



Acute Toxicity Test Results

Samples collected July 2, 2024

Final Report

July 9, 2024

Submitted to: **ALS Environmental**
Yellowknife, NT

SAMPLE INFORMATION

Sample ID/ Internal ID	Dates			Receipt temperature
	Collected	Received	Rainbow trout test initiation	
YL2400777-001 PLPO_Acute_240702/ 2324-2499	2024-07-02 at 0813h	2024-07-03 at 1449h	2024-07-04 at 1255h	2024-07-04 at 1400h 12.8°C

TEST TYPES

- Rainbow trout 96-h LC50 test
- *Daphnia magna* 48-h LC50, EC50 test

RESULTS

Toxicity test results

Sample ID	LC50/EC50 (% v/v)		
	Rainbow trout	<i>Daphnia magna</i>	
	LC50	LC50	EC50
YL2400777-001 PLPO_Acute_240702	>100	>100	>100

LC = Lethal Concentration, EC= Effect Concentration

QA/QC

QA/QC summary	Rainbow trout	<i>Daphnia magna</i>
Reference toxicant LC50 (95% CL)	4.2 (4.0-4.5) g/L KCl ¹	6.2 (5.9-6.5) g/L NaCl ²
Reference toxicant historical mean (2 SD Range)	3.9 (3.0-5.0) g/L KCl	6.2 (5.5-6.8) g/L NaCl
Reference toxicant CV	8.5%	3.5%
Organism health history	Acceptable	Acceptable
Protocol deviations	None	See Below
Water quality range deviations	None	None
Control performance	Acceptable	Acceptable
Test performance	Valid	Valid

¹ Test date 2024-06-12; ² Test date 2024-07-03

LC = Lethal Concentration, CL = Confidence Limit, SD = Standard Deviation, CV = Coefficient of Variation

9 test organisms were found in the CTL test concentration at the 24-hr observation period.

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Reviewed By:
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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.

APPENDIX A – Summary of test conditions

Table 1. Summary of test conditions: 96-h rainbow trout (*Oncorhynchus mykiss*) survival test.

Test species	<i>Oncorhynchus mykiss</i>
Organism source	Fish hatchery
Organism age	Juvenile
Test type	Static
Test duration	96 hours
Test vessel	5-gallon glass aquariums
Test volume	10 - 20 L, depending on size of fish
Test solution depth	Minimum 15 cm
Test concentrations	Five concentrations, plus laboratory control
Test replicates	1 per treatment
Number of organisms	10 per replicate
Control/dilution water	De-chlorinated City of Calgary tap water
Test solution renewal	None
Test temperature	15 ± 1°C
Feeding	None
Light intensity	100 to 500 lux
Photoperiod	16 hours light/8 hours dark
Aeration	6.5 ± 1 mL/min/L
Test Measurements	pH, conductivity, dissolved oxygen, and temperature were measured at test initiation and test completion; salinity measured at test initiation; evaluated for survival daily
Test protocol	Environment Canada (2000), EPS 1/RM/13, with 2007, 2016, & 2023 amendments
Statistical software	None
Test endpoints	96-hour LC50
Test acceptability criteria for controls	Survival ≥ 90%
Reference toxicant	Potassium chloride (KCl)

Table 2. Summary of test conditions: 48-h *Daphnia magna* survival test.

Test species	<i>Daphnia magna</i>
Organism source	In-house culture
Organism age	<24 hours
Test type	Static
Test duration	48 hours
Test vessel	375 mL glass vessels
Test volume	150 mL
Test concentrations	Five concentrations, plus laboratory control
Test replicates	1 per treatment
Number of organisms	10 per replicate
Control/dilution water	De-chlorinated City of Calgary tap water amended with 4 mg/L KCl and with B12 (2 µg/L) and Na ₂ SeO ₄ (2 µg Se/L)
Test solution renewal	None
Test temperature	20 ± 2°C
Feeding	None
Light intensity	400 to 800 lux
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test measurements	pH, conductivity, dissolved oxygen, and temperature measured at test initiation and completion; salinity and hardness measured at test initiation in undiluted sample; evaluated daily for survival
Test protocol	Environment Canada (2000), EPS 1/RM/14 with February 2016 amendments
Statistical software	None
Test endpoints	48-h LC50
Test acceptability criteria for controls	Survival ≥ 90%
Reference toxicant	Sodium chloride (NaCl)

APPENDIX B – Toxicity test data

Method TRD Client ALS106 Reference 2324-2499 Chamber 3

Test Log

Day	Date	Time	Initial	Chem. Cart	Double Counted	Daily Data Review	Sample Information
0	2024-07-04	12:55 *	AC	1	MI	CC	Initial pH: <u>8.1</u> Initial EC (µS/cm): <u>3295</u> Salinity (ppt): <u>3</u>
1	2024-07-05	09:00	CC	-	-	CC	
2	2024-07-06	09:40	BH	-	-	CC	
3	2024-07-07	09:20	KE	-	-	CC	
4	2024-07-08	13:35	BS/BR	1	-	CC	

Note: *, time when the test was loaded with fish

Sample Pre-Aeration

Aeration rate adjusted to 0.5 +/- 1 mL/min/L yes no

Preaeration time	0 hours	0.5 hours	1 hour	1.5 hours	2 hours
DO(mg/L) of 100%	<u>8.6</u>	<u>8.6</u>			
temp (°C) of 100%	<u>16</u>				

DO in mg/L (70% - 100% saturation)**

6.2 mg/L - 8.9 mg/L at 14°C
6.1 mg/L - 8.8 mg/L at 15°C
6.0 mg/L - 8.6 mg/L at 16°C

**corrected for altitude

Test Chemistry and Biology

Conc.	6.25	12.5	25	50	100

pH (units) (range: 5.5-8.5)

Day 0	<u>8.0</u>	<u>8.0</u>	<u>8.0</u>	<u>7.9</u>	<u>7.8</u>
Day 4	<u>7.8</u>	<u>7.8</u>	<u>7.8</u>	<u>7.7</u>	<u>7.5</u>

EC (µS/cm)

Day 0	<u>490</u>	<u>654</u>	<u>964</u>	<u>1515</u>	<u>2660</u>
Day 4	<u>486</u>	<u>653</u>	<u>964</u>	<u>1483</u>	<u>2630</u>

DO (mg/L) (70-100% saturation at test temp.)

Day 0	<u>8.8</u>	<u>8.8</u>	<u>8.6</u>	<u>8.6</u>	<u>8.6</u>
Day 4	<u>8.8</u>	<u>8.8</u>	<u>8.8</u>	<u>8.8</u>	<u>8.8</u>

Temperature (°C) (range: 14-16°C)

Day 0	<u>15</u>	<u>15</u>	<u>16</u>	<u>16</u>	<u>16</u>
Day 4	<u>15</u>	<u>15</u>	<u>15</u>	<u>15</u>	<u>15</u>

Number Alive (In brackets number stressed)

	10	10	10	10	10
Day 0					
Day 1	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>
Day 2	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>
Day 3	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>
Day 4	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>

Unless otherwise noted, behavior is considered to be normal

Test Volume (L): 18

Control Reference Number: 20240704CTLB

Comments :

Reviewed By: AM

Date Reviewed: 2024-07-08

Control Trout Bench Sheet

Client NE Control Reference Number 20240704CTLB Chamber 3

Test Log

Day	Date	Time	Initial	Chem. Cart	Double Counted	Daily Data Review	Sample Reference Number(s):
0	2024-07-04	12:35 *	AC	1	MJ	ec	2324-2491-01
1	2024-07-05	09:00	ec	-	-	NO	2324-2491-02
2	2024-07-06	09:40	RA	-	-	km	2324-2491-03
3	2024-07-07	08:40	KT	-	-	ec	2324-2499
4	2024-07-08	13:25	BS/BO	1	-	ec	2324-2493

*Note: * time when the test was loaded with fish

Control Pre-Aeration

Aeration rate adjusted to 0.5 +/- 1 mL/min/L: yes / no

Test Chemistry and Biology

Conc. CTL

pH (units) (range: 5.5-8.5)
 Day 0 8.0
 Day 4 7.8

EC (uS/cm)
 Day 0 334
 Day 4 342

DO (mg/L) (70-100% saturation at test temp.)
 Day 0 8.8
 Day 4 8.8

Temperature (°C) (range: 14-16°C)
 Day 0 15
 Day 4 15

Number Alive (In brackets number stressed)
 Day 0 10
 Day 1 10
 Day 2 10
 Day 3 10
 Day 4 10

DO in mg/L (70% - 100% saturation)**
 6.2 mg/L - 8.9 mg/L at 14°C
 6.1 mg/L - 8.8 mg/L at 15°C
 6.0 mg/L - 8.6 mg/L at 16°C
 **corrected for altitude

Test Organism Information

Batch 20240516TR

Source Sam Livingston

Tank # Tank 2

Held at 15± 2°C for ≥14 days (must be ≥14 days) Y / N

Percent stock mortality 0
 (7 days prior to test, must be <2%)

Test Volume (L) 16

Acceptable Test Volume Ranges
 (10% of the control)

14 L control allows for 13 L - 15 L test(s)
 16 L control allows for 14 L - 18 L test(s)
 18 L control allows for 16 L - 20 L test(s)

Validity Criteria: must be ≤ 10% mortality and/or stressed behavior in the control
 Unless otherwise noted, behavior is considered to be normal

Control Organism Data

Control Fish	Length (cm)	Weight (g)
1	4.2	0.6
2	3.8	0.4
3	4.0	0.5
4	3.7	0.4
5	4.0	0.4
6	3.3	0.3
7	3.5	0.3
8	4.0	0.5
9	4.2	0.7
10	3.9	0.5

Loading Density (g/L): 0.3
 (must be ≤0.5 g/L)

Mean Length (cm): 3.9

Length Range (cm): 3.3-4.2

Mean Weight (g): 0.5
 (Must be ≥0.3g)

Weight Range (g): 0.3-0.7

Comments/Protocol Deviations:
none

Reviewed By: KO Date Reviewed: 2024-07-08

Method DAD Client ALS 106 Reference 2324-2499

Test Log

Day	Date	Time	Technician	Chem. Cart	Daily Data Review	Sample Information			
0	2024/07/04	1400	CC/LS	2	AM	Initial pH:	<u>8.1</u>		
1	2024/07/05	0904	AP	-	KO	Initial EC (µS/cm):	<u>3295</u>		
2	2024/07/06	1405-0920	AP	8	AP	Salinity (ppt):	<u>3</u>		
Lab Code	CTL	6.25	12.5	25	50	100			

day	pH (units) (range: 6.0-8.5)					
0	<u>8.1</u>	<u>8.1</u>	<u>8.1</u>	<u>8.0</u>	<u>7.9</u>	<u>7.8</u>
2	<u>8.3</u>	<u>8.3</u>	<u>8.3</u>	<u>8.2</u>	<u>8.1</u>	<u>7.9</u>

The pH of the sample was not adjusted prior to test setting, unless noted in the comments below

	EC (µS/cm)					
0	<u>391</u>	<u>582</u>	<u>745</u>	<u>1096</u>	<u>1805</u>	<u>3090</u>
2	<u>400</u>	<u>605</u>	<u>806</u>	<u>1197</u>	<u>2010</u>	<u>3387</u>

	DO (mg/L) (40-100% saturation at test temp.)					
0	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>
2	<u>7.8</u>	<u>7.7</u>	<u>7.8</u>	<u>7.8</u>	<u>7.8</u>	<u>7.8</u>

	Temperature (°C) (range: 18-22 °C)					
0	<u>20</u>	<u>20</u>	<u>20</u>	<u>20</u>	<u>20</u>	<u>20</u>
2	<u>20</u>	<u>20</u>	<u>20</u>	<u>20</u>	<u>20</u>	<u>20</u>

	Number Alive (I, immobile)					
0	<u>10</u>	10	10	10	10	10
1	<u>9</u>	10	10	10	10	10
2	<u>9</u>	10	10	10	10	10

Validity Criteria: must be ≤ 10% mortality and/or abnormal behavior in the control

Notes: Immobile; daphnid can't swim after 60 sec. even if antenna still move

Unless otherwise noted, behaviour is considered to be normal

Culture	Young jar <u>wed c4</u>	Jar(s) mortality 7 days prior to test (must be ≤25%) <u>0.1.</u>
QA (previous month)	Days to first brood (≤12 days) <u>9</u>	Control Validity Criteria
Average number of young produced (≥15 young)	<u>36</u>	Mean % mortality at 48 hours - (must be ≤10%) <u>0</u>
Were test treatments randomized on test tray?	<input checked="" type="radio"/> Yes / <input type="radio"/> No	
Sample	DO (mg/L) of sample prior to aeration: <u>7.9</u>	Temperature (°C) of sample prior to aeration: <u>20</u>
DO % of sample prior to aeration: <u>100%</u>	Is aeration required (<40% or >100%)?	Yes or <input checked="" type="radio"/> No
Duration of aeration (37.5 +/- 12.5 mL/min/L): <u>-</u>	Filtered with 110µm screen prior to testing	Yes or <input checked="" type="radio"/> No
Hardness (mg CaCO ₃ /L) of 100%: <u>1230</u>	Is hardness adjustment required (<25 mg CaCO ₃ /L)?	Yes or <input checked="" type="radio"/> No
Hardness of sample after adjustment (must be between 25 - 30 mg CaCO ₃ /L)	<u>-</u>	
Alkalinity of 100% sample (mg CaCO ₃ /L): <u>-</u>		
Dilution Water	Pail label / preparation date <u>PL106/28</u>	DO Levels (40-100% saturation) - corrected for altitude -
Hardness of dilution water (mg/L) <u>142</u>		3.3 to 8.2 mg/L at 18°C 3.1 to 7.7 mg/L at 21°C
		3.2 to 8.1 mg/L at 19°C 3.0 to 7.6 mg/L at 22°C
		3.2 to 7.9 mg/L at 20°C
Comments/Observations:	<u>★ missload</u>	

Reviewed By: AM Date Reviewed: 2024-07-08

APPENDIX C – Chain-of-custody form



Chain of Custody
 ALS Environmental - Yellowknife
 314 Old Airport Road, Unit 116
 Yellowknife NT Canada X1A
 3T3

191958



Destination Lab: **Nautilus Environmental (Calgary)**
 Address: 10828 27 Street SE Calgary AB Canada T2Z 3V9
 Work Order Number: **YL2400777**
 Original Receipt Date/Time: 02/07/2024 09:10
 Instructions Received



Relinquished By
 Date/Time
 Received By
 Date/Time
 Receipt Temp

Return as Indicated: Results: ALSYK.ClientServices@alsglobal.com Invoice: ALSYK.ClientServices@alsglobal.com Electronic Data: ALSYK.ClientServices@alsglobal.com
 Attention: Oliver Gregg

ALS Sample ID	Client ID	Matrix	Container Type	Test Codes	Method Description	Due Date	Sampling Date and Time	Remarks
YL2400777-001	PLPO_Acute_2 30628 240702 as per client work order	Water	LDPE carboy 2324-2499	TRT-SCR-96, DAP-SCR-48	Survival/Screening Rainbow Trout (96 hours), Survival/Screening Daphnia Magna 48 hours	09-07-2024	02/07/2024 08:13	
YL2400777-001	PLPO_Acute_2 30628	Water	LDPE carboy			09-07-2024	02/07/2024 08:13	

E-RUSH

2024/07/03
 14:49
 Buffalo Air
 JC
 2x20L carboys
 NoS/NoI
 Good Cond.
 12.8°C

END OF REPORT
