



Report of Analysis

812-CE / CL- EPD COMPLIANCE 2017/18

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MOE
BC
Lower Mainland

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Work Order: V18B061

Reported: 2018-03-07
Printed: 2018-03-07

Authorization:

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Manager, PYLET

The results reported pertain only to the samples submitted to and tested by the Environment and Climate Change Canada (ECCC) laboratory indicated in the report.

These ECCC laboratories are accredited by the Canadian Association for Laboratory Accreditation (CALA) to the standard ISO/IEC 17025 for each of the reported analytes, except where indicated by an asterisk (). Please refer to the CALA website (www.cala.ca) to view the full Scope(s) of Accreditation.*

ABSTRACT

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Unit	Description
% by Volume	Percent by volume

Qualifier	Description
NAL	Not Acutely Lethal
*	Non-Accredited Analysis/Analyte
ND	Not Detected at Reporting Limit (RL)
NR	Not Recoverable

SAMPLE DESCRIPTION

Lab ID	Client ID	Station ID	Matrix	Date/Time Sampled	Date Received	Sample Type
V18B061-01	OUTFALL SAMPLE SITE #1		Water	2018-02-21 00:00 Sampled By: Darren Sytewar	PAC 2018-02-23	Grab

REFERENCES

Method ID	Laboratory Method	Reference
V0503W	V_Microtox_IC50ML	EPS 1/RM/24
Toxicology Containers		Temperature °C

-brown: E219092

The fish were held at $15 \pm 2^\circ\text{C}$ for ≥ 2 weeks prior to the use in the test. Fish were not fed < 16 hours before the start of the test. Testing was started ≤ 5 days of sample collection. Tests were conducted in glass aquarium or plastic tanks lined with disposable polyethylene liners. Test solutions were randomly assigned to test locations. The height of the test solution was ≥ 15 cm. Aeration of oil-free compressed air was provided at a rate of 6.5 ± 1 ml/min.L through airstones. Photoperiod was 16 ± 1 hour light / 8 ± 1 hour dark with an intensity of 100-500 lux. All pH and conductivity measurements were performed using instruments that were automatically temperature compensated. Conductivity measurements are corrected for 25°C . Unless otherwise noted, all test conditions and validity criteria as specified by the Environment Canada test method were met.

pH Adjustment: Samples are normally tested without pH adjustment.

Test was performed as prescribed in the laboratory's Standard Operating Procedure and the Environment Canada biological test method, Report EPS 1/RM/13 Second Edition 2000, amended May 2007 and February 2016.

The control solutions were $< 14^{\circ}\text{C}$ at the start of the test. The requirement is $15 \pm 1^{\circ}\text{C}$.

TEST ORGANISM:	Species: Rainbow Trout	<i>Oncorhynchus mykiss</i>	Batch ID: 171227T6/rbt-fw	Tank #: 6
	Source: Aqua Farms		Mortality for 7d immediately preceding test: < 2 %	

Fork Length (cm)	Mean:	Min.:	Max.:	
Weight (g)	Mean:	Min.:	Max.:	Loading Density (g/L):

Start Date: 2018-02-23 Aeration Start Time: Pre-aeration Time (min): Test Start Time: 13:00

Canada

ACUTE LETHALITY TEST USING RAINBOW TROUT (96 HOUR SINGLE CONCENTRATION) - REPORT

LAB SAMPLE ID: V18B061-01

TEST CONDITIONS - CUMULATIVE MORTALITY / OBSERVATIONS [†]

Time Check	Concentration (%)					
	Control		Ctrl 9ppt salt		73	
	mortality	obs.	mortality	obs.	mortality	obs.
24 hr	0		0		0	
48 hr	0		0		0	
72 hr	0		0		0	
96 hr	0		0		0	

[†] Test observation Codes - EPS 1/RM/9 July 1990 amended May 1996 and 2007 Appendix E

** immediate stress, coughing & erratic swimming + behaviour cannot be observed **Integument:** *A* Shedding *B* Mucous *C* Hemorrhaging **Pigmentation:** *D* Light *E* Dark *F* Mottled

General behavior: *G* Quiescent *H* Hyperexcitable *I* Irritated *J* Surfacing *K* Sounding *L* Twitching *M* Tetanic *N* Normal *Δ* Moribund

Swimming: *O* Ceased *P* Erratic *Q* Gyrating *R* Skittering *S* Inverted *T* On side **Respiration:** *U* Rapid *V* Slow *W* Coughing *X* Surface *Z* Irregular

QUALITY CONTROL: Reference Toxicant Test

Test Date: 2018-02-23 96 hr (Static) LC50 = 8.6 mg/L 95% confidence limits: 8.0 & 9.3

Chemical: Phenol Geomean 96 hr LC50 = 10.7 mg/L 95% warning limits: 8.2 & 13.8 (n = 20)

ANALYSIS RESULTS:

Conc. (%)	Control	Ctrl 9ppt salt	73
96 hr Mortality (%)	0	0	0

96 hr (Static) Single Concentration = not acutely lethal @ 73% test concentration.

Where the single concentration (SC) is percent mortality of the test organisms held in a particular test solution over an exposure period of 96 hours.

RESULTS NOTES:

The test solution at 73 % has a salinity of 9.1 ppt.

LIQUID PHASE - TOXICITY TEST USING LUMINESCENT BACTERIA - *Vibrio fischeri* (5 & 15 MINUTE IC50) - REPORT

LAB SAMPLE ID: V18B061-01

Analyst: CB

DESCRIPTION OF SAMPLE:

- 250mL sub-sample from 4x20L carboys of orange liquid

DESCRIPTION OF TEST FACILITIES & CONDITIONS:

Testing was started \leq 72 hours of sample collection. Tests were conducted in glass cuvettes. Test instrument was the Microtox M500. Test results were calculated using Microtox Omni software. All physical chemical measurements were performed using instruments that were automatically temperature compensated where necessary. Unless otherwise noted, all test conditions and validity criteria as specified by the Environment Canada test method were met.

PROTOCOL USED:

Test was performed as prescribed in the lab Standard Operating Procedure and the Environment Canada biological test method, Toxicity Test Using Luminescent Bacteria, Report EPS 1/RM/24 November 1992.

TEST METHOD and/or SOP DEVIATIONS:

The test was conducted at $15 \pm 0.5^{\circ}\text{C}$.

ANALYSIS TYPE:

5 & 15 min IC50 Basic Test Turbidity correction: No Colour correction: No Test Equipment: Model 500 Analyzer

Test Volume: 1 mL Control Replicates: 1 Test Replicates: 1 Start date: 2018-02-27 Start time: 14:20 Test Temp. ($^{\circ}\text{C}$): 15 ± 0.5

TEST CONCENTRATIONS (%):

1. 0	2. 0.195	3. 0.391	4. 0.781	5. 1.563	6. 3.125	7. 6.25	8. 12.5	9. 25	10. 50
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CONTROL/DILUTION WATER TYPE and SOURCE:

Type: 2% NaCl Adjusted Source: DI Water

TEST ORGANISM:

Species: *Vibrio fischeri* Strain: NRRL B-11177 Source: Modern Water Reagent lot #: 17E4123A Receive date: 2017-10-31

Reagent expiry date: 2019-06-01 Reagent reconstitution time: 13:25 Test start time from reagent reconstitution (min): 55

SAMPLE ADJUSTMENTS:

Osmotic Adjustment: 2% NaCl by weight Source: Fisher Scientific (ACS)

QUALITY CONTROL: Reference Toxicant Test

Test Date: 2018-02-27 15 min (Static) IC50 = 20.4 mg/L 95% confidence limits: 15.3 & 27.2

Chemical: Phenol Geomean 15 min (Static) IC50 = 22.8 mg/L 95% warning limits: 16.8 & 30.8 (n = 20)

LIQUID PHASE - TOXICITY TEST USING LUMINESCENT BACTERIA - *Vibrio fischeri* (5 & 15 MINUTE IC50) - REPORT

LAB SAMPLE ID: V18B061-01

ANALYSIS RESULTS:

5 minute (Static) IC50 = not acutely toxic 95% confidence limits: &

Where the inhibition concentration (IC50) is the concentration of material in water that is calculated to cause a 50% decrease in light emission of the test organisms over an exposure period of 5 minutes.

15 minute (Static) IC50 = not acutely toxic 95% confidence limits: &

Where the inhibition concentration (IC50) is the concentration of material in water that is calculated to cause a 50% decrease in light emission of the test organisms over an exposure period of 15 minutes.

15 minute (Static) IC25 = n/a 95% confidence limits: - & -

Where the inhibition concentration (IC25) is the concentration of material in water that is calculated to cause a 25% decrease in light emission of the test organisms over an exposure period of 15 minutes.

The statistical method used to generate the results was the MicrotoxOmni Software for Windows® Version 1.18 95/98/NT: [Copyright by Azur Environmental 1999, issued by Strategic Diagnostics Inc. 2004].

RESULTS NOTES:

The sample was not acutely toxic to *Vibrio fischeri* (Microtox® bacteria) over 5 minutes of exposure.

The sample was not acutely toxic to *Vibrio fischeri* (Microtox® bacteria) over 15 minutes of exposure.

LIQUID PHASE - TOXICITY TEST USING LUMINESCENT BACTERIA - *Vibrio fischeri* (5 & 15 MINUTE IC50) - REPORT

LAB SAMPLE ID: V18B061-01

Microtox Report

Acute Liquid Phase Microtox Test Report

Date: - -

Test Protocol: Basic-type Test

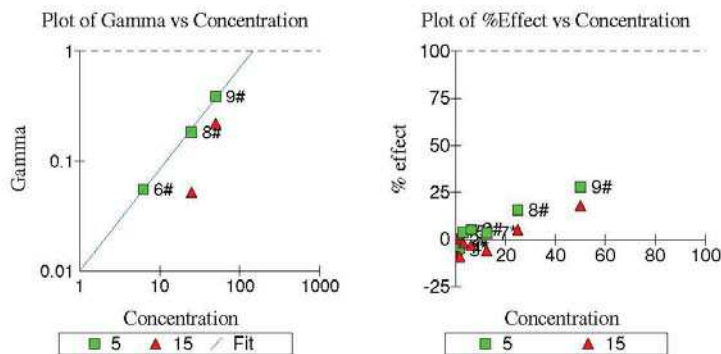
Sample: V18B061-01

Toxicant:

Reagent Lot no.:

Test description: Outfall Sample Site #1 E219092

Data File: V18B061-02 Outfall Sample Site #1 E219092.K5; V18B061-02 Outfall Sample Site #1 E219092.K15;



Sample	Conc	5 Mins Data:				15 Mins Data:			
		Io	It	Gamma	% effect	It	Gamma	% effect	
Control	0.000	92.23	85.76	0.9298 #		82.80	0.8978 #		
1	0.1953	85.64	82.84	-0.0387 *	-4.028%	83.88	-0.0834 *	-9.100%	
2	0.3906	87.66	81.79	-0.0034 *	-0.3428%	78.47	0.0028 *	0.2888%	
3	0.7813	84.92	83.50	-0.0543 *	-5.746%	82.85	-0.0798 *	-8.674%	
4	1.563	86.06	83.63	-0.0431 *	-4.508%	84.29	-0.0833 *	-9.098%	
5	3.125	90.27	80.80	0.0388 *	3.738%	82.55	-0.0182 *	-1.863%	
6	6.250	92.38	81.40	0.0552 #	5.238%	85.40	-0.0288 *	-2.973%	
7	12.50	86.94	78.02	0.0361 *	3.490%	82.67	-0.0558 *	-5.918%	
8	25.00	89.54	70.30	0.1843 #	15.56%	76.39	0.0523 #	4.970%	
9	50.00	89.95	60.34	0.3861 #	27.86%	66.14	0.2209 #	18.10%	

- used in calculation; * - invalid data; D - deleted from calcs.
Autocalc has been used.

Calculations on 5 Mins data:

IC50 Concentration:145.1% (95% confidence range: 33.72 to 623.9)

95% Confidence Factor: 4.301

IC50 value is greater than 100%

Estimating Equation:LOG C =1.078 x LOG G +2.162

Coeff. of Determination (R²):0.9972

Slope: 0.9254

Correction Factor: 0.9298

Calculations on 15 Mins data:

IC50 Concentration:103.4%

Calculated from two data points, therefore no confidence range given.

LIQUID PHASE - TOXICITY TEST USING LUMINESCENT BACTERIA - *Vibrio fischeri* (5 & 15 MINUTE IC50) - REPORT

LAB SAMPLE ID: V18B061-01

Microtox Report Con't

Estimating Equation: $\text{LOG } C = 0.4810 \times \text{LOG } G + 2.014$

Slope: 2.079

Correction Factor: 0.8978

Signature: _____

Printed: 06/03/6-18 03:20 PM

darren.stewart@gov.bc.ca

Laboratory / Laboratoire:

RYLET

[illegible]

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* send results to
- connor.fraleigh@gw.bc.ca
and
- darren.stewart@gw.bc.ca

Sample Type Code/Code du type d'échantillon

- | | |
|----|-------------------|
| # | ENVIRODAT |
| 01 | Discrete Sample |
| 03 | Duplicate Sample |
| 04 | Triplicate Sample |
| 06 | Composite Sample |
| 08 | Trp Blank |
| 09 | Matrix Spike |
| 11 | Pooled Sample |
| 22 | Field Blank |
| 28 | Trp Spike |
- | | |
|---------|--------------------------------|
| Element | Grab: Instantané |
| 01 | Discrete Sample |
| 03 | Duplicate: Duplicate |
| 04 | Triplicate: Triplicate |
| 06 | Composite: Composite |
| 08 | Trp Blank: Blanc transport |
| 09 | Matrix Spike: Matrice enrichie |
| 11 | Pooled: Mise en commun |
| 22 | Field Blank: Blanc terrain |
| 28 | Trp Spike: Enrichi transport |

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Sample Type Code/Code du type d'échantillon

- | | |
|----|-------------------|
| # | ENVIRODAT |
| 01 | Discrete Sample |
| 03 | Duplicate Sample |
| 04 | Triplicate Sample |
| 06 | Composite Sample |
| 08 | Trip Blank |
| 09 | Matrix Spike |
| 11 | Pooled Sample |
| 22 | Field Blank |
| 28 | Trip Spike |
- | | |
|---------|--------------------------------|
| Element | Grab::Instantané |
| | Duplicate::Duplicate |
| | Triplicate::Triplcate |
| | Composite::Composite |
| | Trip Blank::Blanc transport |
| | Matrix Spike::Matrice enrichie |
| | Pooled::Mise en commun |
| | Field Blank::Blanc terrain |
| | Trip Spike::Enrichi transport |

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