MDMER Toxicity Testing on 11A-Runoff

Sample collected on May 14, 2019

Final Report

June 21, 2019

Submitted to: **Nyrstar Myra Falls Ltd**
Campbell River, 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.

SUMMARY

Sample Information and Test Type

Sample ID	11A-Runoff
Sample collection date	May 14, 2019
Sample receipt date	May 15, 2019
Sample receipt temperature	14.2°C
Test types	*Ceriodaphnia dubia* survival and reproduction 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability 7-d *Lemna minor* growth inhibition 72-h *Pseudokirchneriella subcapitata* growth inhibition

Summary of Results

Endpoint	% v/v (95% CL)
Ceriodaphnia. dubia	
Survival LC50	>100
Reproduction IC25	>100
Reproduction IC50	>100
Oncorhynchus mykiss	
Embryo viability EC25	>100
Embryo viability EC50	>100
Lemna minor	
Frond count IC25	83.2 (60.5 – 97)
Frond count IC50	>97
Dry weight IC25	>97
Dry weight IC50	>97
Pseudokirchneriella subcapitata	
Growth IC25	>95.2
Growth IC50	>95.2

LC = Lethal Concentration, IC = Inhibition Concentration, EC = Effective Concentration, CL = Confidence Limits

1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted sub-lethal toxicity tests for Nyrstar Myra Falls Ltd. as part of their requirements under the Metal Diamond Mining Effluent Regulations (MDMER) program. Sample 11A-Runoff was collected on May 14, 2019 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on May 15, 2019. The sample was transported in seven 20-L plastic containers and were received at a temperature of 14.2°C. The sample was stored in the dark at 4 ± 2°C prior to testing. The following sub-lethal toxicity tests were performed:

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

Testing for *C. dubia* and *O. mykiss* were initiated on May 15, 2019. Testing for *L. minor* and *P. subcapitata* were initiated on May 16 and May 17, 2019, respectively This report describes the results of these toxicity tests. Copies of raw laboratory data sheets and statistical analyses for each test species 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 using *C. dubia*, *L. minor* and *P. subcapitata* were conducted according to procedures described by Environment Canada (2007a, 2007b and 2007c). The rainbow trout embryo viability test followed procedures described by Environment Canada (1998) and modified by Canaria et al. (1999). Statistical analyses for all the tests were performed using CETIS (Tidepool Scientific Software, 2013).

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

Test species	*Ceriodaphnia dubia*
Organism source	In-house culture
Organism age	<24 hour old neonates, produced within a 12 hour window
Test type	Static-renewal
Test duration	7 ± 1 day
Test vessel	20-mL glass test tube
Test volume	15 mL
Test solution depth	10 cm
Test concentrations	Seven concentrations, plus laboratory control
Test replicates	10 per treatment
Number of organisms	1 per replicate
Control/dilution water	20% Perrier water and 80% deionized water + 5 µg/L Se and 2 µg/L vitamin B12
Test solution renewal	Daily (100% renewal)
Test temperature	25 ± 1°C
Feeding	Daily with *Pseudokirchneriella subcapitata* and TCC (3:1 ratio)
Light intensity	100 to 600 lux at water surface
Photoperiod	16 hours light / 8 hours dark
Aeration	None
Test measurements	Temperature, dissolved oxygen, pH and conductivity measured daily; hardness and alkalinity of undiluted sample measured at test initiation; survival and reproduction checked daily
Test protocol	Environment Canada (2007a), EPS 1/RM/21
Statistical software	CETIS Version 1.9.4
Test endpoints	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; no ephippia present
Reference toxicant	Sodium chloride (NaCl)

Table 2. Summary of test conditions: rainbow trout (*Oncorhynchus mykiss*) embryo viability test.

Test species	*Oncorhynchus mykiss*
Organism source	Hatchery
Organism age	<30 minutes post fertilization, <24 hour old gametes
Test type	Static-renewal
Test duration	7 days
Test vessel	2-L plastic container
Test volume	2 L
Test solution depth	17 cm
Test concentrations	Five concentrations, plus laboratory control
Test replicates	4 per treatment
Number of organisms	30 per replicate
Control/dilution water	Dechlorinated Metro Vancouver municipal tapwater
Test solution renewal	Daily (80% renewal)
Test temperature	14 ± 1°C
Feeding	None
Light intensity	Dark
Photoperiod	24 hours dark
Aeration	Continuous gentle aeration
Test measurements	Temperature, dissolved oxygen, pH and conductivity measured daily; hardness and alkalinity of undiluted sample measured at test initiation; survival checked daily
Test protocol	Environment Canada (1998), EPS 1/RM/28; Canaria et al. (1999)
Statistical software	CETIS Version 1.9.4
Test endpoint	Embryo viability
Test acceptability criterion for controls	Embryo viability ≥70%
Reference toxicant	Sodium dodecyl sulphate (SDS)

Table 3. Summary of test conditions: *Lemna minor* growth inhibition test.

Test species	*Lemna minor*, strain CPCC# 490
Organism source	In-house axenic culture, obtained from Canadian Phycological Culture Centre, and originally isolated from Wainfleet, Stinking Barn, Niagara Peninsula, Ontario, Canada
Organism age	7- to 10-day old culture
Test type	Static
Test duration	7 days
Test vessel	250-mL glass container
Test volume	100 mL
Test solution depth	4 cm
Test concentrations	Seven concentrations, plus laboratory control
Test replicates	4 per treatment
Number of organisms	Two 3-frond plants per replicate
Control/dilution water	Modified APHA media (deionized water plus 1% of each APHA stock solution A, B and C)
Test solution renewal	None
Test temperature	25 ± 2°C
Feeding	None
Light intensity	4000 to 5600 lux
Photoperiod	24 hours light
Aeration	None
Test measurements	Test area temperature measured daily; temperature, pH and conductivity measured in all concentrations at test initiation; dissolved oxygen of highest concentration measured at test initiation; temperature and pH measured at test termination
Test protocol	Environment Canada (2007b), EPS 1/RM/37
Statistical software	CETIS Version 1.9.4
Test endpoints	Number of fronds and dry weight
Test acceptability criterion for controls	≥ 8-fold increase in number of fronds
Reference toxicant	Potassium chloride (KCl)

Table 4. Summary of test conditions: *Pseudokirchneriella subcapitata* growth inhibition test.

Test species	*Pseudokirchneriella subcapitata*, strain CPCC# 37
Organism source	In-house axenic culture, obtained from Canadian Phycological Culture Center, and originally isolated from Nivelta River, Norway.
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 concentrations	Seven concentrations, plus laboratory control
Test replicates	4 per treatment; 8 for laboratory control
Number of organisms	10,000 cells/mL
Control/dilution water	Deionized water supplemented with 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 measurements	Test area temperature measured daily; temperature and pH measured at test initiation; pH of two control wells measured at test termination
Test protocol	Environment Canada (2007c), EPS 1/RM/25
Statistical software	CETIS Version 1.9.4
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 (added as $ZnSO_4$)

3.0 RESULTS

Results of the toxicity tests are summarized in Tables 5 to 8. There were no adverse effects observed on any of the endpoints for *C. dubia* (Table 5), embryo viability of *O. mykiss* (Table 6), dry weight of *L. minor* (Table 7) or cell yield of *P. subcapitata* (Table 8), resulting in LC and IC values greater than the highest concentration tested. Significant stimulation was observed in *P. subcapitata* cell yield between test concentrations 6% and 95.2% (v/v); percent stimulation ranged between 7.6% and 96.4%.

Inhibitory effects were observed in *L. minor* frond count (Table 7), the IC25 and IC50 were 83.2% and >97% (v/v), respectively.

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

Concentration (% v/v)	Survival (%)	Reproduction (Mean ± SD)
Laboratory Control	100	18.6 ± 3.1
1.56	100	20.0 ± 2.7
3.12	90	17.1 ± 6.9
6.25	100	20.5 ± 1.8
12.5	100	19.7 ± 5.6
25	100	20.1 ± 2.1
50	100	19.5 ± 3.3
100	90	19.6 ± 3.4
Test endpoint (% v/v)		
LC50	>100	--
IC25	--	>100
IC50	--	>100

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

Table 6. Results: rainbow trout (*Oncorhynchus mykiss*) embryo viability test.

Concentration (% v/v)	Embryo Viability (%) (Mean ± SD)
Laboratory Control	93.3 ± 4.7
6.25	90.8 ± 7.4
12.5	92.5 ± 5.0
25	90.0 ± 8.6
50	93.3 ± 6.1
100	86.7 ± 4.7
Test Endpoint (% v/v)	
EC25	>100
EC50	>100

SD = Standard Deviation, EC = Effective Concentration

Table 7. Results: *Lemna minor* growth inhibition test.

Concentration (% v/v)	Frond Growth (No. of Fronds) (Mean ± SD)	Dry Weight (mg) (Mean ± SD)
Laboratory Control	93.8 ± 16.3	7.8 ± 1.2
1.5	100.8 ± 7.3	7.8 ± 0.8
3.0	87.0 ± 6.5	7.0 ± 0.5
6.1	92.2 ± 12.5	7.5 ± 0.8
12.1	83.0 ± 13.9	6.9 ± 0.7
24.2	88.5 ± 7.7	7.0 ± 1.1
48.5	87.5 ± 8.7	6.6 ± 1.3
97	56.2 ± 11.3	5.7 ± 0.8
Test endpoint (% v/v)		
IC25 (95% CL)	83.2 (60.5 – 97)	>97
IC50	>97	>97

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

Table 8. Results: *Pseudokirchneriella subcapitata* growth inhibition test.

Concentration (% v/v)	Cell Yield (x 10^4 cells/mL) (Mean ± SD)	Stimulation (%)
Laboratory Control	34.4 ± 3.0	--
1.5	32.8 ± 3.1	--
3.0	37.0 ± 2.9	7.6
6.0	47.8 ± 6.9*	38.9
11.9	54.2 ± 5.5*	57.8
23.8	60.5 ± 2.1*	76.0
47.6	67.5 ± 5.4*	96.4
95.2	51.8 ± 3.9*	50.6
Test endpoint (% v/v)		
IC25	>95.2	
IC50	>95.2	

SD = Standard Deviation, IC = Inhibition Concentration
* = Indicates cell yield that are significantly greater than the laboratory control

4.0 QA/QC

The health history 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. Uncertainty associated with these tests is best described by the standard deviation around the mean and/or the confidence intervals around the point estimates.

There was a planned deviation from the 7-d rainbow trout embryo viability test methodology. The eggs were exposed using a blocked design (i.e., eggs from one fish was used for replicate A of each test concentration, eggs from the second fish for replicate B, and so on); this approach deviates from the Environment Canada test method, which indicates that the eggs should be pooled prior to testing. However, this modification is considered appropriate because it reduces the risk of non-viable eggs affecting the test results, since in the event that one of the batches of eggs had been non-viable, it would have been possible to exclude data for that replicate. There were no other deviations from the test methodologies.

Results of the reference toxicant tests conducted during the testing program are summarized in Table 9. Results for these tests fell within the range for organism performance of the mean and two standard deviations, based on historical results obtained by the laboratory with these tests. Thus, the sensitivity of the organisms used in these tests was appropriate. The reference toxicant tests were performed under the same conditions as those used for the samples.

Table 9. Reference toxicant test results.

Test Species	Endpoint	Historical Mean (2 SD Range)	CV (%)	Test Date
C. dubia	Survival (LC50): 2.0 g/L NaCl	2.0 (1.8 – 2.2)	5	May 1, 2019
	Reproduction (IC50): 1.2 g/L NaCl	1.6 (1.1 – 2.3)	19	
O. mykiss	Viability (EC50): 6.6 mg/L SDS	4.1 (2.0 – 8.3)	37	May 15, 2019
L. minor	No. Fronds (IC50): 3.8 g/L KCl	3.5 (2.9 – 4.2)	9	May 25, 2019
P. subcapitata	Growth (IC50): 32.9 µg/L Zn	31.0 (25.2 – 38.1)	10	May 28, 2019

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

5.0 REFERENCES

Canaria, E.C., J.R. Elphick and H.C. Bailey. 1999. A simplified procedure for conducting small-scale short-term embryo toxicity tests with salmonids. Environ Toxicol 14:301-307.

Environment Canada. 1998. Biological test method: toxicity tests using early life stages of salmonid fish (rainbow trout). Environmental Protection Series EPS 1/RM/28. Second Edition, July 1998. Environment Canada, Method Development and Application Section, Environmental Technology Centre, Ottawa, ON. 102 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 1.9.4.11 Tidepool Scientific Software, McKinleyville, CA. 275 pp.

APPENDIX A – *Ceriodaphnia dubia* Toxicity Test Data

Ceriodaphnia dubia Summary Sheet

Client: Nyasaar
Work Order No.: 190967

Start Date/Time: May 15/19 1200h
Set up by: SSK/KL

Sample Information:

Sample ID: HA-Runoff
Sample Date: May 14/19
Date Received: May 15/19
Sample Volume: 7x20L

Test Validity Criteria:

1) Mean survival of first generation controls is ≥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.: 180509A,B & 180509C
Age of young (Day 0): <24-h (within 12-h)
Avg No. young in first 3 broods of previous 7 d: 20
Mortality (%) in previous 7 d: 3
Individual female # used ≥8 young on test day: ~~1-9~~ 7, 25, 31, 32, 35, 36, 39, 45

NaCl Reference Toxicant Results:

Reference Toxicant ID: Qtzo
Stock Solution ID: (8NaCl (100g/L NaCl)
Date Initiated: May 1/19

7-d LC50 (95% CL): 2.0 (1.7-2.3) g/L NaCl
7-d IC50 (95% CL): 1.2 (1.1-1.3) g/L NaCl

7-d LC50 Reference Toxicant Mean and Historical Range: 2.0 ~~(1.8-2.2)~~ (1.8-2.2) g/L NaCl CV (%): 5
7-d IC50 Reference Toxicant Mean and Historical Range: 1.6 (1.1-2.3) g/L NaCl CV (%): 19

Test Results:

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

Reviewed by: [signature]
Date reviewed: May 30, 2019

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: Nyrstar
Sample ID: 11A - Runoff
Work Order #: ~~190867~~ 190967 KL

Start Date & Time: May 15/19 @ noon
Stop Date & Time: May 21/19 @ 1600
CER #: 4
Test Species: *Ceriodaphnia dubia*

% (v/v) Concentration: Control	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	24.0	25.0	24.0	25.0	25.0	24.0	25.0	24.0	25.0	24.0	24.0		
DO (mg/L)	8.0	7.3	8.0	7.3	8.1	7.4	8.2	7.3	8.2	7.0	8.3	7.1		
pH	8.2	8.0	8.3	8.0	8.3	7.9	8.2	7.9	8.2	7.7	8.2	7.8		
Cond. (µS/cm)	216	216		216		214		216		210		215		
Initials	SSK	JB		JB		^		^		JW		SSK		

Concentration: 1.56	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)	25.0	24.0	25.0	24.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	24.0		
DO (mg/L)	7.9	6.9	7.9	7.3	8.2	7.2	8.1	7.3	8.1	7.1	8.0	7.1		
pH	8.3	8.0	8.1	8.0	8.2	7.9	8.2	7.8	8.1	7.8	8.1	7.8		
Cond. (µS/cm) ⑤	~~222~~	224		228		226		225		227		231		
Initials	SSK	JB		JB		^		^		JW		SSK		

⑤ 222

Concentration: 12.5	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)	25.0	24.0	25.0	24.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	24.0		
DO (mg/L)	7.9	6.9	8.0	7.3	8.2	7.2	8.1	7.4	8.1	7.0	8.0	7.0		
pH	8.3	8.0	8.3	8.0	8.3	7.9	8.2	7.8	8.2	7.8	8.1	7.8		
Cond. (µS/cm)	253	255		257		262		260		256		269		
Initials	SSK	JB		JB		^		^		JW		SSK		

Concentration: 100	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) ③	~~25.0~~	24.0	25.0	24.0	25.0	25.0	24.0	25.0	24.0	25.0	24.0	24.0		
DO (mg/L) ④	~~8.9~~	7.1	8.1	7.3	8.2	7.3	8.1	7.2	8.1	7.0	8.0	7.0		
pH	8.3	~~8.2~~ 7.8	8.0	7.7	8.2	7.7	8.3	7.7	8.2	7.5	8.3	7.4		
Cond. (µS/cm)	497	499		496		490		486		489		499		
Initials	SSK	JB		JB		^		^		JW		SSK		

③ 25.0 ④ 8.2 KL

Thermometer: 4 DO meter/probe: 1 / 1 pH meter/probe: 1 / 1 Conductivity meter/probe: 1 / 1

	Control	100%		
Hardness*	100	266		
Alkalinity*	106	30		

* mg/L as CaCO3

Analysts: KL, AWD, SSK, JB
Reviewed by: [signature]
Date reviewed: May 30, 2019

Sample Description: Clear, colourless, clearness, no particulates

Comments: Broodboard Used: BS050919A, B (#7, 25, 31, 32, 35, 36, 39) and BS071A (#45)

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: Nyrstar
Sample ID: 11A – Runoff
Work Order: 190967

(%v/v)

Start Date & Time: May 15/19 @ 1600 hrs
Stop Date & Time: May 22/19 @ 1600 hrs
Set up by: KL/SSK

Concentration: CONTROL

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	3	2	3	3	3	3	3	✓	3	3	JW
4	6	6	5	5	5	7	7	4	5	6	JW
5	✓	7	9	✓	9	9	✓	11	✓	✓	JW
6	10	✓	✓	10	✓	✓	11	11	9	8	JW
7											
8			JW								
Total	19	15	~~16~~ 17	18	17	19	21	26	17	17	JW

Concentration: 1.56

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	4	4	3	5	4	4	–	4	3	4	JW
4	7	6	7	7	6	6	7	6	✓	8	JW
5	✓	9	7	✓	✓	11	8	10	6	✓	JW
6	11	✓	✓	11	12	✓	✓	✓	9	11	JW
7											
8											
Total	22	19	17	23	22	24	15	20	18	23	JW

Concentration: 3.12

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	4	4	4	4	4	X	3	4	✓	2	JW
4	7	7	7	6	8		7	6	4	7	JW
5	7	5	12	✓	10		✓	8	4	✓	JW
6	✓	✓	✓	10	✓		13	✓	4	10	JW
7											
8											
Total	18	16	23	20	22	0X	23	18	12	19	JW

Concentration: 6.25

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	4	4	4	4	4	4	3	4	4	3	JW
4	6	6	7	6	7	8	8	6	7	6	JW
5	9	9	7	✓	✓	10	✓	✓	8	✓	JW
6	✓	✓	✓	11	10	✓	12	13	✓	11	JW
7											
8											
Total	19	19	18	21	21	22	23	23	19	20	JW

Concentration: 12.5

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	5	5	6	3	4	2	4	3	4	4	JW
4	8	7	8	7	7	6	7	7	7	7	JW
5	9	12	JW ✓	✓	11	✓	✓	✓	9	✓	JW
6	–	10	JW 14	✓	✓	11	12	8	✓	✓	JW
7											
8							JW				
Total	22	24	28	10	22	19	~~23~~ 23	18	20	11	JW

Concentration: 25

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	4	4	4	4	4	4	2	4	4	3	JW
4	7	6	7	7	7	7	7	5	6	5	JW
5	11	9	11	✓	✓	10	✓	✓	11	8	JW
6	✓	✓	✓	11	10	✓	11	8	✓	✓	JW
7											
8											
Total	22	19	22	22	21	21	20	17	21	16	JW

Concentration: 50

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	2	4	5	4	4	4	4	3	4	2	JW
4	6	5	5	✓	7	✓	5	6	7	5	JW
5	✓	✓	10	✓	✓	10	✓	✓	11	✓	JW
6	10	13	✓	11	13	✓	12	13	✓	10	JW
7											
8											
Total	18	22	20	15	24	14	24	22	22	17	JW

Concentration: 100

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	–	3	✓	✓	✓	✓	✓	2	✓	4	JW
4	3	✓	2	5	5	3	3	✓	4	6	JW
5	5	7	4	5	8	6	8	4	5	10	JW
6	10	14	10	10	10	10	12	14	4X	✓	JW
7											
8											
Total	18	24	16	20	23	19	23	20	13X	20	JW

Concentration:

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

Notes: X = mortality.

Comments: 1. Total # Young only based on the first 3 Broods. Fourth and subsequent broods not included in total count.

2. Ephippia present in Controls (Y), (N) ?

Reviewed by: [signature]

Date reviewed: May 30, 2019

CETIS Analytical Report

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

Analysis ID:	20-7793-0447	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.9.4		
Analyzed:	22 May-19 14:51	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1		
Batch ID:	06-8138-5398	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Kania Lywe		
Start Date:	15 May-19 12:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	20% Perrier Water		
Ending Date:	21 May-19 16:00	**Species:**	Ceriodaphnia dubia	**Brine:**			
Test Length:	6d 4h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:**	<24
Sample ID:	11-6202-5560	**Code:**	45431A58	**Project:**			
Sample Date:	14 May-19 12:30	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls		
Receipt Date:	15 May-19 08:51	**CAS (PC):**		**Station:**	11A-Runoff		
Sample Age:	23h (14.2 °C)	**Client:**	Nyrstar Myra Falls				

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	150079	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	64.89	2.806	n/a	1.541	n/a	35.64
EC10	100	60.99	n/a	1	n/a	1.639
EC15	>100	n/a	n/a	<1	n/a	n/a
EC20	>100	n/a	n/a	<1	n/a	n/a
EC25	>100	n/a	n/a	<1	n/a	n/a
EC40	>100	n/a	n/a	<1	n/a	n/a
EC50	>100	n/a	n/a	<1	n/a	n/a

6d Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	A/B	Mean	%Effect
0	N	10	1.0000	1.0000	1.0000	0.0000	0.00%	0.0%	10/10	1	0.0%
1.56		10	1.0000	1.0000	1.0000	0.0000	0.00%	0.0%	10/10	1	0.0%
3.12		10	0.9000	0.0000	1.0000	0.3162	35.14%	10.0%	9/10	0.98	2.0%
6.25		10	1.0000	1.0000	1.0000	0.0000	0.00%	0.0%	10/10	0.98	2.0%
12.5		10	1.0000	1.0000	1.0000	0.0000	0.00%	0.0%	10/10	0.98	2.0%
25		10	1.0000	1.0000	1.0000	0.0000	0.00%	0.0%	10/10	0.98	2.0%
50		10	1.0000	1.0000	1.0000	0.0000	0.00%	0.0%	10/10	0.98	2.0%
100		10	0.9000	0.0000	1.0000	0.3162	35.14%	10.0%	9/10	0.9	10.0%

6d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.56		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
3.12		1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
6.25		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12.5		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000

6d Survival Rate Binomials

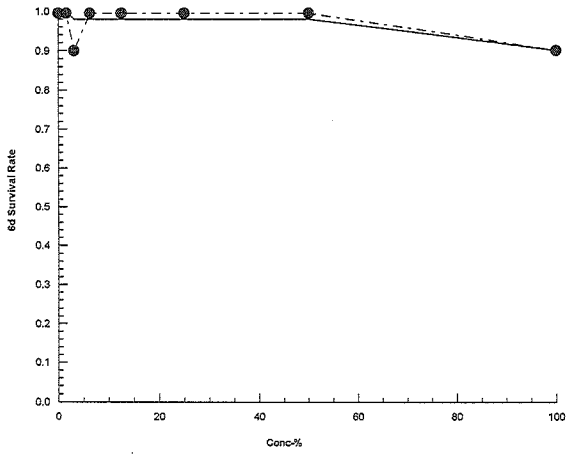
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
1.56		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
3.12		1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
6.25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
12.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
50		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	0/1	1/1

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID:	20-7793-0447	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.9.4
Analyzed:	22 May-19 14:51	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID:	13-2944-8192	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.4
Analyzed:	22 May-19 14:51	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	06-8138-5398	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Kania Lywe
Start Date:	15 May-19 12:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	20% Perrier Water
Ending Date:	21 May-19 16:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Test Length:	6d 4h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture **Age:** <24
Sample ID:	11-6202-5560	**Code:**	45431A58	**Project:**	
Sample Date:	14 May-19 12:30	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 May-19 08:51	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	23h (14.2 °C)	**Client:**	Nyrstar Myra Falls		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	983726	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>100	n/a	n/a	<1	n/a	n/a
IC10	>100	n/a	n/a	<1	n/a	n/a
IC15	>100	n/a	n/a	<1	n/a	n/a
IC20	>100	n/a	n/a	<1	n/a	n/a
IC25	>100	n/a	n/a	<1	n/a	n/a
IC40	>100	n/a	n/a	<1	n/a	n/a
IC50	>100	n/a	n/a	<1	n/a	n/a

Reproduction Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	N	10	18.6	15	26	3.062	16.46%	0.0%	19.39	0.0%
1.56		10	20	15	23	2.708	13.54%	-7.53%	19.39	0.0%
3.12		10	17.1	0	23	6.887	40.28%	8.07%	19.39	0.0%
6.25		10	20.5	18	23	1.78	8.68%	-10.22%	19.39	0.0%
12.5		10	19.7	10	28	5.599	28.42%	-5.91%	19.39	0.0%
25		10	20.1	16	22	2.132	10.61%	-8.07%	19.39	0.0%
50		10	19.5	14	24	3.342	17.14%	-4.84%	19.39	0.0%
100		10	19.6	13	24	3.373	17.21%	-5.38%	19.39	0.0%

Reproduction Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	19	15	17	18	17	19	21	26	17	17
1.56		22	19	17	23	22	21	15	20	18	23
3.12		18	16	23	20	22	0	23	18	12	19
6.25		19	19	18	21	21	22	23	23	19	20
12.5		22	24	28	10	22	19	23	18	20	11
25		22	19	22	22	21	21	20	17	21	16
50		18	22	20	15	24	14	21	22	22	17
100		18	24	16	20	23	19	23	20	13	20

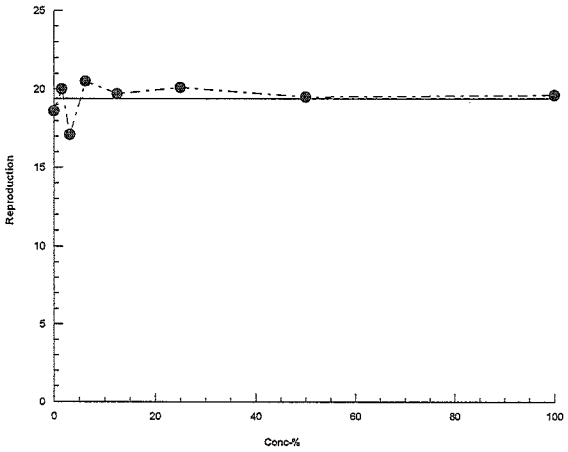
May 30/19

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID:	13-2944-8192	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.4
Analyzed:	22 May-19 14:51	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



Client: Mystar

W.O.#: 190967

Hardness and Alkalinity Datasheet

	Alkalinity						Hardness			
Sample ID	Subsample Date	Date Measured	Sample Volume (mL)	(mL) 0.02N HCL/H_2SO_4 used to pH 4.5	(mL) of 0.02N HCL/H_2SO_4 used to pH 4.2	Total Alkalinity ($mg/LCaCO_3$)	Sample Volume (mL)	Volume of 0.01M EDTA Used (mL)	Total Hardness ($mg/L\ CaCO_3$)	Technician
IIA-Runoff	May 15/19	May 15/19	50	1.6	1.7	30	50	13.3	266	ST
20'L perm	May 15/19	May 15/19	100	10.8	11.0	106	50	5.0	100	K

Notes:

Reviewed by: [signature]

Date Reviewed: May 30, 2019

APPENDIX B – *Oncorhynchus mykiss* Toxicity Test Data

Rainbow Trout Early Life Stage Summary Sheet

Client: Mynstar Myra Falls Start Date/Time: May 15, 2019 @ 1735h

Work Order No.: 190966 Test Species: *Oncorhynchus mykiss*

Sample Information:

Sample ID: 11A-Runoff
Sample Date: May 14, 2019
Date Received: May 15, 2019
Sample Volume: 7x20L

Dilution Water:

Type: Dechlorinated Tap Water
Hardness (mg/L $CaCO_3$): 9
Alkalinity (mg/L $CaCO_3$): 10

Test Organism Information:

Batch No.: 051519
Source: Tedis Trout, Campbell Lake, BC
Loading Density: 1.16 g/L

Number of male broodstock used: 4
Number of female broodstock used: 4
Sperm motility check: Verification of sperm motility using a compound microscope

SDS Reference Toxicant Results:

Reference Toxicant ID: RTE122
Stock Solution ID: 19501
Date Initiated: May 15, 2019
7-d EC50 (95% CL): 6.6 (6.3-6.9) mg/L SDS

Reference Toxicant Mean and Range: 4.1 (2.0-8.3) mg/L SDS
Reference Toxicant CV (%): 37

Test Results:

	Sample ID		
	11A-Runoff		
EC25 % (v/v) (95% CL)	>100		
EC50 % (v/v) (95% CL)	>100		

Reviewed by: JCh Date reviewed: June 20/19

7-d Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: Nyrstar Myra Falls
Sample ID: 11A-Runoff
Work Order #: 1909166

Start Date & Time: May 15, 2019 @ 1735h
Stop Date & Time: May 22, 2019 @ 0945h
CER #: 3
Test Species: *Oncorhynchus mykiss*

Control (% v/v) Concentration	Days													
	0	1		2		3		4		5		6		7
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)	14.0	13.5	13.5	14.0	13.5	14.0	13.6	14.0	13.6	14.0	13.5	14.0	14.0	14.0
DO (mg/L)	10.1	10.1	10.0	10.2	10.1	9.9	10.1	10.1	10.1	10.2	10.3	10.2	10.3	10.1
pH	7.0	6.7	6.8	7.0	7.1	6.8	7.1	7.0	7.1	6.9	6.9	6.7	7.0	6.8
Cond. (µS/cm)	32	32		31		32		32		32		31		33
Initials														

6.25 Concentration	Days													
	0	1		2		3		4		5		6		7
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)	14.0	13.5	13.5	13.5	13.5	14.0	13.6	14.0	13.5	14.0	13.5	14.0	14.0	14.0
DO (mg/L)	9.9	9.9	10.1	10.2	10.3	10.0	9.9	10.1	10.2	10.1	10.2	10.2	10.2	10.1
pH	7.0	7.1	6.9	7.1	7.1	7.2	7.2	7.2	7.1	7.1	6.9	6.9	7.0	7.0
Cond. (µS/cm)	~~67~~	68		66		67		66		67		68		66
Initials														

25 Concentration	Days													
	0	1		2		3		4		5		6		7
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)	14.0	13.5	13.5	13.5	13.5	14.0	13.6	14.0	13.6	14.0	13.5	14.0	14.0	14.0
DO (mg/L)	10.0	9.9	10.2	10.2	10.1	10.0	9.8	10.1	10.2	10.1	10.2	10.1	10.2	10.2
pH	7.2	7.1	6.9	7.2	7.2	7.2	7.3	7.2	7.1	7.4	7.1	7.1	7.1	7.2
Cond. (µS/cm)	162	156		165		166		165		162		166		165
Initials														

100 Concentration	Days													
	0	1		2		3		4		5		6		7
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)	14.0	13.5	13.5	13.5	13.5	14.0	13.6	14.0	13.5	14.0	13.5	14.0	14.0	14.0
DO (mg/L)	10.2	9.9	10.2	10.0	10.1	10.0	9.8	10.0	10.2	10.1	10.2	10.1	10.2	10.2
pH	7.1	7.5	7.1	7.7	7.3	7.6	7.3	7.6	7.1	7.7	7.3	7.6	7.2	7.7
Cond. (µS/cm)	480	481		487		490		491		486		490		491
Initials														

Thermometer: CER#3 **DO meter/probe:** 213 / 213 **pH meter/probe:** 213 / 213 **Conductivity meter/probe:** 213 / 213

	Control	100%		
Hardness*	9	266		
Alkalinity*	10	30		

* mg/L as CaCO3

Analysts: AWD, YM
Reviewed by: JCH
Date reviewed: June 20/19

Sample Description: clear, no colour, no odour, no particulates

Comments:

Embryo Toxicity Test
Daily Mortality

Client: Nyrstar Myra Falls
Sample ID: 11A-Runoff
Work Order #: 190966

Start Date & Time: May 15, 2019 @ 1735h
Stop Date & Time: May 22, 2019 @ 0945h
Test Species: *Oncorhynchus mykiss*

Concentration (% v/v)	Rep	Day of Test - No. of Mortalities 1	2	3	4	5	6	7	Total Dead Eggs	Total Undeveloped	Total No. Embryo	Total Exposed
control	1	0	0	0	0	0	0	0	0	2	28	30
	2							1	1	3	26	30
	3							0	0	1	29	30
	4							0	0	1	29	30
6.25	1							0	0	2	28	30
	2							2	2	4	24	30
	3							0	0	2	28	30
	4							0	0	1	29	30
12.5	1							0	0	1	29	30
	2								0	3	27	30
	3								0	4	26	30
	4								0	1	29	30
25	1								0	0	30	30
	2							2	2	4	24	30
	3							0	0	4	26	30
	4							0	0	2	28	30
50	1								0	0	30	30
	2								0	4	26	30
	3								0	3	27	30
	4								0	1	29	30
100	1							1	1	1	28	30
	2							2	2	3	25	30
	3							0	0	4	26	30
	4					2		1	3	2	25	30
	1											
	2											
	3											
	4											
	1											
	2											
	3											
	4											
Tech Initials		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Comments:

Reviewed by: [illegible] **Date reviewed:** June 20/19

CETIS Analytical Report

Salmonid Embryo Survival and Development Test — **Nautilus Environmental**

Analysis ID:	06-1572-6838	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.9.4
Analyzed:	14 Jun-19 12:18	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	10-5761-4155	**Test Type:**	Development	**Analyst:**	Yvonne Lam
Start Date:	15 May-19 17:35	**Protocol:**	EC/EPS 1/RM/28	**Diluent:**	Dechlorinated Tap Water
Ending Date:	22 May-19 09:45	**Species:**	Oncorhynchus mykiss	**Brine:**	
Test Length:	6d 16h	**Taxon:**	Actinopterygii	**Source:**	Ted's Trout, Campbell Lake **Age:**
Sample ID:	11-6202-5560	**Code:**	45431A58	**Project:**	
Sample Date:	14 May-19 12:30	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 May-19 08:51	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	29h (14.2 °C)	**Client:**	Nyrstar Myra Falls		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1714175	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend Test			0.7391	Non-Significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	75.85	n/a	n/a	1.318	n/a	n/a
EC10	>100	n/a	n/a	<1	n/a	n/a
EC15	>100	n/a	n/a	<1	n/a	n/a
EC20	>100	n/a	n/a	<1	n/a	n/a
EC25	>100	n/a	n/a	<1	n/a	n/a
EC40	>100	n/a	n/a	<1	n/a	n/a
EC50	>100	n/a	n/a	<1	n/a	n/a

Proportion Normal Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	A/B	Mean	%Effect
0	N	4	0.9333	0.8667	0.9667	0.0471	5.05%	0.0%	112/120	0.9333	0.0%
6.25		4	0.9083	0.8000	0.9667	0.0739	8.14%	2.68%	109/120	0.9167	1.79%
12.5		4	0.9250	0.8667	0.9667	0.0500	5.41%	0.89%	111/120	0.9167	1.79%
25		4	0.9000	0.8000	1.0000	0.0861	9.56%	3.57%	108/120	0.9167	1.79%
50		4	0.9333	0.8667	1.0000	0.0609	6.52%	0.0%	112/120	0.9167	1.79%
100		4	0.8667	0.8333	0.9333	0.0471	5.44%	7.14%	104/120	0.8667	7.14%

Proportion Normal Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.9333	0.8667	0.9667	0.9667
6.25		0.9333	0.8000	0.9333	0.9667
12.5		0.9667	0.9000	0.8667	0.9667
25		1.0000	0.8000	0.8667	0.9333
50		1.0000	0.8667	0.9000	0.9667
100		0.9333	0.8333	0.8667	0.8333

Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	28/30	26/30	29/30	29/30
6.25		28/30	24/30	28/30	29/30
12.5		29/30	27/30	26/30	29/30
25		30/30	24/30	26/30	28/30
50		30/30	26/30	27/30	29/30
100		28/30	25/30	26/30	25/30

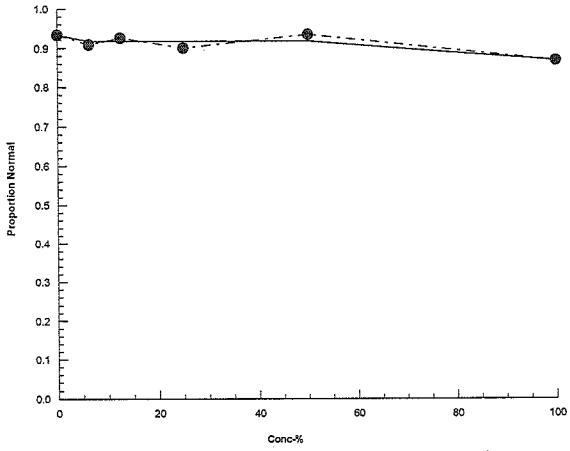
Analyst: YW QA: JGG June 20/19

Salmonid Embryo Survival and Development Test

Nautilus Environmental

Analysis ID:	06-1572-6838	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.9.4
Analyzed:	14 Jun-19 12:18	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



Analyst: WM QA: JCH June 20/19

Client: Nyrstar Myra Falls

W.O.#: 190966

Hardness and Alkalinity Datasheet

	Alkalinity							Hardness			
Sample ID	Subsample Date	Date Measured	Sample Volume (mL)	(mL) 0.02N HCL/H_2SO_4 used to pH 4.5	(mL) of 0.02N HCL/H_2SO_4 used to pH 4.2	Total Alkalinity ($mg/LCaCO_3$)		Sample Volume (mL)	Volume of 0.01M EDTA Used (mL)	Total Hardness ($mg/L\ CaCO_3$)	Technician
11A-Runoff	May 15/19	May 15/19	50	1.6	1.7	30		50	13.3	266	ST
Dechlor	↓	↓	100	1.1	1.2	10		100	0.9	9	″

Notes:

Reviewed by: [signature] Date Reviewed: June 20/19

APPENDIX C – *Lemna minor* Toxicity Test Data

Lemna minor Summary Sheet

Client: Nyrstar Myra Falls
Work Order No.: 190968

Start Date: May 16/19
Set up by: ML/ST

Sample Information:

Sample ID: 11A-Runoff
Sample Date: May 14/19
Date Received: May 15/19
Sample Volume: 7X20L

Test Organism Information:

Culture Date: 050819
Age of culture (Day 0): 8 days
>8X growth in APHA?: Y (46 Fronds)

KCl Reference Toxicant Results:

Reference Toxicant ID: Lm173
Date Initiated: May 25/19

7-d No. of Fronds IC50 (95% CL): 3.8 (3.2-4.3) g/L KCl

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 3.5 (2.9-4.2) g/L KCl CV (%): 9

		Number of Fronds	Dry Weight
Test Results:	IC25 %(v/v) (95% CL)	83.2 (60.5-97)	>97
	IC50 %(v/v) (95% CL)	>97	>97

Reviewed by: [signature]

Date reviewed: June 11, 2019

Plant Growth Inhibition Toxicity Test
Water Quality Measurements

Client : Nyrstar Myra Falls
Setup by: MLT/ST
Sample ID: 11A-Run Off
Test Date: May 16/19
Work Order No.: 190969
CER #: 4
Culture Source: CPCC #490
Test Species: *Lemna minor*
Test Culture Age: 8 days
> 8X Growth? (Y/N): Y (46 Fronds)
Light Intensity Range: lux
Date Measured: May 15/19

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Initials	MG	MG	MG	MG	MG	MG	MG	MG

Sample Characteristics:

	Initial Water Quality		Adjusted Water Quality
Temperature (°C)	25.0	Aeration?: 20 min	24.0
DO (mg/L)	8.1	Nutrients added?[1]: Y	8.0
pH	8.2		8.3
Conductivity (µS)	499		1284

[1] 10 mL of each APHA stock (A,B and C) added to 970 mL sample.

Concentration %(v/v)	Temperature (°C)		pH		Conductivity (µS)
	Day 0	Day 7	Day 0	Day 7	0 h
Control	24.0	25.0	8.3	9.1	883
1.5	24.0	25.0	8.3	9.0	889
3	24.0	25.0	8.3	8.8	891
6.1	24.0	25.0	8.3	8.7	902
12.1	24.0	25.0	8.3	8.8	928
24.2	24.0	25.0	8.3	8.7	951
48.5	24.0	25.0	8.3	8.9	1062
97	24.0	25.0	8.3	8.8	1284
Initials	ST	ST	ST	ST	ST

Thermometer: 4 **Light meter:** 1 **pH meter/probe:** 1/1 **DO meter/probe:** 1/1 **Conductivity meter/probe:** 1/1

Sample Description: clear, colourless, odourless, no particulates

Comments:

Reviewed: [signature] **Date Reviewed:** June 11, 2019

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

Client: Nyrstar Myra Falls
Sample ID: 11A-Runoff
Work Order #: 190969

Start Date: May 16/19
Termination Date: May 23/19
Test set up by: MG/ST

Concentration 0/o(v/v)	Rep	No. of fronds Day 0	No. of fronds Day 7	Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
control	A	6	109										MG
	B	6	117										
	C	6	92										
	D	6	81										
1.5	A	6	102										
	B	6	109										
	C	6	100										
	D	6	116										
3	A	6	101										
	B	6	93										
	C	6	85										
	D	6	93										
6.1	A	6	117										
	B	6	93										
	C	6	92										
	D	6	91										
12.1	A	6	84										
	B	6	89										
	C	6	108										
	D	6	75										
24.2	A	6	90										
	B	6	86										
	C	6	102										
	D	6	100										

Comments:

Reviewed by: [signature]

Date Reviewed: June 11, 2019

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

Client: Nyrstar Myra Falls
Sample ID: 11A-Runoff
Work Order #: 190969

Start Date: May 16/19
Termination Date: May 23/19
Test set up by: ML/ST

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	87										ML
	B	6	100										
	C	6	85										
	D	6	102										
97	A	6	78										
	B	6	62										
	C	6	52										
	D	6	57										
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments:

Reviewed by: [signature]

Date Reviewed: June 11, 2019

7-d *Lemna minor* Weight Data Sheet

Client: Nyrstar Myra Falls
Sample ID: 11A-Runoff
WO #: 190969
0/0(v/v) 0 orange

Start Date: May 16/19
Termination Date: May 23/19
Balance ID: Bal - 1

Concentration	Rep	Pan No.	Pan weight (mg)	Pan + plant (mg)	Initials
control	A	1	1035.04	1043.38	CH/CL
	B	2	1066.25	1075.55	CH/CL
	C	3	1086.96	1093.92	CH/CL
	D	4	1056.83	1063.56	CH/CL
1.5	A	5	1069.91	1076.81	CH/CL
	B	6	1057.97	1066.33	CH/CL
	C	7	1068.15	1075.55	CH/CL
	D	8	1065.49	1074.21	CH/CL
3	A	9	1073.01	1080.55	CH/CL
	B	10	1058.97	1065.68	CH/CL
	C	11	1079.05	1085.58	CH/CL
	D	12	1051.76	1059.02	CH/CL
6.1	A	13	1054.06	1062.70	CH/CL
	B	14	1061.73	1068.63	CH/CL
	C	15	1041.77	1048.82	CH/CL
	D	16	1057.19	1064.74	CH/CL
12.1	A	17	1071.67	1078.18	CH/CL
	B	18	1074.98	1081.58	CH/CL
	C	19	1068.35	1076.30	CH/CL
	D	20	1063.16	1069.54	CH/CL
24.2	A	21	1052.91	1060.71	CH/CL
	B	22	1062.48	1068.01	CH/CL
	C	23	1064.82	1072.75	CH/CL
	D	24	1064.98	1071.90	CH/CL
48.5	A	25	1057.56	1064.00	CH/CL
	B	26	1051.78	1059.64	CH/CL
	C	27	1088.28	1093.12	CH/CL
	D	28	1092.72	1099.84	CH/CL

Comments: 10% re-weigh: ① pan: 7 weight: 1075.51 ② pan: 12 weight: 1058.90
③ pan: 23 weight: 1072.54 ④ pan: 28 weight: 1099.67

Reviewed by: [signature] Date Reviewed: June 11, 2019

7-d *Lemna minor* Weight Data Sheet

Client: Nyrstar Myra Falls
Sample ID: 11A-Runoff
WO #: 190869
Start Date: May 16/19
Termination Date: May 23/19
Balance ID: Bal - 1

% (v/v) Dorange

Concentration	Rep	Pan No.	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1085.89	1092.37	CH/CT
	B	30	1085.43	1091.83	CH CT
	C	31	1053.53	1058.64	CH CT
	D	32	1059.69	1064.69	CH CT
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments:

Reviewed by: [signature]
Date Reviewed: June 11, 2019

CETIS Analytical Report

Report Date:	28 May-19 16:36 (p 1 of 2)
Test Code/ID:	190969 / 16-6149-8060

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	18-7326-0424	**Endpoint:**	Frond Count	**CETIS Version:**	CETISv1.9.4
Analyzed:	28 May-19 16:35	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1

Batch ID:	21-3538-3323	**Test Type:**	Lemna Growth	**Analyst:**	Mimi Tran
Start Date:	16 May-19	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Modified APHA
Ending Date:	23 May-19	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	CPCC#490 **Age:** 8d

Sample ID:	11-6202-5560	**Code:**	45431A58	**Project:**	
Sample Date:	14 May-19 12:30	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 May-19 08:51	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	35h (14.2 °C)	**Client:**	Nyrstar Myra Falls		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Gompertz: μ=α·exp[log[0.5]·[x/δ]^γ]	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
17	-76.41	159.7	163.2	0.4964	Yes	1.237	2.621	0.3228	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	49.57	n/a	74.27	2.017	1.346	n/a
IC10	61.52	n/a	85.95	1.626	1.163	n/a
IC15	70.06	n/a	92.46	1.427	1.081	n/a
IC20	77.05	39.29	96.51	1.298	1.036	2.545
IC25	83.15	60.53	99.27	1.203	1.007	1.652
IC40	98.79	84.77	111.6	1.012	~~0.8959~~	~~1.18~~ MT >97% (v/v)
IC50	~~108.3~~	~~85.01~~	131.4	0.9237	0.7608	1.176

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	91.06	2.389	86.17	95.95	38.11	<1.0E-37	Significant Parameter
γ	3.333	2.131	-1.026	7.692	1.564	0.1287	Non-Significant Parameter
δ	108.3	10.48	86.83	129.7	10.33	<1.0E-37	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	241500	80500	3	635	<1.0E-37	Significant
Lack of Fit	753.4	150.7	5	1.237	0.3228	Non-Significant
Pure Error	2923	121.8	24			
Residual	3676	126.8	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance Test	4.069	14.07	0.7718	Equal Variances
	Mod Levene Equality of Variance	0.7077	2.423	0.6658	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.963	0.9338	0.3306	Normal Distribution
	Anderson-Darling A2 Normality Te	0.5317	2.492	0.1779	Normal Distribution

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID: 18-7326-0424 **Endpoint:** Frond Count **CETIS Version:** CETISv1.9.4
Analyzed: 28 May-19 16:35 **Analysis:** Nonlinear Regression (NLR) **Status Level:** 1

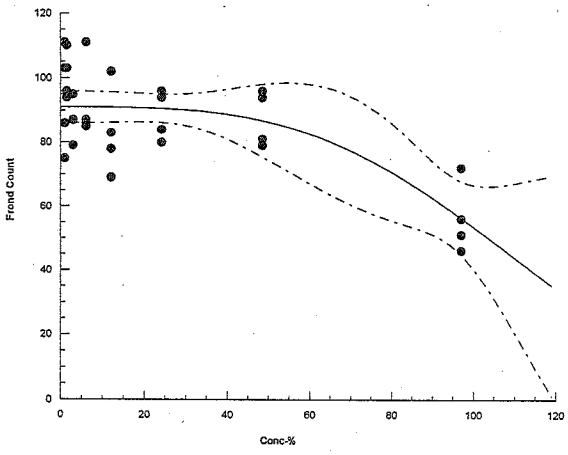
Frond Count Summary

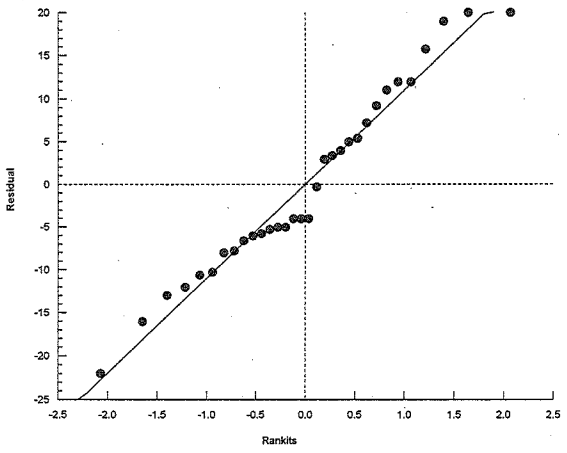
			Calculated Variate						
Conc-%	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	93.75	75	111	8.138	16.28	17.36%	0.0%
1.5		4	100.8	94	110	3.637	7.274	7.22%	-7.47%
3		4	87	79	95	3.266	6.532	7.51%	7.2%
6.1		4	92.25	85	111	6.263	12.53	13.58%	1.6%
12.1		4	83	69	102	6.964	13.93	16.78%	11.47%
24.2		4	88.5	80	96	3.862	7.724	8.73%	5.6%
48.5		4	87.5	79	96	4.368	8.737	9.99%	6.67%
97		4	56.25	46	72	5.633	11.27	20.03%	40.0%

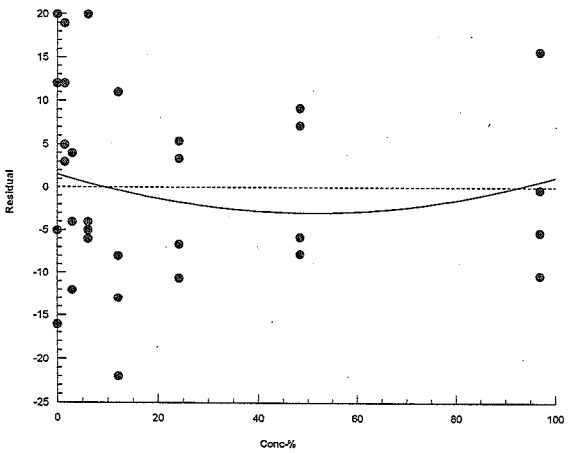
Frond Count Detail

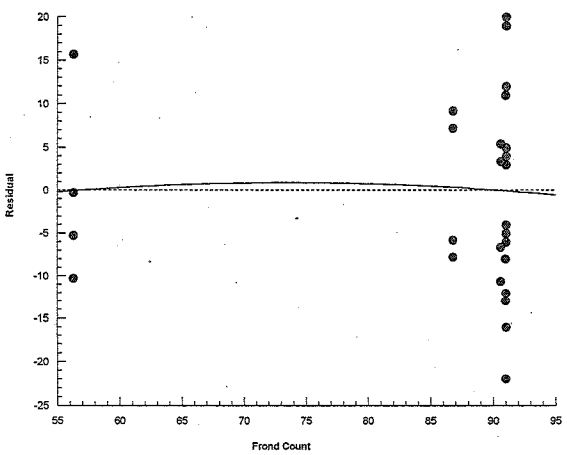
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	103	111	86	75
1.5		96	103	94	110
3		95	87	79	87
6.1		111	87	86	85
12.1		78	83	102	69
24.2		84	80	96	94
48.5		81	94	79	96
97		72	56	46	51

Graphics Model: 3P Log-Gompertz: $\mu=\alpha\cdot\exp[\log[0.5]\cdot[x/\delta]^{\gamma}]$ Distribution: Normal [$\omega=1$]









CETIS Analytical Report

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	06-5484-6893	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETISv1.9.4
Analyzed:	28 May-19 16:36	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Batch ID:	21-3538-3323	**Test Type:**	Lemna Growth	**Analyst:**	Mimi Tran
Start Date:	16 May-19	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Modified APHA
Ending Date:	23 May-19	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	CPCC#490 **Age:** 8d
Sample ID:	11-6202-5560	**Code:**	45431A58	**Project:**	
Sample Date:	14 May-19 12:30	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 May-19 08:51	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	35h (14.2 °C)	**Client:**	Nyrstar Myra Falls		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
2P Exponential: $\mu=\alpha\cdot\exp[\log[0.5]\cdot x/\delta]$	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
4	4.339	-4.264	-1.747	0.3019	Yes	0.4615	2.508	0.8297	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	17.82	2.425	33.93	5.611	2.947	41.24
IC10	36.61	18.41	55.81	2.732	1.792	5.433
IC15	56.47	30.79	84.19	1.771	1.188	3.248
IC20	77.53	42.26	116.8	1.29	0.8562	2.367
IC25	99.95	54.01	152.9	1	0.654	~~1.851~~ 1.497 (MT)
IC40	177.5	95.49	285	0.5634	0.3509	1.047
IC50	~~240.8~~	131.5	401.3	0.4152	0.2492	0.7607

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	7.531	0.2035	7.115	7.946	37	<1.0E-37	Significant Parameter
δ	240.8	67.84	102.3	379.4	3.55	0.0013	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	1604	802	2	1018	<1.0E-37	Significant
Lack of Fit	2.445	0.4075	6	0.4615	0.8297	Non-Significant
Pure Error	21.19	0.883	24			
Residual	23.64	0.7879	30			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance Test	3.524	14.07	0.8327	Equal Variances
	Mod Levene Equality of Variance	0.7677	2.423	0.6196	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9604	0.9338	0.2809	Normal Distribution
	Anderson-Darling A2 Normality Te	0.6625	2.492	0.0840	Normal Distribution

Total Dry Weight-mg Summary

			Calculated Variate						
Conc-%	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	7.833	6.73	9.3	0.6047	1.209	15.44%	0.0%
1.5		4	7.845	6.9	8.72	0.4205	0.841	10.72%	-0.16%
3		4	7.01	6.53	7.54	0.2352	0.4704	6.71%	10.5%
6.1		4	7.535	6.9	8.64	0.3936	0.7873	10.45%	3.8%
12.1		4	6.86	6.38	7.95	0.3661	0.7323	10.67%	12.42%
24.2		4	7.043	5.53	7.92	0.5512	1.102	15.65%	10.09%
48.5		4	6.565	4.84	7.86	0.644	1.288	19.62%	16.18%
97		4	5.747	5	6.48	0.4008	0.8015	13.95%	26.62%

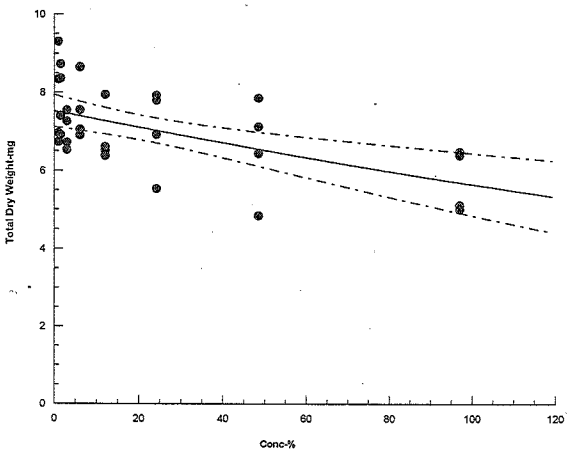
Lemna Growth Inhibition Test — **Nautilus Environmental**

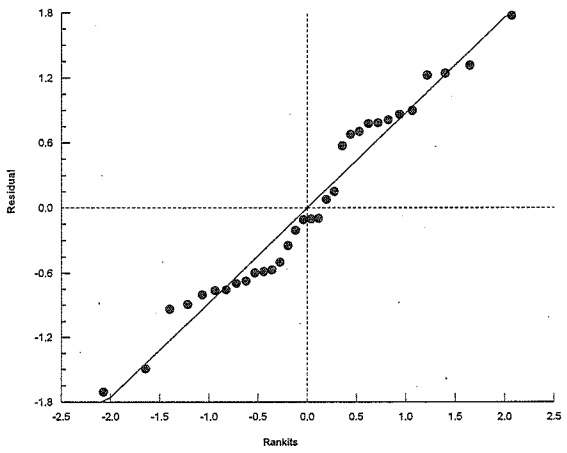
Analysis ID:	06-5484-6893	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETISv1.9.4
Analyzed:	28 May-19 16:36	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1

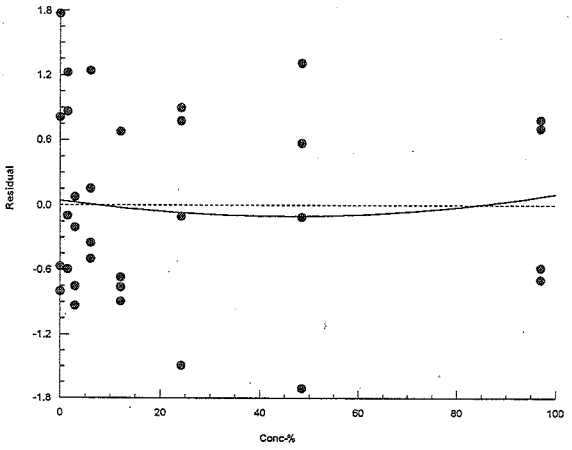
Total Dry Weight-mg Detail

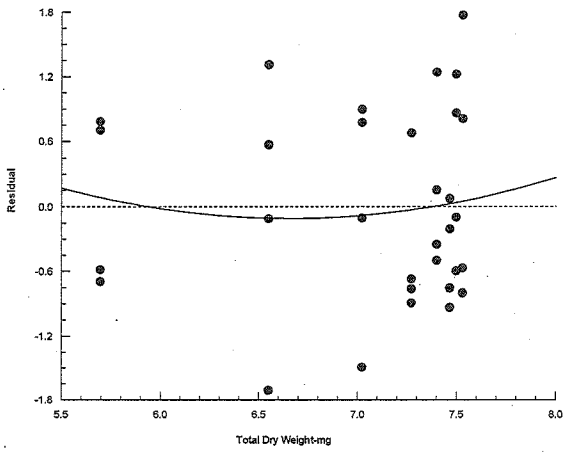
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	8.34	9.3	6.96	6.73
1.5		6.9	8.36	7.4	8.72
3		7.54	6.71	6.53	7.26
6.1		8.64	6.9	7.05	7.55
12.1		6.51	6.6	7.95	6.38
24.2		7.8	5.53	7.92	6.92
48.5		6.44	7.86	4.84	7.12
97		6.48	6.4	5.11	5

Graphics Model: 2P Exponential: $\mu=\alpha\cdot\exp[\log[0.5]\cdot x/\delta]$ Distribution: Normal [ω=1]









APPENDIX D – *Pseudokirchneriella subcapitata* Toxicity Test Data

Pseudokirchneriella subcapitata Summary Sheet

Client: Nyrstar
Work Order No.: 190968

Start Date: May 17/19
Set up by: ML

Sample Information:

Sample ID: 11A-Runoff
Sample Date: May 14/19
Date Received: May 15/19
Sample Volume: 7x20L

Test Organism Information:

Culture Date: May 10/19
Age of culture (Day 0): 7d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC183
Stock Solution ID: 197n02
Date Initiated: May ~~27~~ 28/19

72-h IC50 (95% CL): 32.9 (29.9 - 36.0) mg/L Zn

72-h IC50 Reference Toxicant Mean and Range: 31.0 (25.2 - 38.1) mg/L Zn CV (%): 10

Test Results:

	Algal Growth
IC25 %(v/v) (95% CL)	795.2
IC50 %(v/v) (95% CL)	795.2

Reviewed by: [signature]

Date reviewed: June 12/19

72-h Algal Growth Inhibition Toxicity Test
Water Quality Measurements

Client : Nyrstar **Setup by:** MG

Sample ID: 11-A Runoff **Test Date/Time:** May 17/19 @ 1200h

Work Order No.: 190968 **CER #:** 4

Test Species: *Pseudokirchneriella subcapitata*

Culture Date: May 10/19 **Age of Culture:** 7d **Culture Health:** Good

Culture Count: 1 450 2 420 **Average:** ~~425~~ 435 MG **Culture Cell Density (c1):** 435 MG × 10⁴ cells/mL

v1 = 220,000 cells/ml x 15 ml / (c1) 435 ~~425~~ MG × 10⁴ cells/ml = 5.18 MG mL 5.0 mL

Time Zero Counts: 1 20 2 22 **Average:** 21 × MG

No. of Cells/mL: 21 × 10⁴ **Initial Density:** # cells/mL ÷ 220 µL x 10 µL = 9545 cells/mL

Concentration %(v/v)	Water Quality		Incubator Temperature (°C)				Microplates rotated 2X per day?			
	pH	Temp (°C)								
	0 h	0 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Control	7.3	24.0	29.0	25.0	25.0	25.0	✓	✓	✓	✓
1.5	7.4	24.0	↓	↓	↓	↓	✓	✓	✓	✓
3	7.4	24.0					✓	✓	✓	✓
6	7.5	24.0					✓	✓	✓	✓
11.9	7.7	24.0					✓	✓	✓	✓
23.8	7.7	24.0					✓	✓	✓	✓
47.6	7.8	24.0					✓	✓	✓	✓
95.2	7.9	24.0	↓	↓	↓	↓	✓	✓	✓	✓
Initials	MG	MG	MG	MG	~	MG	MG	MG	~	MG

Initial control pH: Well 1: 7.3 Well 2: 7.3

Final control pH: Well 1: 7.2 Well 2: 7.2

Light intensity (lux): 4220 **Date measured:** May 17/19

Thermometer: 4 **Light meter:** 1 **pH meter/probe:** 1/1

Sample Description: clear, colourless, odourless, no particulates

Comments:

Reviewed: JCh **Date reviewed:** June 12/19

Pseudokirchneriella subcapitata Toxicity Test Data Sheet
72-h Algal Cell Counts

Client: Nyrstar | Start Date/Time: May 17/19 @ 1200h
Work Order #: 190968 | Termination Date: May 20/19 @ 1200h
Sample ID: IHA Runoff | Test set up by: MD
%(v/v)

Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	32					MD
	B	39					"
	C	36					"
	D	33					"
	E	40					"
	F	35					"
	G	36					"
	H	32					"
1.5	A	31					"
	B	38					"
	C	34					"
	D	32					"
3	A	39					"
	B	41					"
	C	38					"
	D	34					"
6	A	44					"
	B	50					"
	C	43					"
	D	58					"
11.9	A	48					"
	B	54					"
	C	60					"
	D	59					"
23.8	A	59					"
	B	61					"
	C	64					"
	D	62					"
47.6	A	65					"
	B	76					"
	C	64					"
	D	69					"
95.7	A	54					"
	B	56					"
	C	47					"
	D	54					"

Comments:

Reviewed by: JOL | Date Reviewed: June 12/19

Pseudokirchneriella subcapitata Algal Counts

Client: Nyrstar Myra Falls
WO#: 190968
Sample ID: 11A-Runoff

Start Date/Time: 17-May-19 @ 1200h
Termination Date/Time: 20-May-19 @ 1200h

Initial Cell Density: 9545 cell/mL

									210000
									0.22
									0.01
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		9545.455
Control	A	32				32	31.0	mean	34.4
	B	39				39	38.0	SD	3.020761
	C	36				36	35.0	CV	8.776065
	D	33				33	32.0		
	E	40				40	39.0		
	F	35				35	34.0		
	G	36				36	35.0		
	H	32				32	31.0		
1.5	A	31				31	30.0		
	B	38				38	37.0		
	C	34				34	33.0		
	D	32				32	31.0		
3	A	39				39	38.0		
	B	41				41	40.0		
	C	38				38	37.0		
	D	34				34	33.0		
6	A	44				44	43.0		
	B	50				50	49.0		
	C	43				43	42.0		
	D	58				58	57.0		
11.9	A	48				48	47.0		
	B	54				54	53.0		
	C	60				60	59.0		
	D	59				59	58.0		
23.8	A	59				59	58.0		
	B	61				61	60.0		
	C	64				64	63.0		
	D	62				62	61.0		
47.6	A	65				65	64.0		
	B	76				76	75.0		
	C	64				64	63.0		
	D	69				69	68.0		
95.2	A	54				54	53.0		
	B	56				56	55.0		
	C	47				47	46.0		
	D	54				54	53.0		

Reviewed by: JGh

Date reviewed: June 12/19

CETIS Analytical Report

EC Alga Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	20-3947-1346	**Endpoint:**	Cell Yield	**CETIS Version:**	CETISv1.9.4
Analyzed:	28 May-19 17:50	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	01-1148-1374	**Test Type:**	Cell Growth	**Analyst:**	Mimi Tran
Start Date:	17 May-19 12:00	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water + nutrients
Ending Date:	20 May-19 12:00	**Species:**	Pseudokirchneriella subcapitata	**Brine:**	
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture **Age:** 7d
Sample ID:	11-6202-5560	**Code:**	45431A58	**Project:**	
Sample Date:	14 May-19 12:30	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 May-19 08:51	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	71h (14.2 °C)	**Client:**	Nyrstar Myra Falls		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1158058	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend Test			0.7232	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	n/a	n/a
IC10	>95.2	n/a	n/a	<1.05	n/a	n/a
IC15	>95.2	n/a	n/a	<1.05	n/a	n/a
IC20	>95.2	n/a	n/a	<1.05	n/a	n/a
IC25	>95.2	n/a	n/a	<1.05	n/a	n/a
IC40	>95.2	n/a	n/a	<1.05	n/a	n/a
IC50	>95.2	n/a	n/a	<1.05	n/a	n/a

Cell Yield Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	N	8	34.38	31	39	3.021	8.79%	0.0%	48.23	0.0%
1.5		4	32.75	30	37	3.096	9.45%	4.73%	48.23	0.0%
3		4	37	33	40	2.944	7.96%	-7.64%	48.23	0.0%
6		4	47.75	42	57	6.898	14.45%	-38.91%	48.23	0.0%
11.9		4	54.25	47	59	5.5	10.14%	-57.82%	48.23	0.0%
23.8		4	60.5	58	63	2.082	3.44%	-76.0%	48.23	0.0%
47.6		4	67.5	63	75	5.447	8.07%	-96.36%	48.23	0.0%
95.2		4	51.75	46	55	3.948	7.63%	-50.55%	48.23	0.0%

Cell Yield Detail

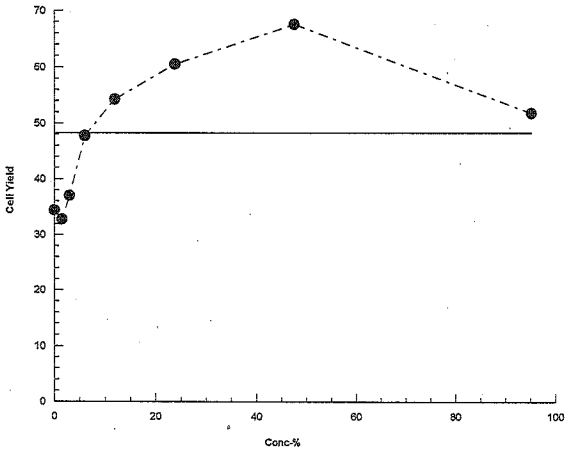
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	31	38	35	32	39	34	35	31
1.5		30	37	33	31				
3		38	40	37	33				
6		43	49	42	57				
11.9		47	53	59	58				
23.8		58	60	63	61				
47.6		64	75	63	68				
95.2		53	55	46	53				

EC Alga Growth Inhibition Test

Nautilus Environmental

Analysis ID:	20-3947-1346	**Endpoint:**	Cell Yield	**CETIS Version:**	CETISv1.9.4
Analyzed:	28 May-19 17:50	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



CETIS Analytical Report

EC Alga Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	16-2324-7672	**Endpoint:**	Cell Yield	**CETIS Version:**	CETISv1.9.4
Analyzed:	28 May-19 17:50	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	01-1148-1374	**Test Type:**	Cell Growth	**Analyst:**	Mimi Tran
Start Date:	17 May-19 12:00	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water + nutrients
Ending Date:	20 May-19 12:00	**Species:**	Pseudokirchneriella subcapitata	**Brine:**	
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture **Age:** 7d
Sample ID:	11-6202-5560	**Code:**	45431A58	**Project:**	
Sample Date:	14 May-19 12:30	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 May-19 08:51	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	71h (14.2 °C)	**Client:**	Nyrstar Myra Falls		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	3	6	4.243	33.33	19.03%

Dunnett Multiple Comparison Test

Control vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	1.5	-0.6274	2.526	6.543	10	CDF	0.9890	Non-Significant Effect
	3	1.014	2.526	6.543	10	CDF	0.5445	Non-Significant Effect
	6*	5.164	2.526	6.543	10	CDF	5.9E-05	Significant Effect
	11.9*	7.674	2.526	6.543	10	CDF	<1.0E-37	Significant Effect
	23.8*	10.09	2.526	6.543	10	CDF	<1.0E-37	Significant Effect
	47.6*	12.79	2.526	6.543	10	CDF	<1.0E-37	Significant Effect
	95.2*	6.708	2.526	6.543	10	CDF	<1.0E-37	Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend Test			0.7232	Non-Significant Trend in Controls

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5196.76	742.395	7	41.5	<1.0E-37	Significant Effect
Error	500.875	17.8884	28			
Total	5697.64		35			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	6.627	18.48	0.4687	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9835	0.9166	0.8550	Normal Distribution

Cell Yield Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	8	34.38	31.85	36.9	34.5	31	39	1.068	8.79%	0.00%
1.5		4	32.75	27.82	37.68	32	30	37	1.548	9.45%	4.73%
3		4	37	32.32	41.68	37.5	33	40	1.472	7.96%	-7.64%
6		4	47.75	36.77	58.73	46	42	57	3.449	14.45%	-38.91%
11.9		4	54.25	45.5	63	55.5	47	59	2.75	10.14%	-57.82%
23.8		4	60.5	57.19	63.81	60.5	58	63	1.041	3.44%	-76.00%
47.6		4	67.5	58.83	76.17	66	63	75	2.723	8.07%	-96.36%
95.2		4	51.75	45.47	58.03	53	46	55	1.974	7.63%	-50.55%

Analyst: MT QA: JOH June 12/19

APPENDIX E – Chain-of-Custody Form

Nautilus Environmental

Chain of Custody (electronic)

X	British Columbia: 8664 Commerce Court, Burnaby, BC V5A 4N3	Tel: 604-420-8773
	Washington: 5009 Pacific Highway East, Suite 2, Tacoma, WA 98424	Tel: 253-922-4296
	California: 5550 Morehouse Drive, Suite 150, San Diego, CA 92121	Tel: 858-587-7333

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Sample Collection By:		
	Report to:	**Invoice to:**
Company	Nyrstar Myra Falls Ltd	same
Address	PO BOX 8000	
City/State/Zip	Campbell River, BC	
Contact	Craig Schweitzer	Grace Augustin
Phone	250-287-9271 EXT. 3397	250-287-9271 ext. 3221
Email	craig.schweitzer@nyrstar.com	Grace.augustin@nyrstar.com
	nicole.pesonen@nyrstar.com	Nicole.pesonen@nyrstar.com

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	# OF CONTAINERS	COMMENTS	ANALYSES REQUIRED: 7-d RBT embryo	7-d Ceriodaphnia	72-hr Selenastrum (or Pseudokirchneriella subcapitata)	Lemna Minor	RBT LC50	Daphnia Magna LC 50						Receipt Temperature (°C)
1	0 11A-RUNOFF	2019-05-14	12:30	water	plastic	7x20L		X	X	X	X	X	X						14.2
2																			
3																			
4																			
5																			
6																			
7																			
8								19 09 16	19 09 17	19 09 68	19 09 69	19 09 70	19 09 71						
9								19 09 66	19 09 67	19 09 68	19 09 69	19 09 70	19 09 71						
10								19 09 66	19 09 67	19 09 68	19 09 69	19 09 70	19 09 71						

PROJECT INFORMATION	SAMPLE RECEIPT		RELIQUINSHED BY (CLIENT)	RELIQUINSHED BY (COURIER)
Client: Nyrstar Myra Falls	**Total # Containers:**	7	**Signature:**	**Signature:**
P.O. No.: 4501745322	**Good Condition?**	Y	**Print: Craig Schweitzer**	**Print:**
Shipped Via: Purolator	**Matches Schedule?**	Y	**Company: Nyrstar Myra Falls Ltd**	**Company:**
			Time/Date: March 5 15:00	**Time/Date:**
SPECIAL INSTRUCTIONS/COMMENTS:			**RECEIVED BY (COURIER)**	**RECEIVED BY (LABORATORY)**
Please send results to both emails listed above.			**Signature:**	**Signature:** TM
0 Clear, colourless, odourless, no particulates.			**Print:**	**Print:** Tyrone Hamilton
			Company:	**Company:** Maxxam
			Time/Date:	**Time/Date:** May 15/19 @ 08:51

Additional costs may be required for sample disposal or storage. Net 30 unless otherwise contracted.

END OF REPORT