5	64.71	72.29	68.5	66	71	1.19	3.48%	-46.52%
11.9		4	86.25	68.9	103.6	83	77	102	5.452	12.64%	-84.49%
23.8		4	155.8	140	171.5	155	146	167	4.956	6.36%	-233.2%
47.6		4	162.3	132.8	191.7	167	137	178	9.259	11.41%	-247.1%
95.2		4	148.8	135.2	162.3	150	138	157	4.27	5.74%	-218.2%

QA *tole*
 March 13/14

CETIS Analytical Report

Report Date: 02 Mar-14 16:20 (p 2 of 2)
 Test Code: 14078a | 14-6494-7294

EC Alga Growth Inhibition Test

Nautilus Environmental

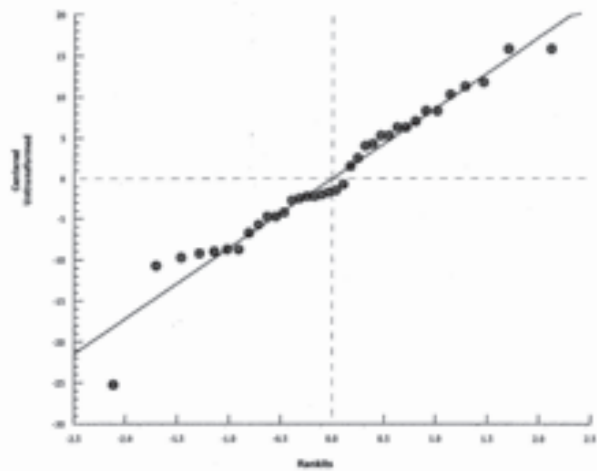
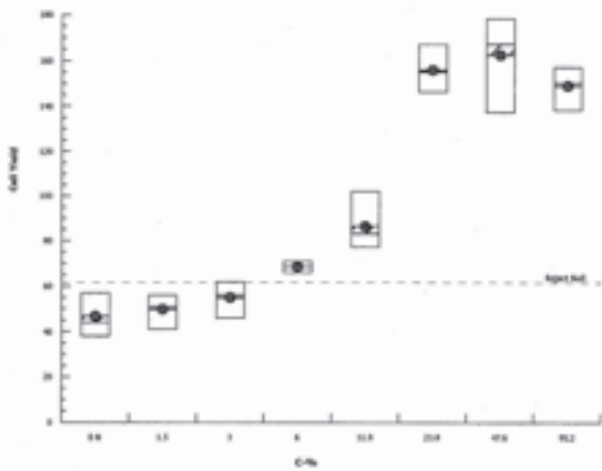
Analysis ID: 15-2090-6552 Endpoint: Cell Yield
 Analyzed: 02 Mar-14 16:19 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
 Official Results: Yes

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	41	53	55	46	42	57	38	42
1.5		54	58	41	48				
3		48	59	62	53				
6		68	70	67	71				
11.9		84	77	82	102				
23.8		149	161	167	146				
47.6		174	160	137	178				
95.2		157	146	154	138				

Graphics



Pseudokirchneriella subcapitata Summary Sheet

Client: ALS
Work Order No.: 14078

Start Date: Feb 27/14
Set up by: EMM

Sample Information:

Sample ID: L1426336-2 (NFI)
Sample Date: Feb 25/14 @ 14:35 h
Date Received: Feb 27/14 @ 1030h
Sample Volume: 2x20L

Test Organism Information:

Culture Date: Feb 21/14
Age of culture (Day 0): 6d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC105
Stock Solution ID: 13Zn01
Date Initiated: Feb 21/14
72-h IC50 (95% CL): 24.7 (15.2-33.8) ^{16.1-31.7 emm} µg/L Zn

72-h IC50 Reference Toxicant Mean and Range: 22.7 (15.2-33.8) µg/L Zn CV (%): 22

Test Results:	Algal Growth
IC25 %(v/v) (95% CL)	<u>795.2</u>
IC50 %(v/v) (95% CL)	<u>795.2</u>

Reviewed by: JGU

Date reviewed: March 13/14

72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client: ALS Setup by: EMM
 Sample ID: NF1(L14763362) Test Date/Time: Feb 27/14 @ 1600
 Work Order No.: 14078 Test Species: Pseudokirchneriella subcapitata

Culture Date: Feb 21/14 Age of Culture: 6d Culture Health: Good
 Culture Count: 1562 2589 Average: 575.5 Culture Cell Density (c1): 575.5 x 10⁴ cells/ml

$$v1 = \frac{220,000 \text{ cells/ml} \times 100 \text{ ml}}{(c1) \ 575.5 \text{ cells/ml}} = 4 \text{ ml}$$

Time Zero Counts: 124 220 Average: 22

No. of Cells/mL: 22 x 10⁴ Initial Density: # cells/mL ÷ 220 µL x 10 µL = 10 000 cells/ml

Concentration %(v/v)	Water Quality Measurements					Microplates rotated 2X per day?			
	pH	Temp (°C)				0 h	24 h	48 h	72 h
		0 h	0 h	24 h	48 h				
Control	6.9	24.0	25.0	25.0	25.5	✓	✓	/	/
1.5	7.0	24.5	↓	↓	↓	✓	✓	/	/
3.0	7.0	24.5				✓	✓	/	/
6.0	7.1	25.0				✓	✓	/	/
11.9	7.2	26.0				✓	✓	/	/
23.8	7.2	25.5				✓	✓	/	/
47.6	7.3	25.5				✓	✓	/	/
95.2	7.4	25.5				✓	✓	/	/
Initials	EMM	EMM				EMM	M	A	EMM

Initial control pH: Well 1: 6.8 Well 2: 6.8

Final control pH: Well 1: 6.5 Well 2: 6.5

Light intensity (lux): 3700 Date measured: Feb 27/14

Sample Description: clear

Comments: _____

Reviewed: JGH Date reviewed: March 13/14

***Pseudokirchneriella subcapitata* Toxicity Test Data Sheet**
72-h Algal Cell Counts

Client: ALS Start Date/Time: Feb 27/14 @ 1600
 Work Order #: 14678 Termination Date: March 2/14 @ 1600
 Sample ID: NFI (44723362) Test set up by: Emm/JW
 % (v/v)

Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	44					EMM
	B	54					
	C	52					
	D	60					
	E	42					
	F	45					
	G	58					
	H	46					
1.5	A	53					
	B	46					
	C	54					
	D	47					
3.0	A	66					
	B	69					
	C	54					
	D	64					
6.0	A	90					
	B	81					
	C	74					
	D	77					
11.9	A	56	60				
	B	64	62				
	C	78	74				
	D	76	73				
23.8	A	83					
	B	77					
	C	91					
	D	89					
47.6	A	117					
	B	92					
	C	121					
	D	124					
95.2	A	135					
	B	148					
	C	127					
	D	123					

Comments: _____

Reviewed by: JGh Date Reviewed: March 13/14

Pseudokirchneriella subcapitata Algal Counts

Client: ALS
 WO#: 14078
 Sample ID: NF1 (L1426336-2)

Start Date/Time: 27-Feb-14 @1600h
 Termination Date: 02-Mar-14 @1600h

Initial Cell Density: 10000 cell/mL
 220000
 0.22
 0.01
 10000

Concentration % v/v	Rep	Count 1 (x 10 ⁴)	Count 2 (x 10 ⁴)	Count 3 (x 10 ⁴)	Count 4 (x 10 ⁴)	Mean (x 10 ⁴)	Cell Yield (x 10 ⁴) cell/mL		
Control	A	44				44	43.0	mean	49.1
	B	54				54	53.0	SD	6.812541
	C	52				52	51.0	CV	13.86777
	D	60				60	59.0		
	E	42				42	41.0		
	F	45				45	44.0		
	G	58				58	57.0		
	H	46				46	45.0		
1.5	A	53				53	52.0		
	B	46				46	45.0		
	C	54				54	53.0		
	D	47				47	46.0		
3	A	66				66	65.0		
	B	69				69	68.0		
	C	54				54	53.0		
	D	64				64	63.0		
5.9	A	90				90	89.0		
	B	81				81	80.0		
	C	74				74	73.0		
	D	77				77	76.0		
11.9	A	56	60			58	57.0		
	B	64	62			63	62.0		
	C	78	74			76	75.0		
	D	76	73			74.5	73.5		
23.8	A	83				83	82.0		
	B	77				77	76.0		
	C	91				91	90.0		
	D	89				89	88.0		
47.6	A	117				117	116.0		
	B	92				92	91.0		
	C	121				121	120.0		
	D	124				124	123.0		
95.2	A	135				135	134.0		
	B	148				148	147.0		
	C	127				127	126.0		
	D	123				123	122.0		

Job
 March 13/14

CETIS Analytical Report

Report Date: 02 Mar-14 16:29 (p 1 of 2)
 Test Code: 14078b | 17-5330-0750

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 09-7802-2192	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-14 16:28	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 05-3776-6416	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 27 Feb-14 16:00	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 02 Mar-14 16:00	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 6d
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 49h (4.3 °C)	Station: L1426336-2(NF1)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	392813	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.9049	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>95.2	N/A	N/A	<1.05	NA	NA
IC10	>95.2	N/A	N/A	<1.05	NA	NA
IC15	>95.2	N/A	N/A	<1.05	NA	NA
IC20	>95.2	N/A	N/A	<1.05	NA	NA
IC25	>95.2	N/A	N/A	<1.05	NA	NA
IC40	>95.2	N/A	N/A	<1.05	NA	NA
IC50	>95.2	N/A	N/A	<1.05	NA	NA

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	49.13	41	59	2.409	6.813	13.87%	0.0%
1.5		4	49	45	53	2.041	4.082	8.33%	0.25%
3		4	62.25	53	68	3.25	6.5	10.44%	-26.72%
6		4	79.5	73	89	3.476	6.952	8.75%	-61.83%
11.9		4	67	57	75	4.453	8.907	13.29%	-36.39%
23.8		4	84	76	90	3.162	6.325	7.53%	-70.99%
47.6		4	112.5	91	123	7.309	14.62	12.99%	-129.0%
95.2		4	132.3	122	147	5.513	11.03	8.34%	-169.2%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	43	53	51	59	41	44	57	45
1.5		52	45	53	46				
3		65	68	53	63				
6		89	80	73	76				
11.9		57	62	75	74				
23.8		82	76	90	88				
47.6		116	91	120	123				
95.2		134	147	126	122				

CETIS Analytical Report

Report Date: 02 Mar-14 16:29 (p 2 of 2)
Test Code: 14078b | 17-5330-0750

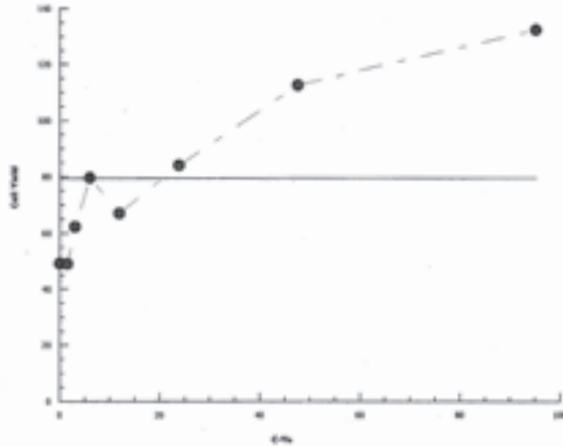
EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 09-7802-2192 Endpoint: Cell Yield
Analyzed: 02 Mar-14 16:28 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 02 Mar-14 16:28 (p 1 of 2)
 Test Code: 14078b | 17-5330-0750

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 04-8432-7857	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-14 16:28	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 05-3776-6416	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 27 Feb-14 16:00	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 02 Mar-14 16:00	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 6d
Sample ID: 17-8615-7390	Code: 6A76994E	Client: ALS
Sample Date: 25 Feb-14 14:35	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 49h (4.3 °C)	Station: L1426336-2(NF1)	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	26.7%	1.5	3	2.121	66.67

Dunnett Multiple Comparison Test

Control	vs C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	1.5	-0.02409	2.526	13.11	10	0.9313	CDF	Non-Significant Effect
	3*	2.529	2.526	13.11	10	0.0497	CDF	Significant Effect
	6*	5.854	2.526	13.11	10	<0.0001	CDF	Significant Effect
	11.9*	3.445	2.526	13.11	10	0.0058	CDF	Significant Effect
	23.8*	6.721	2.526	13.11	10	<0.0001	CDF	Significant Effect
	47.6*	12.21	2.526	13.11	10	<0.0001	CDF	Significant Effect
	95.2*	16.02	2.526	13.11	10	<0.0001	CDF	Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.9049	Non-significant Trend in Controls

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	28064.38	4009.197	7	55.84	<0.0001	Significant Effect
Error	2010.375	71.79911	28			
Total	30074.75		35			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.108	18.48	0.5272	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9701	0.9166	0.4274	Normal Distribution

Cell Yield Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	49.13	43.43	54.82	48	41	59	2.409	13.87%	0.0%
1.5		4	49	42.5	55.5	49	45	53	2.041	8.33%	0.25%
3		4	62.25	51.91	72.59	64	53	68	3.25	10.44%	-26.72%
6		4	79.5	68.44	90.56	78	73	89	3.476	8.75%	-61.83%
11.9		4	67	52.83	81.17	68	57	75	4.453	13.29%	-36.39%
23.8		4	84	73.94	94.06	85	76	90	3.162	7.53%	-70.99%
47.6		4	112.5	89.24	135.8	118	91	123	7.309	12.99%	-129.0%
95.2		4	132.3	114.7	149.8	130	122	147	5.513	8.34%	-169.2%

CETIS Analytical Report

Report Date: 02 Mar-14 16:28 (p 2 of 2)
 Test Code: 14078b | 17-5330-0750

EC Alga Growth Inhibition Test

Nautilus Environmental

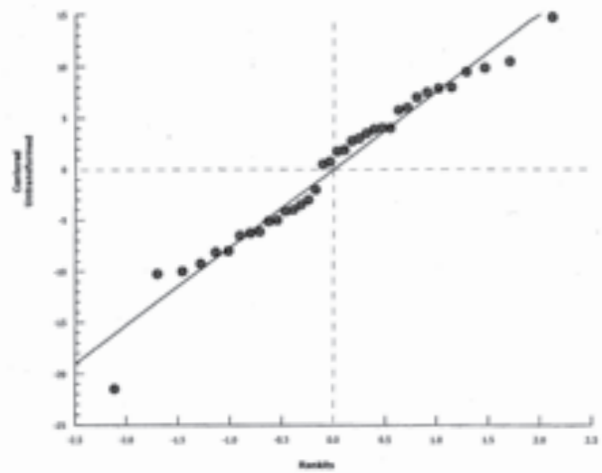
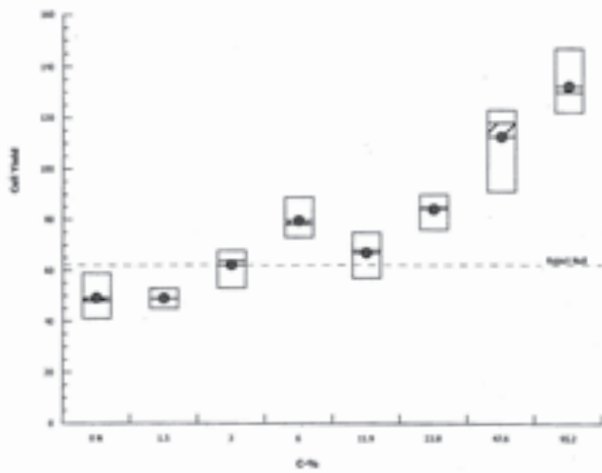
Analysis ID: 04-8432-7857 Endpoint: Cell Yield
 Analyzed: 02 Mar-14 16:28 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
 Official Results: Yes

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	43	53	51	59	41	44	57	45
1.5		52	45	53	46				
3		65	68	53	63				
6		89	80	73	76				
11.9		57	62	75	74				
23.8		82	76	90	88				
47.6		116	91	120	123				
95.2		134	147	126	122				

Graphics



Pseudokirchneriella subcapitata Summary Sheet

Client: ALS
Work Order No.: 14678

Start Date: Feb 27/14
Set up by: EMM

Sample Information:

Sample ID: L1426336-3 (NF2)
Sample Date: Feb 25/14 @ 1305h
Date Received: Feb 27/14 @ 1030h
Sample Volume: 2x20L

Test Organism Information:

Culture Date: Feb 2/14
Age of culture (Day 0): 6d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC105
Stock Solution ID: 137n01
Date Initiated: Feb 2/14
72-h IC50 (95% CL): 24.7 (16.1 - 31.7) ^{err} ~~(15.2 - 33.8)~~ µg/L Zn

72-h IC50 Reference Toxicant Mean and Range: 22.7 (15.2 - 33.8) µg/L Zn CV (%): 22

Test Results:	Algal Growth
IC25 %(v/v) (95% CL)	^{err} <u>12.0 (9.5 - 14.5) 8.5 (7.5 - 9.7)</u>
IC50 %(v/v) (95% CL)	<u>12.0 (9.5 - 14.5)</u>

Reviewed by: JGh

Date reviewed: March 17/14

72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client: ALS Setup by: EMM
 Sample ID: NF2 (L1426363) Test Date/Time: Feb 27/14 @ 1600
 Work Order No.: 14078 Test Species: Pseudokirchneriella subcapitata

Culture Date: Feb 21/14 Age of Culture: 6d Culture Health: Good
 Culture Count: 1562 2589 Average: 575.5 Culture Cell Density (c1): 575.5 x 10⁴ cells/ml

$$v1 = \frac{220,000 \text{ cells/ml} \times 100 \text{ ml}}{(c1) \ 575.5 \times 10^4 \text{ cells/ml}} = 4 \text{ ml}$$

Time Zero Counts: 124 220 Average: 22

No. of Cells/mL: 22 x 10⁴ Initial Density: # cells/mL ÷ 220 µL x 10 µL = 10000 cells/ml

Concentration %(v/v)	Water Quality Measurements					Microplates rotated 2X per day?			
	pH	Temp (°C)							
	0 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Control	6.9	24.5	25.0	25.0	25.5	✓	✓	/	/
1.5	7.0	24.5	↓	↓	↓	✓	✓	/	/
3.0	7.0	24.5	↓	↓	↓	✓	✓	/	/
6.0	7.1	24.5	↓	↓	↓	✓	✓	/	/
11.9	7.2	24.5	↓	↓	↓	✓	✓	/	/
23.8	7.3	25.0	↓	↓	↓	✓	✓	/	/
47.6	7.3	25.0	↓	↓	↓	✓	✓	/	/
95.2	7.4	25.0	↓	↓	↓	✓	✓	/	/
Initials	EMM	EMM	EMM	M	M	EMM	EMM	M	M

Initial control pH: Well 1: 6.8 Well 2: 6.8

Final control pH: Well 1: 6.5 Well 2: 6.5

Light intensity (lux): 3800 Date measured: Feb 27/14

Sample Description: clear

Comments: _____

Reviewed: JGh Date reviewed: March 14/14

***Pseudokirchneriella subcapitata* Toxicity Test Data Sheet**
72-h Algal Cell Counts

Client: ALS Start Date/Time: Feb 27/14 @ 1600
 Work Order #: 14678 Termination Date: March 2/14 @ 1600
 Sample ID: NE2 (U426336-3) Test set up by: Emm/JW
 % (v/v)

Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	43					Emm
	B	40					
	C	48					
	D	42					
	E	45					
	F	45					
	G	44					
	H	41					
1.5	A	52					
	B	56					
	C	37	39				
	D	58					
3.0	A	59					
	B	55					
	C	51					
	D	62					
6.0	A	48					
	B	39					
	C	52					
	D	43					
11.9	A	23					
	B	28					
	C	16	14				
	D	24					
23.8	A	4	2	2			
	B	2					
	C	1					
	D	1					
47.6	A	0					
	B	0					
	C	0					
	D	0					
95.2	A	0					
	B	1					
	C	0					
	D	0					

Comments: _____

Reviewed by: JW Date Reviewed: March 14/14

***Pseudokirchneriella subcapitata* Algal Counts**

Client: ALS
 WO#: 14078
 Sample ID: NF2 (L1426336-3)

Start Date/Time: 27-Feb-14 @1600h
 Termination Date: 02-Mar-14 @1600h

Initial Cell Density: 10000 cell/mL
 220000
 0.22
 0.01
 10000

Concentration % v/v	Rep	Count 1 (x 10 ⁴)	Count 2 (x 10 ⁴)	Count 3 (x 10 ⁴)	Count 4 (x 10 ⁴)	Mean (x 10 ⁴)	Cell Yield (x 10 ⁴) cell/mL		
Control	A	43				43	42.0	mean	42.5
	B	40				40	39.0	SD	2.56348
	C	48				48	47.0	CV	6.031717
	D	42				42	41.0		
	E	45				45	44.0		
	F	45				45	44.0		
	G	44				44	43.0		
	H	41				41	40.0		
1.5	A	52				52	51.0		
	B	56				56	55.0		
	C	37	39			38	37.0		
	D	58				58	57.0		
3	A	59				59	58.0		
	B	55				55	54.0		
	C	51				51	50.0		
	D	62				62	61.0		
6	A	48				48	47.0		
	B	39				39	38.0		
	C	52				52	51.0		
	D	43				43	42.0		
11.9	A	23				23	22.0		
	B	28				28	27.0		
	C	16	14			15	14.0		
	D	24				24	23.0		
23.8	A	4	2	2		2.6666667	1.7		
	B	2				2	1.0		
	C	1				1	0.0		
	D	1				1	0.0		
47.6	A	0				0	-1.0		
	B	0				0	-1.0		
	C	0				0	-1.0		
	D	0				0	-1.0		
95.2	A	0				0	-1.0		
	B	1				1	0.0		
	C	0				0	-1.0		
	D	0				0	-1.0		

JBB
 March 14/14

CETIS Analytical Report

Report Date: 02 Mar-14 16:36 (p 1 of 2)
 Test Code: 14078c | 13-3187-1210

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 11-4088-9740	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-14 16:36	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 19-9503-0983	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 27 Feb-14 16:00	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 02 Mar-14 16:00	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 6d
Sample ID: 07-0729-5812	Code: 2A287A44	Client: ALS
Sample Date: 25 Feb-14 13:05	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 51h (3.3 °C)	Station: L1426336-3(NF2)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2075691	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.9049	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	4.299	3.104	7.652	23.26	13.07	32.21
IC10	6.005	3.087	7.311	16.65	13.68	32.4
IC15	6.48	3.946	7.809	15.43	12.81	25.34
IC20	6.988	4.944	8.354	14.31	11.97	20.23
IC25	7.53	5.715	8.93	13.28	11.2	17.5
IC40	9.388	7.783	11.21	10.65	8.922	12.85
IC50	10.85	9.083	13.25	9.22	7.549	11.01

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	42.5	39	47	0.9063	2.563	6.03%	0.0%
1.5		4	50	37	57	4.509	9.018	18.04%	-17.65%
3		4	55.75	50	61	2.394	4.787	8.59%	-31.18%
6		4	44.5	38	51	2.843	5.686	12.78%	-4.71%
11.9		4	21.5	14	27	2.723	5.447	25.33%	49.41%
23.8		4	0.75	0	2	0.4787	0.9574	127.7%	98.24%
47.6		4	0	0	0	0	0		100.0%
95.2		4	0	0	0	0	0		100.0%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	42	39	47	41	44	44	43	40
1.5		51	55	37	57				
3		58	54	50	61				
6		47	38	51	42				
11.9		22	27	14	23				
23.8		2	1	0	0				
47.6		0	0	0	0				
95.2		0	0	0	0				

CETIS Analytical Report

Report Date: 02 Mar-14 16:36 (p 2 of 2)
Test Code: 14078c | 13-3187-1210

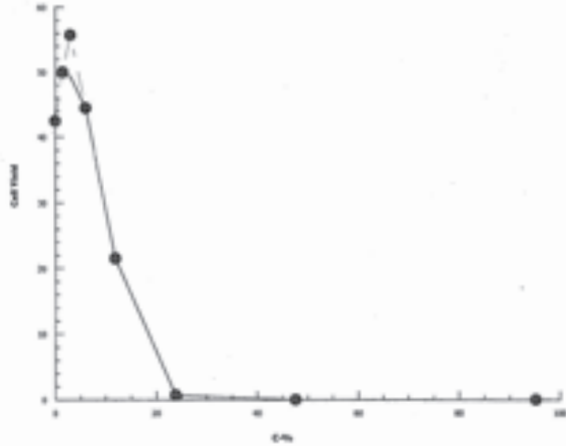
EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 11-4088-9740 Endpoint: Cell Yield
Analyzed: 02 Mar-14 16:36 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 02 Mar-14 16:41 (p 1 of 2)
 Test Code: 14078c(a) | 16-1132-3261

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 07-6181-9388	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-14 16:41	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 05-6146-3800	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 27 Feb-14 16:00	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 02 Mar-14 16:00	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 6d
Sample ID: 07-0729-5812	Code: 2A287A44	Client: ALS
Sample Date: 25 Feb-14 13:05	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 51h (3.3 °C)	Station: L1428336-3(NF2)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	279983	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.9049	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	6.348	5.826	6.859	15.75	15.02	17.16
IC10	6.828	6.243	7.333	14.65	13.64	16.02
IC15	7.34	6.721	8.051	13.62	12.42	14.88
IC20	7.886	7.083	8.828	12.68	11.33	14.12
IC25	8.467	7.468	9.692	11.81	10.32	13.39
IC40	10.45	8.642	12.96	9.57	7.716	11.57
IC50	12	9.474	14.49	8.332	6.9	10.55

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	42.5	39	47	0.9063	2.563	6.03%	0.0%
1.5		4	42	42	42	0	0	0.0%	1.18%
3		4	42	42	42	0	0	0.0%	1.18%
6		4	42	42	42	0	0	0.0%	1.18%
11.9		4	21.5	14	27	2.723	5.447	25.33%	49.41%
23.8		4	0.75	0	2	0.4787	0.9574	127.7%	98.24%
47.6		4	0	0	0	0	0		100.0%
95.2		4	0	0	0	0	0		100.0%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	42	39	47	41	44	44	43	40
1.5		42	42	42	42				
3		42	42	42	42				
6		42	42	42	42				
11.9		22	27	14	23				
23.8		2	1	0	0				
47.6		0	0	0	0				
95.2		0	0	0	0				

CETIS Analytical Report

Report Date: 02 Mar-14 16:41 (p 2 of 2)
Test Code: 14078c(a) | 16-1132-3261

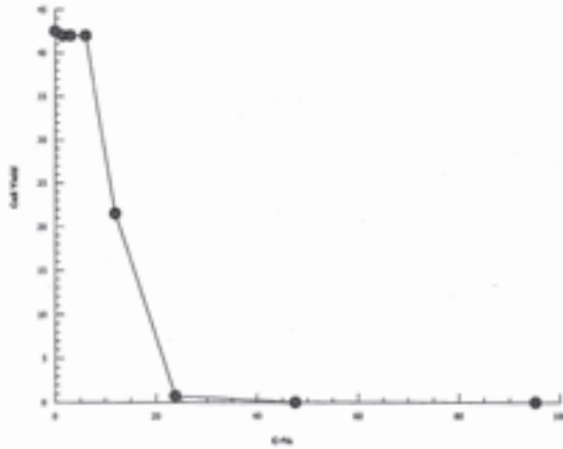
EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 07-6181-9388 Endpoint: Cell Yield
Analyzed: 02 Mar-14 16:41 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



***Pseudokirchneriella subcapitata* Summary Sheet**

Client: ALS
 Work Order No.: 14078

Start Date: Feb 27/14
 Set up by: EMM

Sample Information:

Sample ID: L1426336-34 (X1)
 Sample Date: Feb 25/14 @ 1110
 Date Received: Feb 27/14 @ 1030
 Sample Volume: 2x20L

Test Organism Information:

Culture Date: Feb 21/14
 Age of culture (Day 0): 6d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC105
 Stock Solution ID: 13Zn01
 Date Initiated: Feb 21/14
 72-h IC50 (95% CL): 24.7 (15.7 - 33.8) ^{em} µg/L Zn

72-h IC50 Reference Toxicant Mean and Range: 22.7 (15.2 - 33.8) ^{em} µg/L Zn CV (%): 22

Test Results:	Algal Growth ^{em}
IC25 %(v/v) (95% CL)	em 7.5 9.7 (8.4 - 11.0) 10.2 (7.9 - 11.8)
IC50 %(v/v) (95% CL)	15.01 (13.5 - 16.1) ^{em} 15.4 (13.2 - 16.6)

Reviewed by: JGU

Date reviewed: March 17/14

72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client: ALS Setup by: EMM
 Sample ID: X1 (14426336-4) Test Date/Time: Feb 27/14 @ 1530
 Work Order No.: 14078 Test Species: Pseudokirchneriella subcapitata

Culture Date: Feb 27/14 Age of Culture: bd Culture Health: Good
 Culture Count: 1 562 2 589 Average: 575.5 Culture Cell Density (c1): 575.5 x 10⁴ cells/ml

$$v1 = \frac{220,000 \text{ cells/ml} \times 100 \text{ ml}}{(c1) \ 575.5 \times 10^4 \text{ cells/ml}} = 4 \text{ ml}$$

Time Zero Counts: 1 24 2 20 Average: 22

No. of Cells/mL: 22 x 10⁴ Initial Density: # cells/mL + 220 μ L x 10 μ L = 10000 cells/ml

Concentration %(v/v)	Water Quality Measurements					Microplates rotated 2X per day?			
	pH	Temp (°C)				0 h	24 h	48 h	72 h
		0 h	0 h	24 h	48 h				
Control	6.9	24.0	25.0	25.0	25.5	✓	✓	/	/
1.5	6.9	24.0				✓	✓	/	/
3.0	6.9	24.0				✓	✓	/	/
6.0	7.0	24.0				✓	✓	/	/
11.9	7.0	24.0				✓	✓	/	/
23.8	7.3	24.0				✓	✓	/	/
47.6	7.5	24.0				✓	✓	/	/
95.2	7.5	24.0	↓	↓	↓	✓	✓	/	/
Initials	EMM	EMM	EMM	m	m	EMM	EMM	m	m

Initial control pH: Well 1: 6.8 Well 2: 6.8
 Final control pH: Well 1: 6.5 Well 2: 6.5
 Light intensity (lux): 3800 Date measured: Feb 27/14

Sample Description: A_{em}
 Comments: clear

Reviewed: JGk Date reviewed: March 14/14

***Pseudokirchneriella subcapitata* Toxicity Test Data Sheet**
72-h Algal Cell Counts

Client: ALS Start Date/Time: Feb 27/14 @ 1530
 Work Order #: 14078 Termination Date: MARCH 2/14 @ 1530
 Sample ID: X1 (11426336-4) Test set up by: FMM/JW
 % (v/v)

Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	43					FMM
	B	48					
	C	42					
	D	55					
	E	56					
	F	44					
	G	54					
	H	51					
1.5	A	42					
	B	58	60				
	C	40					
	D	51					
3.0	A	51					
	B	58					
	C	55					
	D	55					
6.0	A	56					
	B	59					
	C	46					
	D	47					
11.9	A	34					
	B	36					
	C	30					
	D	35					
23.8	A	11					
	B	8					
	C	10					
	D	12					
47.6	A	0					
	B	2					
	C	1					
	D	0					
95.2	A	2	0				
	B	6	2	3			
	C	0	0				
	D	2	2				

Comments: _____

Reviewed by: JGK Date Reviewed: March 14/14

Pseudokirchneriella subcapitata Algal Counts

Client: ALS
 WO#: 14078
 Sample ID: X1 (L1426336-4)

Start Date/Time: 27-Feb-14 @1600h
 Termination Date: 02-Mar-14 @1600h

Initial Cell Density: 10000 cell/mL
 220000
 0.22
 0.01
 10000

Concentration % v/v	Rep	Count 1 (x 10 ⁴)	Count 2 (x 10 ⁴)	Count 3 (x 10 ⁴)	Count 4 (x 10 ⁴)	Mean (x 10 ⁴)	Cell Yield (x 10 ⁴) cell/mL		
Control	A	43				43	42.0	mean	48.1
	B	48				48	47.0	SD	5.667892
	C	42				42	41.0	CV	11.77744
	D	55				55	54.0		
	E	56				56	55.0		
	F	44				44	43.0		
	G	54				54	53.0		
	H	51				51	50.0		
1.5	A	42				42	41.0		
	B	58	60			59	58.0		
	C	40				40	39.0		
	D	51				51	50.0		
3	A	51				51	50.0		
	B	58				58	57.0		
	C	55				55	54.0		
	D	55				55	54.0		
6	A	56				56	55.0		
	B	59				59	58.0		
	C	46				46	45.0		
	D	47				47	46.0		
11.9	A	34				34	33.0		
	B	36				36	35.0		
	C	30				30	29.0		
	D	35				35	34.0		
23.8	A	11				11	10.0		
	B	8				8	7.0		
	C	10				10	9.0		
	D	12				12	11.0		
47.6	A	0				0	-1.0		
	B	2				2	1.0		
	C	1				1	0.0		
	D	0				0	-1.0		
95.2	A	2	0			1	0.0		
	B	6	2	3		3.6666667	2.7		
	C	0	0			0	-1.0		
	D	2	2			2	1.0		

JOL
 March 14/14

CETIS Analytical Report

Report Date: 02 Mar-14 16:51 (p 1 of 2)
 Test Code: 14078d | 03-4486-3229

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 08-0057-9365	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-14 16:51	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 15-1545-1848	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 27 Feb-14 16:00	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 02 Mar-14 16:00	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 6d
Sample ID: 19-2140-4869	Code: 72864FC5	Client: ALS
Sample Date: 25 Feb-14 11:10	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 53h (4 °C)	Station: L1426336-4(X1)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	439728	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.3987	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	6.649	2.431	6.782	15.04	14.74	41.14
IC10	7.359	5.37	7.651	13.59	13.07	18.62
IC15	8.134	6.299	8.616	12.29	11.61	15.88
IC20	8.981	7.355	9.687	11.13	10.32	13.6
IC25	9.907	8.394	10.95	10.09	9.134	11.91
IC40	12.93	11.31	14.07	7.732	7.108	8.842
IC50	15.01	13.47	16.08	6.662	6.22	7.423

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	48.13	41	55	2.004	5.668	11.78%	0.0%
1.5		4	47	39	58	4.378	8.756	18.63%	2.34%
3		4	53.75	50	57	1.436	2.872	5.34%	-11.69%
6		4	51	45	58	3.24	6.481	12.71%	-5.97%
11.9		4	32.75	29	35	1.315	2.63	8.03%	31.95%
23.8		4	9.25	7	11	0.8539	1.708	18.46%	80.78%
47.6		4	0.25	0	1	0.25	0.5	200.0%	99.48%
95.2		4	1	0	3	0.7071	1.414	141.4%	97.92%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	42	47	41	54	55	43	53	50
1.5		41	58	39	50				
3		50	57	54	54				
6		55	58	45	46				
11.9		33	35	29	34				
23.8		10	7	9	11				
47.6		0	1	0	0				
95.2		0	3	0	1				

CETIS Analytical Report

Report Date: 02 Mar-14 16:51 (p 2 of 2)

Test Code: 14078d | 03-4486-3229

EC Alga Growth Inhibition Test

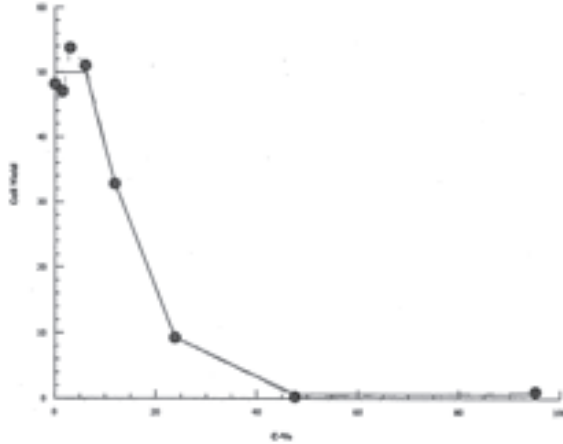
Nautilus Environmental

Analysis ID: 08-0057-9365
Analyzed: 02 Mar-14 16:51

Endpoint: Cell Yield
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 02 Mar-14 16:55 (p 1 of 2)
 Test Code: 14078d(a) | 06-5207-0372

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 06-5990-8232	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-14 16:55	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 16-0001-8244	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 27 Feb-14 16:00	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 02 Mar-14 16:00	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 6d
Sample ID: 19-2140-4869	Code: 72864FC5	Client: ALS
Sample Date: 25 Feb-14 11:10	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 53h (4 °C)	Station: L1426336-4(X1)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	550508	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.3987	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	6.582	N/A	6.947	15.19	14.4	NA
IC10	7.367	5.209	7.929	13.57	12.61	19.2
IC15	8.235	6.125	9.034	12.14	11.07	16.33
IC20	9.192	6.895	10.32	10.88	9.69	14.5
IC25	10.25	7.86	11.77	9.758	8.496	12.72
IC40	13.37	10.97	14.57	7.481	6.863	9.112
IC50	15.43	13.18	16.55	6.483	6.043	7.588

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	48.13	41	55	2.004	5.668	11.78%	0.0%
1.5		4	47	39	58	4.378	8.756	18.63%	2.34%
3		4	48	48	48	0	0	0.0%	0.26%
6		4	48	48	48	0	0	0.0%	0.26%
11.9		4	32.75	29	35	1.315	2.63	8.03%	31.95%
23.8		4	9.25	7	11	0.8539	1.708	18.46%	80.78%
47.6		4	0.25	0	1	0.25	0.5	200.0%	99.48%
95.2		4	1	0	3	0.7071	1.414	141.4%	97.92%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	42	47	41	54	55	43	53	50
1.5		41	58	39	50				
3		48	48	48	48				
6		48	48	48	48				
11.9		33	35	29	34				
23.8		10	7	9	11				
47.6		0	1	0	0				
95.2		0	3	0	1				

CETIS Analytical Report

Report Date: 02 Mar-14 16:55 (p 2 of 2)
Test Code: 14078d(a) | 06-5207-0372

EC Alga Growth Inhibition Test

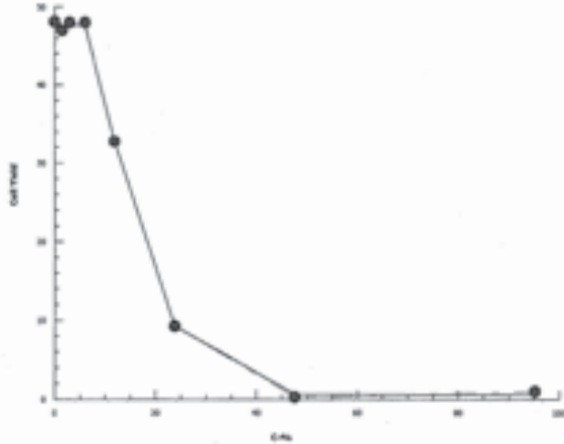
Nautilus Environmental

Analysis ID: 06-5990-8232
Analyzed: 02 Mar-14 16:55

Endpoint: Cell Yield
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



Pseudokirchneriella subcapitata Summary Sheet

Client: ALS
Work Order No.: 14078

Start Date: Feb 28/14
Set up by: Emm

Sample Information:

Sample ID: L1426336-5 (X14)
Sample Date: Feb 25/14 @ 0900h
Date Received: Feb 27/14 @ 1030h
Sample Volume: 2x20L

Test Organism Information:

Culture Date: Feb 21/14
Age of culture (Day 0): 7d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC105
Stock Solution ID: 137n01
Date Initiated: Feb 21/14

72-h IC50 (95% CL): 24.7 (16.1 - 31.7)^{em} /ug/L Zn

72-h IC50 Reference Toxicant Mean and Range: 22.7 (15.2 - 33.8) /ug/L Zn CV (%): 22

Test Results:

	Algal Growth
IC25 %(v/v) (95% CL)	19.3 (16.8 - 21.8)
IC50 %(v/v) (95% CL)	30.3 (26.4 - 33.6)

Reviewed by: Joh

Date reviewed: March 17/14

72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client : ALS Setup by: EMM
 Sample ID: X14 (144263365) Test Date/Time: Feb 28/14 @ 0830
 Work Order No.: 14078 Test Species: Pseudokirchneriella subcapitata

Culture Date: Feb 21/14 Age of Culture: 7d Culture Health: Good
 Culture Count: 1 750 2 764 Average: 757 Culture Cell Density (c1): 757 x 10⁴ cells/ml

$$v1 = \frac{220,000 \text{ cells/ml} \times 100 \text{ ml}}{(c1) \quad 757 \times 10^4 \text{ cells/ml}} = 3 \text{ ml}$$

Time Zero Counts: 1 21 2 27 Average: 24

No. of Cells/mL: 24 x 10⁴ Initial Density: # cells/mL ÷ 220 µL x 10 µL = 10909 cells/ml

Concentration %(v/v)	Water Quality Measurements					Microplates rotated 2X per day?			
	pH	Temp (°C)				0 h	24 h	48 h	72 h
		0 h	24 h	48 h	72 h				
Control	7.0	24.0	25.0	25.5	25.5	✓	/	/	/
1.5	7.0	↓	↓	↓	↓	✓	/	/	/
3.0	7.1	↓	↓	↓	↓	✓	/	/	/
6.0 ^{emp} 6.0	7.2	↓	↓	↓	↓	✓	/	/	/
11.9	7.4	↓	↓	↓	↓	✓	/	/	/
23.8	7.6	↓	↓	↓	↓	✓	/	/	/
47.6	7.8	↓	↓	↓	↓	✓	/	/	/
95.2	7.8	↓	↓	↓	↓	✓	/	/	/
						✗			
Initials	EMM	EMM	m	m	EMM	EMM	m	m	EMM

Initial control pH: Well 1: 6.8 Well 2: 6.8

Final control pH: Well 1: 6.5 Well 2: 6.5

Light intensity (lux): 3800 Date measured: Feb 21/14

Sample Description: clear

Comments: _____

Reviewed: JHU Date reviewed: March 14/14

Pseudokirchneriella subcapitata Toxicity Test Data Sheet
72-h Algal Cell Counts

Client: ALS Start Date/Time: Feb 28/14 @ 0830
 Work Order #: 7 14078 Termination Date: March 3/14 @ 0835
 Sample ID: X14 (11426336-5) Test set up by: EMM

Concentration %(v/v)	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	46					EMM
	B	53					
	C	52					
	D	56					
	E	49					
	F	43					
	G	57					
	H	52					
1.5	A	48					
	B	59					
	C	54					
	D	62					
3.0	A	64					
	B	60					
	C	54					
	D	65					
6.0	A	71					
	B	81					
	C	80					
	D	101	110				
11.9	A	52					
	B	61					
	C	56					
	D	57					
23.8	A	36					
	B	28					
	C	32					
	D	36					
47.6	A	13					
	B	9	7				
	C	16					
	D	14					
95.2	A	0					
	B	0					
	C	0					
	D	0					

Comments: _____
 Reviewed by: JGU Date Reviewed: March 14/14

Pseudokirchneriella subcapitata Algal Counts

Client: ALS
 WO#: 14078
 Sample ID: X14 (L1426336-5)

Start Date/Time: 28-Feb-14 @0830h
 Termination Date: 03-Mar-14 @0830h

Initial Cell Density: 10909 cell/mL
 240000
 0.22
 0.01
 10909.09

Concentration % v/v	Rep	Count 1 (x 10 ⁴)	Count 2 (x 10 ⁴)	Count 3 (x 10 ⁴)	Count 4 (x 10 ⁴)	Mean (x 10 ⁴)	Cell Yield (x 10 ⁴) cell/mL		
Control	A	46				46	44.9	mean	49.9
	B	53				53	51.9	SD	4.780914
	C	52				52	50.9	CV	9.579246
	D	56				56	54.9		
	E	49				49	47.9		
	F	43				43	41.9		
	G	57				57	55.9		
	H	52				52	50.9		
1.5	A	48				48	46.9		
	B	59				59	57.9		
	C	54				54	52.9		
	D	62				62	60.9		
3	A	64				64	62.9		
	B	60				60	58.9		
	C	54				54	52.9		
	D	65				65	63.9		
6	A	71				71	69.9		
	B	84				84	82.9		
	C	80				80	78.9		
	D	101	110			105.5	104.4		
11.9	A	52				52	50.9		
	B	61				61	59.9		
	C	56				56	54.9		
	D	57				57	55.9		
23.8	A	36				36	34.9		
	B	28				28	26.9		
	C	32				32	30.9		
	D	36				36	34.9		
47.6	A	13				13	11.9		
	B	9	7			8	6.9		
	C	16				16	14.9		
	D	14				14	12.9		
95.2	A	0				0	-1.1		
	B	0				0	-1.1		
	C	0				0	-1.1		
	D	0				0	-1.1		

JGh
 March 14/14

CETIS Analytical Report

Report Date: 04 Mar-14 16:48 (p 1 of 2)
 Test Code: 14078e | 07-0869-5137

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 14-2586-3492	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 04 Mar-14 16:47	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 06-3545-2069	Test Type: Cell Growth	Analyst: Yvonne Lam
Start Date: 28 Feb-14 08:30	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 03 Mar-14 08:30	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 7d
Sample ID: 18-2798-0256	Code: 6CF4C3E0	Client: ALS
Sample Date: 25 Feb-14 09:00	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 71h (2.8 °C)	Station: L1426336-5(X14)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1504063	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.9049	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	8.323	6.873	14.42	12.01	6.933	14.55
IC10	11.42	7.743	14.26	8.758	7.014	12.91
IC15	12.9	10.13	15.17	7.75	6.591	9.871
IC20	14.16	11.91	16.57	7.063	6.034	8.396
IC25	15.53	13.2	18.19	6.441	5.499	7.575
IC40	20.42	17.49	23.98	4.898	4.17	5.717
IC50	24.58	20.67	28.89	4.068	3.462	4.838

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	50	42	56	1.69	4.781	9.56%	0.0%
1.5		4	54.75	47	61	3.065	6.131	11.2%	-9.5%
3		4	59.75	53	64	2.496	4.992	8.35%	-19.5%
6		4	84	70	104	7.2	14.4	17.14%	-68.0%
11.9		4	55.5	51	60	1.848	3.697	6.66%	-11.0%
23.8		4	32	27	35	1.915	3.83	11.97%	36.0%
47.6		4	11.75	7	15	1.702	3.403	28.97%	76.5%
95.2		4	0	0	0	0	0		100.0%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	45	52	51	55	48	42	56	51
1.5		47	58	53	61				
3		63	59	53	64				
6		70	83	79	104				
11.9		51	60	55	56				
23.8		35	27	31	35				
47.6		12	7	15	13				
95.2		0	0	0	0				

QA: JGh
March 11

CETIS Analytical Report

Report Date: 04 Mar-14 16:48 (p 2 of 2)
Test Code: 14078e | 07-0869-5137

EC Alga Growth Inhibition Test

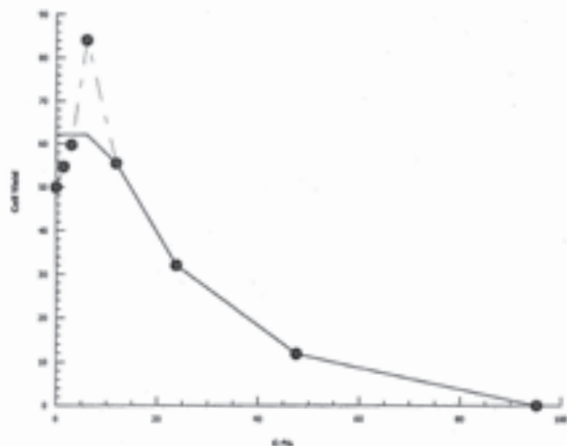
Nautilus Environmental

Analysis ID: 14-2586-3492
Analyzed: 04 Mar-14 16:47

Endpoint: Cell Yield
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 04 Mar-14 16:53 (p 1 of 2)
 Test Code: 14078e(a) | 16-6598-6747

EC Alga Growth Inhibition Test			Nautilus Environmental
Analysis ID: 12-8245-3026	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7	
Analyzed: 04 Mar-14 16:53	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes	
Batch ID: 19-3933-4226	Test Type: Cell Growth	Analyst: Yvonne Lam	
Start Date: 28 Feb-14 08:30	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water	
Ending Date: 03 Mar-14 08:30	Species: Pseudokirchneriella subcapitata	Brine:	
Duration: 72h	Source: In-House Culture	Age:	
Sample ID: 18-2798-0256	Code: 6CF4C3E0	Client: ALS	
Sample Date: 25 Feb-14 09:00	Material: Effluent	Project:	
Receive Date: 27 Feb-14 10:30	Source: ALS		
Sample Age: 71h (2.8 °C)	Station: L1426336-5(X14)		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2117751	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.9049	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	13.13	N/A	13.42	7.619	7.449	NA
IC10	14.47	12.07	15.13	6.912	6.611	8.283
IC15	15.94	13.54	17.03	6.274	5.873	7.385
IC20	17.55	15.07	19.23	5.699	5.2	6.635
IC25	19.31	16.76	21.8	5.179	4.587	5.966
IC40	25.5	21.64	28.61	3.921	3.495	4.62
IC50	30.29	26.39	33.61	3.301	2.975	3.789

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	50	42	56	1.69	4.781	9.56%	0.0%
1.5		4	50	50	50	0	0	0.0%	0.0%
3		4	50	50	50	0	0	0.0%	0.0%
6		4	50	50	50	0	0	0.0%	0.0%
11.9		4	50	50	50	0	0	0.0%	0.0%
23.8		4	32	27	35	1.915	3.83	11.97%	36.0%
47.6		4	11.75	7	15	1.702	3.403	28.97%	76.5%
95.2		4	0	0	0	0	0		100.0%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	45	52	51	55	48	42	56	51
1.5		50	50	50	50				
3		50	50	50	50				
6		50	50	50	50				
11.9		50	50	50	50				
23.8		35	27	31	35				
47.6		12	7	15	13				
95.2		0	0	0	0				

CETIS Analytical Report

Report Date: 04 Mar-14 16:53 (p 2 of 2)
Test Code: 14078e(a) | 16-6596-6747

EC Alga Growth Inhibition Test

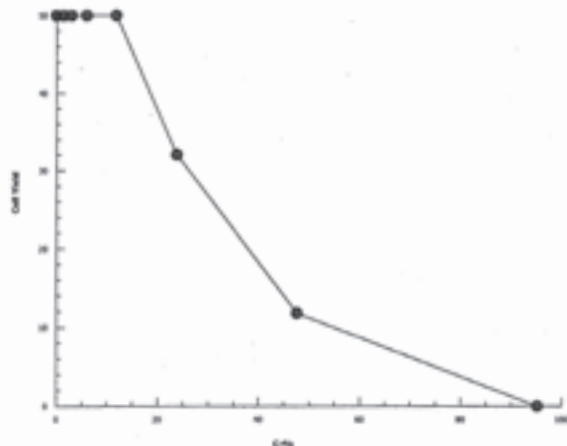
Nautilus Environmental

Analysis ID: 12-8245-3026
Analyzed: 04 Mar-14 16:53

Endpoint: Cell Yield
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



***Pseudokirchneriella subcapitata* Summary Sheet**

Client: ALS
Work Order No.: 14078

Start Date: Feb 28/14
Set up by: Emm

Sample Information:

Sample ID: L1426336-6 (X3A)
Sample Date: Feb 25/14 @ 1135h
Date Received: Feb 27/14 @ 1030h
Sample Volume: 2x20L

Test Organism Information:

Culture Date: Feb 21/14
Age of culture (Day 0): 7d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC105
Stock Solution ID: 13Zn01
Date Initiated: Feb 21/14

72-h IC50 (95% CL): 24.7 (16.1 - 31.7) µg/L Zn.

72-h IC50 Reference Toxicant Mean and Range: 22.7 (15.2 - 33.8) µg/L Zn CV (%): 22

Test Results:

	Algal Growth
IC25 %(v/v) (95% CL)	7.8 (3.6 - 11.1)
IC50 %(v/v) (95% CL)	18.4 (13.4 - 22.2)

Reviewed by: Joh

Date reviewed: March 17/14

72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client: ALS Setup by: EMM
 Sample ID: X3A (U426336-6) Test Date/Time: Feb 28/14 @ 0830
 Work Order No.: 14078 Test Species: Pseudokirchneriella subcapitata

Culture Date: Feb 2/14 Age of Culture: 7d Culture Health: Good
 Culture Count: 1750 2764 Average: 757 Culture Cell Density (c1): 757 x 10⁴ cells/ml

$$v1 = \frac{220,000 \text{ cells/ml} \times 100 \text{ ml}}{(c1) \ 757 \times 10^4 \text{ cells/ml}} = 3 \text{ ml}$$

Time Zero Counts: 121 227 Average: 24

No. of Cells/mL: 24 x 10⁴ Initial Density: # cells/mL + 220 μL x 10 μL = 10909 cells/ml

Concentration %(v/v)	Water Quality Measurements					Microplates rotated 2X per day?			
	pH	Temp (°C)				0 h	24 h	48 h	72 h
		0 h	0 h	24 h	48 h				
Control	7.0	24.0	25.0	25.5	25.5	✓	✓	✓	✓
1.5	7.0	24.0	↓	↓	↓	✓	✓	✓	✓
3.0	7.2	24.0	↓	↓	↓	✓	✓	✓	✓
6.0	7.4	24.0	↓	↓	↓	✓	✓	✓	✓
11.9	7.5	24.0	↓	↓	↓	✓	✓	✓	✓
23.8	7.5	24.0	↓	↓	↓	✓	✓	✓	✓
47.6	7.5	24.0	↓	↓	↓	✓	✓	✓	✓
95.2	7.6	24.0	↓	↓	↓	✓	✓	✓	✓
Initials	EMM	EMM	A	P	EMM	EMM	EMM	EMM	EMM

Initial control pH: Well 1: 6.8 Well 2: 6.8

Final control pH: Well 1: 6.5 Well 2: 6.5

Light intensity (lux): 3900 Date measured: Feb 2/14

Sample Description: clear

Comments: _____

Reviewed: JGU Date reviewed: March 14/14

Pseudokirchneriella subcapitata Toxicity Test Data Sheet
72-h Algal Cell Counts

Client: ALS Start Date/Time: Feb 28/14 @ 0930
 Work Order #: 14078 Termination Date: March 3/14 @ 0830
 Sample ID: X3A(H142636-6) Test set up by: EMM
 % (v/v) (L1426336-6)

Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	54					EMM
	B	63					
	C	56					
	D	55					
	E	54					
	F	59					
	G	49					
	H	51					
1.5	A	52					
	B	59					
	C	49					
	D	50					
3.0	A	53					
	B	52					
	C	65					
	D	60					
6.0	A	36	39				
	B	38	44				
	C	43	42				
	D	62	66				
11.9	A	39					
	B	36					
	C	37					
	D	32					
23.8	A	27					
	B	23					
	C	24					
	D	23					
47.6	A	2	0				
	B	0	0				
	C	4	3	4			
	D	1	1				
95.2	A	0					
	B	0					
	C	1	0	0			
	D	0					

Comments: _____

Reviewed by: JGU Date Reviewed: March 14/14

***Pseudokirchneriella subcapitata* Algal Counts**

Client: ALS
 WO#: 14078
 Sample ID: X3A (L1426336-6)

Start Date/Time: 28-Feb-14 @0830h
 Termination Date: 03-Mar-14 @0830h

Initial Cell Density: 10909 cell/mL
 240000
 0.22
 0.01
 10909.09

Concentration % v/v	Rep	Count 1 (x 10 ⁴)	Count 2 (x 10 ⁴)	Count 3 (x 10 ⁴)	Count 4 (x 10 ⁴)	Mean (x 10 ⁴)	Cell Yield (x 10 ⁴) cell/mL		
Control	A	54				54	52.9	mean	54.0
	B	63				63	61.9	SD	4.389517
	C	56				56	54.9	CV	8.123606
	D	55				55	53.9		
	E	54				54	52.9		
	F	59				59	57.9		
	G	49				49	47.9		
	H	51				51	49.9		
1.5	A	52				52	50.9		
	B	59				59	57.9		
	C	49				49	47.9		
	D	50				50	48.9		
3	A	53				53	51.9		
	B	52				52	50.9		
	C	65				65	63.9		
	D	60				60	58.9		
6	A	36	39			37.5	36.4		
	B	38	44			41	39.9		
	C	43	42			42.5	41.4		
	D	62	66			64	62.9		
11.9	A	39				39	37.9		
	B	30				30	28.9		
	C	37				37	35.9		
	D	32				32	30.9		
23.8	A	27				27	25.9		
	B	23				23	21.9		
	C	24				24	22.9		
	D	23				23	21.9		
47.6	A	2	0			1	-0.1		
	B	0	0			0	-1.1		
	C	4	3	4		3.6666667	2.6		
	D	1	1			1	-0.1		
95.2	A	0				0	-1.1		
	B	0				0	-1.1		
	C	1	0	0		0.3333333	-0.8		
	D	0				0	-1.1		

JGU
 March 14/14

CETIS Analytical Report

Report Date: 05 Mar-14 09:57 (p 1 of 2)
 Test Code: 14078f | 04-5755-2077

EC Alga Growth Inhibition Test			Nautilus Environmental		
Analysis ID: 15-1589-2421	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7			
Analyzed: 05 Mar-14 9:57	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			
Batch ID: 12-1510-8147	Test Type: Cell Growth	Analyst: Emma Marus			
Start Date: 28 Feb-14 08:30	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water			
Ending Date: 03 Mar-14 08:30	Species: Pseudokirchneriella subcapitata	Brine:			
Duration: 72h	Source: In-House Culture	Age: 7d			
Sample ID: 05-2872-4256	Code: 1F83B120	Client: ALS			
Sample Date: 25 Feb-14 11:35	Material: Effluent	Project:			
Receive Date: 27 Feb-14 10:30	Source: ALS				
Sample Age: 69h (3.9 °C)	Station: L1426336-6(X3A)				

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	390736	200	Yes	Two-Point Interpolation

Residual Analysis						
Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)	
Control Trend	Mann-Kendall Trend			0.1788	Non-significant Trend in Controls	

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	3.696	N/A	8.224	27.05	12.16	NA
IC10	4.557	2.59	8.788	21.94	11.38	38.61
IC15	5.575	2.972	9.41	17.94	10.63	33.65
IC20	6.662	3.267	10.31	15.01	9.7	30.61
IC25	7.848	3.599	11.06	12.74	9.045	27.78
IC40	12.77	8.633	16.61	7.83	6.02	11.58
IC50	18.45	13.38	22.15	5.421	4.515	7.474

Cell Yield Summary			Calculated Variate						
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	54.13	48	62	1.552	4.39	8.11%	0.0%
1.5		4	51.5	48	58	2.255	4.509	8.76%	4.85%
3		4	56.5	51	64	3.069	6.137	10.86%	-4.39%
6		4	45	36	63	6.096	12.19	27.1%	16.86%
11.9		4	33.5	29	38	2.102	4.203	12.55%	38.11%
23.8		4	23.25	22	26	0.9465	1.893	8.14%	57.04%
47.6		4	0.75	0	3	0.75	1.5	200.0%	98.61%
95.2		4	0	0	0	0	0		100.0%

Cell Yield Detail									
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	53	62	55	54	53	58	48	50
1.5		51	58	48	49				
3		52	51	64	59				
6		36	40	41	63				
11.9		38	29	36	31				
23.8		26	22	23	22				
47.6		0	0	3	0				
95.2		0	0	0	0				

CETIS Analytical Report

Report Date: 05 Mar-14 09:57 (p 2 of 2)
Test Code: 14078f | 04-5755-2077

EC Alga Growth Inhibition Test

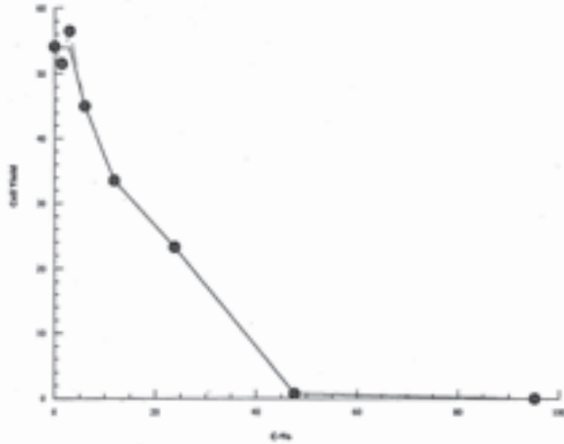
Nautilus Environmental

Analysis ID: 15-1589-2421
Analyzed: 05 Mar-14 9:57

Endpoint: Cell Yield
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



***Pseudokirchneriella subcapitata* Summary Sheet**

Client: ALS
Work Order No.: 14078

Start Date: Feb 28/14
Set up by: EMM

Sample Information:

Sample ID: L1426336-7 (R3)
Sample Date: Feb 25/14 @ 1630h
Date Received: Feb 27/14 @ 1030h
Sample Volume: 2x20L

Test Organism Information:

Culture Date: Feb 21/14
Age of culture (Day 0): 7d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC105
Stock Solution ID: 13Zn01
Date Initiated: Feb 21/14

72-h IC50 (95% CL): 24.7 (16.1-31.7) µg/L Zn

72-h IC50 Reference Toxicant Mean and Range: 22.7 (15.2-33.8) µg/L^{Zn} CV (%): 22

Test Results:

	Algal Growth
IC25 %(v/v) (95% CL)	35.8 (30.1 - 44.9)
IC50 %(v/v) (95% CL)	53.1 (38.5 - 63.4)

Reviewed by: JOu

Date reviewed: March 17/14

72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client : ALS Setup by: EMM
 Sample ID: P3(61426336-7) Test Date/Time: Feb 28/14 @ 0830
 Work Order No.: 14078 Test Species: Pseudokirchneriella subcapitata

Culture Date: Feb 21/14 Age of Culture: 7d Culture Health: Good
 Culture Count: 1 750 2 764 Average: 757 Culture Cell Density (c1): 757 x 10⁴ cells/ml

$$v1 = \frac{220,000 \text{ cells/ml} \times 100 \text{ ml}}{(c1) \quad 757 \times 10^4 \text{ cells/ml}} = 3 \text{ ml}$$

Time Zero Counts: 1 21 2 27 Average: 24

No. of Cells/mL: 24 x 10⁴ Initial Density: # cells/mL ÷ 220 µL x 10 µL = 10909 cells/ml

Concentration %(v/v)	Water Quality Measurements					Microplates rotated 2X per day?			
	pH	Temp (°C)				0 h	24 h	48 h	72 h
		0 h	0 h	24 h	48 h				
Control	7.0	24.0	25.0	25.5	25.5	✓	/	/	/
1.5	7.1	↓	↓	↓	↓	✓	/	/	/
3.0	7.1	↓	↓	↓	↓	✓	/	/	/
6.0	7.3	↓	↓	↓	↓	✓	/	/	/
11.9	7.5	↓	↓	↓	↓	✓	/	/	/
23.8	7.4	↓	↓	↓	↓	✓	/	/	/
47.6	7.6	↓	↓	↓	↓	✓	/	/	/
95.2	7.6	↓	↓	↓	↓	✓	/	/	/
Initials	EMM	EMM	EMM	EMM	EMM	EMM	EMM	EMM	EMM

Initial control pH: Well 1: 6.8 Well 2: 6.8

Final control pH: Well 1: 6.5 Well 2: 6.5

Light intensity (lux): 3800 Date measured: Feb 21/14

Sample Description: clear

Comments: _____

Reviewed: John Date reviewed: March 14/14

***Pseudokirchneriella subcapitata* Toxicity Test Data Sheet**
72-h Algal Cell Counts

Client: ALS Start Date/Time: Feb 28/14 @ 0830h
 Work Order # 170 14078 Termination Date: March 3/14 @ 0830h
 Sample ID: B3 (L1426336-7) Test set up by: EMM
 % (v/v)

Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	41					EMM
	B	47					
	C	45					
	D	50					
	E	50					
	F	48					
	G	46					
	H	38					
1.5	A	56					
	B	51					
	C	42					
	D	55					
3.0	A	94					
	B	97					
	C	84					
	D	75					
6.0	A	98					
	B	107					
	C	109					
	D	110					
11.9	A	91					
	B	88					
	C	79					
	D	86					
23.8	A	58					
	B	69					
	C	54					
	D	62					
47.6	A	21	20				
	B	21	23				
	C	34	36				
	D	30	31				
95.2	A	5					
	B	7					
	C	8					
	D	3	4				

Comments: _____

Reviewed by: JGU Date Reviewed: March 14/14

Pseudokirchneriella subcapitata Algal Counts

Client: ALS
 WO#: 14078
 Sample ID: R3 (L1426336-7)

Start Date/Time: 28-Feb-14 @0830h
 Termination Date: 03-Mar-14 @0830h

Initial Cell Density: 10909 cell/mL
 240000
 0.22
 0.01
 10909.09

Concentration % v/v	Rep	Count 1 (x 10 ⁴)	Count 2 (x 10 ⁴)	Count 3 (x 10 ⁴)	Count 4 (x 10 ⁴)	Mean (x 10 ⁴)	Cell Yield (x 10 ⁴) cell/mL		
Control	A	41				41	39.9	mean	44.5
	B	47				47	45.9	SD	4.240536
	C	45				45	43.9	CV	9.521999
	D	50				50	48.9		
	E	50				50	48.9		
	F	48				48	46.9		
	G	46				46	44.9		
	H	38				38	36.9		
1.5	A	56				56	54.9		
	B	51				51	49.9		
	C	42				42	40.9		
	D	55				55	53.9		
3	A	94				94	92.9		
	B	97				97	95.9		
	C	84				84	82.9		
	D	75				75	73.9		
6	A	98				98	96.9		
	B	107				107	105.9		
	C	109				109	107.9		
	D	110				110	108.9		
11.9	A	91				91	89.9		
	B	88				88	86.9		
	C	79				79	77.9		
	D	86				86	84.9		
23.8	A	58				58	56.9		
	B	69				69	67.9		
	C	54				54	52.9		
	D	62				62	60.9		
47.6	A	21	20			20.5	19.4		
	B	21	23			22	20.9		
	C	34	36			35	33.9		
	D	30	31			30.5	29.4		
95.2	A	5				5	3.9		
	B	7				7	5.9		
	C	8				8	6.9		
	D	3	4			3.5	2.4		

JGU
 March 14/14

CETIS Analytical Report

Report Date: 05 Mar-14 10:04 (p 1 of 2)
 Test Code: 14078g | 21-2831-2742

EC Alga Growth Inhibition Test			Nautilus Environmental		
Analysis ID: 00-6843-9117	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7			
Analyzed: 05 Mar-14 10:04	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			
Batch ID: 03-0974-8538	Test Type: Cell Growth	Analyst: Emma Marus			
Start Date: 28 Feb-14 08:30	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water			
Ending Date: 03 Mar-14 08:30	Species: Pseudokirchneriella subcapitata	Brine:			
Duration: 72h	Source: In-House Culture	Age: 7d			
Sample ID: 13-8828-3648	Code: 52BF8700	Client: ALS			
Sample Date: 25 Feb-14 16:30	Material: Effluent	Project:			
Receive Date: 27 Feb-14 10:30	Source: ALS				
Sample Age: 64h (3.4 °C)	Station: L1426336-7(R3)				

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1370559	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.9049	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	14.25	13.13	16.52	7.016	6.052	7.616
IC10	17.04	14.44	22.65	5.87	4.414	6.925
IC15	20.33	15.83	27.04	4.92	3.699	6.318
IC20	23.98	17.43	27.52	4.17	3.634	5.738
IC25	25.89	20.56	29.41	3.863	3.401	4.863
IC40	32.51	28.33	36.81	3.076	2.717	3.53
IC50	37.81	32.95	43.85	2.645	2.281	3.035

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	44.63	37	49	1.499	4.241	9.5%	0.0%
1.5		4	50	41	55	3.189	6.377	12.75%	-12.04%
3		4	86.5	74	96	5.008	10.02	11.58%	-93.84%
6		4	105	97	109	2.739	5.477	5.22%	-135.3%
11.9		4	85	78	90	2.55	5.099	6.0%	-90.48%
23.8		4	59.75	53	68	3.198	6.397	10.71%	-33.89%
47.6		4	25.75	19	34	3.497	6.994	27.16%	42.3%
95.2		4	4.75	2	7	1.109	2.217	46.68%	89.36%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	40	46	44	49	49	47	45	37
1.5		55	50	41	54				
3		93	96	83	74				
6		97	106	108	109				
11.9		90	87	78	85				
23.8		57	68	53	61				
47.6		19	21	34	29				
95.2		4	6	7	2				

QA: *JGM*
March All

CETIS Analytical Report

Report Date: 05 Mar-14 10:04 (p 2 of 2)
Test Code: 14078g | 21-2831-2742

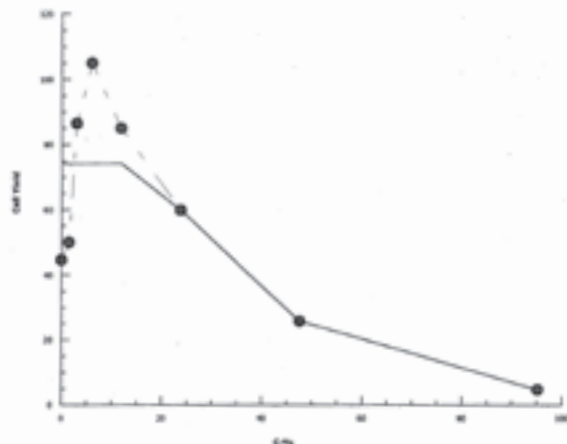
EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 00-6843-9117 Endpoint: Cell Yield
Analyzed: 05 Mar-14 10:04 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 05 Mar-14 10:11 (p 1 of 2)
 Test Code: 14078g(a) | 06-7087-9815

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID: 11-4998-9586	Endpoint: Cell Yield	CETIS Version: CETISv1.8.7
Analyzed: 05 Mar-14 10:11	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 02-3145-7835	Test Type: Cell Growth	Analyst: Emma Marus
Start Date: 28 Feb-14 08:30	Protocol: EC/EPS 1/RM/25	Diluent: Deionized Water
Ending Date: 03 Mar-14 08:30	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 72h	Source: In-House Culture	Age: 7d
Sample ID: 13-8828-3648	Code: 52BF8700	Client: ALS
Sample Date: 25 Feb-14 16:30	Material: Effluent	Project:
Receive Date: 27 Feb-14 10:30	Source: ALS	
Sample Age: 64h (3.4 °C)	Station: L1426336-7(R3)	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1436493	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend			0.9049	Non-significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	25.83	22.89	27.04	3.871	3.699	4.368
IC10	28.03	24.92	30.67	3.567	3.26	4.013
IC15	30.41	26.87	34.76	3.288	2.877	3.722
IC20	32.99	28.35	39.51	3.032	2.531	3.527
IC25	35.77	30.06	44.9	2.795	2.227	3.326
IC40	45.58	34.73	57.44	2.194	1.741	2.88
IC50	53.07	38.53	63.45	1.884	1.576	2.596

Cell Yield Summary

C-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	44.63	37	49	1.499	4.241	9.5%	0.0%
1.5		4	45	45	45	0	0	0.0%	-0.84%
3		4	45	45	45	0	0	0.0%	-0.84%
6		4	45	45	45	0	0	0.0%	-0.84%
11.9		4	45	45	45	0	0	0.0%	-0.84%
23.8		4	45	45	45	0	0	0.0%	-0.84%
47.6		4	25.75	19	34	3.497	6.994	27.16%	42.3%
95.2		4	4.75	2	7	1.109	2.217	46.68%	89.36%

Cell Yield Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	40	46	44	49	49	47	45	37
1.5		45	45	45	45				
3		45	45	45	45				
6		45	45	45	45				
11.9		45	45	45	45				
23.8		45	45	45	45				
47.6		19	21	34	29				
95.2		4	6	7	2				

CETIS Analytical Report

Report Date: 05 Mar-14 10:11 (p 2 of 2)
Test Code: 14078g(a) | 06-7087-9815

EC Alga Growth Inhibition Test

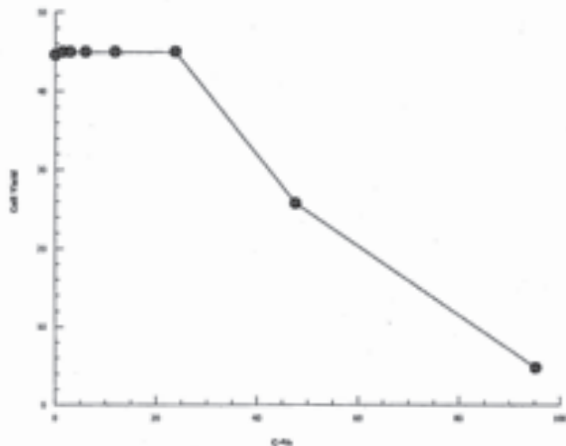
Nautilus Environmental

Analysis ID: 11-4998-9586
Analyzed: 05 Mar-14 10:11

Endpoint: Cell Yield
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



APPENDIX D - Rainbow Trout (*Oncorhynchus mykiss*) LC50 Toxicity Test Data

Rainbow Trout Summary Sheet

Client: ALS

Start Date/Time: February 28/14 @ 1045

Work Order No.: 14079

Test Species: Oncorhynchus mykiss

Sample Information:

Sample ID: L1426836-1 (R10)
Sample Date: February 25/14 @ 1500
Date Received: February 27/14 @ 1030
Sample Volume: 2 x 20L
Other: N/A

Test Validity Criteria:

≥ 90% control survival

WQ Ranges:

T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L CaCO₃): 11
Alkalinity (mg/L CaCO₃): 9

Test Organism Information:

Batch No.: 011414
Source: Vancouver Island Trout Hatchery
No. Fish/Volume (L): 10/12
Loading Density: 0.39
Mean Length ± SD (mm): 37 ± 2 Range: 33 - 42
Mean Weight ± SD (g): 0.47 ± 0.11 Range: 0.30 - 0.65

NaNO₂ Reference Toxicant Results:

Reference Toxicant ID: RTNE55
Stock Solution ID: 13NE02
Date Initiated: February 19/14
96-h LC50 (95% CL): 4.7 (3.8 - 5.8) mg/L NaNO₂

Reference Toxicant Mean and Historical Range: 5.4 (2.2 - 13.4) mg/L NaNO₂
Reference Toxicant CV (%): 57

Test Results: The 96-h LC50 is >100% (WN).

Reviewed by: Jeb

Date reviewed: March 12/14

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: ALS
 Sample I.D.: L1426336-1 (R10)
 W.O. #: 14079
 RBT Batch #: 011414
 Date Collected/Time: February 25/14 @ 1500
 Date Setup/Time: February 28/14 @ 1045
 Sample Setup By: YUV

Number Fish/Volume: 10/12 L
 7-d % Mortality: 0.15
 Total Pre-aeration Time (mins): 30
 Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Y

D.O. meter: DO: 1/2/3
 pH meter: pH: 1/2/3
 Cond. Meter: C: 1/2/3

Undiluted Sample WQ			
Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C	14.5	/	14.0
pH	7.3	/	7.4
D.O. (mg/L)	10.0	/	10.1
Cond. (µS/cm)	297	/	299

Concentration	# Survivors										Temperature (°C)			Dissolved Oxygen (mg/L)			pH			Conductivity (µS/cm)						
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24		48	72	96			
(% v/v)	1	2	4	10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.6	9.6	9.6	9.6	7.0	7.1	7.0	7.0	7.0	0	0	0	0	96
Cont				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.7	9.7	9.7	9.7	7.0	7.0	7.0	7.0	7.0	33	34	33	33	34
6.25				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.8	9.8	9.8	9.8	7.0	7.0	7.0	7.0	7.0	56	63	63	63	63
12.5				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.9	9.9	9.9	9.9	7.0	7.0	7.0	7.0	7.0	81	87	87	87	87
25				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.9	9.9	9.9	9.9	7.2	7.2	7.2	7.2	7.2	119	119	119	119	119
50				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.8	9.8	9.8	9.8	7.2	7.2	7.2	7.2	7.2	174	181	181	181	181
100				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.9	9.9	9.9	9.9	7.4	7.4	7.4	7.4	7.4	299	304	304	304	304
Initials				AS	AS	AS	AS																			

WQ Ranges: T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Sample Description/Comments: Clear w/ slight greenish colour

Fish Description at 96 h All fish appear OK Number of Stressed Fish at 96 h 0

Other Observations: _____

Reviewed by: Joh Date Reviewed: March 12/14

Rainbow Trout Summary Sheet

Client: ALS

Start Date/Time: February 28/14 @ 1100

Work Order No.: 14079

Test Species: Oncorhynchus mykiss

Sample Information:

Sample ID: L1426336-2 (NFI)
Sample Date: February 25/14 @ 1435
Date Received: February 27/14 @ 1030
Sample Volume: 2 x 20L
Other: N/A

Test Validity Criteria:

≥ 90% control survival

WQ Ranges:

T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L CaCO₃): 11
Alkalinity (mg/L CaCO₃): 9

Test Organism Information:

Batch No.: 011414
Source: Vancouver Island Trout Hatchery
No. Fish/Volume (L): 10/12
Loading Density: 0.37
Mean Length ± SD (mm): 37 ± 3 Range: 30 - 39
Mean Weight ± SD (g): 0.44 ± 0.11 Range: 0.21 - 0.63

NaNO₂ Reference Toxicant Results:

Reference Toxicant ID: RTN655
Stock Solution ID: 13N602
Date Initiated: February 19/14
96-h LC₅₀ (95% CL): 4.7 (3.8 - 5.8) mg/L NaNO₂

Reference Toxicant Mean and Historical Range: 5.4 (2.2 - 13.4) mg/L NaNO₂
Reference Toxicant CV (%): 57

Test Results: The 96-h LC₅₀ is > 100% (N).

Reviewed by: JGh

Date reviewed: March 12/14

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: ALS

Sample I.D. L1426336-2 (NFI)

W.O. # 14079

RBT Batch #: 011414

Date Collected/Time: February 25/14 @ 14:35

Date Setup/Time: February 28/14 @ 11:00

Sample Setup By: WUL

D.O. meter: DO: 1/2/3

pH meter: pH: 1/2/3

Cond. Meter: C: 1/2/3

Number Fish/Volume: 10/12 L

7-d % Mortality: 0.15

Total Pre-aeration Time (mins): 30

Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Y

Undiluted Sample WQ		
Parameters	Initial WQ	Adjustment
Temp °C	14.0	14.0
pH	7.3	7.4
D.O. (mg/L)	10.3	10.2
Cond. (µS/cm)	307	307

Concentration	# Survivors										Temperature (°C)						Dissolved Oxygen (mg/L)						pH						Conductivity (µS/cm)
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96		
(% v/v)	1	2	4	10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.6	9.6	9.6	9.6	9.6	7.1	7.1	7.1	7.1	7.1	0	0	0	0	0		
Cont				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.1	9.8	9.6	9.9	9.6	7.0	7.1	6.9	7.1	7.1	33	33	33	33	39		
6.25				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.1	9.9	9.6	9.9	9.5	7.0	7.3	7.1	7.4	7.3	56	63	63	63	63		
12.5				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.8	9.7	9.8	9.6	7.0	7.4	7.3	7.5	7.5	70	76	76	76	76		
25				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.7	9.8	9.6	7.1	7.6	7.5	7.7	7.7	105	111	111	111	111		
50				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.6	9.9	9.8	7.2	7.9	7.6	7.9	7.9	170	177	177	177	177		
100				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.8	9.7	9.9	9.9	7.4	7.9	7.8	8.2	8.2	307	313	313	313	313		
Initials																													

WQ Ranges: T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Sample Description/Comments: clear w/ slight greenish colour

Fish Description at 96 h All fish appear ok Number of Stressed Fish at 96 h 0

Other Observations: _____

Reviewed by: JGU

Date Reviewed: March 12/14

Rainbow Trout Summary Sheet

Client: ALS

Start Date/Time: February 28/14 @ 1115

Work Order No.: 14079

Test Species: Oncorhynchus mykiss

Sample Information:

Sample ID: L1426336-3 (WF2)
Sample Date: February 25/14 @ 1305
Date Received: February 27/14 @ 1030
Sample Volume: 2x20L
Other: N/A

Test Validity Criteria:

≥ 90% control survival

WQ Ranges:

T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L CaCO₃): 11
Alkalinity (mg/L CaCO₃): 9

Test Organism Information:

Batch No.: 011414
Source: Vancouver Island Trout Hatchery
No. Fish/Volume (L): 10/12
Loading Density: 0.42
Mean Length ± SD (mm): 38 ± 2
Mean Weight ± SD (g): 0.50 ± 0.08

Range: 35 - 41
Range: 0.36 - 0.62

NaNO₂ Reference Toxicant Results:

Reference Toxicant ID: RTNE55
Stock Solution ID: 13NE02
Date Initiated: February 19/14
96-h LC50 (95% CL): 4.7 (3.8 - 5.8) mg/L NaNO₂

Reference Toxicant Mean and Historical Range: 5.4 (2.2 - 13.4) mg/L NaNO₂
Reference Toxicant CV (%): 57

Test Results: The 96-h LC50 is > 1008 (µM).

Reviewed by: JOB

Date reviewed: March 12/14

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#:

Sample I.D.

W.O. #

RBT Batch #:

Date Collected/Time:

Date Setup/Time:

Sample Setup By:

D.O. meter:

pH meter:

Cond. Meter:

ALS
 L1426336-3 (NF2)
 14079
 01/14/14
 February 25/14 @ 1305
 February 28/14 @ 1115
 YML

DO: 1/2/3
 pH: 1/2/3
 C: 1/2/3

Number Fish/Volume:

7-d % Mortality:

Total Pre-aeration Time (mins):

Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N):

10/12 L
 0.15
 30
 Y

Undiluted Sample WQ		
Parameters	Initial WQ	Adjustment
Temp °C	14.0	14.0
pH	7.0	7.1
D.O. (mg/L)	10.2	10.2
Cond. (µS/cm)	320	323

Concentration	# Survivors						Temperature (°C)						Dissolved Oxygen (mg/L)						pH						Conductivity (µS/cm)		
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24		48	72
(% v/v)																											
Cont				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.1	9.8	9.7	9.8	10.0	7.0	7.1	6.9	7.1	7.1	0	0	0	0	0
6.25				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.6	9.8	9.9	7.0	7.3	7.1	7.4	7.5	0	0	0	0	0
12.5				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.7	9.9	9.9	7.0	7.6	7.4	7.5	7.5	0	0	0	0	0
25				10	10	9	9	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.6	9.9	9.9	7.1	7.9	7.6	7.6	7.6	0	0	0	0	0
50				10	10	8	8	14.0	14.0	14.0	14.0	14.0	10.2	9.8	9.7	10.0	10.0	7.1	7.1	7.9	7.9	7.9	0	0	0	0	0
100				10	10	6	6	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.7	9.9	10.1	7.1	7.1	8.0	8.1	8.1	0	0	0	0	0
Initials																											

WQ Ranges: T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Sample Description/Comments: clear w/ slight greenish colour

Fish Description at 96 h remaining trout appear ok Number of Stressed Fish at 96 h 0

Other Observations:

Reviewed by: JG Date Reviewed: March 12/14

Rainbow Trout Summary Sheet

Client: ALS

Start Date/Time: February 28/14 @ 1200

Work Order No.: 14079

Test Species: Oncorhynchus mykiss

Sample Information:

Sample ID: L1426336-4 (X1)
Sample Date: February 25/14 @ 1110
Date Received: February 27/14 @ 1030
Sample Volume: 2 x 20L
Other: N/A

Test Validity Criteria:

≥ 90% control survival

WQ Ranges:

T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L CaCO₃): 11
Alkalinity (mg/L CaCO₃): 9

Test Organism Information:

Batch No.: 011414
Source: Vancouver Island Trout Hatchery
No. Fish/Volume (L): 10/12
Loading Density: 0.39
Mean Length ± SD (mm): 37 ± 1 Range: 35 - 39
Mean Weight ± SD (g): 0.47 ± 0.08 Range: 0.39 - 0.64

NaNO₂ Reference Toxicant Results:

Reference Toxicant ID: RTNE55
Stock Solution ID: 13Nt02
Date Initiated: February 19/14
96-h LC50 (95% CL): 4.7 (3.8 - 5.8) mg/L NaNO₂

Reference Toxicant Mean and Historical Range: 5.4 (2.2 - 13.4) mg/L NaNO₂
Reference Toxicant CV (%): 57

Test Results: The 96-h LC50 is > 100% (N/A).

Reviewed by: JGU

Date reviewed: March 12/14

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: ALS

Sample I.D. L1426336-4 (X1)

W.O. # 14079

RBT Batch #: 011414

Date Collected/Time: February 25/14 @ 110

Date Setup/Time: February 28/14 @ 12:00

Sample Setup By: MMU

D.O. meter: DO: 1/2/3

pH meter: pH: 1/2/3

Cond. Meter: C: 1/2/3

Number Fish/Volume: 10/12 L

7-d % Mortality: 0.15

Total Pre-aeration Time (mins): 30

Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Y

Undiluted Sample WQ			
Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C	14.0		14.0
pH	7.3	/	7.3
D.O. (mg/L)	10.2	/	10.2
Cond. (µS/cm)	336		339

Concentration	# Survivors										Temperature (°C)						Dissolved Oxygen (mg/L)						pH						Conductivity (µS/cm)	
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	96	
(% v/v)																														
<u>cont</u>				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.6	9.6	9.6	9.6	9.6	7.0	7.0	7.0	7.0	7.0	7.1	7.1	7.1	7.1	7.1	33	39	
<u>6.25</u>				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.7	9.7	9.7	9.7	9.7	7.1	7.1	7.1	7.1	7.1	7.5	7.5	7.5	7.5	7.5	55	61	
<u>12.5</u>				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.6	9.6	9.6	9.6	9.6	7.1	7.1	7.1	7.1	7.1	7.5	7.5	7.5	7.5	7.5	75	81	
<u>25</u>				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.6	9.6	9.6	9.6	9.6	7.1	7.1	7.1	7.1	7.1	7.5	7.5	7.5	7.5	7.5	107	114	
<u>50</u>				10	10	9	9	14.0	14.0	14.0	14.0	14.0	9.7	9.7	9.7	9.7	9.7	7.2	7.2	7.2	7.2	7.2	7.8	7.8	7.8	7.8	7.8	183	190	
<u>100</u>				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.7	9.7	9.7	9.7	9.7	7.3	7.3	7.3	7.3	7.3	8.2	8.2	8.2	8.2	8.2	339	345	
Initials				A	A	SBF	SBF	MMU	MMU	MMU	MMU	MMU	SBF	SBF	SBF	SBF	SBF	MMU	MMU	MMU	MMU	MMU	SBF	SBF	SBF	SBF	SBF	MMU	SBF	

WQ Ranges: T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Sample Description/Comments: clear

Fish Description at 96 h remaining fish appear OK Number of Stressed Fish at 96 h 0

Other Observations: _____

Reviewed by: JGA

Date Reviewed: March 12/14

Rainbow Trout Summary Sheet

Client: ALS

Start Date/Time: February 28/14 @ 1430

Work Order No.: 14079

Test Species: Oncorhynchus mykiss

Sample Information:

Sample ID: L1426336-5 (X14)
Sample Date: February 25/14 @ 0900
Date Received: February 27/14 @ 1030
Sample Volume: 2 x 20L
Other: N/A

Test Validity Criteria:

≥ 90% control survival

WQ Ranges:

T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L CaCO₃): 11
Alkalinity (mg/L CaCO₃): 9

Test Organism Information:

Batch No.: 011414
Source: Vancouver Island Trout Hatchery
No. Fish/Volume (L): 10/12
Loading Density: 0.43
Mean Length ± SD (mm): 39 ± 2 Range: 35 - 42
Mean Weight ± SD (g): 0.52 ± 0.09 Range: 0.35 - 0.60

NaNO₂ Reference Toxicant Results:

Reference Toxicant ID: RTWE55
Stock Solution ID: 13Nt02
Date Initiated: February 19/14
96-h LC50 (95% CL): 4.7 (3.8 - 5.8) mg/L NaNO₂

Reference Toxicant Mean and Historical Range: 5.4 (2.2 - 13.4) mg/L NaNO₂
Reference Toxicant CV (%): 57

Test Results: The 96-h LC50 is >100% (UN).

Reviewed by: JGlu

Date reviewed: March 14/14

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: ALS

Sample I.D. L1426336-5 (X14)

W.O. # 14079

RBT Batch #: 01/14/14

Date Collected/Time: February 25/14 @ 0900

Date Setup/Time: February 28/14 @ 1430

Sample Setup By: WNV/KS2

D.O. meter: DO: 1/2/3

pH meter: pH: 1/2/3

Cond. Meter: C: 1/2/3

Number Fish/Volume: 10/12 L

7-d % Mortality: 0.15

Total Pre-aeration Time (mins): 30

Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Y

Undiluted Sample WQ		Adjustment		30 min WQ	
Parameters	Initial WQ	Adjustment			
Temp °C	14.0	/			14.0
pH	7.2	/			7.3
D.O. (mg/L)	10.2	/			10.1
Cond. (µS/cm)	826	/			829

Concentration	# Survivors						Temperature (°C)						Dissolved Oxygen (mg/L)						pH						Conductivity (µS/cm)						
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24		48	72	96			
(% v/v)																															
Cont				10	10	10	10	10.0	14.0	14.0	14.0	14.0	10.0	9.8	9.6	9.3	9.3	7.1	6.9	6.8	7.0	7.0	33	33	33	33	39				
6.25				10	10	10	10	14.0	14.0	14.0	14.0	15.0	10.1	9.9	9.7	9.6	9.6	7.1	7.1	7.4	7.5	7.5	144	144	144	144	206	153			
12.5				10	10	10	10	14.0	14.0	14.0	14.0	15.0	10.1	9.8	9.7	9.6	9.6	7.2	7.4	7.6	7.6	7.6	194	194	194	201					
25				10	10	10	10	14.0	14.0	14.0	14.0	15.0	10.1	9.9	9.6	9.6	9.6	7.5	7.5	7.8	7.9	7.9	298	298	298	310					
50				10	10	10	10	14.0	14.0	14.0	14.0	15.0	10.1	9.8	9.7	9.9	9.7	7.3	7.3	7.8	8.0	8.0	476	476	476	482					
100				10	10	10	10	14.0	14.0	14.0	14.0	15.0	10.1	9.9	9.7	9.9	9.5	7.3	6.2	7.9	8.3	8.3	528	528	528	828					
Initials																															

WQ Ranges: T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Sample Description/Comments: turbid, pale yellow

Fish Description at 96 h All fish appear ok

Number of Stressed Fish at 96 h 0

Other Observations: _____

Reviewed by: JG

Date Reviewed: March 14/14

Rainbow Trout Summary Sheet

Client: ALS

Start Date/Time: February 28/14 @ 1435

Work Order No.: 14079

Test Species: Oncorhynchus mykiss

Sample Information:

Sample ID: L1426336-6 (X3A)
Sample Date: February 25/14 @ 1135
Date Received: February 27/14 @ 1030
Sample Volume: 2x 20L
Other: N/A

Test Validity Criteria:

≥ 90% control survival

WQ Ranges:

T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L CaCO₃): 11
Alkalinity (mg/L CaCO₃): 9

Test Organism Information:

Batch No.: 011414
Source: Vancouver Island Trout Hatchery
No. Fish/Volume (L): 10/12
Loading Density: 0.36
Mean Length ± SD (mm): 38 ± 1 Range: 37 - 40
Mean Weight ± SD (g): 0.47 ± 0.07 Range: 0.37 - 0.55

NaNO₂ Reference Toxicant Results:

Reference Toxicant ID: RTNE55
Stock Solution ID: 13NE02
Date Initiated: February 19/14
96-h LC50 (95% CL): 4.7 (3.8 - 5.8) mg/L NaNO₂

Reference Toxicant Mean and Historical Range: 5.4 (2.2 - 13.4) mg/L NaNO₂
Reference Toxicant CV (%): 57

Test Results: The 96-h LC50 is > 100% (N/A).

Reviewed by: JGh

Date reviewed: March 14/14

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: ALS
 Sample I.D.: L1426336-6 (X3A)
 W.O. #: 14079
 RBT Batch #: 01/14/14
 Date Collected/Time: February 25/14 @ 11:35
 Date Setup/Time: February 28/14 @ 14:35
 Sample Setup By: KSW/ML
 D.O. meter: DO: 1/2/3
 pH meter: pH: 1/2/3
 Cond. Meter: C: 1/2/3

Number Fish/Volume: 10/12 L
 7-d % Mortality: 0.15
 Total Pre-aeration Time (mins): 30
 Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Y

Undiluted Sample WQ			
Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C	14.0	/	14.0
pH	7.3	/	7.4
D.O. (mg/L)	10.2	/	10.2
Cond. (µS/cm)	336	/	338

Concentration	# Survivors						Temperature (°C)						Dissolved Oxygen (mg/L)						pH						Conductivity (µS/cm)			
	1	2	4	24	48	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	96
(% v/v)																												
Cont				10	10	9	14.0	14.0	14.0	14.0	14.0	10.0	9.8	9.6	9.8	9.9	7.1	6.9	6.9	7.1	7.1	34	34	34	34	34	39	39
6.25				10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.6	9.7	9.8	7.1	7.1	7.1	7.3	7.6	67	67	67	67	67	73	73
12.5				10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.8	9.6	9.7	9.7	7.2	7.4	7.2	7.4	7.5	74	74	74	74	74	81	81
25				10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.7	9.7	9.6	7.2	7.6	7.5	7.5	7.7	115	115	115	115	115	121	121
50				10	10	10	14.0	14.0	14.0	14.0	14.0	10.2	9.8	9.6	9.7	9.6	7.2	7.7	7.7	7.7	7.8	187	187	187	187	187	193	193
100				10	10	6	14.0	14.0	14.0	14.0	14.0	10.2	9.9	9.7	9.8	9.7	7.4	8.2	8.2	8.2	8.2	338	338	338	338	338	343	343
Initials																												

WQ Ranges: T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Sample Description/Comments: Clear, colourless

Fish Description at 96 h: All remaining fish appear OK Number of Stressed Fish at 96 h: 0

Other Observations: 09

Reviewed by: JGW Date Reviewed: March 13/14

Rainbow Trout Summary Sheet

Client: ALS

Start Date/Time: February 28/14 @ 1440

Work Order No.: 14079

Test Species: Oncorhynchus mykiss

Sample Information:

Sample ID: L1426336-7 (R3)
Sample Date: February 25/14 @ 1630
Date Received: February 27/14 @ 1030
Sample Volume: 2 x 20L
Other: N/A

Test Validity Criteria:

≥ 90% control survival

WQ Ranges:

T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L CaCO₃): 11
Alkalinity (mg/L CaCO₃): 9

Test Organism Information:

Batch No.: 011414
Source: Vancouver Island Trout Hatchery
No. Fish/Volume (L): 10/12
Loading Density: 0.42
Mean Length ± SD (mm): 38 ± 1 Range: 35 - 40
Mean Weight ± SD (g): 0.50 ± 0.06 Range: 0.41 - 0.59

NaNO₂ Reference Toxicant Results:

Reference Toxicant ID: RTNE55
Stock Solution ID: 13N602
Date Initiated: February 19/14
96-h LC50 (95% CL): 4.7 (3.8 - 5.8) mg/L NaNO₂

Reference Toxicant Mean and Historical Range: 5.4 (2.2 - 13.4) mg/L NaNO₂
Reference Toxicant CV (%): 57

Test Results: The 96-h LC50 is >100% (N/A).

Reviewed by: JGL

Date reviewed: March 13/14

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: ALS

Sample I.D. L1426336-7 (R3)

W.O. # 14079

RBT Batch #: 01/14/14

Date Collected/Time: February 25/14 @ 1630

Date Setup/Time: February 28/14 @ 1440

Sample Setup By: KJL/WW

D.O. meter: DO: 1/2/3

pH meter: pH: 1/2/3

Cond. Meter: C: 1/2/3

Number Fish/Volume: 10/12 L

7-d % Mortality: 0.15

Total Pre-aeration Time (mins): 30

Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Y

Undiluted Sample WQ			
Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C	14.0		14.0
pH	7.4		7.4
D.O. (mg/L)	10.1		10.1
Cond. (µS/cm)	661		673

Concentration	# Survivors										Temperature (°C)						Dissolved Oxygen (mg/L)						pH						Conductivity (µS/cm)	
	1	2	4	24	48	72	96	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	96	
(% v/v)																														
Cont				10	10	10	10	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	
6.25				10	10	10	10	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	
12.5				10	10	10	10	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	
25				10	10	10	10	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	
50				10	10	10	10	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	
100				10	10	10	10	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	
Initials				A	M			WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		

WQ Ranges: T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Sample Description/Comments: clear, colourless

Fish Description at 96 h All fish appear ok.

Other Observations: _____

Reviewed by: Joh

Date Reviewed: March 13/14

APPENDIX E - Chain of Custody Form

Subcontract Request Form
Subcontract To:
NAUTILUS ENVIRONMENTAL

 8664 COMMERCE COURT
 BURNABY, BC V5A 4N7

 7d Cerio Survival - 14076
 7d Lemna growth - ~~14078~~ 14077
 72h P. Subcapitata growth ^{SBF} ~~14079~~ 14078
 96h RBT LC50 ^{SBF} ~~14080~~ 14079

NOTES: Please reference on final report and invoice: PO# L1426336
 ALS requires QC data to be provided with your final results.

 Please see enclosed 2 sample(s) in 14 Container(s)

SAMPLE NUMBER	CLIENT ID	ANALYTICAL REQUIRED	DATE SAMPLED	DUE DATE	Priority Flag
L1426336-1	R10	Special Request- Nautilus Environmental (SPECIAL REQUEST-NL 14)	2/25/2014 15:00	3/5/2014	P T: 3.6
L1426336-2	NF1	Special Request- Nautilus Environmental (SPECIAL REQUEST-NL 14)	2/25/2014 14:35	3/5/2014	P T: 4.3
L1426336-3	NF2	Special Request- Nautilus Environmental (SPECIAL REQUEST-NL 14)	2/25/2014 13:05	3/5/2014	P T: 3.3
^{emh} ① L1426336-4	X1	Special Request- Nautilus Environmental (SPECIAL REQUEST-NL 14)	2/25/2014 11:10	3/5/2014	P T: 4.0
① L1426336-5	X14	Special Request- Nautilus Environmental (SPECIAL REQUEST-NL 14)	2/25/2014 09:00	3/5/2014	P T: 2.8
① L1426336-6	X3A	Special Request- Nautilus Environmental (SPECIAL REQUEST-NL 14)	2/25/2014 11:35	3/5/2014	P T: 3.9
① L1426336-7	R3	Special Request- Nautilus Environmental (SPECIAL REQUEST-NL 14)	2/25/2014 16:30	3/5/2014	P T: 3.4

① ^{R3} X1, X14, X3A subcontracted
 emh - sent via havex for Cerio 7d.

EMM

1/3



L1426336

VANCOUVER

Subcontract Request Form

Subcontract To:

NAUTILUS ENVIRONMENTAL

8664 COMMERCE COURT
BURNABY, BC V5A 4N7

Subcontract Info Contact:

Dorota Jamro (604) 253-4188

Analysis and reporting info contact:

Can Dang
8081 LOUGHEED HWY
SUITE 100
BURNABY, BC V5A 1W9

Phone: (604) 253-4188

Email: can.dang@alsglobal.com

Please email confirmation of receipt to:

can.dang@alsglobal.com

Shipped By: _____

Date Shipped: _____

Received By: JBF/NY

Date Received: Feb 27/14 - 10:30h.

Verified By: _____

Date Verified: _____

Temperature: ~ 3.5°

Sample Integrity Issues: N/A



Chain of Custody (COC) / Analytical Request Form

Canada Toll Free: 1 800 668 9878

L 1426336-COCF

COC Number: 14 -

Page of

Report To

Company: EDI
 Contact: Meighan Kearns
 Address: 2195 - 2nd Avenue
 Whitehorse, YT Y1A 3T8
 Phone: 867-393-4882

Invoice To Same as Report To Yes No
 Copy of Invoice with Report Yes No

Company: EDI
 Contact: S Jenner

Project Information

ALS Quote #: Q38556
 Job #: 13-Y-0452
 PO / AFE:
 LSD:

Report Format / Distribution

Select Report Format: PDF EXCEL EDD (DIGITAL)
 Quality Control (QC) Report with Report Yes No
 Criteria on Report - provide details below if box checked
 Select Distribution: EMAIL MAIL FAX

Email 1 or Fax: mkearns@edynamics.com
 Email 2: address@uncoffed@box.yk.ca

Invoice Distribution

Select Invoice Distribution: EMAIL MAIL FAX
 Email 1 or Fax: sjenner@edynamics.com
 Email 2:

Oil and Gas Required Fields (client use)

Approver ID:
 GL Account:
 Activity Code:
 Location:

ALS Contact:

ALS Lab Work Order # (lab use only)

Sample Identification and/or Coordinates
 (This description will appear on the report)

ALS Sample # (lab use only)	ALS Lab Work Order # (lab use only)	Date (dd-mm-yy)	Time (hh:mm)	Sample Type
K10		25-FEB-14	15:00	Surf (14)
API			14:35	
NF2			13:05	
X1			11:10	
X1A			9:00	
X3A			1:35	
R3			18:20	

Drinking Water (DW) Samples (client use)

Are samples taken from a Regulated DW System? Yes No
 Are samples for human drinking water use? Yes No

Special Instructions / Specify Criteria to add on report (client use)

Use CH2M_EQUIS for EDD.
 Priority

SHIPMENT RELEASE (client use)

Released by: _____ Date: _____ Time: _____

INITIAL SHIPMENT RECEPTION (lab use only)

Received by: _____ Date: 26 Feb 14 Time: 2:00

Analysis Request

Indicate Filled (F), Preserved (P) or Filtered and Preserved (FP) below

Number of Containers

Analysis Request

Indicate Filled (F), Preserved (P) or Filtered and Preserved (FP) below

Number of Containers

SAMPLE CONDITION AS RECEIVED (lab use only)

Frozen SIF Observations Yes No
 Ice packs Yes No Custody seal intact Yes No
 Cooling Inhibited

INITIAL COOLER TEMPERATURES °C: 6.7 2.3 7.0
 FINAL COOLER TEMPERATURES °C:

FINAL SHIPMENT RECEPTION (lab use only)

Received by: _____ Date: _____ Time: _____



Chain of Custody

British Columbia
 8664 Commerce Court
 Burnaby, British Columbia, Canada V5A 4N3
 Phone 604-420-8773

Date Feb 27/14 Page 1 of 1

Sample Collection By:			Invoice To:									
Report to:	Company <u>Nautilus Environmental</u> Address <u>8664 Commerce Court</u> City/State/Zip <u>Burnaby, BC, V5A 4N3</u> Contact <u>Emma Marus</u> Phone <u>604-420-8773</u> Email <u>emma@nautilusenvironmental.com</u>	Company <u>Nautilus Environmental</u> Address <u>8664 Commerce Court</u> City/State/Zip <u>Burnaby, BC, V5A 4N3</u> Contact <u>Emma Marus</u> Phone <u>604-420-8773</u> Email <u>emma@nautilusenvironmental.com</u>	NO. OF CONTAINERS	COMMENTS	ANALYSES REQUIRED							
1	SAMPLE ID <u>X1</u>	DATE <u>Feb 25/14</u>	TIME <u>1110</u>	MATRIX <u>water</u>		CONTAINER TYPE <u>1L</u>	NO. OF CONTAINERS <u>7 x 1L</u>	COMMENTS <u>INTERVAL # 14076 411</u>	X	3d Ceritrophica survival + IRCLD# 1402154 1400155	Receipt Temperature (°C)	
2	<u>X14</u>	↓	<u>0900</u>	↓		↓	<u>6</u>		X			<u>11.3</u>
3	<u>X3A</u>	↓	<u>1135</u>	↓		↓	<u>7 x 1L</u>	↓	X			<u>10.9</u>
4											<u>9.8</u>	
5												
6												
7												
8												
9												
10												
PROJECT INFORMATION						RELINQUISHED BY (CLIENT)						
Client:				Total No. of Containers						(Signature)	(Time)	RELINQUISHED BY (COURIER)
PO No.:				Received Good Condition?						(Printed Name)	(Date)	
Shipped Via:				Matches Test Schedule?						(Company)	(Date)	
SPECIAL INSTRUCTIONS/COMMENTS: one/7 sample container for X14 arrived at IRC broken. Sample lost. * Test cancelled, as per clients request.						(Signature) <u>Feb 27/14</u> (Printed Name) <u>Emma Marus</u> (Company) <u>Nautilus Environmental</u> (Date) <u>1300</u>						
						RECEIVED BY (COURIER)						
						(Signature) <u>Melissa Hebert</u> (Printed Name) <u>Melissa Hebert</u> (Company) <u>IRC</u> (Date) <u>27 Feb 14</u> <u>via NoveX</u>						
						RECEIVED BY (LABORATORY)						
						(Signature)						
						(Printed Name)						
						(Company)						

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.



TESTING LOCATION (Please Circle)

Chain of Custody

British Columbia
8664 Commerce Court
Burnaby, British Columbia, Canada V5A 4N3
Phone 604.420.8773

Date Feb 27/14 Page 1 of 1

Sample Collection By:				Invoice To:				ANALYSES REQUIRED										Receipt Temperature (°C)	
Report to:				Company															
Nautilus Environmental				Nautilus Environmental															
8664 Commerce Court				8664 Commerce Court															
Burnaby, BC, V5A 4N3				Burnaby, BC, V5A 4N3															
Emma Marus				Emma Marus															
604-420-8773				604-420-8773															
emma@nautilusenvironmental.com				emma@nautilusenvironmental.com															
SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS													
03	Feb 25/14	1330	water	plastic	34 x 2L full	W0A14076	X 7 day centrifuge structure										9.30		
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
PROJECT INFORMATION				SAMPLE RECEIPT				RELINQUISHED BY (CLIENT)				RELINQUISHED BY (COURIER)							
Client:				Total No. of Containers				(Signature)				(Signature)							
PO No.:				Received Good Condition?				Emma Marus				(Printed Name)							
Shipped Via:				Matches Test Schedule?				Feb 27/14				(Date)							
								1330				(Date)							
								Nautilus Environmental				(Company)							
SPECIAL INSTRUCTIONS/COMMENTS:				RECEIVED BY (COURIER)				RECEIVED BY (LABORATORY)											
R3 to replace sample "X1" for testing.				(Signature)				Novex				(Signature)							
				(Printed Name)				(Date)				(Printed Name)				(Date)			
				(Company)				(Date)				(Company)				(Date)			

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.



Canada Toll Free: 1 800 668 9878

L1426336-COFC

www.alsglobal.com

Report To		Report Format / Distribution			below (Rush Turnaround Time (TAT) is not available for all tests)				
Company: EDI		Select Report Format: <input checked="" type="checkbox"/> PDF <input checked="" type="checkbox"/> EXCEL <input checked="" type="checkbox"/> EDD (DIGITAL)			R <input type="checkbox"/> Regular (Standard TAT if received by 3 pm - business days) P <input checked="" type="checkbox"/> Priority (2-4 bus. days if received by 3pm) 50% surcharge - contact ALS to confirm TAT E <input type="checkbox"/> Emergency (1-2 bus. days if received by 3pm) 100% surcharge - contact ALS to confirm TAT E2 <input type="checkbox"/> Same day or weekend emergency - contact ALS to confirm TAT and surcharge				
Contact: Meghan Kearns		Quality Control (QC) Report with Report <input type="checkbox"/> Yes <input type="checkbox"/> No			Specify Date Required for E2, E or P.				
Address: 2195 - 2nd Avenue Whitehorse, YT Y1A 3T8		<input type="checkbox"/> Criteria on Report - provide details below if box checked			Analysis Request Indicate Filtered (F), Preserved (P) or Filtered and Preserved (FP) below				
Phone: 867-393-4882		Select Distribution: <input type="checkbox"/> EMAIL <input type="checkbox"/> MAIL <input type="checkbox"/> FAX							
Invoice To: Same as Report To <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		Invoice Distribution			Number of Containers <div style="border: 1px solid black; padding: 5px;"> Ceriodaphnia dubia survival and reproduction Lemna growth inhibition Pseudokirchneriella subcapitata growth inhibition Rainbow trout acute LC50 </div>				
Copy of Invoice with Report <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		Select Invoice Distribution: <input type="checkbox"/> EMAIL <input type="checkbox"/> MAIL <input type="checkbox"/> FAX							
Company: EDI		Email 1 or Fax: mkearns@edynamics.com							
Contact: S Jenner		Email 2: adrienne.turcotte@edv.yk.ca							
		Email 1 or Fax: sjenner@edynamics.com							
Project Information		Oil and Gas Required Fields (client use)							
ALS Quote #: Q38556		Approver ID:		Cost Center:					
Job #: 13-Y-0452		GL Account:		Routing Code:					
PO / AFE:		Activity Code:							
LSD:		Location:							
ALS Lab Work Order # (lab use only):		ALS Contact:		Sampler:					
ALS Sample # (lab use only):	Sample Identification and/or Coordinates <small>(This description will appear on the report)</small>	Date <small>(dd-mm-yy)</small>	Time <small>(hh:mm)</small>	Sample Type					
	K10	25-FEB-14	15:00	Surface Water	X ↓ ↓ ↓ ↓ ↓ ↓				
	NPI		14:35						
	NF2		13:05						
	X1		11:10						
	<151		9:00						
	X 3A		1:35						
	R3		16:30						
Drinking Water (DW) Samples ¹ (client use)		Special Instructions / Specify Criteria to add on report (client use)			SAMPLE CONDITION AS RECEIVED (lab use only)				
Are samples taken from a Regulated DW System? <input type="checkbox"/> Yes <input type="checkbox"/> No		Priority			Frozen <input type="checkbox"/> SIF Observations: Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> Ice packs Yes <input type="checkbox"/> No <input type="checkbox"/> Custody seal intact: Yes <input type="checkbox"/> No <input type="checkbox"/> Cooling initiated <input type="checkbox"/>				
Are samples for human drinking water use? <input type="checkbox"/> Yes <input type="checkbox"/> No					INITIAL COOLER TEMPERATURES °C: 6.7 7.3 7.0 FINAL COOLER TEMPERATURES °C:				
SHIPMENT RELEASE (client use)		INITIAL SHIPMENT RECEPTION (lab use only)			FINAL SHIPMENT RECEPTION (lab use only)				
Released by:	Date:	Time:	Received by:	Date:	Time:	Received by:	Date:	Time:	
				26-FEB-14	2:00				