MDMER Toxicity Testing on 11A-Runoff

Sample collected on October 28 and November 13, 2019

Final Report

December 20, 2019

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

8664 Commerce Court, Burnaby, BC V5A 4N7

TABLE OF CONTENTS

List of Tables

List of Appendices

SIGNATURE PAGE

Report By:
Jeslin Wijaya, B.Sc
Laboratory Biologist

Reviewed By:
Armando Tang, R.P. Bio
Senior Reviewer

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	October 28 and November 13, 2019
Sample receipt date	October 29 and November 15, 2019
Sample receipt temperature	10.7 and 13.3°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	55.1 (37.3 – 74.8)
Reproduction IC50	>100
Oncorhynchus mykiss	
Embryo viability EC25	>100
Embryo viability EC50	>100
Lemna minor	
Frond count IC25	31.0 (11.4 – 62.8)
Frond count IC50	73.3 (29.6 – 96.3)
Dry weight IC25	30.8 (5.0 – 52.7)
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 October 28, 2019 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on October 29, 2019. The sample was transported in eight 20-L plastic containers and were received at a temperature of 10.7°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

A second sample was collected on November 13, 2019 and was delivered on November 15, 2019. The sample was received at a temperature of 13.3°C. The following sub-lethal toxicity tests were performed:

- 7-d *Lemna minor* growth inhibition
- 72-h *Pseudokirchneriella subcapitata* growth inhibition

Testing for *C. dubia* and *O. mykiss* were initiated on October 29 and 30, 2019, respectively. Testing for *L. minor* and *P. subcapitata* were initiated on November 15, 2019. 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 forms are 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[1] (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)

[1]TCC = Trout chow and cerophyl

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 survival of *C. dubia* (Table 5), embryo viability of *O. mykiss* (Table 6) or cell yield of *P. subcapitata* (Table 8), resulting in LC and IC values greater than the highest concentration tested.

Reduction in *C. dubia* reproduction was observed, resulting in an IC25 and IC50 of 55.1% and >100%, respectively. Moreover, adverse effects were observed in both *L. minor* test endpoints (Table 7). The frond count IC25 and IC50 were 31.0% and 73.3% (v/v), respectively; dry weight IC25 and IC50 were 30.8% and >97% (v/v), respectively.

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

Concentration (% v/v)	Survival (%)	Reproduction (Mean ± SD)
Laboratory Control	90	22.1 ± 7.1
1.56	100	24.8 ± 5.9
3.12	90	25.8 ± 3.4
6.25	100	22.7 ± 3.4
12.5	90	21.9 ± 4.4
25	90	21.2 ± 5.9
50	100	20.3 ± 6.3
100	100	14.2 ± 2.2
Test endpoint (% v/v)		
LC50	>100	--
IC25 (95% CL)	--	55.1 (37.3 – 74.8)
IC50	--	>100

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

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

Concentration (% v/v)	Embryo Viability (%) (Mean ± SD)
Laboratory Control	90.0 ± 9.8
6.25	95.0 ± 5.8
12.5	91.7 ± 10.4
25	95.0 ± 4.3
50	93.3 ± 9.0
100	85.0 ± 14.0
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	103.8 ± 19.6	9.2 ± 1.7
1.5	98.0 ± 5.6	8.7 ± 0.8
3.0	96.0 ± 24.3	8.3 ± 2.7
6.1	101.8 ± 12.1	8.8 ± 1.5
12.1	101.8 ± 3.6	8.1 ± 0.6
24.2	84.2 ± 10.4	7.4 ± 0.9
48.5	66.0 ± 15.3	6.0 ± 0.6
97	42.2 ± 5.0	5.3 ± 0.3
Test endpoint (% v/v)		
IC25 (95% CL)	31.0 (11.4 – 62.8)	30.8 (5.0 – 52.7)
IC50 (95% CL)	73.3 (29.6 – 96.3)	>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)
Laboratory Control	37.2 ± 3.1
1.5	49.5 ± 4.5[†]
3.0	53.8 ± 4.4[†]
6.0	67.8 ± 2.5[†]
11.9	74.5 ± 5.1[†]
23.8	101.2 ± 2.5[†]
47.6	101.0 ± 9.4[†]
95.2	35.2 ± 2.9
Test endpoint (% v/v)	
IC25	>95.2
IC50	>95.2

SD = Standard Deviation, IC = Inhibition Concentration
[†] = The data did not fit the hormesis regression model; therefore, the cell yield was adjusted to that of the control value and analyzed using linear interpolation.

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): 2.1 g/L NaCl	2.0 (1.9 – 2.2)	4	October 16, 2019
	Reproduction (IC50): 2.0 g/L NaCl	1.7 (1.2 – 2.4)	16	
O. mykiss	Viability (EC50): 3.7 mg/L SDS	4.3 (2.3 – 8.2)	33	October 30, 2019
L. minor	No. Fronds (IC50): 3.6 g/L KCl	3.5 (3.0 – 4.1)	8	November 20, 2019
P. subcapitata	Growth (IC50): 26.6 µg/L Zn	31.6 (25.8 – 38.7)	10	November 22, 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: Nyrstar Myra Falls Ltd. Start Date/Time: Oct 29/19 @ 1100h
Work Order No.: 192165 Set up by: MLF/SSK

Sample Information:

Sample ID: 11A - Runoff
Sample Date: Oct 28/19
Date Received: Oct 29/19
Sample Volume: 8 X 20L

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.: BB102319A
Age of young (Day 0): <24-h (within 12-h)
Avg No. young in first 3 broods of previous 7 d: 23
Mortality (%) in previous 7 d: 10
Individual female # used ≥8 young on test day: # 31-33, 35, 39, 40, 42, 43

NaCl Reference Toxicant Results:

Reference Toxicant ID: Cd236
Stock Solution ID: 19NaCl3
Date Initiated: Oct 16/19

7-d LC50 (95% CL): 2.1 (1.5 - 3.0) g/L NaCL
7-d IC50 (95% CL): 2.0 (1.7 - 2.2) g/L NaCL

7-d LC50 Reference Toxicant Mean and Historical Range: 2.0 (1.9 - 2.2) g/L NaCL CV (%): 4
7-d IC50 Reference Toxicant Mean and Historical Range: 1.7 (1.2 - 2.4) g/L NaCL CV (%): 16

Test Results:

	Survival	Reproduction
LC50 % (v/v) (95% CL)	>100	55.1 (37.3 - 74.8)
IC25 % (v/v) (95% CL)		~~59.9 (39.8 - 82.3)~~ SSK
IC50 % (v/v) (95% CL)		>100

Reviewed by: [signature] Date reviewed: Nov 14, 2019

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: Nyrstar Myra Falls Ltd.
Sample ID: 11A - Run off
Work Order #: 192168

Start Date & Time: Oct 29/19 @ 1100h
Stop Date & Time: NOV 05/19 @ 1200h
CER #: 4
Test Species: *Ceriodaphnia dubia*

%(v/v)

Concentration	Days													
control	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	25.0	24.0	25.0	25.0	25.0	~~24.0~~ 25.0 SAK	25.0	24.0	25.0	24.0	25.0	25.0	25.0
DO (mg/L)	8.0	7.0	8.0	7.0	8.0	7.0	8.0	7.4	8.1	7.3	8.1	7.1	8.0	7.0
pH	8.2	7.8	7.9	7.7	8.0	7.7	8.1	8.0	8.2	7.8	8.2	7.8	8.2	7.8
Cond. (µS/cm)	215	217		214		~~215~~ 223 SAK		216		210		215		220
Initials	MF	MF		SSK		MF		A		A		MF		SSK

Concentration	Days													
156	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	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	7.9	7.0	7.9	7.0	7.7	6.7	8.0	7.5	8.1	7.3	8.1	7.1	8.0	7.0
pH	8.2	7.8	8.0	7.8	8.0	7.7	8.0	7.9	8.0	7.9	8.0	7.7	8.1	7.9
Cond. (µS/cm)	221	223		226		233		231		234		223		227
Initials	MF	MF		SAK		SAK		A		A		SAK		SSK

Concentration	Days													
12.5	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	25.0	~~24.0~~ 25.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	7.9	7.0	7.9	6.9	7.9	6.6	8.0	7.4	8.1	7.0	8.2	7.1	8.0	7.0
pH	8.2	7.8	8.0	7.8	8.0	7.6	8.0	7.7	8.0	7.6	8.0	7.7	8.1	7.9
Cond. (µS/cm)	263	265		269		266		268		270		265		267
Initials	MF	MF		SAK		SAK		A		A		SAK		SSK

Concentration	Days													
100	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	25.0	24.0	25.0	24.0	25.0	24.0	25.0	25.0	25.0
DO (mg/L)	8.2	7.0	8.0	7.0	8.1	6.6	8.0	7.2	8.2	7.0	8.3	7.1	08.2	7.0
pH	⊙7.5	7.2	7.4	7.3	7.3	7.0	7.3	7.3	7.4	7.3	7.5	7.2	7.2	7.3
Cond. (µS/cm)	576	581		579		579		573		571		583		575
Initials	MF	MF		SAK		SAK		A		A		SAK		SSK

⊙ 7.3

SAK 08.2

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

	Control	100%		
Hardness*	100	230		
Alkalinity*	90	22		

* mg/L as CaCO3

SSK

Analysts: MF, SSK, AWD, SAK

Reviewed by:

Date reviewed: NOV 4, 2019

Sample Description: clear, colourless, odourless, some organic particulate matter present.

Comments: Broodboard Used: DB102319A (#31-33, 35, 39, 40, 42, 43)

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: Nyrstar Myra Falls Ltd.
Sample ID: 11A - Runoff
Work Order: 192168

Start Date & Time: Oct 29/19 @ 1100h
Stop Date & Time: NOV 05/19 @ 1200h
Set up by: SSK/MLF

(%v/v)

Concentration: CONTROL

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	✓	✓	✓	✓	✓	✓	✓	4	3	✓	MLF
4	✓	3	✓	✓	3	4	✓	✓	✓	✓	A
5	3	✓	3	4	✓	✓	3	7	7	4	A
6	2	10	7	12	12	6	11	14	11	12	MLF
7	1	12	13	10	11	10	12	12	8	10	SSK
8											
Total	32	25	23	26	26	20	26	25	21	26	SSK

Concentration: 1.56

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	✓	3	✓	✓	✓	3	✓	4	✓	✓	MLF
4	2	6	6	7	6	✓	✓	✓	7	A	A
5	✓	✓	✓	✓	✓	6	3	5	3	✓	8
6	3	10	12	11	12	9	12	11	12	12	MLF
7	11	12	13	12	13	10	12	12	11	11	SSK
8											
Total	16	19	31	30	31	18	27	20	26	30	SSK

Concentration: 3.12

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	✓	✓	✓	✓	✓	✓	3	✓	✓	✓	MLF
4	✓	✓	5	7	7	5	✓	7	6	✓	2
5	6	5	5	5	3	3	6	✓	✓	6	A
6	10	12	12	13	14	15	14	12	14	12	MLF
7	9	9	11X	12	11	12	11	13	11	9	SSK
8											
Total	25	26	22X	25	24	23	23	32	31	27	SSK

Concentration: 6.25

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	4	✓	2	✓	✓	✓	✓	✓	3	✓	MLF
4	6	5	✓	3	✓	✓	✓	3	6	8	A
5	6	✓	3	4	5	3	3	5	✓	6	A
6	13	11	13	14	7	12	12	13	13	12	MLF
7	10	11	9	10	9	9	7	9	8	11	SSK
8											
Total	23	19	21	22	19	25	22	23	22	31	SSK

Concentration: 12.5

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	3	✓	✓	✓	✓	2	✓	✓	2	✓	MLF
4	✓	✓	✓	✓	3	✓	✓	✓	6	✓	A
5	3	✓	3	3	✓	6	5	3	✓	5	A
6	14	11	✓	12	✓	15X	13	8	14	13	MLF
7	8	9	10	8	8	1	10	10	11	9	SSK
8											
Total	20	26	13	23	17	22X	27	22	22	27	SSK

Concentration: 25

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	✓	✓	✓	2	4	4	3	2	3	✓	MLF
4	6	8	5	✓	✓	✓	8	✓	✓	6	A
5	✓	✓	✓	4	6	5	✓	5	4	X	A
6	11	11	11	14	14	13	10	14	15	1	MLF
7	12	9	8	8	7	7	9	7	7	1	SSK
8											
Total	29	23	24	20	24	22	21	21	22	6	SSK

Concentration: 50

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	4	✓	2	2	✓	✓	✓	3	✓	✓	MLF
4	✓	5	✓	✓	✓	5	2	✓	2	3	A
5	✓	✓	5	5	3	✓	✓	4	5	✓	A
6	11	12	10	8	✓	11	12	12	10	12	MLF
7	10	12	10	10	5	10	9	7	8	9	SSK
8											
Total	25	29	17	15	8	26	23	19	17	24	SSK

Concentration: 100

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	SSK
3	3	✓	✓	✓	4	✓	✓	3	✓	✓	MLF
4	✓	✓	5	✓	✓	✓	6	5	2	6	A
5	✓	✓	2	✓	3	✓	2	✓	✓	✓	0
6	4	4	✓	5	2	2	✓	3	3	MLF	
7	9	7	6	9	5	9	9	9	10	8	SSK
8											
Total	12	13	15	12	14	13	17	17	12	17	SSK

70 SSK

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: NOV. 14, 2019

Client: Nyrstar Myra Falls Ltd

W.O.#: 192168

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	Oct29/19	Oct29/19	50	1.2	1.3	22		100①	2.3	230	MD
25% Pernier	Oct29/19	Oct29/19	100	9.2	9.4	90		50	5.0	100	SSK

Notes: ① Diluted to 100mL using DI water.

Reviewed by: [signature]

Date Reviewed: Nov. 14, 2019

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

Batch ID:	04-9836-0408	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Sakshi Singh		
Start Date:	29 Oct-19 11:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	20% Perrier Water		
Ending Date:	05 Nov-19 12:00	**Species:**	Ceriodaphnia dubia	**Brine:**			
Test Length:	7d 1h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:**	<24
Sample ID:	13-3821-2767	**Code:**	4FC3819F	**Project:**			
Sample Date:	28 Oct-19 08:45	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls		
Receipt Date:	29 Oct-19 08:57	**CAS (PC):**		**Station:**	11A-Runoff		
Sample Age:	26h (10.7 °C)	**Client:**	Nyrstar Myra Falls				

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
06-5638-1810	7d Survival Rate	Linear Interpolation (ICPIN)		EC5	>100	n/a	n/a	<1	1
				EC10	>100	n/a	n/a	<1	
				EC15	>100	n/a	n/a	<1	
				EC20	>100	n/a	n/a	<1	
				EC25	>100	n/a	n/a	<1	
				EC40	>100	n/a	n/a	<1	
			✓	EC50	>100	n/a	n/a	<1	
19-0772-9383	Reproduction	NLR: 3P Log-Gompertz	✓	IC5	9.409	n/a	20	10.63	1
			✓	IC10	19.68	6.193	34.48	5.081	
			✓	IC15	30.69	14.82	48.24	3.258	
			✓	IC20	42.48	25.51	61.27	2.354	
			✓	IC25	55.11	37.33	74.84	1.814	
			✓	IC40	99.29	66.38	139.1	1.007	
				IC50	135.8	77.94	218.6	0.7365	

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	0.9000	0.6738	1.0000	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
1.56		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%
3.12		10	0.9000	0.6738	1.0000	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
6.25		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%
12.5		10	0.9000	0.6738	1.0000	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
25		10	0.9000	0.6738	1.0000	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
50		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%
100		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%

Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	22.1	17.05	27.15	3	26	2.233	7.062	31.96%	0.00%
1.56		10	24.8	20.55	29.05	16	31	1.879	5.94	23.95%	-12.22%
3.12		10	25.8	23.4	28.2	22	32	1.062	3.36	13.02%	-16.74%
6.25		10	22.7	20.24	25.16	19	31	1.086	3.433	15.13%	-2.71%
12.5		10	21.9	18.73	25.07	13	27	1.402	4.433	20.24%	0.90%
25		10	21.2	16.98	25.42	6	29	1.867	5.903	27.84%	4.07%
50		10	20.3	15.81	24.79	8	29	1.984	6.273	30.90%	8.14%
100		10	14.2	12.66	15.74	12	17	0.6799	2.15	15.14%	35.75%

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

7d 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	0.0000	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	0.0000	1.0000	1.0000	1.0000	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	0.0000	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	0.0000
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	1.0000	1.0000

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	3	25	23	26	26	20	26	25	21	26
1.56		16	19	31	30	31	18	27	20	26	30
3.12		25	26	22	25	24	23	23	32	31	27
6.25		23	19	21	22	19	25	22	23	22	31
12.5		20	26	13	23	17	22	27	22	22	27
25		29	23	24	20	24	22	21	21	22	6
50		25	29	17	15	8	26	23	19	17	24
100		12	13	15	12	14	13	17	17	12	17

7d 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	0/1	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	0/1	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	0/1	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	0/1
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	1/1	1/1

CETIS Analytical Report

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

Analysis ID:	06-5638-1810	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.9.4		
Analyzed:	07 Nov-19 16:14	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1		
Batch ID:	04-9836-0408	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Sakshi Singh		
Start Date:	29 Oct-19 11:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	20% Perrier Water		
Ending Date:	05 Nov-19 12:00	**Species:**	Ceriodaphnia dubia	**Brine:**			
Test Length:	7d 1h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:**	<24
Sample ID:	13-3821-2767	**Code:**	4FC3819F	**Project:**			
Sample Date:	28 Oct-19 08:45	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls		
Receipt Date:	29 Oct-19 08:57	**CAS (PC):**		**Station:**	11A-Runoff		
Sample Age:	26h (10.7 °C)	**Client:**	Nyrstar Myra Falls				

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	16407	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	>100	n/a	n/a	<1	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

7d 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	0.9000	0.0000	1.0000	0.3162	35.14%	0.0%	9/10	0.95	0.0%
1.56		10	1.0000	1.0000	1.0000	0.0000	0.00%	-11.11%	10/10	0.95	0.0%
3.12		10	0.9000	0.0000	1.0000	0.3162	35.14%	0.0%	9/10	0.95	0.0%
6.25		10	1.0000	1.0000	1.0000	0.0000	0.00%	-11.11%	10/10	0.95	0.0%
12.5		10	0.9000	0.0000	1.0000	0.3162	35.14%	0.0%	9/10	0.95	0.0%
25		10	0.9000	0.0000	1.0000	0.3162	35.14%	0.0%	9/10	0.95	0.0%
50		10	1.0000	1.0000	1.0000	0.0000	0.00%	-11.11%	10/10	0.95	0.0%
100		10	1.0000	1.0000	1.0000	0.0000	0.00%	-11.11%	10/10	0.95	0.0%

7d 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	0.0000	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	0.0000	1.0000	1.0000	1.0000	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	0.0000	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	0.0000
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	1.0000	1.0000

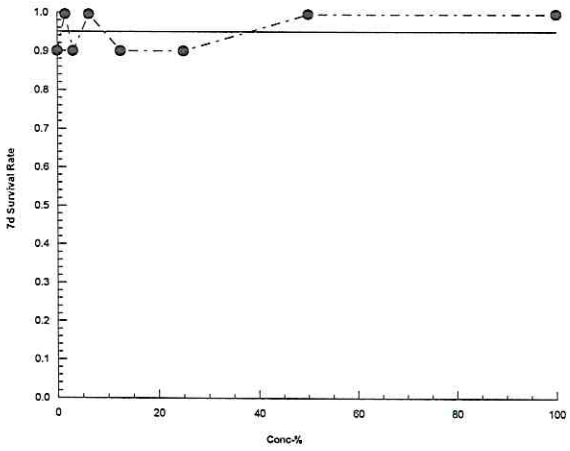
7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	0/1	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	0/1	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	0/1	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	0/1
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	1/1	1/1

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

Analysis ID:	06-5638-1810	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.9.4
Analyzed:	07 Nov-19 16:14	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID:	19-0772-9383	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.4		
Analyzed:	14 Nov-19 17:10	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1		
Batch ID:	04-9836-0408	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Sakshi Singh		
Start Date:	29 Oct-19 11:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	20% Perrier Water		
Ending Date:	05 Nov-19 12:00	**Species:**	Ceriodaphnia dubia	**Brine:**			
Test Length:	7d 1h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:**	<24
Sample ID:	13-3821-2767	**Code:**	4FC3819F	**Project:**			
Sample Date:	28 Oct-19 08:45	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls		
Receipt Date:	29 Oct-19 08:57	**CAS (PC):**		**Station:**	11A-Runoff		
Sample Age:	26h (10.7 °C)	**Client:**	Nyrstar Myra Falls				

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Gompertz: $\mu=\alpha\cdot\exp[\log[0.5]\cdot[x/\delta]^{\gamma}]$	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%)
10	-117.6	241.5	248.2	0.3373	Yes	0.7322	2.344	0.6017	Non-Significant Lack of Fit

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	9.409	n/a	20	10.63	4.999	n/a
IC10	19.68	6.193	34.48	5.081	2.9	16.15
IC15	30.69	14.82	48.24	3.258	2.073	6.75
IC20	42.48	25.51	61.27	2.354	1.632	3.92
IC25	55.11	37.33	74.84	1.814	1.336	2.679
IC40	99.29	66.38	139.1	1.007	0.7187	1.507
IC50	135.8	77.94	218.6	0.7365	0.4575	1.283

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	24.61	0.9607	22.7	26.52	25.62	<1.0E-37	Significant Parameter
γ	0.9754	0.3532	0.2719	1.679	2.761	0.0072	Significant Parameter
δ	135.8	31.91	72.22	199.3	4.255	5.9E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	38590	12860	3	639	<1.0E-37	Significant
Lack of Fit	75.03	15.01	5	0.7322	0.6017	Non-Significant
Pure Error	1455	20.49	71			
Residual	1530	20.13	76			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value Test	3.51	3.302	0.0206	Outlier Detected
Variances	Mod Levene Equality of Variance	1.857	2.143	0.0899	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9679	0.9688	0.0440	Non-Normal Distribution
	Anderson-Darling A2 Normality Te	0.7095	2.492	0.0640	Normal Distribution
Control Trend	Mann-Kendall Trend Test	8		0.5627	Non-Significant Trend in Controls

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

Analysis ID:	19-0772-9383	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.4
Analyzed:	14 Nov-19 17:10	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1

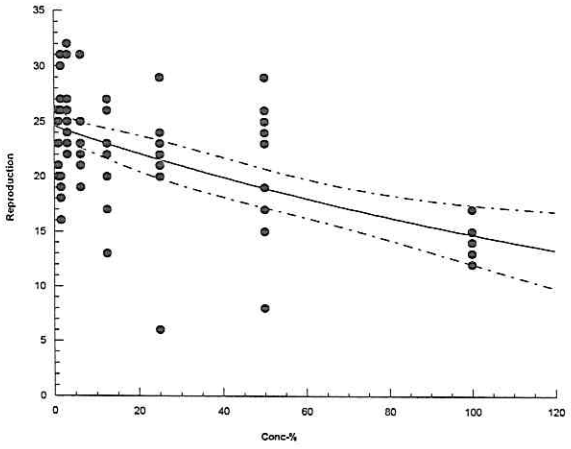
Reproduction Summary

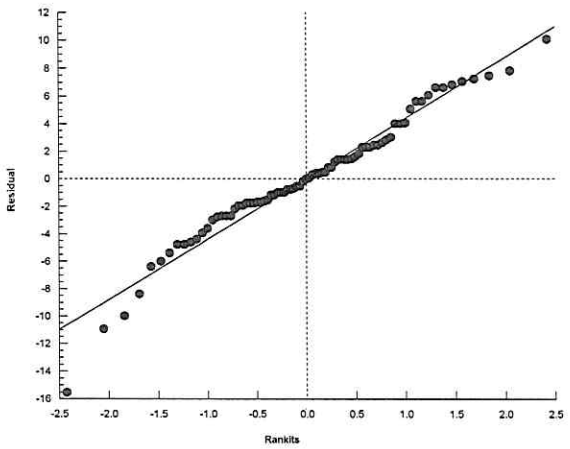
			Calculated Variate						
Conc-%	**Code**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0	N	9	24.22	20	26	0.7778	2.333	9.63%	0.0%
1.56		10	24.8	16	31	1.879	5.94	23.95%	-2.39%
3.12		10	25.8	22	32	1.062	3.36	13.02%	-6.51%
6.25		10	22.7	19	31	1.086	3.433	15.13%	6.28%
12.5		10	21.9	13	27	1.402	4.433	20.24%	9.59%
25		10	21.2	6	29	1.867	5.903	27.84%	12.48%
50		10	20.3	8	29	1.984	6.273	30.90%	16.19%
100		10	14.2	12	17	0.6799	2.15	15.14%	41.38%

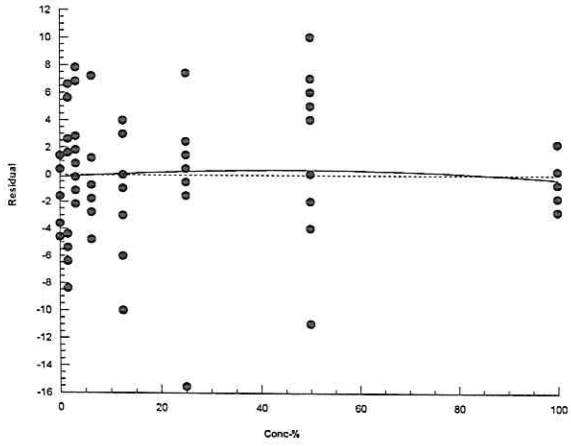
Reproduction Detail

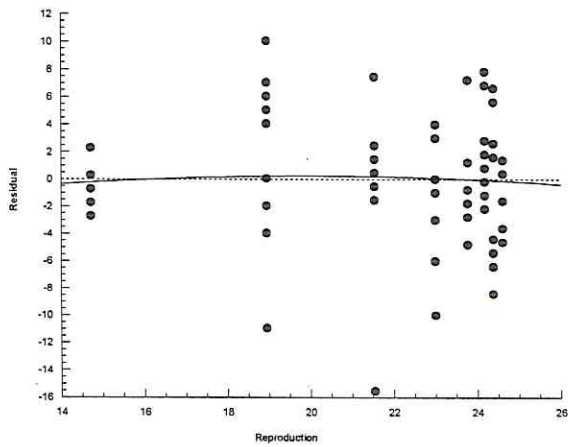
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	25	23	26	26	20	26	25	21	26	
1.56		16	19	31	30	31	18	27	20	26	30
3.12		25	26	22	25	24	23	23	32	31	27
6.25		23	19	21	22	19	25	22	23	22	31
12.5		20	26	13	23	17	22	27	22	22	27
25		29	23	24	20	24	22	21	21	22	6
50		25	29	17	15	8	26	23	19	17	24
100		12	13	15	12	14	13	17	17	12	17

Graphics Model: 3P Log-Gompertz: μ=α·exp[log[0.5]·[x/δ]^γ] Distribution: Normal [ω=1]









APPENDIX B – *Oncorhynchus mykiss* Toxicity Test Data

Rainbow Trout Early Life Stage Summary Sheet

Client: Nyrstar Myra Falls Start Date/Time: October 30, 2019 @ 1455h

Work Order No.: 192167 Test Species: *Oncorhynchus mykiss*

Sample Information:

Sample ID: 11A-RUNOFF
Sample Date: October 28, 2019
Date Received: October 29, 2019
Sample Volume: 8 x 20L

Dilution Water:

Type: Dechlorinated Tap Water
Hardness (mg/L $CaCO_3$): 8
Alkalinity (mg/L $CaCO_3$): 7

Test Organism Information:

Batch No.: 103019
Source: Lyndon ~~Trout~~ FISH Hatcheries, New Dundee, ON
Loading Density: 0.80 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: RTE126
Stock Solution ID: 19502
Date Initiated: October 30, 2019
7-d EC50 (95% CL): 3.7 (3.5-4.0) mg/L SDS

Reference Toxicant Mean and Range: 4.3 (2.3-8.2) mg/L SDS
Reference Toxicant CV (%): 33

Test Results:

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

Reviewed by: [signature] Date reviewed: NOV 22, 2019

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

Client: Nyrstar Myra Falls
Sample ID: 11A Runoff
Work Order #: 192167

Start Date & Time: Oct 30/19 @ 1455h
Stop Date & Time: Nov. 6/19 @ 1020h
CER #: 10
Test Species: *Oncorhynchus mykiss*

% (v/v) Concentration: CONT	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	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5
DO (mg/L)	10.1	10.0	10.0	10.1	9.8	10.0	9.7	10.1	9.9	10.2	9.9	10.0	9.9	10.0
pH	6.9	7.0	7.2	7.0	6.9	6.9	6.8	6.8	6.8	6.7	6.8	6.8	6.9	6.8
Cond. (µS/cm)	29	30		30		30		29		29		29		29
Initials	A	A		A		A		A		MM		MM		MM

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	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5
DO (mg/L)	10.1	10.1	10.0	9.8	9.9	10.0	9.8	10.1	9.8	10.1	9.9	10.0	9.9	10.0
pH	7.0	7.1	7.2	7.1	7.1	7.1	7.1	6.8	6.8	6.7	6.9	6.9	6.9	6.9
Cond. (µS/cm)	71	73		77		75		74		74		76		74
Initials	A	A		A		A		A		MM		MM		MM

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	14.0	14.0	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5
DO (mg/L)	10.1	10.1	10.1	9.9	9.9	9.9	9.8	10.1	9.8	10.2	9.9	9.8	10.0	10.1
pH	7.2	7.2	7.2	7.2	7.1	7.2	7.1	6.8	6.7	6.9	7.1	7.0	7.1	7.0
Cond. (µS/cm)	~~7.2~~ 183	186		181		183		182		184		186		189
Initials	A	A		A		A		A		MM		MM		MM

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)	14.5	14.0	14.0	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5
DO (mg/L)	~~10.3~~ 10.1	10.1	10.0	9.9	10.0	9.8	9.7	10.1	9.9	10.0	9.9	9.8	10.0	10.0
pH	7.3	7.4	7.3	7.3	7.2	7.2	7.2	6.9	7.0	7.2	7.3	7.2	7.3	7.2
Cond. (µS/cm)	563	568		567		568		568		567		571		572
Initials	A	A		A		A		A		MM		MM		MM

Thermometer: CER#10 **DO meter/probe:** 213 / 213 **pH meter/probe:** 213 / 213 **Conductivity meter/probe:** 213 / 213

	Control	100%		
Hardness*	8	284		
Alkalinity*	7	20		

* mg/L as $CaCO_3$

Analysts: AWD, MM
Reviewed by: MM
Date reviewed: NOV 22, 2019

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

Comments:

Embryo Toxicity Test
Daily Mortality

Client: Nyrstar Myra Falls
Sample ID: 11A Runoff
Work Order #: 192167

Start Date & Time: October 30, 2019 @ 1455h
Stop Date & Time: November 6, 2019 @ 1020h
Test Species: *Oncorhynchus mykiss*

Concentration (% v/v)	Rep	Day of Test - No. of Mortalities							Total Dead Eggs	Total Undeveloped	Total No. Embryo	Total Exposed
		1	2	3	4	5	6	7				
Control	1	0	0	0	0	0	0	0	0	5	25	30
	2							3	3	3	24	30
	3							0	0	0	30	30
	4							0	0	1	29	30
6.25	1							0	0	1	29	30
	2							1	1	0	29	30
	3							0	0	0	30	30
	4						↓	0	0	4	26	30
12.5	1						4	0	4	3	23	30
	2						0	1	1	1	29	30
	3		↓				↓	0	0	0	30	30
	4		1				↓	0	1	0	29	30
25	1		0			↓	1	0	1	0	29	30
	2					1	0	0	1	1	29	30
	3					0		0	0	0	30	30
	4							0	0	3	27	30
50	1					1		1	1	0	29	30
	2					1		0	1	0	29	29
	3					0		0	0	0	30	30
	4					0	↓	0	0	6	24	30
100	1					1	1	0	2	0	29	31
	2					3	2	1	6	1	23	30
	3					0	0	0	0	0	30	30
	4	↓	↓	↓	↓	0	0	0	0	9	21 ~~30~~ 4	30
	1											
	2											
	3											
	4											
	1											
	2											
	3											
	4											
Tech Initials		AF	AF	AF	AF	MW	MW	MW	MW	MW	MW	MW

Comments:

Reviewed by: [signature]

Date reviewed: NOV 22, 2019

Client: Nyrstar

W.O.#: 192167

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	Oct. 30/19	Oct. 30/19	50	1.1	1.2	20	50	14.2	284	SPT
Dechlor	Oct. 30/19	Oct. 30/19	100	0.8	0.9	7	100	0.8	8	MM

Notes:

Reviewed by: [signature] Date Reviewed: Nov. 22, 2019

CETIS Analytical Report

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

Analysis ID:	03-2852-7595	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.9.4
Analyzed:	21 Nov-19 13:30	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	04-8865-5029	**Test Type:**	Development	**Analyst:**	Yvonne Lam
Start Date:	30 Oct-19 14:55	**Protocol:**	EC/EPS 1/RM/28	**Diluent:**	Dechlorinated Tap Water
Ending Date:	06 Nov-19 10:20	**Species:**	Oncorhynchus mykiss	**Brine:**	
Test Length:	6d 19h	**Taxon:**	Actinopterygii	**Source:**	Lyndon Fish Hatcheries **Age:**
Sample ID:	13-3821-2767	**Code:**	4FC3819F	**Project:**	
Sample Date:	28 Oct-19 08:45	**Material:**	Water Sample	**Source:**	Nyrstar Myra Falls
Receipt Date:	29 Oct-19 08:57	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	54h (10.7 °C)	**Client:**	Nyrstar Myra Falls		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2054894	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	75.33	n/a	n/a	1.327	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	0.9000	0.8000	1.0000	0.0981	10.90%	0.0%	108/120	0.9301	0.0%
6.25		4	0.9500	0.8667	1.0000	0.0577	6.08%	-5.56%	114/120	0.9301	0.0%
12.5		4	0.9172	0.7667	1.0000	0.1038	11.31%	-1.91%	111/121	0.9301	0.0%
25		4	0.9500	0.9000	1.0000	0.0430	4.53%	-5.56%	114/120	0.9301	0.0%
50		4	0.9330	0.8000	1.0000	0.0901	9.66%	-3.67%	111/119	0.9301	0.0%
100		4	0.8505	0.7000	1.0000	0.1405	16.52%	5.5%	103/121	0.8505	8.55%

Proportion Normal Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.8333	0.8000	1.0000	0.9667
6.25		0.9667	0.9667	1.0000	0.8667
12.5		0.7667	0.9355	1.0000	0.9667
25		0.9667	0.9333	1.0000	0.9000
50		0.9667	0.9655	1.0000	0.8000
100		0.9355	0.7667	1.0000	0.7000

Proportion Normal Binomials

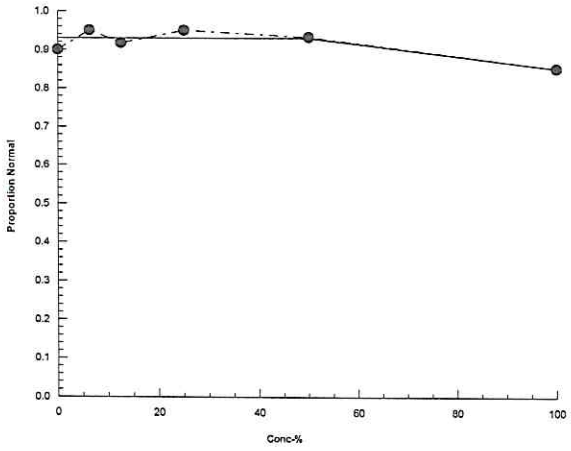
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	25/30	24/30	30/30	29/30
6.25		29/30	29/30	30/30	26/30
12.5		23/30	29/31	30/30	29/30
25		29/30	28/30	30/30	27/30
50		29/30	28/29	30/30	24/30
100		29/31	23/30	30/30	21/30

Analyst: _____ QA: NN · 22/19

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

Analysis ID:	03-2852-7595	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.9.4
Analyzed:	21 Nov-19 13:30	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



Analyst: WW QA: NN 22/19

APPENDIX C – *Lemna minor* Toxicity Test Data

Lemna minor Summary Sheet

Client: Nyrstar Myra Falls
Work Order No.: ~~11A~~ 192170 JW

Start Date: 15 - NOV - 19
Set up by: MLJ

Sample Information:

Sample ID: 11A - RUNOFF
Sample Date: 13 - NOV - 19
Date Received: 15 - NOV - 19
Sample Volume: 2 x 20L

Test Organism Information:

Culture Date: 110719
Age of culture (Day 0): 8 days
>8X growth in APHA?: Y (59 fronds)

KCl Reference Toxicant Results:

Reference Toxicant ID: LM 181
Date Initiated: 20 - NOV - 19

7-d No. of Fronds IC50 (95% CL): 3.6 (3.3 - 4.0) g/L KCl

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 3.5 (3.0 - 4.1) g/L KCl CV (%): 8

Test Results:		Number of Fronds	Dry Weight
	IC25 %(v/v) (95% CL)	31.0 (11.4 - 62.8)	30.8 (5.0 - 52.7)
	IC50 %(v/v) (95% CL)	73.3 (29.6 - 96.3)	>97

Reviewed by: JCH

Date reviewed: Dec. 6/19

Plant Growth Inhibition Toxicity Test
Water Quality Measurements

Client :	Nyrstar Myra Falls	**Setup by:**	MLT
Sample ID:	11A - RUNOFF	**Test Date:**	Nov 15, 2019
Work Order No.:	192170	**CER #:**	6
Culture Source:	CPCC #490	**Test Species:**	*Lemna minor*
Test Culture Age:	8 days	**> 8X Growth? (Y/N):**	Y (59 fronds)
Light Intensity Range:	4670 - 5350 lux	**Date Measured:**	Nov 14, 2019

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

Sample Characteristics:	**Initial Water Quality**		**Adjusted Water Quality**
Temperature (°C)	24.0	**Aeration?:** 20 min	24.0
DO (mg/L)	8.2	**Nutrients added?[1]:** Y →	8.0
pH	7.8		8.2
Conductivity (µS)	494		1273

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

Concentration	**Temperature (°C)**		**pH**		**Conductivity (µS)**
% (v/v)	**Day 0**	**Day 7**	**Day 0**	**Day 7**	**0 h**
Control	24.0	25.0	8.2	9.0	914
1.5	24.0	25.0	8.2	8.7	923
3	24.0	25.0	8.2	8.7	929
6.1	24.0	25.0	8.2	8.8	943
12.1	24.0	25.0	8.2	cs 8.9.0	966
24.2	24.0	25.0	8.2	8.6	1012
48.5	24.0	25.0	8.2	8.6	1106
97	24.0	25.0	8.2	8.7	1273
Initials	MLT		MLT		MLT

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

Sample Description: clear, colorless liquid, fine grey particulates, no odour.

Comments:

Reviewed: JDH **Date Reviewed:** Dec. 6/19

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

Client: Nyrstar Myra Falls
Sample ID: 11A - RUNOFF
Work Order #: 192170

Start Date: NOV . 15 , 2019
Termination Date: NOV . 22 , 2019
Test set up by: MLT

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	81										MLT
	B	6	114										
	C	6	123										
	D	6	121										
1.5	A	6	102										
	B	6	99										
	C	6	103										
	D	6	112										
3	A	6	99										
	B	6	116										
	C	6	69										
	D	6	124										
6.1	A	6	104										
	B	6	124										
	C	6	95										
	D	6	108										
12.1	A	6	107										
	B	6	106										
	C	6	105										
	D	6	113										
24.2	A	6	84										
	B	6	90										
	C	6	82										
	D	6	105										↓

Comments:

Reviewed by: JBM

Date Reviewed: Dec. 6/19

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

Client: Nyrstar Myra Falls
Sample ID: 11A - RUNOFF
Work Order #: 192170

Start Date: Nov 15, 2019
Termination Date: Nov 22, 2019
Test set up by: MLT

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	53			X							MLT
	B	6	89			X							
	C	6	68			X							
	D	6	78			X							
97	A	6	53		X	X							
	B	6	52		X	X							
	C	6	45		X	X							
	D	6	43		X	X							
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments:

Reviewed by: JCM

Date Reviewed: Dec. 6/19

7-d *Lemna minor* Weight Data Sheet

Client: Myra Falls
Sample ID: 11A-runoff
WO #: 192170
% (v/v)

Start Date: Nov 15 / 19
Termination Date: Nov 22 / 19
Balance ID: Bal - 1

Concentration	Rep	Pan No.	Pan weight (mg)	Pan + plant (mg)	Initials
(MF green)	A	1	1048.36	1055.05	ST /
	B	2	1017.75	1027.45	/
CONTROL	C	3	1062.22	1072.28	/
	D	4	1062.51	1072.76	/
	A	5	1077.00	1085.76	/
1.5	B	6	1051.48	1059.27	/
	C	7	1087.08	1095.54	/
	D	8	1069.27	1078.98	/
	A	9	1074.33	1081.87	/
3	B	10	1044.70	1054.33	/
	C	11	1064.88	1069.82	/
	D	12	1076.10	1087.32	/
	A	13	1051.04	1059.26	/
6.1	B	14	1059.42	1069.99	/
	C	15	1065.77	1072.90	/
	D	16	1045.28	1054.70	/
	A	17	1030.63	1038.85	/
12.1	B	18	1057.849	1064.5.04	/
	C	19	1046.09	1053.88	/
	D	20	1066.88	1075.89	/
	A	21	1027.64	1034.33	/
24.2	B	22	1032.12	1039.79	/
	C	23	1029.78	1036.39	/
	D	24	1040.85	1049.32	/
	A	25	1065.19	1070.56	/
48.5	B	26	1054.12	1060.77	/
	C	27	1067.89	1073.45	/
	D	28	1036.55	1042.853	↓ /

Comments: 10% re-weighs: ① pan # 3 Weight: 1072.15 mg
② pan # 18 Weight: 1064.92 mg
③ pan # 23 Weight: 1036.41 mg
④ pan # 30 Weight: 1055.52 mg

Reviewed by: JCM

Date Reviewed: Dec. 6/19

7-d *Lemna minor* Weight Data Sheet

Client: Myra Falls
Sample ID: HA - runoff
WO #: 192170
1/1 (V/V)

Start Date: NOV 15 / 19
Termination Date: NOV 22 / 19
Balance ID: Bal - 1

Concentration	Rep	Pan No.	Pan weight (mg)	Pan + plant (mg)	Initials
(MF green) 97	A	29	1070.45	1075.51	ST
	B	30	1050.05	1055.585	
	C	31	1083.37	1088.83	
	D	32	1052.68	1057.70	↓
	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: JCh

Date Reviewed: Dec. 6/19

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	11-3369-0443	**Endpoint:**	Frond Count	**CETIS Version:**	CETISv1.9.4
Analyzed:	05 Dec-19 14:27	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	09-4759-2212	**Test Type:**	Lemna Growth	**Analyst:**	Jeslin Wijaya
Start Date:	15 Nov-19	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Modified APHA
Ending Date:	22 Nov-19	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	CPCC#490 **Age:** 8d
Sample ID:	10-5577-6941	**Code:**	3EEDE0AD	**Project:**	
Sample Date:	13 Nov-19 09:55	**Material:**	Effluent	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 Nov-19 09:14	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	38h (13.3 °C)	**Client:**	Nyrstar Myra Falls		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	926924	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	12.57	n/a	22.72	7.956	4.4	n/a
IC10	15.98	n/a	31.99	6.257	3.126	n/a
IC15	20.25	n/a	37.5	4.937	2.667	n/a
IC20	25.39	8.544	45.23	3.938	2.211	11.7
IC25	30.98	11.4	62.77	3.228	1.593	8.774
IC40	54.14	23.48	77.88	1.847	1.284	4.259
IC50	73.3	29.65	96.3	1.364	1.038	3.373

Frond Count Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	N	4	103.8	75	117	19.55	18.84%	0.0%	103.8	0.0%
1.5		4	98	93	106	5.598	5.71%	5.54%	99.38	4.22%
3		4	96	63	118	24.34	25.36%	7.47%	99.38	4.22%
6.1		4	101.8	89	118	12.12	11.91%	1.93%	99.38	4.22%
12.1		4	101.8	99	107	3.594	3.53%	1.93%	99.38	4.22%
24.2		4	84.25	76	99	10.4	12.35%	18.8%	84.25	18.8%
48.5		4	66	47	83	15.3	23.18%	36.39%	66	36.39%
97		4	42.25	37	47	4.992	11.81%	59.28%	42.25	59.28%

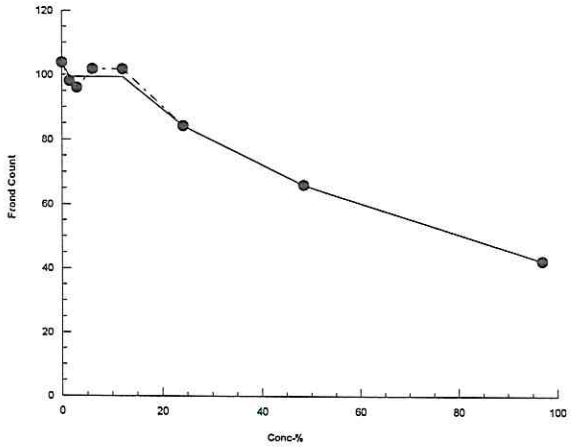
Frond Count Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	75	108	117	115
1.5		96	93	97	106
3		93	110	63	118
6.1		98	118	89	102
12.1		101	100	99	107
24.2		78	84	76	99
48.5		47	83	62	72
97		47	46	39	37

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	11-3369-0443	**Endpoint:**	Frond Count	**CETIS Version:**	CETISv1.9.4
Analyzed:	05 Dec-19 14:27	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



Analyst: JW QA: JCH Dec. 6/19

CETIS Analytical Report

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	12-6194-1419	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETISv1.9.4
Analyzed:	05 Dec-19 14:32	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	09-4759-2212	**Test Type:**	Lemna Growth	**Analyst:**	Jeslin Wijaya
Start Date:	15 Nov-19	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Modified APHA
Ending Date:	22 Nov-19	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	CPCC#490 **Age:** 8d
Sample ID:	10-5577-6941	**Code:**	3EEDE0AD	**Project:**	
Sample Date:	13 Nov-19 09:55	**Material:**	Effluent	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 Nov-19 09:14	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	38h (13.3 °C)	**Client:**	Nyrstar Myra Falls		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1404012	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	1.338	n/a	26.96	74.75	3.71	n/a
IC10	10.17	n/a	34.36	9.836	2.91	n/a
IC15	16.46	n/a	38.7	6.075	2.584	n/a
IC20	24.44	n/a	43.08	4.091	2.321	n/a
IC25	30.76	5.002	52.68	3.251	1.898	19.99
IC40	76.23	16.56	n/a	1.312	n/a	6.037
IC50	>97	n/a	n/a	<1.031	n/a	n/a

Total Dry Weight-mg Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	N	4	9.175	6.69	10.25	1.672	18.23%	0.0%	9.175	0.0%
1.5		4	8.68	7.79	9.71	0.7974	9.19%	5.4%	8.68	5.4%
3		4	8.332	4.94	11.22	2.718	32.62%	9.18%	8.584	6.45%
6.1		4	8.835	7.13	10.57	1.487	16.84%	3.71%	8.584	6.45%
12.1		4	8.143	7.55	9.01	0.6413	7.88%	11.25%	8.143	11.25%
24.2		4	7.36	6.61	8.47	0.8831	12.00%	19.78%	7.36	19.78%
48.5		4	5.962	5.37	6.65	0.6	10.06%	35.01%	5.962	35.01%
97		4	5.26	5.02	5.5	0.2551	4.85%	42.67%	5.26	42.67%

Total Dry Weight-mg Detail

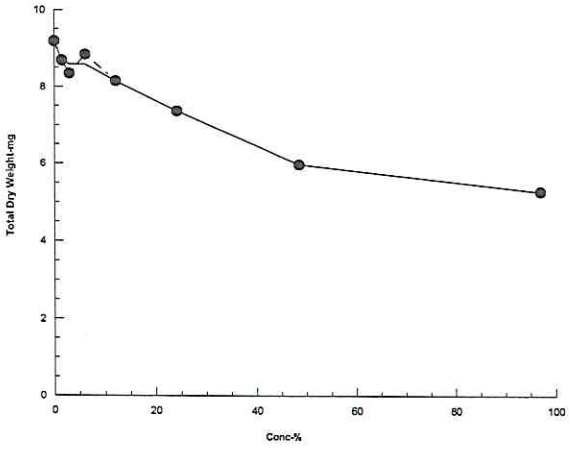
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	6.69	9.7	10.06	10.25
1.5		8.76	7.79	8.46	9.71
3		7.54	9.63	4.94	11.22
6.1		8.22	10.57	7.13	9.42
12.1		8.22	7.55	7.79	9.01
24.2		6.69	7.67	6.61	8.47
48.5		5.37	6.65	5.56	6.27
97		5.06	5.5	5.46	5.02

Analyst: JN QA: Dec. 6/19

Lemna Growth Inhibition Test **Nautilus Environmental**

Analysis ID:	12-6194-1419	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETISv1.9.4
Analyzed:	05 Dec-19 14:32	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



Analyst: JW QA: JGK Dec. 6/19

APPENDIX D – *Pseudokirchneriella subcapitata* Toxicity Test Data

Pseudokirchneriella subcapitata Summary Sheet

Client: Nyrstar Myra Falls
Work Order No.: 192169

Start Date: Nov 15/19
Set up by: MG

Sample Information:

Sample ID: 11A-Runoff
Sample Date: Nov 13/19
Date Received: Nov 15/19
Sample Volume: 23 x 20L
JW

Test Organism Information:

Culture Date: Nov 8/19
Age of culture (Day 0): 7d

Zinc Reference Toxicant Results:

Reference Toxicant ID: SC191
Stock Solution ID: 19Zn02
Date Initiated: Nov 22/19

72-h IC50 (95% CL): 26.6 (23.5 - 30.0) mg/L Zn

72-h IC50 Reference Toxicant Mean and Range: 31.6 (25.8 - 38.7) mg/L Zn CV (%): 10

Test Results:

	Algal Growth
IC25 %(v/v) (95% CL)	>95.2
IC50 %(v/v) (95% CL)	>95.2

Reviewed by: [signature]

Date reviewed: Dec 17, 2019

72-h Algal Growth Inhibition Toxicity Test
Water Quality Measurements

Client: Nyrstar Myra Falls Ltd. | Setup by: MG

Sample ID: 11A-Runoff | Test Date/Time: Nov 15/19 @ 1300h.

Work Order No.: 197169 | CER #: 4

Test Species: *Pseudokirchneriella subcapitata*

Culture Date: Nov 8/19 | Age of Culture: 7d | Culture Health: Good.

Culture Count: 1 390 2 410 | Average: 400 | Culture Cell Density (c1): 400 x 10⁴ cells/mL

$v1 = \frac{220{,}000 \text{ cells/ml} \times 100 \text{ ml}}{(c1)\ 400 \times 10^4 \text{ cells/ml}} = 5.5 \text{ mL}$

Time Zero Counts: 1 23 2 24 | Average: 23.5

No. of Cells/mL: 23.5 x 10⁴ | Initial Density: # cells/mL ÷ 220 µL x 10 µL = 10682 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	7.0	24.0	25.0	25.0	26.0	25.0	✓	✓	✓	✓
1.5	7.0	24.0	↓	↓	↓	↓	✓	✓	✓	✓
3	7.0	24.0					✓	✓	✓	✓
6	7.2	24.0					✓	✓	✓	✓
11.9	7.2	24.0					✓	✓	✓	✓
23.8	7.3	24.0					✓	✓	✓	✓
47.6	7.4	24.0					✓	✓	✓	✓
95.2	7.5	24.0	↓	↓	↓	↓	✓	✓	✓	✓
Initials	MG	MG	MG	~	~	MG	MG	~	~	MG

Initial control pH: Well 1: 7.0 | Well 2: 7.0

Final control pH: Well 1: 7.1 | Well 2: 7.1

Light intensity (lux): 4100. | Date measured: Nov 15/19

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

Sample Description: clear, colourless, odourless, fine gray particulates.

Comments:

Reviewed: [signature] | Date reviewed: Dec. 17, 2019

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

Client: Nyrstar Myra Falls Ltd
Start Date/Time: Nov 15/19 @ 1300h
Work Order #: 192169
Termination Date: Nov 18/19 @ 1300h
Sample ID: 11A-Runoff
Test set up by: MLT
%(v/v)

Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	A	44					MLT
	B	38					
	C	35					
	D	39					
	E	37					
	F	39					
	G	34					
	H	40					
1.5	A	50					
	B	45					
	C	51					
	D	56					
3	A	59					
	B	58					
	C	50					
	D	52					
6	A	69					
	B	66					
	C	68					
	D	72					
11.9	A	74					
	B	69					
	C	79					
	D	80					
23.8	A	103					
	B	99					
	C	102					
	D	105					
47.6	A	109					
	B	111					
	C	92					
	D	96					
95.2	A	38					
	B	37					
	C	38					
	D	32					✓

Comments:

Reviewed by: [signature]
Date Reviewed: Dec. 17, 2019

Pseudokirchneriella subcapitata Algal Counts

Client:	Nyrstar Myra Falls	Start Date/Time:	15-Nov-19 @ 1300h
WO#:	192169	Termination Date/Time	18-Nov-19 @ 1300h
Sample ID:	11A-Runoff		
		Initial Cell Density:	10682 cell/mL

									235000
									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		10681.82
Control	A	44				44	42.9	mean	37.2
	B	38				38	36.9	SD	3.105295
	C	35				35	33.9	CV	8.351649
	D	39				39	37.9		
	E	37				37	35.9		
	F	39				39	37.9		
	G	34				34	32.9		
	H	40				40	38.9		
1.5	A	50				50	48.9		
	B	45				45	43.9		
	C	51				51	49.9		
	D	56				56	54.9		
3	A	59				59	57.9		
	B	58				58	56.9		
	C	50				50	48.9		
	D	52				52	50.9		
6	A	69				69	67.9		
	B	66				66	64.9		
	C	68				68	66.9		
	D	72				72	70.9		
11.9	A	74				74	72.9		
	B	69				69	67.9		
	C	79				79	77.9		
	D	80				80	78.9		
23.8	A	103				103	101.9		
	B	99				99	97.9		
	C	102				102	100.9		
	D	105				105	103.9		
47.6	A	109				109	107.9		
	B	111				111	109.9		
	C	92				92	90.9		
	D	96				96	94.9		
95.2	A	38				38	36.9		
	B	37				37	35.9		
	C	38				38	36.9		
	D	32				32	30.9		

Reviewed by: _____ Date reviewed: Dec. 17, 2019

CETIS Analytical Report

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

Analysis ID:	09-1494-1302	**Endpoint:**	Cell Yield	**CETIS Version:**	CETISv1.9.4
Analyzed:	12 Dec-19 19:12	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	13-6698-9720	**Test Type:**	Cell Growth	**Analyst:**	Mimi Tran
Start Date:	15 Nov-19 13:00	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water + nutrients
Ending Date:	18 Nov-19 13:00	**Species:**	Pseudokirchneriella subcapitata	**Brine:**	
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture **Age:** 7d
Sample ID:	10-5577-6941	**Code:**	3EEDE0AD	**Project:**	
Sample Date:	13 Nov-19 09:55	**Material:**	Effluent	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 Nov-19 09:14	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	51h (13.3 °C)	**Client:**	Nyrstar Myra Falls		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	279931	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value Test	2.43	2.991	0.4132	No Outliers Detected
Control Trend	Mann-Kendall Trend Test	2.43		0.7195	Non-Significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	51.1	50.63	51.46	1.957	1.943	1.975
IC10	54.85	53.84	55.63	1.823	1.798	1.857
IC15	58.87	57.25	60.13	1.699	1.663	1.747
IC20	63.18	60.88	64.98	1.583	1.539	1.643
IC25	67.79	64.72	70.22	1.475	1.424	1.545
IC40	83.74	77.74	88.55	1.194	1.129	1.286
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	37.25	33	43	3.105	8.34%	0.0%	69.29	0.0%
1.5		4	49.5	44	55	4.509	9.11%	-32.89%	69.29	0.0%
3		4	53.75	49	58	4.425	8.23%	-44.3%	69.29	0.0%
6		4	67.75	65	71	2.5	3.69%	-81.88%	69.29	0.0%
11.9		4	74.5	68	79	5.066	6.80%	-100.0%	69.29	0.0%
23.8		4	101.2	98	104	2.5	2.47%	-171.8%	69.29	0.0%
47.6		4	101	91	110	9.416	9.32%	-171.1%	69.29	0.0%
95.2		4	35.25	31	37	2.872	8.15%	5.37%	35.25	49.12%

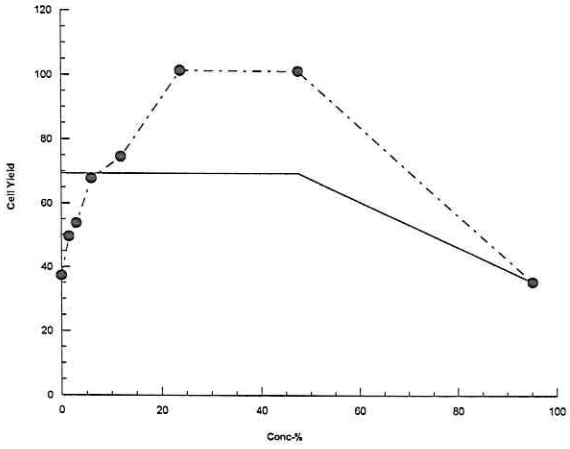
Cell Yield Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	43	37	34	38	36	38	33	39
1.5		49	44	50	55				
3		58	57	49	51				
6		68	65	67	71				
11.9		73	68	78	79				
23.8		102	98	101	104				
47.6		108	110	91	95				
95.2		37	36	37	31				

EC Alga Growth Inhibition Test **Nautilus Environmental**

Analysis ID:	09-1494-1302	**Endpoint:**	Cell Yield	**CETIS Version:**	CETISv1.9.4
Analyzed:	12 Dec-19 19:12	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



CETIS Analytical Report

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

Analysis ID:	08-1453-6244	**Endpoint:**	Cell Yield	**CETIS Version:**	CETISv1.9.4
Analyzed:	12 Dec-19 19:15	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	07-5930-5270	**Test Type:**	Cell Growth	**Analyst:**	Mimi Tran
Start Date:	15 Nov-19 13:00	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water + nutrients
Ending Date:	18 Nov-19 13:00	**Species:**	Pseudokirchneriella subcapitata	**Brine:**	
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture **Age:** 7d
Sample ID:	10-5577-6941	**Code:**	3EEDE0AD	**Project:**	
Sample Date:	13 Nov-19 09:55	**Material:**	Effluent	**Source:**	Nyrstar Myra Falls
Receipt Date:	15 Nov-19 09:14	**CAS (PC):**		**Station:**	11A-Runoff
Sample Age:	51h (13.3 °C)	**Client:**	Nyrstar Myra Falls		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2104718	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value Test	3.542	2.991	0.0031	Outlier Detected
Control Trend	Mann-Kendall Trend Test	3.542		0.7195	Non-Significant Trend in Controls

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	90.17	n/a	n/a	1.109	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	37.25	33	43	3.105	8.34%	0.0%	37.25	0.0%
1.5		4	37	37	37	0	0.00%	0.67%	37	0.67%
3		4	37	37	37	0	0.00%	0.67%	37	0.67%
6		4	37	37	37	0	0.00%	0.67%	37	0.67%
11.9		4	37	37	37	0	0.00%	0.67%	37	0.67%
23.8		4	37	37	37	0	0.00%	0.67%	37	0.67%
47.6		4	37	37	37	0	0.00%	0.67%	37	0.67%
95.2		4	35.25	31	37	2.872	8.15%	5.37%	35.25	5.37%

Cell Yield Detail

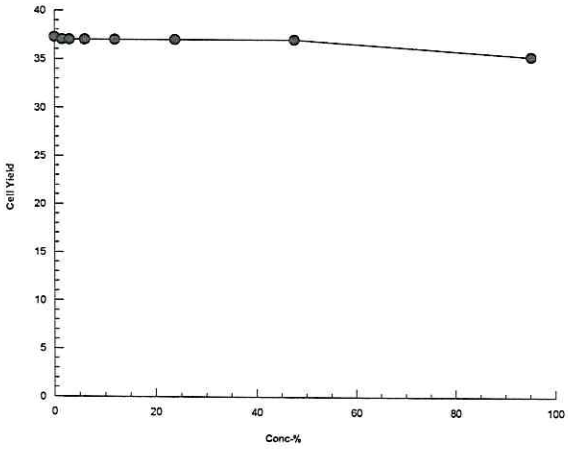
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	43	37	34	38	36	38	33	39
1.5		37	37	37	37				
3		37	37	37	37				
6		37	37	37	37				
11.9		37	37	37	37				
23.8		37	37	37	37				
47.6		37	37	37	37				
95.2		37	36	37	31				

Analyst: MC QA: Dec. 17/19

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

Analysis ID:	08-1453-6244	**Endpoint:**	Cell Yield	**CETIS Version:**	CETISv1.9.4
Analyzed:	12 Dec-19 19:15	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



Analyst: MU QA: Dec-17/19

APPENDIX E – Chain-of-Custody Forms

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

October 28th 2019 pg 1/1

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	Accounts Payable
Phone	250-287-9271 EXT. 3397	250-287-9271 ext. 3221
Email	craig.schweitzer@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	11A-RUNOFF	28/10/2019	0845	water	plastic	8	***One extra bucket for acute toxicity re-run for daphnia	X	X	X	X	X	X						10.7
2																			
3																			
4																			
5																			
6																			
7																			
8								19 21 67	19 21 68	19 21 69	19 21 70	19 21 71	19 21 72						
9								19 21	19 21	19 21	19 21	19 21	19 21						
10								19	19	19	19	19	19						

PROJECT INFORMATION	SAMPLE RECEIPT		RELIQUINSHED BY (CLIENT)	RELIQUINSHED BY (COURIER)
Client: Nyrstar Myra Falls	Total # Containers:	8	Signature: Ralph Arndt	Signature:
P.O. No.: 4501745322	Good Condition?	Y	Print: ~~Craig Schweitzer~~ Ralph Arndt	Print:
Shipped Via: Purolator	Matches Schedule?	Y	Company: Nyrstar Myra Falls Ltd	Company:
			Time/Date: ~~March 5 15:00~~ Oct 28 2019	Time/Date:
			RECEIVED BY (CLIENT)	RECEIVED BY (LABORATORY)
			Signature:	Signature: TH
			Print:	Print: Tyrone Hamilton
			Company:	Company: Nautilus
			Time/Date:	Time/Date: Oct 29/19 @ 8:57

SPECIAL INSTRUCTIONS/COMMENTS:

Please send results to both emails listed above.

***One extra bucket for acute toxicity re-run for daphnia

Sample Description: clear, colourless, & odourless liquid with small organic particulate matter.

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

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

Sample Collection By: CS

	Report to:	Invoice to:
Company	Nyrstar Myra Falls Ltd	Nyrstar Myra Falls
Address	PO BOX 8000	PO BOX 8000
City/State/Zip	Campbell River, BC	Campbell River, BC
Contact	Ralph Arndt	Grace Augustin
Phone	250-287-9271 EXT. 3397	250-287-9271 EXT. 3221
Email	nicole.pesonen@nyrstar.com, craig.schweitzer@nyrstar.com, ralph.arndt@nyrstar.com	myrafalls.accountspayable@nyrstar.com

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	# OF CONTAINERS	COMMENTS	ANALYSES REQUIRED: RBT LC50	Daphnia magna LC50	SWIM Entry		7-d L.minor EC50	72-h P.Subcapitata EC50						Receipt Temperature (°C)
1	11A-RUNOFF	2019-11-13	09:55	water	20 L plastic pail	2 x 20L	Sample by RA	X	X	X		X	X						13.3
2																			
3																			
4																			
5																			
6																			
7																			
8								19 2304	19 23 05			19 21 70	19 21 69						
9								19	19			19 21	19 21						
10								19	19			19 21	19 21						

PROJECT INFORMATION	SAMPLE RECEIPT		RELIQUINSHED BY (CLIENT)	RELIQUINSHED BY (COURIER)
Client: Nyrstar Myra Falls	Total # Containers:	2	Signature: SP	Signature:
P.O. No.: 4501745322	Good Condition?	Y	Print: Shane Pollard	Print:
Shipped Via: Purolator	Matches Schedule?	Y	Company: Nyrstar Myra Falls Ltd	Company:
			Time/Date: 09-10-2019 5:30 PM	Time/Date:
SPECIAL INSTRUCTIONS/COMMENTS:			RECEIVED BY (COURIER)	RECEIVED BY (LABORATORY)
			Signature:	Signature: TM
			Print:	Print: Tyme
			Company:	Company: Nmlabs
			Time/Date:	Time/Date: Nov. 15/19 @ 9:14

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

END OF REPORT