Toxicity Testing for 11A Runoff

Sample collected April 23, 2018

Final Report

June 7, 2018

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

8664 Commerce Court, Burnaby, BC V5A 4N7

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SIGNATURE PAGE

Report By:
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Reviewed By:
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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 sample tested.

SUMMARY

Sample Information and Test Type

Sample ID	11A Runoff
Sample collection date	April 23, 2018
Sample receipt date	April 24, 2018
Sample receipt temperature	12.0°C
Test type	7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability

Summary of Results

Endpoint	**% v/v**
Oncorhynchus mykiss	
Embryo viability EC25	>100
Embryo viability EC50	>100

EC = Effective Concentration

1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted a sub-lethal toxicity test for Nyrstar Myra Falls as part of their requirements under the Metal Mining Effluent Regulations (MMER) and Environmental Effects Monitoring (EEM) program. Sample 11A Runoff was collected on April 23, 2018 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on April 24, 2018. The sample was transported in four 20-L plastic containers and received at a temperature of 12.0°C. The sample was stored in the dark at 4 ± 2°C prior to testing.

This report describes the results of the 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability test conducted on the sample. Testing was initiated on April 26, 2018. Copies of raw laboratory data sheets and statistical analyses are provided in Appendix A. The chain-of-custody form is provided in Appendix B.

2.0 METHODS

The method for the 7-d rainbow trout embryo viability test is summarized in Table 1, and followed procedures described by Environment Canada (1998) and modified by Canaria *et al.* (1999). Statistical analyses for the test were performed using CETIS (Tidepool Scientific Software, 2013).

Table 1. 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 1.8.7
Test endpoint	Embryo viability
Test acceptability criterion for controls	Embryo viability ≥70%
Reference toxicant	Sodium dodecyl sulphate (SDS)

3.0 RESULTS

Results of the toxicity test are summarized in Table 2. There were no adverse effects observed on the embryo viability of *O. mykiss*, with the EC25/EC50 values >100% (v/v).

Table 2. Results: rainbow trout (*Oncorhynchus mykiss*) embryo viability test.

Concentration (% v/v)	Embryo Viability (%) (Mean ± SD)
Laboratory Control	87.5 ± 14.2
6.25	87.5 ± 12.9
12.5	83.6 ± 10.1
25	85.8 ± 6.9
50	80.0 ± 16.6
100	81.7 ± 23.8
Test Endpoint (% v/v)	
EC25	>100
EC50	>100

SD = Standard Deviation, EC = Effective Concentration

4.0 QA/QC

The health history of the test organisms used in the exposure was acceptable and met the requirements of the Environment Canada protocol. The test met all control acceptability criteria and water quality parameters remained within ranges specified in the protocol throughout the test. Uncertainty associated with the test is best described by the standard deviation around the mean and/or the confidence intervals around the point estimates.

There was a deviation in the 7-d rainbow trout embryo viability test. The eggs were exposed using a blocked design (eggs from each of the four female fish were distributed separately in each of replicates A to D) rather than pooled, as specified in the test protocol. The modification was used because the egg quality from each female varied considerably, and blocking would minimize the effects of poor quality eggs from one particular female fish. This did not seem to affect the results of the test since control criterion was met at the end of the seven day exposure.

Results of the reference toxicant test conducted during the testing program are summarized in Table 3. Results for this test fell within the range for organism performance of the mean and two standard deviations, based on historical results obtained by the laboratory with this test. Thus, the sensitivity of the organisms used in this test was appropriate. The reference toxicant test was performed under the same conditions as those used for the sample.

Table 3. Reference toxicant test results.

Test Species	Endpoint	Historical Mean (2 SD Range)	CV (%)	Test Date
O. mykiss	Viability (EC50): 2.3 mg/L SDS	4.3 (2.2 – 8.7) mg/L SDS	42	April 26, 2018

SD = Standard Deviation, CV = Coefficient of Variation, 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.

Tidepool Scientific Software. 2013. CETIS comprehensive environmental toxicity information system, version 1.8.7.16 Tidepool Scientific Software, McKinleyville, CA. 275 pp.

APPENDIX A – *Oncorhynchus mykiss* Toxicity Test Data

Rainbow Trout Early Life Stage Summary Sheet

Client: Nyrstar Myra Falls Start Date/Time: April 26, 2018 @ 1840h

Work Order No.: 180621 Test Species: *Oncorhynchus mykiss*

Sample Information:

Sample ID: IIA Runoff
Sample Date: April 23, 2018
Date Received: April 24, 2018
Sample Volume: 4 x 20L

Dilution Water:

Type: Dechlorinated Tap Water
Hardness (mg/L $CaCO_3$): 11
Alkalinity (mg/L $CaCO_3$): 13

Test Organism Information:

Batch No.: 042618
Source: Tedis Trout Fish Farm, Campbell Lake
Loading Density: 1.08 g/L

Number of male broodstock used: 3
Number of female broodstock used: 4
Sperm motility check: Verification of sperm motility using a compound microscope

SDS Reference Toxicant Results:

Reference Toxicant ID: RTE105
Stock Solution ID: 18501
Date Initiated: April 26, 2018
7-d EC50 (95% CL): 2.3 (2.3 - 2.4) mg/L SDS

Reference Toxicant Mean and Range: 4.3 (2.2 - 8.7) mg/L SDS
Reference Toxicant CV (%): 42

Test Results:

	Sample ID		
	IIA Runoff		
EC25 % (v/v) (95% CL)	>100		
EC50 % (v/v) (95% CL)	>100		

Reviewed by: JCh Date reviewed: May 29/18

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

Client: Nynas
Sample ID: IIA + Runoff
Work Order #: 180621

Start Date & Time: April 26, 2018 @ 1640h
Stop Date & Time: May 3, 2018 @ 1020h
CER #: 3
Test Species: *Oncorhynchus mykiss*

Control Concentration (% v/v)	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	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.5	14.5
DO (mg/L)	10.1	10.2	10.0	10.2	9.9	10.1	9.8	10.2	10.1	10.0	10.0	10.0	10.1	10.1
pH	6.8	6.7	6.8	6.2	7.0	6.7	7.2	6.7	6.9	6.7	6.8	6.7	6.8	6.9
Cond. (µS/cm)	34	35		34		34		36		35		35		36
Initials	YML	AD		AD		AD		YML		YML		YML		YML

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	14.0	14.0	14.0	14.0	14.3	14.0	14.0	14.0	14.0	14.0	14.0	14.5	14.5
DO (mg/L)	10.1	10.1	10.0	10.1	9.9	10.2	9.9	10.0	10.1	10.0	10.1	10.0	10.2	10.2
pH	7.2	7.1	7.3	7.0	7.1	7.0	7.2	7.1	7.3	7.1	7.2	7.0	7.0	7.0
Cond. (µS/cm)	92	88		100		106		90		93		95		95
Initials	YML	AD		AD		AD		YML		YML		YML		YML

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	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.5	14.5
DO (mg/L)	10.1	10.2	9.9	10.1	9.8	10.2	9.8	10.0	10.1	9.9	10.1	9.9	10.2	10.1
pH	7.1	7.1	7.4	7.1	7.3	7.0	7.2	7.2	7.4	7.2	7.3	7.1	7.1	7.3
Cond. (µS/cm)	244	249		241		236		232		230		249		245
Initials	YML	AD		AD		AD		YML		YML		YML		YML

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	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.5	14.5
DO (mg/L)	10.1	10.2	9.9	10.2	9.9	10.2	9.8	10.0	10.0	9.7	10.1	9.9	10.2	10.1
pH	7.6	7.2	7.3	7.2	7.5	7.2	7.4	7.3	7.4	7.4	7.4	7.5	7.3	7.4
Cond. (µS/cm)	712	720		721		720		711		713		712		720
Initials	YML	AD		AD		AD		YML		YML		YML		YML

Thermometer: 3 **DO meter/probe:** 2/3 / 2/3 **pH meter/probe:** 2/3 / 2/3 **Conductivity meter/probe:** 2/3 / 2/3

	Control	100%		
Hardness*	11	360		
Alkalinity*	13	32		

* mg/L as CaCO3

Analysts: AWD, YML

Reviewed by: JBL

Date reviewed: May 29/18

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

Comments:

Embryo Toxicity Test
Daily Mortality

Client: Nyrstar Myra Falls
Sample ID: IIA Runoff
Work Order #: 180621

Start Date & Time: April 26, 2018 @ 1840h
Stop Date & Time: May 3, 2018 @ 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	1	29	30
	2				0	3	1	4	8	2	20	30
	3				0	0	0	0	0	1	29	30
	4				2	0	0	0	2	1	27	30
6.25	1				0	0	0	0	0	0	30	30
	2				0	2	2	2	6	3	21	30
	3				0	0	1	0	1	3	26	30
	4				1	0	0	0	1	1	28	30
12.5	1				0	0	1	0	1	2	27	30
	2				1	2	0	3	6	0	24	30
	3				0	0	0	0	0	2	28	30
	4				1	1	3	3	9	0	22	31
25	1				0	0	0	0	1	1	28	30
	2				2	1	1	2	6	0	25	31
	3				0	1	0	0	1	2	27	30
	4				1	0	2	2	5	1	23	29
50	1				0	0	0	0	0	0	30	30
	2				1	1	0	2	4	0	26	30
	3				1	1	1	3	6	3	21	30
	4				1	4	0	5	11	0	19	30
100	1			0	0	0		0	0	1	30	31
	2				1	3	1	8	13	3	14	30
	3				2	1	0	0	3	1	26	30
	4				1	0	0	0	1	0	29	30
	1											
	2											
	3											
	4											
	1											
	2											
	3											
	4											
Tech Initials		MM	A	A	MM/AB	MM	MM	A	MM	MM	MM	MM

Comments:

Reviewed by: JGR **Date reviewed:** May 29/18

CETIS Analytical Report

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

Analysis ID:	08-3704-2437	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 May-18 12:34	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	16-8062-9307	**Test Type:**	Development	**Analyst:**	Yvonne Lam
Start Date:	26 Apr-18 18:40	**Protocol:**	EC/EPS 1/RM/28	**Diluent:**	Dechlorinated Tap Water
Ending Date:	03 May-18 10:20	**Species:**	Oncorhynchus mykiss	**Brine:**	
Duration:	6d 16h	**Source:**	Ted's Trout, Campbell Lake	**Age:**	
Sample ID:	07-0383-1514	**Code:**	29F39DDA	**Client:**	Nyrstar Myra Falls
Sample Date:	23 Apr-18 11:00	**Material:**	Effluent	**Project:**	
Receive Date:	24 Apr-18 08:55	**Source:**	Nyrstar Myra Falls		
Sample Age:	80h (12 °C)	**Station:**	11A Runoff		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1643855	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	33.23	N/A	N/A	3.01	NA	NA
EC10	>100	N/A	N/A	<1	NA	NA
EC15	>100	N/A	N/A	<1	NA	NA
EC20	>100	N/A	N/A	<1	NA	NA
EC25	>100	N/A	N/A	<1	NA	NA
EC40	>100	N/A	N/A	<1	NA	NA
EC50	>100	N/A	N/A	<1	NA	NA

Proportion Normal Summary

			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	4	0.875	0.6667	0.9667	0.0712	0.1424	16.27%	0.0%	105	120
6.25		4	0.875	0.7	1	0.06437	0.1287	14.71%	0.0%	105	120
12.5		4	0.8358	0.7097	0.9333	0.05068	0.1014	12.13%	4.49%	101	121
25		4	0.8582	0.7931	0.9333	0.03453	0.06906	8.05%	1.92%	103	120
50		4	0.8	0.6333	1	0.08278	0.1656	20.69%	8.57%	96	120
100		4	0.8169	0.4667	0.9677	0.1191	0.2383	29.17%	6.64%	99	121

Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0.9667	0.6667	0.9667	0.9
6.25		1	0.7	0.8667	0.9333
12.5		0.9	0.8	0.9333	0.7097
25		0.9333	0.8065	0.9	0.7931
50		1	0.8667	0.7	0.6333
100		0.9677	0.4667	0.8667	0.9667

Proportion Normal Binomials

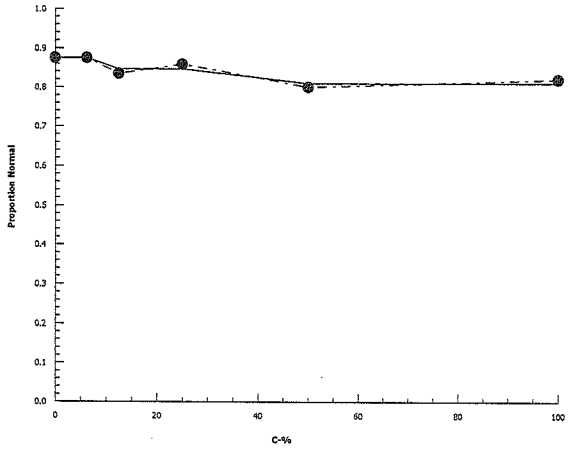
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	29/30	20/30	29/30	27/30
6.25		30/30	21/30	26/30	28/30
12.5		27/30	24/30	28/30	22/31
25		28/30	25/31	27/30	23/29
50		30/30	26/30	21/30	19/30
100		30/31	14/30	26/30	29/30

Analyst: YL QA: May 29/18

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

Analysis ID:	08-3704-2437	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 May-18 12:34	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: JGh QA: May 29/18

Client: Nyrstar Myra Falls

W.O.#: 180621

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	Apr.26/18	Apr.26/18	50	1.7	1.8	32	~~50~~ 10①	3.6	360	YML
							~~YML~~			
Dechlor	Apr.26/18	Apr.26/18	100	1.4	1.5	13	100	1.1	11	YML

Notes: ① Diluted to 100 mL w/ DI water

Reviewed by: JOL

Date Reviewed: May 29/18

APPENDIX B – 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

WO # 180621

####

Sample Collection By: CS, KB

	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	Nicole Pesonen	Ruth Kish
Phone	250-287-9271 EXT. 3397	250-287-9271 EXT. 3221
Email	nicole.pesonen@nyrstar.com, katie.babin@nyrstar.com, craig.schweitzer@nyrstar.com	ruth.kish@nyrstar.com

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	# OF CONTAINERS	COMMENTS	ANALYSES REQUIRED: 7-d RBT embryo	Receipt Temperature (°C)
1	11A Runoff	Apr 23/18	11:00	water	plastic	4 (2 coolers)	4x2L	X	12.0
2									
3									
4									
5									
6									
7									
8									
9									
10									

PROJECT INFORMATION	SAMPLE RECEIPT		RELIQUINSHED BY (CLIENT)	RELIQUINSHED BY (COURIER)
Client: Nyrstar Myra Falls	**Total # Containers:**	4	**Signature:**	**Signature:**
P.O. No.: 4501557413	**Good Condition?**	Y	**Print:** K. Babin	**Print:**
Shipped Via: Purolater	**Matches Schedule?**	Y	**Company:** Nyrstar Myra Falls Ltd	**Company:**
			Time/Date: 18-02-23	**Time/Date:**
sample desc - clear, no colour, no odour, no particulates			**RECEIVED BY (COURIER)**	**RECEIVED BY (LABORATORY)**
			Signature:	**Signature:** AW
			Print:	**Print:** ANDREA WELSINK
			Company:	**Company:** NAUTILUS
			Time/Date:	**Time/Date:** Apr 24/18 @ 8:35

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

END OF REPORT