# ATTACHMENT A 2019 AEMP SAMPLING SCHEDULE

Table A-1 2019 AEMP Sampling Schedule

								Ice Co	over																		Open Wa	ter									
Station				Α	pril								May											Au	gust										Septembe	er	
	22	23	24	25	26	27 2	28	29	30	1	4	5		8	9	10	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29 30	31	1	2	3	4	5
NF1	An																							Anpsb													
NF2																An									Anpsb												
NF3		An																																	Anpsb		
NF4		An																							Anpsb												
NF5		An															Anpsb																				
MF1-1																An								Anpsb													
MF1-3						Α	n <sup>(c)</sup>																	Anpsb													
MF1-5						- 1	۸n																Anpsb <sup>(a)</sup>														
MF2-1				An																					Anpsb												1
MF2-3			An <sup>(d)</sup>																			Anpsb															1
FF2-2																An(b)						Anpsb															
FF2-5																An						Anpsb															1
MF3-1																An																			Anpsb		1
MF3-2									An																					Anpsb(c)							1
MF3-3															An															Anpsb							
MF3-4															An(b)														Anpsb								
MF3-5															An														Anpsb								
MF3-6										An																			Anpsb								
MF3-7										An																		Anpsb									
FF1-1											Mn								Mnpsb																		
FF1-2											Mn										Mnpsb																
FF1-3											Mn									Mnpsb																	
FF1-4											Mn <sup>(a)</sup>												Mnspb														1
FF1-5											Mn										Mnspb																
FFB-1													Mn															Mnpsb									
FFB-2													Mn														Mnpsb <sup>(a)</sup>										1
FFB-3													Mn															Mnpsb									
FFB-4												Mn(c)															Mnpsb	•									1
FFB-5												Mn															Mnpsb										1
FFA-1														Mn																						Mnpsb	
FFA-2														Mn																							Mnpsl
FFA-3														Mn																						Mnpsb	
FFA-4	1 1	t	<u> </u>					<u> </u>	<u> </u>	1		1		Mn																		1				Mnpsb	<b>†</b>
FFA-5														Mn																						Mnpsb	
LDS-1					Mn																													Mnp			
LDS-2	1 1	t	<u> </u>		Mn			<u> </u>	<u> </u>	1		1																				1		Mnp			<b>†</b>
LDS-3	1 1	t	<u> </u>					<u> </u>	<u> </u>	1		1				Mn																1		Mnp			<b>†</b>
LDS-4	1 1	1								t		1	1	1						1			1		1	1	1					1					Mn
LDG-48	1 1													Mn									1			1	1	l		1		Mn					<del></del>

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A = water quality surface, middle depth and bottom depth samples collected; M = mid-depth sample collected; n = nutrient sample collected; p = plankton sample collected; b = benthic invertebrate sample collected; QA/QC = quality assurance/quality control.

Note: QA/QC samples are colour coded: Grab Water (GW), Equipment Blank (EBW), Field Blank (FBW), Trip Blank (TBW), and Duplicate 1/Duplicate 2 (DUP1/DUP2).

NF = near-field; MF = mid-field; FF = far-field; LDG = Lac de Gras; LDS = Lac du Sauvage.

a) Field Blanks only collected for total ammonia samples.

b) Trip Blanks only collected for total ammonia samples.

c) Equipment Blanks only collected for total ammonia samples.

d) Duplicates only collected for total ammonia samples.

### **ATTACHMENT B**

# QUALITY ASSURANCE AND QUALITY CONTROL METHODS AND RESULTS

## QUALITY ASSURANCE AND QUALITY CONTROL METHODS AND RESULTS

#### Introduction

Quality assurance and quality control (QA/QC) practices determine data integrity and are relevant to all aspects of a study, from sample collection to data analysis and reporting. Quality assurance encompasses management and technical practices designed to generate consistent, high quality data. Quality control is an aspect of quality assurance and includes the techniques used to assess data quality and the corrective actions to be taken when the data quality objectives are not met. Details of the QA/QC practices applied during the Aquatic Effects Monitoring Program (AEMP) are described in the *Quality Assurance Project Plan (QAPP) Version 3.1* (Golder 2017a). This appendix describes QA/QC practices applied during the 2019 AEMP, evaluates quality control data, and describes the implications of QC results to the interpretation of study results.

#### **Quality Assurance**

#### Field Staff Training and Operations

Diavik Diamond Mines (2012) Inc. (DDMI) field staff are trained to be proficient in standardized field sampling procedures, data recording, and equipment operations applicable to water quality sampling. Field work was completed according to specified instructions and standard operating procedures (SOP) as follows:

- ENVI-923-0119 AEMP SOP Combined Open Water and Ice Cover
- ENVI-915-0119 SOP SNP Sampling
- ENVI-902-0119 SOP Quality Assurance Quality Control
- ENVI-900-0119 SOP Chain of Custody
- ENVI-903-0119 SOP Dissolved Oxygen Analysis
- ENVI-904-0119 SOP pH Analysis
- ENVI-906-0119 SOP Turbidity Analysis
- ENVI-904-0119 SOP Total Suspended Solids
- ENVI-918-0119 SOP Field Meter
- ENVI-684-0317 SOP YSI ProDSS

These SOPs include guidelines for field record-keeping and sample tracking, guidance for use and calibration of sampling equipment, relevant technical procedures, and sample labelling, shipping and tracking protocols.

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#### Laboratory Analyses

Samples were sent for analysis to Bureau Veritas Laboratories (BV Labs; formerly Maxxam Analytics), a laboratory accredited by the Canadian Association of Laboratory Accreditation (CALA). Duplicate samples for ammonia analysis were also sent to ALS Laboratories (ALS), another CALA accredited lab. Under the accreditation program, performance assessments are completed annually for laboratory procedures, analytical methods, and internal quality control.

In previous years, samples have been analyzed by BV Labs at the Burnaby, British Columbia (BC) location. However, during the ice-cover season, samples from 23 stations were analyzed at BV Labs in Burnaby and samples from the remaining 15 stations were analyzed by BV Labs at the Calgary, Alberta (AB) location, due to equipment issues at the Burnaby laboratory. All of the 2019 open-water samples were analyzed in Calgary. A lab comparison study was undertaken by BV Labs in 2019 as a result of multiple locations completing the analyses. The study is included in Annex A. Based on a review of the 2019 data, there was no indication that this change resulted in data quality issues for ammonia. The Calgary laboratory uses the same methods as the Burnaby laboratory, and has recently constructed a new clean lab. In addition, moving the analyses to the Calgary location had many benefits, including reducing risks of contamination due to subsampling and splitting of samples, improved/shorter shipping routes, which also resulted in shorter turnaround times, and reduced risk of missing hold times.

Quality assurance completed by the DDMI Environmental Sampling team encompasses all quality-related activities related to aquatic testing and analysis, and relevant technical support.

DDMI's quality assurance places an emphasis on four aspects:

- infrastructure (instruments, testing capabilities, calibrations, SOPs)
- control measures (internal/external)
- personnel (competence, ethics and integrity)
- data management

#### Field and Office Operations

A quality assurance system was established as an organized system of data control, analysis and filing. Relevant elements of this system are as follows:

- · pre-field meetings to discuss specific work instructions with field crews
- field crew check-in with task managers every 24 to 48 hours to report work completed during that period
- designating two crew members responsible for:
  - collecting all required samples
  - downloading and storing electronic data
  - completing chain-of-custody and analytical request forms; labelling and documentation
  - processing, where required, and delivering samples to analytical laboratory in a timely manner

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- cross-checking chain-of-custody forms and analysis request forms by the task manager to verify that the correct analysis packages had been requested
- review of field sheets by the task manager for completeness and accuracy
- reviewing laboratory data immediately after receipt from the analytical laboratory
- · creating backup files before data analysis
- completing appropriate logic checks and verifying accuracy of calculations

#### **Quality Control**

Quality control is a specific aspect of quality assurance and includes the techniques used to assess data quality and the remedial measures to be taken when the data quality objectives are not met. The field QC program included collection of field blanks, trip blanks, equipment blanks, and duplicate samples to assess potential sample contamination, and within-station variation (i.e., sampling precision). Quality control samples were submitted to BV Labs for analysis of the full list of variables, and to ALS for analysis of ammonia.

Field blanks consisted of samples prepared in the field using laboratory-provided de-ionized water to fill a set of sample bottles, which were then submitted to the appropriate laboratory for the same analyses as the original water samples. Trip blanks consisted of sample bottles filled with high-grade de-ionized water from the laboratory. They accompanied the other samples through sample collection, handling, shipping and analysis, but remained sealed. Equipment blanks consisted of de-ionized water exposed to all aspects of sample collection and analysis, using the same procedures used in the field, including contact with all sampling devices (i.e., beta bottle) and other equipment (i.e., filters, tubing). Equipment blanks provide information regarding potential cross-contamination between samples and contamination introduced by field equipment.

The field, trip and equipment blanks were used to detect potential sample contamination during collection, shipping and analysis. Although concentrations of all variables should be below their respective detection limit (DL) in these blanks, their concentrations were considered notable if they were greater than five times the corresponding DL. This threshold is based on the Practical Quantitation Limit defined by the United States Environmental Protection Agency (US EPA 1994, 2007; BC MOE 2009), which takes into account the potential for data accuracy errors when variable concentrations approach or are below DLs.

Notable results observed in the blanks were evaluated relative to concentrations observed in the lake-water samples to determine whether sample contamination was limited to the QC sample. If, based on this comparison, sample contamination was not isolated to the QC sample, the field data were flagged and further interpretation of results was made with this limitation in mind.

Duplicate samples consisted of two samples collected from the same location at the same time, using the same sampling and sample handling procedures. They were labelled and preserved individually and submitted separately to the analytical laboratory for identical analyses. Duplicate samples are used to check within-station variation and the precision of field sampling and analytical methods. Differences between concentrations measured in duplicate water samples were calculated as the relative percent difference

(RPD) for each variable. Before calculating the RPD, concentrations below the DL were replaced with 0.5 times the DL value. The RPD was calculated using the following formula:

RPD = (|difference in concentration between duplicate samples| / mean concentration) x 100

The RPD value for a given variable was considered notable if:

- it was greater than 40%; and
- concentrations in one or both samples were greater than or equal to five times the DL.

These criteria are similar to those used by BV Labs for internal QC of laboratory duplicate samples, and take into account the potential for data accuracy error as variable concentrations approach DLs.

The number of variables which exceeded the assessment criteria was compared to the total number of variables analyzed to evaluate analytical precision. The analytical precision was rated as follows:

- high, if less than 10% of the total number of variables were notably different from one another;
- moderate, if 10% to 30% of the total number of variables were notably different from one another; and
- low, if more than 30% of the total number of variables were notably different from one another.

#### **Quality Control Results**

#### **Detection Limits**

Water quality samples were submitted to BV Labs, an accredited analytical laboratory, for analyses of variables (e.g., major ions, nutrients, metals) in water samples. BV Labs has a dedicated inductively coupled plasma-mass spectrometer (ICP-MS) specifically for ultra-low trace metal analysis. The ultra-low analytical DLs can only be obtained on water samples with very low particulate matter (i.e., turbidity less than 0.5 nephelometric turbidity unit [NTU]).

BV Labs used analyte-specific DLs to report results for water quality variables analyzed in 2019. The DLs used by BV Labs in 2019 are listed in Tables B-1 and B-2 (see also Section 2.2, Table 2-2 of the 2019 *Effluent and Water Chemistry Report* [Appendix II]). Deviations from the target DLs and a discussion of potential effects on data quality are as follows:

- The DL for total organic carbon (0.2 mg/L) was raised to 0.5 mg/L in 13 samples from the ice-cover season due to an incorrect lab test code being assigned during sample log-in at the Calgary laboratory. The error was corrected for the open-water season. Use of the elevated DL does not affect data quality, because concentrations in all 13 samples were greater than the DL.
- The DL for total dissolved solids, measured (1.0 mg/L) was raised in 16 samples (12 to 1.1 mg/L, three to 1.2 mg/L, and one to 1.3 mg/L) due to insufficient sample volume. Use of the elevated DLs does not affect data quality, because concentrations in all 16 samples were greater than the DL.
- Similar to previous years, sulphate was analyzed at a DL of 0.5 mg/L (versus a requested DL of 0.05 mg/L) due to limitations of the current analytical method. BV Labs is currently investigating ways to provide the requested DL. In 2019, samples that were less than the DL for the ICP-MS method (i.e., DL

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of 0.5 mg/L) were reanalyzed using inductively coupled plasma atomic emission spectroscopy (ICP-OES) with a DL of 0.05 mg/L. Only QC blanks results were less than the DL. As a result, use of the elevated DL does not affect data quality.

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- The DLs for nitrate + nitrite were elevated above the requested values (i.e., 2 μg-N/L requested and 2.2 μg-N/L reported) in 122 samples (i.e., 37 in ice-cover and 85 in open-water) due to an issue with the calculation used for the DL. BV Labs is currently investigating ways to adjust this calculation. Use of the elevated DL is not expected to affect data quality because the majority of the samples were greater than the DL and the elevated DL is close to the requested DL.
- The DL for total Kjeldahl nitrogen and total nitrogen (i.e., 20 μg-N/L) was raised to 200 μg-N/L in one sample (MF2-1T) due to insufficient sample volume and sample matrix interference, respectively.
- The DLs for total dissolved nitrogen were elevated above the requested values (i.e., 20 μg-N/L requested and 55 μg-N/L reported) in 31 samples from the ice-cover season due to an incorrect lab test code being assigned during sample log-in at the Calgary laboratory. The error was corrected for the open-water samples. Use of the elevated DL is not expected to affect data quality, because concentrations in the majority of the samples were greater than the DL with the exception of two QC samples.
- The DLs for total and dissolved sulphur were elevated above the requested values (i.e., 0.1 mg/L requested and 0.5 mg/L reported) due to limitations of the current analytical method. BV Labs is currently investigating ways to provide the requested DL. In 2019, samples that were less than the DL for the ICP-MS method (i.e., DL of 0.5 mg/L) were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-OES) with a DL of 0.1 mg/L. Only QC blanks and LDS stations were less than the DL. As a result, use of the elevated DL does not affect data quality.

#### Blank Samples

Of the 93 variables analyzed during the ice-cover season, six variables (i.e., dissolved calcium, nitrate, nitrate + nitrite, total dissolved nitrogen, total zinc, and dissolved copper) were measured in QC blank samples at a concentration above the data quality objective (DQO) of less than five times the DL (Table B-1). Details of the ice-cover blank sample DQO exceedances are as follows:

- Dissolved calcium, nitrate, and nitrate + nitrite exceeded the DQO in the field blank sample collected at MF3-1B.
- Total dissolved nitrogen exceeded the DQO in the equipment blank prepared at NF2B. Further details
  on QC issues relating to nitrogen variables are provided in the QA/QC Attachment of the Eutrophication
  Indicators Report (Appendix XIII).
- Total zinc and dissolved copper exceeded the DQO in the equipment blank at MF3-3B.

Exceedances of the DQO occurred in 1.3% of the ice-cover blank sample results and, therefore, the blank results indicated acceptable data quality. Overall, concentrations of dissolved calcium reported in the blank samples were well below those measured in the lake-water samples, whereas concentrations of nitrate, nitrate + nitrite, total dissolved nitrogen, total zinc, and dissolved copper were similar to those measured in the lake-water samples. Nitrogen variables are evaluated in the QA/QC Attachment of the *Eutrophication Indicators Report* (Appendix XIII). The potential contamination identified for copper and zinc was relatively

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minor and did not interfere with the determination of Action Levels, as concentrations in the NF area were below reference conditions for Lac de Gras despite potential contamination identified in blank samples.

During the open-water season, the concentrations of two (i.e., soluble reactive phosphorus and total aluminum) of the 93 variables measured in QC blanks was greater than five times the DL (Table B-1). Details of the open-water blank sample DQO exceedances are as follows:

- Soluble reactive phosphorus exceeded the DQO in the travel blank assigned to FFA-3M.
- Total aluminum exceeded the DQO in one field blank collected at MF3-7B.

Exceedances of the DQO occurred in 0.4% of the open-water blank sample results and, therefore, the blank results indicated acceptable data quality. The concentration of soluble reactive phosphorus reported in the blank sample was above the majority of concentrations measured in the lake-water samples, whereas total aluminum was below the majority of concentrations measured in the lake-water samples. Phosphorus variables are evaluated in the QA/QC Attachment of the *Eutrophication Indicators Report* (Appendix XIII). An Action Level was triggered for total aluminum similar to previous years. The potential contamination identified for aluminum was relatively minor and did not interfere with the determination of Action Levels.

As the DQO exceedances for these parameters were infrequent, it is unlikely that the contamination found in blank samples affected the reliability of the data used in the AEMP Effluent and Water Chemistry Report.

#### **Field Duplicate Samples**

A total of 9 out of 93 water quality variables analyzed in 2019 exceeded the DQO of both the 40% RPD and five times DL criteria for field duplicate samples at least once (Table B-2). These variables included ammonia, total dissolved nitrogen, soluble reactive phosphorus, total aluminum, total zinc, dissolved aluminum, dissolved beryllium, dissolved copper, and dissolved zinc. In total, 2.5% of field duplicate data assessed in the duplicate comparison exceeded the DQO, which indicates a high level of analytical precision for the 2019 samples. These results were considered notable, because the differences in concentrations between duplicate samples for these analytes (i.e., RPD of 40% to 172%) were appreciably greater than the QC objectives used by BV Labs to identify unacceptable differences between laboratory duplicate samples (i.e., RPD of 20% to 25%). Laboratory duplicates consist of two independently analyzed portions of the same sample and would, therefore, be expected to have lower variability among paired duplicate samples than field duplicates, which consist of two separate grab samples.

Overall, duplicate sample results indicated that data were of acceptable quality. Generally, concentrations in duplicate samples with DQO exceedances were within the range of values reported at other nearby AEMP stations, indicating that the QC issues identified with these variables did not likely interfere with the evaluation of Mine-related effects.

Table B-1 Blank Sample Results, 2019

					Ice-Cover						Open-	-Water			
			NF2B-1	MF1-1T-3	MF3-1B-2	MF3-3B-1	FF1-3M-2	NF1T-2	MF1-5T-2	MF2-3B-1	MF3-1M-3	MF3-2B-1	MF3-7B-2	FFB-2M-2	FFA-3M-3
Parameter	Unit	DL	10-May-19	10-May-19	10-May-19	9-May-19	4-May-19	22-Aug-19	21-Aug-19	20-Aug-19	3-Sep-19	28-Aug-19	26-Aug-19	25-Aug-19	4-Sep-19
			Equipment Blank	Travel Blank	Field Blank	Equipment Blank	Field Blank	Field Blank	Field Blank	Equipment Blank	Travel Blank	Equipment Blank	Field Blank	Field Blank	Travel Blank
Conventional Parameters			•	•									•		•
Total alkalinity as CaCO <sub>3</sub>	mg/L	0.5	<0.5	<0.5	<0.5	<0.5	0.54	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
Specific conductivity	μS/cm	1	<1	<1	<1	<1	1.1	<1	-	<1	<1	-	<1	-	<1
Total hardness as CaCO <sub>3</sub>	mg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
pH - lab	-	-	4.59	4.67	4.95	4.62	5.63	4.52	-	4.48	4.53	-	4.43	-	4.43
Total dissolved solids, calculated	mg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.6	-	<0.5	<0.5	-	<0.5	-	<0.5
Total dissolved solids, measured	mg/L	1	2.4	1.2	4.4	1.6	4.3	<1	-	<1	<1	-	<1	-	<1
Total suspended solids	mg/L	1	<1	<1	1.1	<1	<1	<1	-	<1	<1	-	<1	-	<1
Total organic carbon	mg/L	0.2	0.21	<0.2	<0.2	<0.2	<0.2	0.23	-	0.28	<0.2	-	0.27	-	0.3
Turbidity - lab	NTU	0.1	<0.1	<0.1	<0.1	<0.1	0.11	0.14	-	<0.1	<0.1	-	<0.1	-	<0.1
Major Ions															
Bicarbonate	mg/L	0.5	<0.5	<0.5	<0.5	<0.5	0.66	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
Calcium (dissolved)	mg/L	0.01	0.011	<0.01	0.055	<0.01	<0.01	0.012	-	<0.01	<0.01	-	<0.01	-	<0.01
Carbonate	mg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
Chloride	mg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.55	-	<0.5	<0.5	-	<0.5	-	<0.5
Fluoride	mg/L	0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.011	-	<0.01	<0.01	-	<0.01	-	<0.01
Hydroxide	mg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
Magnesium (dissolved)	mg/L	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	-	<0.005
Potassium (dissolved)	mg/L	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	-	<0.01
Sodium (dissolved)	mg/L	0.01	<0.01	<0.01	0.012	0.013	<0.01	<0.01	-	0.023	<0.01	-	<0.01	-	<0.01
Sulphate	mg/L	0.5	<0.05	<0.05	<0.05	0.18	<0.05	<0.05	-	0.077	<0.05	-	<0.05	-	<0.05
Nutrients															
Ammonia <sup>(a)</sup>	μg-N/L	5	<5	5.2	11.1	<5	<5	<5	(b)	<5	<5	(b)	<5	(b)	<5
Nitrate	μg-N/L	2	<2	<2	18	3.7	<2	<2	-	<2	<2	-	<2	-	<2
Nitrite	μg-N/L	1	<1	<1	<1	<1	<1	<1	-	<1	<1	-	<1	-	<1
Nitrate + nitrite	μg-N/L	2	<2.2	<2.2	18	3.7	<2	<2	-	<2	<2	-	<2	-	<2
Total Kjeldahl nitrogen	μg-N/L	20	<20	24	23	<20	58	57	-	62	54	-	53	-	46
Total dissolved nitrogen	μg-N/L	20	130	<55	37	<55	28	<20	-	<20	<20	-	<20	-	<20
Total nitrogen	μg-N/L	20	<20	36	23	<20	58	57	-	53	54	-	53	-	46
Soluble reactive phosphorus	μg-P/L	1	<1	<1	<1	<1	<1	2.3	-	<1	1.6	-	<1	-	7.3
Total dissolved phosphorus	μg-P/L	2	<2	<2	<2	<2	<2	<2	-	2.2	<2	-	<2	-	<2
Total phosphorus	μg-P/L	2	<2	<2	<2	<2	<2	<2	-	<2	<2	-	<2	-	<2
Total Metals															
Aluminum	μg/L	0.2	0.43	0.52	0.64	0.95	0.57	<0.2	-	<0.2	<0.2	-	1.75	-	<0.2
Antimony	μg/L	0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	-	<0.02
Arsenic	μg/L	0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	-	<0.02
Barium	μg/L	0.02	0.055	<0.02	<0.02	<0.02	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	-	<0.02
Beryllium	μg/L	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	-	<0.01
Bismuth	μg/L	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	-	<0.005
Boron	μg/L	5	<5	<5	<5	<5	<5	<5	-	<5	<5	-	<5	-	<5
Cadmium	μg/L	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	-	<0.005
Calcium	mg/L	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	-	<0.01

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Table B-1 Blank Sample Results, 2019 (continued)

Table B-1 Blank	Sample Results	5, 2019 (C	Jillilueu)												
					Ice-Cover						Open	-Water			
			NF2B-1	MF1-1T-3	MF3-1B-2	MF3-3B-1	FF1-3M-2	NF1T-2	MF1-5T-2	MF2-3B-1	MF3-1M-3	MF3-2B-1	MF3-7B-2	FFB-2M-2	FFA-3M-3
Parameter	Unit	DL	10-May-19	10-May-19	10-May-19	9-May-19	4-May-19	22-Aug-19	21-Aug-19	20-Aug-19	3-Sep-19	28-Aug-19	26-Aug-19	25-Aug-19	4-Sep-19
			Equipment Blank	Travel Blank	Field Blank	Equipment Blank	Field Blank	Field Blank	Field Blank	Equipment Blank	Travel Blank	Equipment Blank	Field Blank	Field Blank	Travel Blank
Chromium	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	< 0.05	-	< 0.05	<0.05	-	<0.05	-	<0.05
Cobalt	μg/L	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	-	<0.005
Copper	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-	<0.05	<0.05	-	< 0.05	-	<0.05
Iron	μg/L	1	<1	<1	<1	<1	<1	<1	-	<1	<1	-	<1	-	<1
Lead	μg/L	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	-	0.006
Lithium	μg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
Magnesium	mg/L	0.005	<0.05	<0.05	<0.05	<0.05	<0.05	< 0.05	-	< 0.05	<0.05	-	<0.05	-	<0.05
Manganese	μg/L	0.05	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	< 0.005	-	< 0.005
Mercury	μg/L	0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	-	<0.002
Molybdenum	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-	<0.05	<0.05	-	< 0.05	-	<0.05
Nickel	μg/L	0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	-	<0.02
Potassium	mg/L	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	-	<0.01
Selenium	μg/L	0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	-	<0.04
Silicon	μg/L	50	51	<50	<50	63	<50	<50	-	<50	<50	-	<50	-	<50
Silver	μg/L	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	-	<0.005
Sodium	mg/L	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	0.017	-	<0.01	-	0.02
Strontium	μg/L	0.05	0.104	<0.05	<0.05	<0.05	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	-	<0.05
Sulphur	mg/L	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	-	<0.1	<0.1	-	<0.1	-	<0.1
Thallium	μg/L	0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	-	<0.002
Tin	μg/L	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	-	<0.01
Titanium	μg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
Uranium	μg/L	0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	-	<0.002
Vanadium	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	-	<0.05
Zinc	μg/L	0.1	0.1	<0.1	<0.1	0.53	<0.1	<0.1	-	<0.1	<0.1	-	0.41	-	<0.1
Zirconium	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	< 0.05	-	< 0.05	<0.05	-	<0.05	-	<0.05
Dissolved Metals	·														
Aluminum	μg/L	0.2	<0.2	<0.2	0.29	<0.2	<0.2	0.62	-	<0.2	<0.2	-	<0.2	-	0.23
Antimony	μg/L	0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	-	<0.02
Arsenic	μg/L	0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	-	<0.02
Barium	µg/L	0.02	<0.02	<0.02	0.057	<0.02	<0.02	<0.02	-	0.033	<0.02	-	<0.02	-	<0.02
Beryllium	μg/L	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	-	<0.01
Bismuth	µg/L	0.005	<0.005	<0.005	<0.005	< 0.005	<0.005	<0.005	-	< 0.005	< 0.005	-	< 0.005	-	<0.005
Boron	μg/L	5	<5	<5	<5	<5	<5	<5	-	<5	<5	-	<5	-	<5
Cadmium	µg/L	0.005	<0.005	<0.005	<0.005	< 0.005	<0.005	<0.005	-	< 0.005	< 0.005	-	< 0.005	-	<0.005
Chromium	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	-	<0.05
Cobalt	µg/L	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	-	<0.005
Copper	µg/L	0.05	<0.05	<0.05	<0.05	0.327	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	-	<0.05
Iron	µg/L	1	<1	<1	<1	<1	<1	<1	-	<1	<1	-	<1	-	<1
Lead	μg/L	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	<0.005	<0.005	-	0.0052	-	<0.005
Lithium	μg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
Manganese	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	-	<0.05
Mercury	μg/L	0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	-	<0.002
Molybdenum	µg/L	0.05	0.056	<0.05	<0.05	<0.05	<0.05	<0.05	_	<0.05	<0.05	-	<0.05	_	<0.05

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Table B-1 Blank Sample Results, 2019 (continued)

					Ice-Cover						Open-	Water			
			NF2B-1	MF1-1T-3	MF3-1B-2	MF3-3B-1	FF1-3M-2	NF1T-2	MF1-5T-2	MF2-3B-1	MF3-1M-3	MF3-2B-1	MF3-7B-2	FFB-2M-2	FFA-3M-3
Parameter	Unit	DL	10-May-19	10-May-19	10-May-19	9-May-19	4-May-19	22-Aug-19	21-Aug-19	20-Aug-19	3-Sep-19	28-Aug-19	26-Aug-19	25-Aug-19	4-Sep-19
			Equipment Blank	Travel Blank	Field Blank	Equipment Blank	Field Blank	Field Blank	Field Blank	Equipment Blank	Travel Blank	Equipment Blank	Field Blank	Field Blank	Travel Blank
Nickel	μg/L	0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.031	-	<0.02	<0.02	-	<0.02	-	<0.02
Selenium	μg/L	0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	-	<0.04
Silicon	μg/L	50	54	53	<50	50	<50	<50	-	<50	<50	-	<50	-	<50
Silver	μg/L	0.005	<0.005	<0.005	<0.005	<0.005	< 0.005	<0.005	-	<0.005	<0.005	-	<0.005	-	< 0.005
Strontium	μg/L	0.05	<0.05	<0.05	0.057	<0.05	<0.05	< 0.05	-	0.062	<0.05	-	<0.05	-	<0.05
Sulphur	mg/L	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	-	<0.1	<0.1	-	<0.1	-	<0.1
Thallium	μg/L	0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	-	<0.002
Tin	μg/L	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	-	<0.01
Titanium	μg/L	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	-	<0.5
Uranium	μg/L	0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	-	<0.002
Vanadium	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	< 0.05	-	<0.05	<0.05	-	<0.05	-	<0.05
Zinc	μg/L	0.1	<0.1	<0.1	0.13	<0.1	<0.1	<0.1	-	<0.1	<0.1	-	0.11	-	<0.1
Zirconium	μg/L	0.05	<0.05	<0.05	<0.05	<0.05	< 0.05	< 0.05	-	< 0.05	<0.05	-	<0.05	-	< 0.05

a) Based on the results of the ammonia investigation, the ALS ammonia dataset was used in the ice-cover season and the BV ammonia dataset was used in the open-water season (see the "Ammonia Investigation" section below).

b) Results were removed due to laboratory quality control issues identified in 2019 (see the "Ammonia Investigation" section below).

Note: Bold values represent an exceedance of the data quality objective for blank samples (concentration greater than 5 times the DL).

NTU = nephelometric turbidity units;  $\mu$ g-N/L = micrograms nitrogen per litre;  $\mu$ g-P/L = micrograms phosphorus per litre;  $\mu$ S/cm = microsiemens per centimetre; DL = detection limit; CaCO<sub>3</sub> = calcium carbonate; NF = near-field; MF = mid-field; FF = far-field.

Table B-2 Duplicate Sample Results, 2019

			N	F3B		MF	3-5T		FFA	<b>A-5M</b>		NF	F5M		MF1	-1B-4		FF1-	1M-4	
Parameter	Unit	MDL	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD
			23-Apr-19	23-Apr-19		9-May-19	9-May-19		8-May-19	8-May-19		15-Aug-19	15-Aug-19		22-Aug-19	22-Aug-19		17-Aug-19	17-Aug-19	
Conventional Parameters		•					•	•						•						
Total Alkalinity	mg/L	0.5	6.99	6.57	6.2%	5.5	7.52	31.0%	4.21	4.24	0.7%	5.23	4.6	12.8%	5.52	5.17	6.5%	5.32	5.01	6.0%
Specific Conductivity - lab	μS/cm	1	57	57	0.0%	36.5	36.1	1.1%	27.9	28.1	0.7%	36.4	35.7	1.9%	35.1	35.1	0.0%	26.3	26.3	0.0%
Total Hardness as CaCO3	mg/L	0.5	13.9	13.5	2.9%	10.3	10.2	1.0%	7.82	7.78	0.5%	9.44	9.38	0.6%	9.36	9.26	1.1%	7.5	7.45	0.7%
pH	-	-	6.71	6.73	4.6%	6.69	6.86	38.7%	6.5	6.57	16.1%	6.76	6.86	22.9%	5.89	5.82	16.1%	5.79	5.68	25.2%
Total Dissolved Solids, Calculated	mg/L	0.5	27	27	0.0%	18	19	5.4%	14	14	0.0%	17.3	17.2	0.6%	17.9	17.1	4.6%	13.4	13.1	2.3%
Total Dissolved Solids, Measured	mg/L	1	49.2	53.5	8.4%	21.2	25.2	17.2%	20.8	17.2	18.9%	23.6	24	1.7%	24.8	25.6	3.2%	17.2	16.8	2.4%
Total Suspended Solids	mg/L	1	1	<1	-	<1	<1	-	<1	<1	-	1.1	2.7	-	<1	<1	-	1.1	1.1	-
Total Organic Carbon	mg/L	0.2	2	1.9	5.1%	2.8	2.8	0.0%	1.5	1.7	12.5%	2.3	2.3	0.0%	2.3	2.5	8.3%	2.2	2.1	4.7%
Turbidity	NTU	0.1	0.22	0.15	-	<0.1	<0.1	-	<0.1	<0.1	-	0.2	0.5	-	0.22	0.17	-	0.4	0.35	-
Major Ions	•	•	•	•		•	•	•	•	•		•	•	•	•	•	•	•	•	
Bicarbonate	mg/L	0.5	8.53	8.02	6.2%	6.71	9.17	31.0%	5.14	5.17	0.6%	6.38	5.61	12.8%	6.73	6.3	6.6%	6.49	6.11	6.0%
Calcium	mg/L	0.01	3.05	3.01	1.3%	1.94	1.95	0.5%	1.57	1.62	3.1%	2.1	2.52	18.2%	2.27	2.03	11.2%	1.62	1.58	2.5%
Carbonate	mg/L	0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-
Chloride	mg/L	0.5	7.4	7.49	1.2%	3.1	3.1	0.0%	2.6	2.6	0.0%	3.6	3.4	5.7%	3.5	3.5	0.0%	2	1.9	-
Fluoride	mg/L	0.01	0.036	0.034	-	0.036	0.036	-	0.03	0.03	-	0.034	0.03	-	0.031	0.032	-	0.032	0.028	-
Hydroxide	mg/L	0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-
Magnesium	mg/L	0.005	1.4	1.38	1.4%	1.21	1.23	1.6%	0.977	0.994	1.7%	1.04	1.09	4.7%	1.08	1.05	2.8%	0.895	0.919	2.6%
Potassium	mg/L	0.01	1.16	1.15	0.9%	1.1	1.11	0.9%	0.869	0.888	2.2%	0.974	1.03	5.6%	0.997	0.984	1.3%	0.782	0.791	1.1%
Sodium	mg/L	0.01	4.25	4.25	0.0%	2.07	2.09	1.0%	1.67	1.68	0.6%	2.2	2.14	2.8%	2.38	2.35	1.3%	1.44	1.44	0.0%
Sulphate	mg/L	0.05	4.89	5.52	12.1%	5.1	5	2.0%	3.8	3.8	0.0%	4.1	4.1	0.0%	4.2	4	4.9%	3.4	3.4	0.0%
Nutrients	•	•	•	•		•	•	•	•	•		•	•	•	•	•	•	•	•	
Ammonia <sup>(a)</sup>	μg-N/L	5	47.3	32.3	37.7%	18.7	17.7	-	25.8	16	46.9%	16	12	-	8.4	11	-	22	7	-
Nitrate	μg-N/L	2	133	136	2.2%	<2	<2	-	5.3	6	-	17	13	26.7%	12	12	0.0%	<2	<2	-
Nitrite	μg-N/L	1	<1	<1	-	<1	<1	-	1.4	1.1	-	1.5	1.4	-	<1	<1	-	<1	<1	-
Nitrate + nitrite	μg-N/L	2	133	136	2.2%	<2.2	<2.2	-	6.8	7.1	-	18	14	25.0%	12	12	0.0%	<2	<2	-
Total Kjeldahl Nitrogen	μg-N/L	20	174	200	13.9%	200	200	0.0%	150	150	0.0%	250	260	3.9%	220	210	4.7%	170	200	16.2%
Total Dissolved Nitrogen	μg-N/L	20	280	288	2.8%	190	200	5.1%	130	280	73.2%	180	200	10.5%	300	200	40.0%	150	160	6.5%
Total Nitrogen	μg-N/L	20	309	339	9.3%	240	220	8.7%	190	170	11.1%	270	270	0.0%	230	220	4.4%	170	200	16.2%
Soluble Reactive Phosphorus	μg-P/L	1	1.2	2.1	-	<1	<1	-	<1	<1	-	1.3	<1	-	1.8	2	-	2.4	5.6	80.0%
Total Dissolved Phosphorous	μg-P/L	2	<2	<2	-	<2	<2	-	<2	<2	-	<2	<2	-	<2	<2	-	<2	<2	-
Total Phosphorous	μg-P/L	2	2.4	2.1	-	<2	<2		<2	<2	-	2.2	<2	_	<2	<2	-	<2	<2	
Total Metals																				
Aluminum	μg/L	0.2	8.63	8.09	6.5%	1.91	1.85	3.2%	1.76	1.81	2.8%	2.55	4.08	46.2%	1.45	1.22	17.2%	1.4	2.39	52.2%
Antimony	μg/L	0.02	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	<0.02	-
Arsenic	μg/L	0.02	0.307	0.3	2.3%	0.17	0.177	4.0%	0.05	0.052	-	0.22	0.196	11.5%	0.277	0.266	4.1%	0.214	0.195	9.3%
Barium	μg/L	0.02	5.01	4.99	0.4%	2.76	2.75	0.4%	2.13	2.14	0.5%	2.89	2.4	18.5%	2.37	2.32	2.1%	1.86	1.78	4.4%
			1	1	1	1			1	1		1	1	1	1	1	<u> </u>	1	1	

Table B-2 Duplicate Sample Results, 2019 (continued)

		NF	3B		MF	3-5T		FF.A	\-5M		NF	5M		MF1	-1B-4		FF1-	1M-4	
Unit	MDL	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD
		23-Apr-19	23-Apr-19		9-May-19	9-May-19		8-May-19	8-May-19		15-Aug-19	15-Aug-19		22-Aug-19	22-Aug-19		17-Aug-19	17-Aug-19	<u> </u>
μg/L	0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	<0.01	-
μg/L	0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-
μg/L	5	<5	<5	-	<5	<5	-	<5	<5	-	15.3	<5	-	<5	<5	-	<5	<5	-
μg/L	0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-
μg/L	10	3130	3090	1.3%	2010	2020	0.5%	1510	1540	2.0%	2060	2040	1.0%	2020	2020	0.0%	1550	1560	0.6%
μg/L	0.05	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	0.078	0.065	-	<0.05	<0.05	-	<0.05	<0.05	-
μg/L	0.005	0.014	0.014	-	0.012	0.012	-	0.013	0.012	-	0.0115	0.0123	-	0.0158	0.0116	-	0.0084	0.0084	-
μg/L	0.05	0.55	0.533	3.1%	0.645	0.642	0.5%	0.522	0.515	1.4%	0.5	0.564	12.0%	0.548	0.554	1.1%	0.515	0.573	10.7%
μg/L	1	2.2	1.9	-	<1	<1	-	<1	<1	-	2.6	2.4	-	2.6	3.5	-	2.5	2.3	-
μg/L	0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-
μg/L	0.5	2.36	2.34	-	2.47	2.43	-	1.57	1.51	-	2.19	1.09	-	<0.5	<0.5	-	0.72	<0.5	-
μg/L	5	3940	3620	8.5%	505	511	1.2%	678	684	0.9%	2360	2380	0.8%	2360	2600	9.7%	2420	2670	9.8%
μg/L	0.05	1.47	1.39	5.6%	1.28	1.26	1.6%	0.982	0.959	2.4%	1.04	1.04	0.0%	1.05	1.03	1.9%	0.882	0.866	1.8%
μg/L	0.002	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	<0.002	-	0.0036	0.0031	-
μg/L	0.05	1.1	1.08	1.8%	0.313	0.313	0.0%	0.232	0.236	-	0.576	0.583	1.2%	0.438	0.596	30.6%	0.12	0.112	-
μg/L	0.02	0.758	0.764	0.8%	1.08	1.15	6.3%	0.935	0.923	1.3%	0.554	0.566	2.1%	0.623	0.622	0.2%	0.611	0.593	3.0%
μg/L	10	1180	1150	2.6%	1120	1150	2.6%	851	858	0.8%	975	966	0.9%	959	972	1.3%	786	781	0.6%
μg/L	0.04	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	<0.04	-
μg/L	50	279	285	2.1%	<50	<50	-	<50	<50	-	<50	<50	-	<50	<50	-	<50	<50	-
μg/L	0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-
μg/L	10	4340	4240	2.3%	2100	2140	1.9%	1630	1640	0.6%	2190	2150	1.8%	2350	2330	0.9%	1430	1430	0.0%
μg/L	0.05	50.3	49.4	1.8%	19.3	19.6	1.5%	14.4	14.4	0.0%	23.6	24.1	2.1%	25.4	25.9	1.9%	14.7	14.7	0.0%
μg/L	500	1750	1640	-	1550	1590	-	1250	1240	-	1490	1340	-	1510	1470	-	820	780	-
μg/L	0.002	<0.002	<0.002	-	0.002	0.002	-	0.002	0.002	-	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	<0.002	-
μg/L	0.01	<0.01	0.015	-	0.012	0.028	-	0.029	<0.01	-	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	<0.01	-
μg/L	0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-
μg/L	0.002	0.117	0.122	4.2%	0.05	0.052	3.9%	0.038	0.039	2.6%	0.0712	0.0703	1.3%	0.0713	0.0722	1.3%	0.0333	0.033	0.9%
μg/L	0.05	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-
μg/L	0.1	0.13	0.11	-	0.36	0.28	-	0.47	0.3	-	0.44	0.17	-	0.15	0.18	-	1.21	0.6	67.4%
μg/L	0.05	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-
•	•	•	•	•	•	•	•	•	•	•	•	•					•		-
μg/L	0.2	4.63	4.72	1.9%	1.53	1.61	5.1%	2.05	2.34	13.2%	5.16	6.21	18.5%	3.45	0.28	170%	5.5	2.52	74.3%
μg/L	0.02	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	<0.02	-	0.02	<0.02	-	<0.02	<0.02	-	<0.02	<0.02	-
μg/L	0.02	0.277	0.294	6.0%	0.12	0.125	4.1%	0.067	0.067	-	0.316	0.283	11.0%	0.278	0.281	1.1%	0.15	0.175	15.4%
μg/L	0.02	4.87	4.87	0.0%	2.63	2.65	0.8%	2.31	2.24	3.1%	2.49	2.42	2.9%	2.36	2.43	2.9%	1.97	1.78	10.1%
	ру/L ру/L ру/L ру/L ру/L ру/L ру/L ру/L	μg/L 0.01 μg/L 0.005 μg/L 5 μg/L 0.005 μg/L 10 μg/L 0.05 μg/L 0.05 μg/L 0.05 μg/L 0.05 μg/L 0.05 μg/L 0.5 μg/L 0.5 μg/L 0.05 μg/L 0.5 μg/L 0.05	Unit         MDL         Sample           μg/L         0.01         <0.01	µg/L         0.01         <0.01         <0.01         <0.01           µg/L         0.005         <0.005	Unit   MDL   Sample   Duplicate   23-Apr-19   23-Apr-19       μg/L   0.01   <0.01   <0.01   <0.005       μg/L   5   <5   <5   <-5   <-5       μg/L   10   3130   3090   1.3%     μg/L   0.005   <0.005   <0.005   <-0.005   <-0.005   <-0.005       μg/L   10   3130   3090   1.3%     μg/L   0.05   <0.05   <0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.05   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0.005   <-0	Unit   MDL   Sample   23-Apr-19   24-2005   20.002   20.004   20.004   20.004   20.004   20.004   20.004   20.005	Unit   MDL   Sample   Duplicate   23-Apr-19   23-Apr-19   23-Apr-19   23-Apr-19   3-May-19   3-M	Unit   MDL   Sample   Duplicate   23-Apr-19   23-Apr-19   23-Apr-19   2-0.01   -0.01   -0.01   -0.01   -0.01   -0.01   -0.01   -0.01   -0.005	Unit   MDL   Sample   Duplicate   23-Apr-19   23-Apr-19   23-Apr-19   3-May-19   9-May-19   9-May-19   8-May-19   1-	Unit   MDL   Sample   Duplicate   23-Apr-19   23-Apr-19   23-Apr-19   9-May-19   9-May-19   9-May-19   8-May-19   8-May-19   8-May-19   9-May-19   9-Ma	Unit   Mol.   Sample   Duplicate   Z3-Apr-19   Z3-A	Unit   MDL   Sample   Duplicate   23-Apr-19   23-Ap	Unit   MOL   Sample   Duplicate   PAB   Sample   Duplicate   Salay-19   Sample   Sample   Salay-19   Sample   Salay-19   Sample   Salay-19   Salay-19	Unit   MDL   Sample   Duplicate   RPD   Sample   Duplicate   RPD   Sample   Sampl	Unit   Mol.   Sample   Duplicate   PPD   Takug-19   Tak	Unit   Min   May   Sample   Duplicate   Page   Sample   Duplicate   Sample   Duplicate   Sample   Sa	Unit   Mode   Mode	Unit   MDL   MDL   Sample   Duplicate   PD   Sample   Sample   Duplicate   PD   Sample   Duplicate   PABy-19   Sample   D	Part   Part

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Table B-2 Duplicate Sample Results, 2019 (continued)

			NF	3B		MF	3-5T		FF.	A-5M		NF	5M		MF1	-1B-4		FF1-	-1M-4	
Parameter	Unit	MDL	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD	Sample	Duplicate	RPD
			23-Apr-19	23-Apr-19		9-May-19	9-May-19		8-May-19	8-May-19		15-Aug-19	15-Aug-19		22-Aug-19	22-Aug-19		17-Aug-19	17-Aug-19	
Beryllium	μg/L	0.01	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	0.103	165%	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	<0.01	-
Bismuth	μg/L	0.005	<0.005	<0.005	-	<0.005	<0.005	-	0.009	0.007	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-
Boron	μg/L	5	<5	<5	-	<5	<5	-	<5	<5	-	6.6	<5	-	<5	<5	-	<5	<5	-
Cadmium	μg/L	0.005	<0.005	<0.005	-	<0.005	<0.005	-	0.006	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	1	<0.005	<0.005	-
Chromium	μg/L	0.05	<0.05	0.055	-	<0.05	<0.05	-	<0.05	<0.05	-	0.063	0.067	-	<0.05	<0.05	1	<0.05	<0.05	-
Cobalt	μg/L	0.005	0.006	0.006	-	0.012	0.011	-	0.021	0.024	-	<0.005	<0.005	-	0.0089	0.0095	-	0.0143	0.0133	-
Copper	μg/L	0.05	0.508	0.491	3.4%	0.64	0.65	1.6%	0.496	5.7	168%	0.559	0.493	12.5%	0.462	0.55	17.4%	0.541	0.499	8.1%
Iron	μg/L	1	<1	<1	-	<1	<1	-	<1	<1	-	2.1	1.6	-	1.5	2.6	1	1.5	1.5	-
Lead	μg/L	0.005	<0.005	<0.005	-	<0.005	<0.005	-	0.008	0.009	-	<0.005	<0.005	-	<0.005	<0.005	ı	<0.005	<0.005	-
Lithium	μg/L	0.5	2.22	2.15	-	1.91	1.89	-	1.78	1.74	-	2.92	2.92	0.0%	4.42	3.78	15.6%	1.37	0.64	-
Manganese	μg/L	0.05	1.13	1.16	2.6%	0.297	0.251	16.8%	0.678	0.667	1.6%	1.28	0.989	25.7%	0.701	0.847	18.9%	0.978	0.903	8.0%
Mercury	μg/L	0.002	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	<0.002	-	<0.002	<0.002	-
Molybdenum	μg/L	0.05	1.05	1.06	0.9%	0.361	0.311	14.9%	0.239	0.251	4.9%	0.633	0.511	21.3%	0.569	0.523	8.4%	0.189	0.157	-
Nickel	μg/L	0.02	0.708	0.752	6.0%	1.14	1.13	0.9%	0.919	0.952	3.5%	0.555	0.588	5.8%	0.601	0.604	0.5%	0.689	0.709	2.9%
Selenium	μg/L	0.04	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	<0.04	-	<0.04	<0.04	-
Silicon	μg/L	50	277	253	9.1%	<50	<50	-	<50	<50	-	<50	<50	-	<50	<50	-	<50	<50	-
Silver	μg/L	0.005	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-
Strontium	μg/L	0.05	49.4	49.4	0.0%	18.2	18.2	0.0%	15	14.9	0.7%	24.4	24.2	0.8%	25.8	25.7	0.4%	13.1	13.5	3.0%
Sulphur	μg/L	500	1610	1610	-	1610	1660	-	1360	1410	-	1380	1360	-	1430	1420	-	880	940	-
Thallium	μg/L	0.002	<0.002	<0.002	-	0.002	0.002	-	0.005	0.003	-	<0.002	<0.002	-	<0.002	<0.002	1	<0.002	<0.002	-
Tin	μg/L	0.01	<0.01	<0.01	-	<0.01	0.041	-	<0.01	0.013	-	<0.01	<0.01	-	<0.01	<0.01	-	<0.01	<0.01	-
Titanium	μg/L	0.5	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-	<0.5	<0.5	-
Uranium	μg/L	0.002	0.084	0.088	4.7%	0.048	0.046	4.3%	0.038	0.033	14.1%	0.0657	0.0609	7.6%	0.068	0.066	3.0%	0.0367	0.0304	18.8%
Vanadium	μg/L	0.05	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-
Zinc	μg/L	0.1	<0.1	<0.1	-	0.28	0.27	-	14.8	1.88	155%	1.33	0.1	172%	<0.1	0.16	1	1.48	0.66	76.6%
Zirconium	μg/L	0.05	<0.05	<0.05	_	<0.05	<0.05	-	<0.05	<0.05	_	<0.05	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-
Calculated Quantities																				
RPD values over 20%	%	-	-	-	0.0	-	-	0.0	-	-	5.4	-	-	2.2	-	-	2.2	-	-	5.4
RPD values over 20%	#	-	-	-	0	-	-	0	-	-	5	-	-	2	-	-	2	-	-	5

a) Based on the results of the ammonia investigation, the ALS ammonia dataset was used in the ice-cover season and the BV ammonia dataset was used in the open-water season (see the "Ammonia Investigation" section below).

Note: Bold RPD values are greater than 40%, and concentrations in one or both samples that were greater than or equal to five times the DL.

RPD = relative percent difference; - = not applicable; NTU = nephelometric turbidity unit; µg-N/L = micrograms nitrogen per litre; µg-P/L = micrograms phosphorus per litre; µg-P/L = micrograms phosp

#### **Ammonia Investigation**

The reader is directed to Appendix 4B of the 2014 to 2016 Aquatic Effects Re-evaluation Report Version 1.1 (Golder 2019a), Appendix B of the 2017 Effluent and Water Chemistry Report (Golder 2018) and Appendix B of the 2018 Effluent and Water Chemistry Report (Golder 2019b) for a review of the history of the ammonia contamination issue for the AEMP prior to 2019. The following text provides a summary of efforts that took place in 2019 and the selection of ammonia data used for analysis in the 2019 AEMP Annual Report.

Data quality issues with ammonia continue to be a concern in 2019, with incidental occurrences in blank samples, and relatively large variability between duplicate samples. In 2019, DDMI once again sent lake water quality samples to both BV Labs and ALS for analysis of ammonia. A comparison of the available ammonia data for Lac de Gras is shown in Figure B-1.

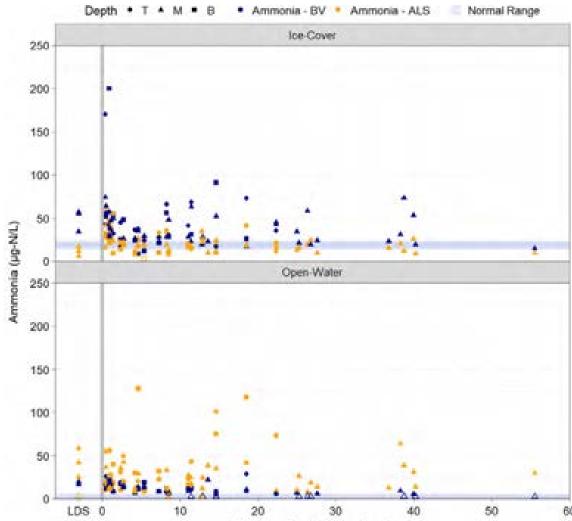


Figure B-1 Ammonia Concentrations in Lac de Gras Measured by Maxxam and ALS, 2019

Note: Samples from NF5T, FF1-5M, LDS-2M, and LDS-4M analyzed by BV Labs in open-water were excluded from the plot due to preservative contamination (see Table B-3 below).

Distance from Diffuser (km)

T = top depth; M = middle depth; B = bottom depth; µg-N/L = micrograms nitrogen per litre; <DL = less than detection limit.

Contamination of the preservative used for the BV Labs ice-cover season ammonia samples was identified by BV Labs. The ice-cover data was found to contain erroneously elevated results and was, therefore, deemed unreliable. A letter from BV Labs describing this issue is included in Annex B (following Attachment B). BV Labs recommended that the ALS ice-cover data be reported. Since the ALS data did not appear to have a similar contamination issue, as the majority of QC samples met the DQO, this recommendation was accepted and the ice-cover season ammonia data generated by ALS were used in the data analyses, tables, and figures in the 2019 AEMP Annual Report.

Initial open-water results showed that the ammonia bottles provided by BV Labs also contained contaminated preservative. As a result, BV Labs conducted the ammonia analysis on water from the unpreserved general chemistry bottle. However, there was no alternate container for seven samples (three of which were QC samples) and the original data for contaminated samples was reported in error (Table B-3). These samples were removed from the dataset. To further investigate data quality issues identified for ammonia, BV Labs completed an inter-laboratory comparison study evaluating differences in ammonia results for the 2019 open-water samples analyzed by BV Labs and ALS. The study is included in Annex C. The key results of the study were that:

- (1) The open-water BV Labs data, analyzed from the unpreserved general chemistry data, were useable.
- (2) Evidence suggests the ALS open-water data were potentially subject to a variable degree of contamination from an unknown source.
- (3) Open-water season ammonia data generated by BV Labs had fewer data quality issues and was recommended for reporting.

Based on these results, the recommendation to use the BV Labs data for the open-water season was accepted: BV labs open-water data was used in the data analyses, tables, and figures completed in support of the 2019 AEMP Annual Report.

Table B-3 BV Lab Ammonia Results Excluded from Analyses, Open-Water Season 2019

Sample ID	BV Sample ID	Sample Type
NF5T	WI5323	-
MF1-5T-2	WJ3219	Field Blank
MF3-2B-1	WK6304	Equipment Blank
FF1-5M	WJ4135	-
FFB-2M-2	WK2068	Field Blank
LDS-2M	WK8078	-
LDS-4M	WL7085	-

NF = near-field; MF = mid-field; FF = far-field; LDS = Lac Du Sauvage.

The DL used for ammonia (0.005 mg/L) is at the absolute limit of instrument sensitivity, and concentrations measured close to the DL are subject to large uncertainty as a result. Especially at low levels, ammonia presents issues with respect to potential contamination because it is airborne. Previous studies have shown that airborne ammonia contamination can be introduced over time into unopened containers (Golder 2019b). The 2019 open-water interlaboratory comparison study also included initiatives that BV Labs is undertaking to minimize sample contamination:

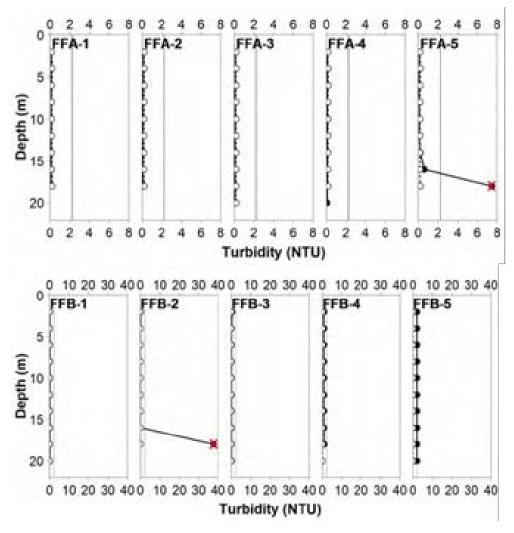
- Proofing 12 vials per lot under worst-case conditions prior to use. Previously 2 vials were proofed.
- Approaching regulators to allow sodium bisulphate as an alternate preservative instead of 50% sulphuric
  acid, because it is equally effective at lowering pH and, as a solid, is much less corrosive than sulphuric
  acid.
- Considering the use of unpreserved samples for low level ammonia, because within the typical surface water pH range (6 to 8), ammonia is greater than 99.9% to 99.0% ionized, so volatilization is not an issue.

The recommendations and conclusions outlined in this study are those of BV Labs and do not necessarily reflect DDMI's plan for the AEMP. However, the information gathered by these studies is valuable and will be used in future decision-making related to the handling of the ammonia analysis and reporting for the AEMP. DDMI will continue to work with the analytical laboratory to determine a path forward for the ammonia analysis for future monitoring. More work is planned in 2020 to help determine the path forward. Duplicate samples will again be provided to BV Labs and ALS for analysis in the ice-cover season of 2020.

#### **Abnormal Depth Profile Data**

Occasionally, measurements of turbidity taken near the lake bottom indicated that the probe had come into contact with bottom sediments. These values were not included in the graphical analysis of field measured data (Section 3.3), but are shown in Figure B-2, for reference. In total, two turbidity values (at FFA-5 and FFB-2) were removed from the depth profile plots shown in Section 3.3. All erroneous values were measured at 18 m depth, which is close to the target depth of 20 m for the AEMP stations.

Figure B-2 FFA and FFB Depth Profile Data, 2019



NTU = nephelometric turbidity unit; FF = far-field.

#### References

- BC MOE (British Columbia Ministry of the Environment). 2009 (with updates). British Columbia Laboratory Manual. Inductively Coupled Plasma-Mass Spectrometry (PBM Method). Surrey, BC, Canada.
- Golder (Golder Associates Ltd.). 2017a. Diavik Diamond Mine Aquatic Effects Monitoring Program Quality Assurance Project Plan (QAPP). Version 3.1. Prepared for Diavik Diamond Mines (2012) Inc., Yellowknife, NT. June 2017.
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- Golder. 2019a. 2014 to 2016 Aquatic Effects Re-evaluation Report. Version 1.1. Prepared for Diavik Diamond Mines (2012) Inc. (DDMI). Yellowknife, NT. June 2019.
- Golder. 2019b. AEMP 2018 Annual Report. Submitted to Diavik Diamond Mines (2012) Inc., Yellowknife, Northwest Territories. March 2019.
- US EPA (United States Environmental Protection Agency). 1994. Method 200.8. Revision 5.4:

  Determination of Trace Elements in Water and Wastes by Inductively Coupled Plasma-Mass Spectrometry. Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, OH, USA.
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### **ATTACHMENT C**

# INITIAL EFFLUENT AND WATER QUALITY DATA SCREENING

19115664/8000

#### INITIAL EFFLUENT AND WATER QUALITY DATA SCREENING

#### Introduction

Data screening is the initial phase of data handling when analyzing chemistry datasets that are subject to occasional extreme values. Extreme values are frequently incorrect, reflecting field or laboratory errors, data transcription or calculation errors, or extreme natural variability. Data screening is undertaken prior to data analysis and interpretation to verify that the data quality objectives established by the *Quality Assurance Project Plan (QAPP) Version 3.1* (Golder 2017a) and the *AEMP Design Plan Version 4.1* (Golder 2017b) have been met. The purpose of data screening is to identify unusually large or small values (referred to as anomalous data), verify or correct them if possible, and make a decision whether to retain or exclude remaining anomalous data from further analysis.

The data screening approach used in this report includes a numerical method to aid in the identification of anomalous data, followed by visual/logical assessment of the identified values. This approach removes the subjectivity of classifying values based on visual evaluation of data alone. This initial screening is primarily applicable to chemistry data, because anomalous results are less common in biological (e.g., taxonomy) data and are typically resolved through contacting the taxonomist.

#### **Methods**

Initial screening of the annual AEMP datasets was completed using a method based on Chebyshev's theorem (Mann 2010) combined with the visual examination of scatter-plots (Golder 2017b). The method is applied by first identifying data that lie outside the 4.47 SD on a scatter-plot of annual data, and then visually verifying the anomalous values based on potential spatial trends. If data were visually anomalous, the value was investigated to see if it was reported in error, or if it was consistent with associated variables (e.g., total dissolved solids and major ion concentrations) and data collected in previous years. No data were identified as anomalous based on visual evaluation alone.

In cases where the above numerical screening identified an elevated value in the NF area or at the mixing zone boundary as anomalous, the identified value was conservatively retained in the dataset used for analysis if the SD distance from the mean was less than two times the 4.5 SD criterion discussed above. Hence, only very extreme values, which were greater than approximately 9 SD from the mean, were removed from further analysis of NF area data, upon visual confirmation of screening results. Finally, in cases where the annual datasets contained a large proportion of non-detect data (i.e., censored values), only values that were greater than or equal to 5 times the detection limit (DL) were considered anomalous and were removed from the analysis if visual screening confirmed the numerical screening results.

#### Results

Results of the initial data screening are summarized herein for effluent, mixing zone and AEMP datasets (Tables C-1 to C-3; Figures C-1 to C-10). Results consist of a table of anomalous values removed from each dataset and scatter-plots, which allow visual review of anomalous data and provide transparency. Overall, the number of anomalous values identified by the data screening procedure was very small compared to the amount of data summarized, accounting for less than half of one percent of the total data.

#### **SNP**

Table C-1 List of Anomalous Values Removed from SNP Analyses, SNP 1645-18 and 1645-18B (Effluent)

C-2

Variable	Station	Value	Unit	Date	Standard Deviation Distance (a)
Acidity (pH 8.3)	1645-18	9	mg/L	02-Nov-2018	5.61
Total Phosphorus	1645-18	105	μg-P/L	18-Feb-2019	5.21
Total Aluminum	1645-18	2,120	μg/L	31-Jul-2019	7.24
Total Boron	1645-18	66.4	μg/L	17-Aug-2019	5.09
Total Chromium	1645-18	2.08	μg/L	06-Jul-2019	5.43
Total Iron	1645-18	473	μg/L	06-Jul-2019	7.71
Total Potassium	1645-18	184	mg/L	10-Oct-2019	7.34
Total Zinc	1645-18	6.47	μg/L	17-Jul-2019	7.19
Turbidity	1645-18B	1.8	NTU	06-Jul-2019	4.58
Total Kjeldahl Nitrogen	1645-18B	7,100	μg-N/L	01-May-2019	7.00
Total Aluminum	1645-18B	1,730	μg/L	31-Jul-2019	6.85
Total Chromium	1645-18B	1.85	μg/L	06-Jul-2019	4.83
Total Copper	1645-18B	1.39	μg/L	14-Nov-2018	4.91
Total Iron	1645-18B	467	μg/L	06-Jul-2019	7.70
Total Lead	1645-18B	0.034	μg/L	14-Nov-2018	5.03
Total Zinc	1645-18B	6.12	μg/L	17-Jul-2019	7.31

a) Number of standard deviations from the mean calculated for the 2019 monitoring period.

μg/L = micrograms per litre; μg-N/L= micrograms nitrogen per litre; μg-P/L= micrograms phosphorus per litre.

Table C-2 List of Anomalous Values Removed from SNP Analyses, SNP 1645-19A, 1645 19B and 1645-19C (Mixing Zone)

Variable	Station	Value	Unit	Date	Standard Deviation Distance <sup>(a)</sup>
Total Kjeldahl Nitrogen	1645-19	1,300	μg-N/L	19-Jul-2019	11.00
Total Beryllium	1645-19	0.44	μg/L	19-Jul-2019	11.59
Total Chromium	1645-19	2.24	μg/L	17-Dec-2018	11.32
Total Silver	1645-19	0.046	μg/L	29-Dec-2018	11.22
Dissolved Iron	1645-19	41.9	μg/L	23-Mar-2019	10.43

a) Number of standard deviations from the mean calculated for the 2019 monitoring period.

 $<sup>\</sup>mu$ g/L = micrograms per litre;  $\mu$ g-P/L= micrograms phosphorus per litre.

Figure C-1 Anomalous Data Removed from SNP Analyses Completed for Acidity (pH 8.3), Dissolved Iron, Total Aluminum, and Total Beryllium

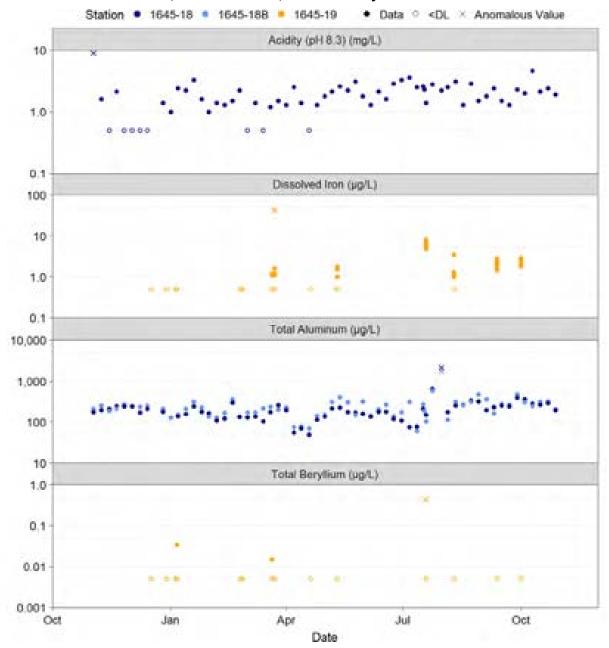


Figure C-2 Anomalous Data Removed from SNP Analyses Completed for Total Boron, Total Chromium, Total Copper, and Total Iron

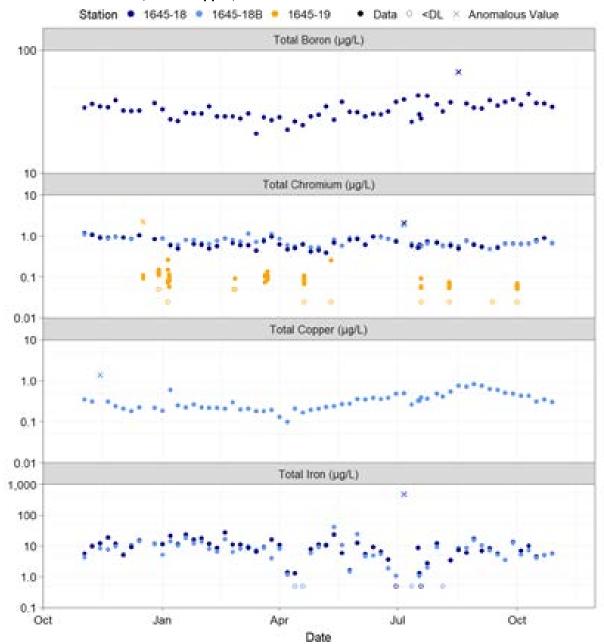
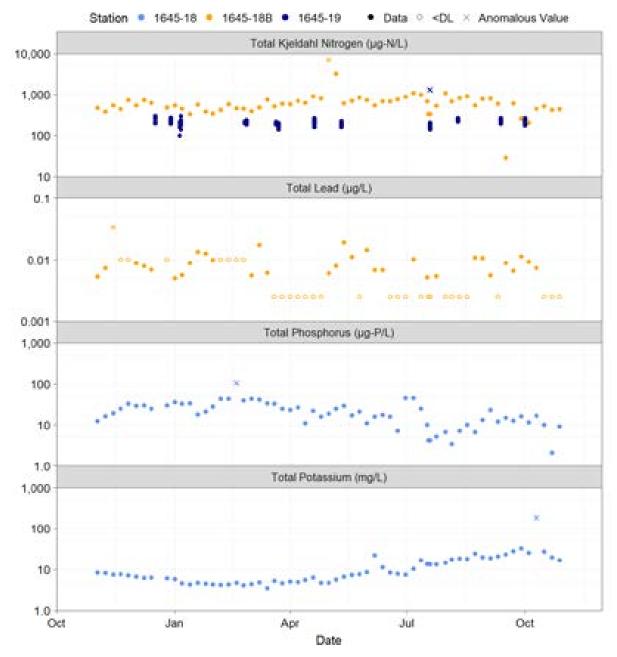
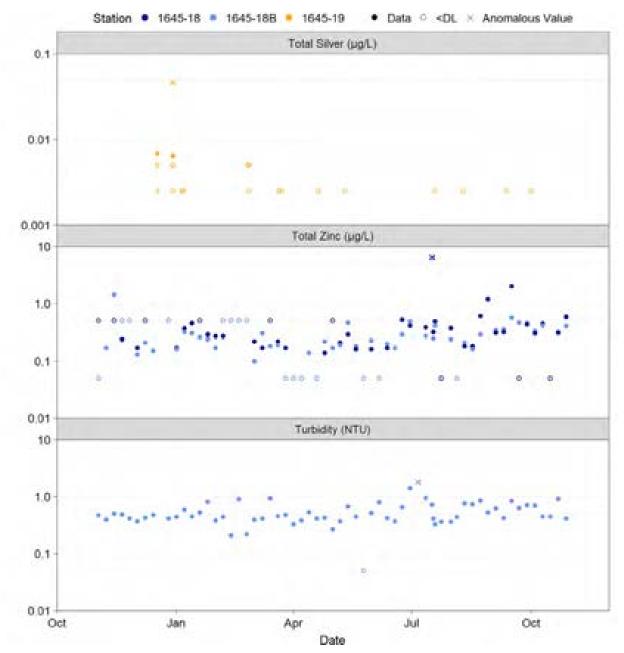


Figure C-3 Anomalous Data Removed from SNP Analyses Completed for Total Kjeldahl Nitrogen, Total Lead, Total Phosphorus, and Total Potassium



μg/L = micrograms per litre; μg-N/L = micrograms nitrogen per litre; μg-P/L = micrograms phosphorus per litre; DL= detection limit.

Figure C-4 Anomalous Data Removed from SNP Analyses Completed for Total Silver, Total Zinc, and Turbidity



#### **AEMP**

Table C-3 List of Anomalous Values Removed from AEMP Analyses

Variable	Station	Season	Value	Unit	Date	Standard Deviation Distance <sup>(a)</sup>
Total Arsenic	MF3-5B	IC	0.63	μg/L	09-May-2019	4.55
Total Cobalt	MF1-5B	IC	0.123	μg/L	28-Apr-2019	4.76
Total Cobalt	MF3-5B	IC	0.169	μg/L	09-May-2019	6.79
Total Iron	MF3-5B	IC	138	μg/L	09-May-2019	8.16
Total Manganese	MF1-5B	IC	298	μg/L	28-Apr-2019	7.41
Total Nickel	MF1-5B	IC	2.36	μg/L	28-Apr-2019	5.72
Total Tin	MF3-5M	IC	0.184	μg/L	09-May-2019	6.50
Total Zinc	FFA-M	IC	5.01	μg/L	08-May-2019	7.82
Soluble Reactive Phosphorus	FFB-M	IC	8.9	μg-P/L	06-May-2019	7.55
Total Dissolved Phosphorous	MF1-3T	IC	36.3	μg-P/L	28-Apr-2019	8.33
Dissolved Chromium	FF2-2T	IC	0.193	μg/L	10-May-2019	4.90
Dissolved Cobalt	MF1-5B	IC	0.083	μg/L	28-Apr-2019	6.12
Dissolved Cobalt	MF3-5B	IC	0.069	μg/L	09-May-2019	4.94
Dissolved Iron	MF3-5B	IC	5.8	μg/L	09-May-2019	5.61
Dissolved Lead	FF2-5M	IC	0.043	μg/L	10-May-2019	8.25
Dissolved Manganese	MF1-5B	IC	281	μg/L	28-Apr-2019	8.33
Dissolved Molybdenum	MF3-5M	IC	6.44	μg/L	09-May-2019	7.48
Dissolved Nickel	MF1-5B	IC	2.42	μg/L	28-Apr-2019	5.78
Dissolved Vanadium	FF2-2T	IC	0.92	μg/L	10-May-2019	4.93
Dissolved Zinc	FFA-M	IC	14.8	μg/L	08-May-2019	8.32
Total Cobalt	LDG-48M	OW	0.069	μg/L	31-Aug-2019	5.46
Total Copper	MF2-1M	OW	0.742	μg/L	23-Aug-2019	4.71
Total Tin	FF1-M	OW	0.062	μg/L	19-Aug-2019	8.43
Soluble Reactive Phosphorus	FF2-5M	OW	7.9	μg-P/L	20-Aug-2019	4.59
Dissolved Cobalt	LDG-48M	OW	0.075	μg/L	31-Aug-2019	7.30
Dissolved Iron	LDG-48M	OW	9.2	μg/L	31-Aug-2019	5.24
Dissolved Lithium	MF1-1B	OW	4.42	μg/L	22-Aug-2019	4.79

a) Number of standard deviations from the mean calculated for the 2019 monitoring period.

 $\mu$ g/L = micrograms per litre;  $\mu$ g-N/L = micrograms nitrogen per litre;  $\mu$ g-P/L = micrograms phosphorus per litre; IC = ice-cover; OW = open-water; MF = mid-field; FF = far-field; LDG = Lac De Gras.

Figure C-5 Anomalous Data Removed from AEMP Analyses Completed for Dissolved Chromium, Dissolved Cobalt, Dissolved Iron, Dissolved Lead, and Dissolved Manganese, Ice-Cover Season, 2019

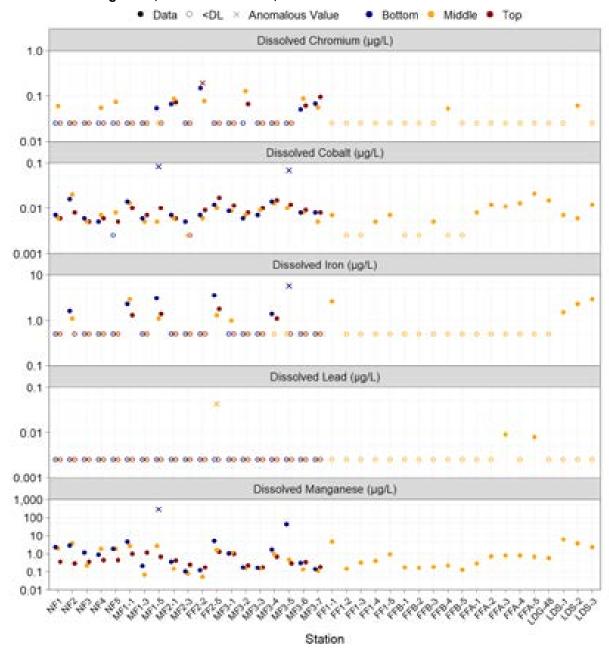


Figure C-6 Anomalous Data Removed from AEMP Analyses Completed for Dissolved Molybdenum, Dissolved Nickel, Dissolved Vanadium, Dissolved Zinc, and Soluble Reactive Phosphorus, Ice-Cover Season, 2019

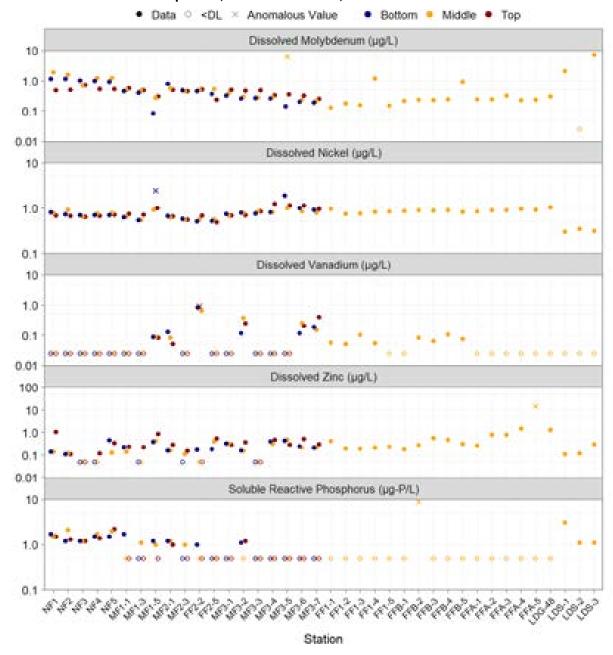


Figure C-7 Anomalous Data Removed from AEMP Analyses Completed for Total Arsenic, Total Cobalt, Total Dissolved Phosphorus, Total Iron, and Total Manganese, Ice-Cover Season, 2019

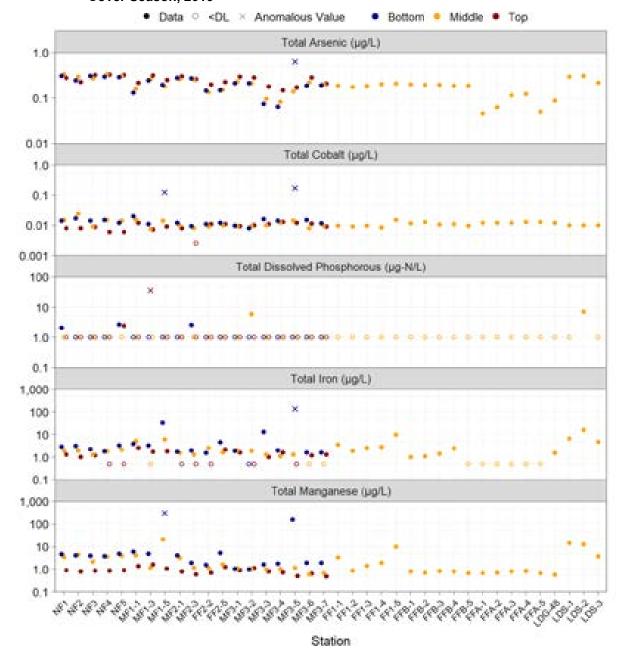
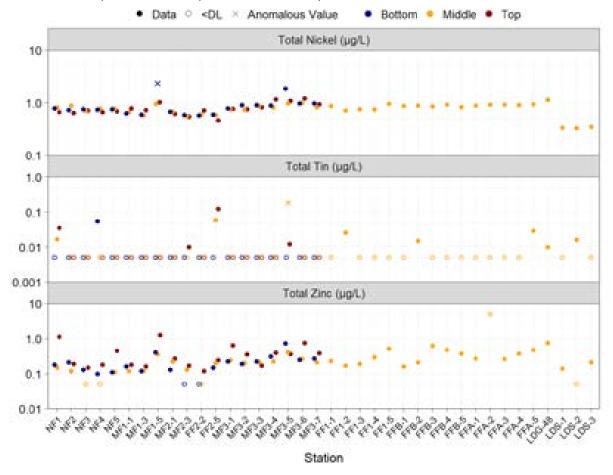
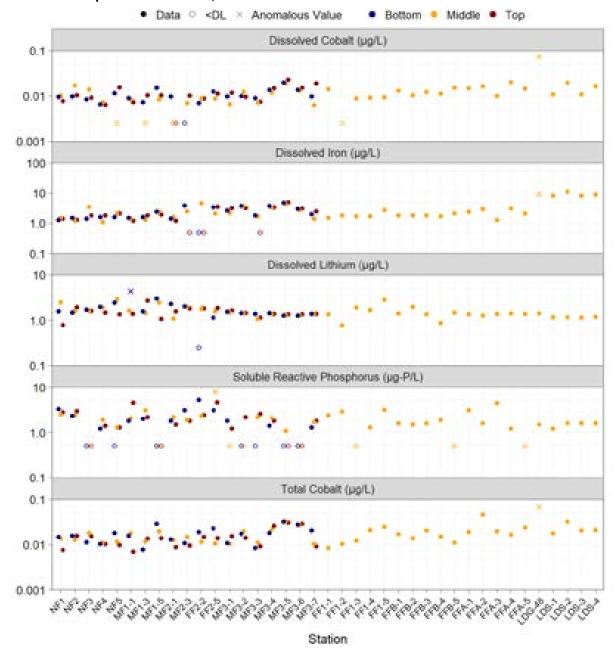


Figure C-8 Anomalous Data Removed from AEMP Analyses Completed for Total Nickel, Total Tin, and Total Zinc, Ice-Cover Season, 2019



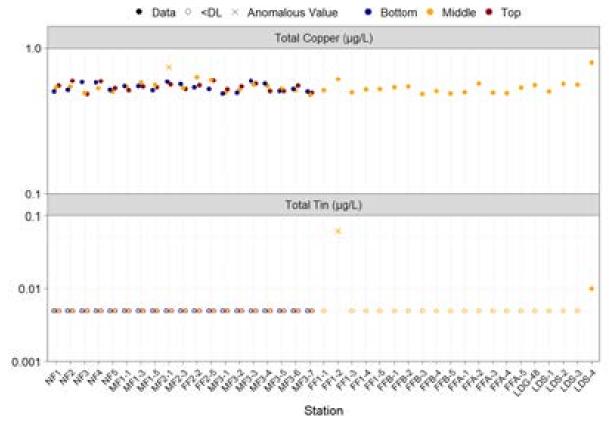
 $\mu$ g/L = micrograms per litre;  $\mu$ g-P/L = micrograms phosphorus per litre; DL= detection limit.

Figure C-9 Anomalous Data Removed from AEMP Analyses Completed for Dissolved Cobalt, Dissolved Iron, Dissolved Lithium, Soluble Reactive Phosphorus, and Total Cobalt, Open-Water Season, 2019



 $\mu g/L$  = micrograms per litre;  $\mu g$ -P/L = micrograms phosphorus per litre; DL= detection limit.

Figure C-10 Anomalous Data Removed from AEMP Analyses Completed for Total Copper and Total Tin, Open-Water Season, 2019



 $\mu g/L$  = micrograms per litre;  $\mu g$ -P/L = micrograms phosphorus per litre; DL= detection limit.

#### References

- Golder (Golder Associates Ltd.) . 2017a. Diavik Diamond Mine Aquatic Effects Monitoring Program Quality Assurance Project Plan (QAPP). Version 3.1. Prepared for Diavik Diamond Mines (2012) Inc., Yellowknife, NT. June 2017.
- Golder. 2017b. Diavik Diamond Mine Inc. Aquatic Effects Monitoring Program Design Plan Version 4.1. Prepared for Diavik Diamond Mines (2012) Inc. Yellowknife, NT, Canada. June 2017.
- Mann PS. 2010. Introductory Statistics. 7th Edition. John Wiley and Sons, Inc. Hoboken, NJ.

### **ATTACHMENT D**

# 2019 WATER QUALITY RAW DATA – AEMP AND SNP (SNP 1645-18/18B AND SNP 1645-19)

These data are provided electronically as an Excel file.

# ATTACHMENT E 2019 TOXICITY TESTING RAW DATA

These data are provided electronically as an Excel file.



## Ceriodaphnia dubia Bioassay Report

Job and Sample ID: B8A8117 - UY0944

Date sample collected: 2018/12/11 Test Initiation Date: 2018/12/12

Test End Date: 2018/12/18

Dates when subsamples used: 2018/12/12

2018/12/16 2018/12/13 2018/12/17 2018/12/18

2018/12/14

2018/12/15

Dissolved Oxygen prior to test (mg/L): 8.6

pH prior to test (pH units): 7.2 Temperature (°C): 25

531

Conductivity (µS/cm):

Test Performed By: NM9, DBA

	Test Results		Signit	icant or non-signi	ficant	Method	Data Transforms	Outliers* ( concentration-replicate)
Sample Test Results (%	7 day Survival Result	Effect		Non-Significant		Fisher Exact Test	Log X	None
vol/vol)	7 day Biomass Result	Effect		Non-Significant		Equal Variance t Two-Sample Test	Log X	None
Refere	nce Toxicant Resul	lts	Endpoint	95% LCL	95% UCL	Method	Data Transforms	Outliers* ( concentration-replicate)
Reference	7 day Survival Result	LC50	1.87	1.51	2.31	Spearman-Karber	Log X	None
Toxicant Test Results (g/L)	7 day Biomass Result	IC50	1.18	1.03	1.38	Regression: 4P Log- Logistic+Hormesis	Log X	None
Control Chart	7 day Survival Result	LC50	1.51	0.977	2.33	Shewart	n/a	n/a
Data (g/L)	7 day Biomass Result	IC50	1.20	0.855	1.69	Silewait	11/4	пуа

<sup>\*</sup> If outliers were removed, describe in comments.

om		

Reference toxicant analyzed 2018/12/12

## **Test Organisms**

Species: Ceriodaphnia dubia Source of test organisms: Aquatic Biosystems, Colorado Age of organisms at test initiation: ≤ 24 hours, within 12 hours

Unusual Appearance, behaviour or treatment prior to use in test: None Mean % mortality of brood organisms during 7-day period proceding test: Number of neonates produced by each organism in its third or fourth brood: ≥8 neonates Mean number of neonates per adult during first 3 broods in 7  $\,$ 37 Observations of ephippia: None

All test organisms used to initiate this test were taken from a series of individual cultures, that originated from the same mass culture.

The 4th brood or subsequent broods produced during the test are not included in the final statistical analysis.

**Test Facilities and Apparatus** 

Name address of test laboratory: Maxxam Analytics. 9331 48st NW, Edmonton, AB Fisherbrand 20 x 150 mm lime glass test tubes Test vessels used:

Control/Dilution Water

Consists of: 16 L RODI from in-house system, to which the following are added:

4 L Perrier Brand carbonated spring water 1 mL cyanocobalamin (Vitamin B-12) 1 mL Sodium Selenate Decahydrate

Test Method

Reference method used for testing:  $Biological \ Test \ Method: Test \ of \ Reproduction \ and \ Survival \ Using \ the \ Cladoceran \ \textit{Ceriodaphnia dubia} \ . \ Environment \ Canada, EPS \ 1/RM/21$ 

Second Edition - February 2007

Did the following occur during sample preparations:

Filtered: No
Adjusted for hardness: No
Adjusted for pH: No
Frequency of observations: 24 ± 1 hour
Frequency of water quality measurements: Daily
Design and description of any specialized procedure: N/A

Program used for statistical calculations: Comprehensive Environmental Toxicity Information System (CETIS). Tidepool Scientific Software. Version 1.9.3.0

Reference method used for statistical calculations: Guidance Document on Statistical Methods for Environmental Toxicity Tests. Environment Canada, EPS 1/RM/46 - March 2005.

#### **Test Conditions and Procedures**

Number of test solutions: 20

Number of test concentrations: 1 and a negative control

 Concentrations tested (%):
 100

 Units of tested concentrations:
 % vol/vol

 Number of replicates:
 10

 Volume of test solutions:
 ≥ 15 mL

 Depth of test solutions:
 ≥ 5 cm

 Individuals per test vessel:
 1

 Was pre-aeration performed:
 Yes

Procedure: Oil-free compressed air is dispensed through airline tubing and a disposable pipette

Rate: ≤ 100 bubbles / minute

Duration: 20 minutes

Dates where pre-aeration occurred: 2018/12/12 2018/12/16

2018/12/13 2018/12/17

2018/12/14 2018/12/15

Aeration during testing: None

Refer to comments section for any deviations.

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

Refer to comments section for any deviations regarding reference toxicity testing.

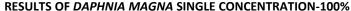
#### Ceriodaphnia dubia Survival and Reproduction Observations

Note: Mortalities are indicated by an "X" in the column and row of the concentration, replicate and day of occurance. If organism was not used for testing, this will be indicated by "N/A" in mortality and neonate columns for the replicate.

Concentration	Doulieste							Da	ay of Testing								Cumulative	Cumulative	
(%/%vol)	numbor		1		2		3	4	5		6		7			8	Mean %	Mean	SD of mean reproduction
(70) 70001)	Hullibei	neonates	Mortality	neonates	Mortality	neonates	Mortality	neonates Mort	ality neonat	es Mortality	neonates	Mortality	neonates	Mortality	neonates	Mortality	Mortality	Reproduction	
	1	0		0		0		8	13		18								
	2	0		0		0		6	14		0								
	3	0		0		0		5	11		16								
	4	0		0		0		8	10		0								
Control	5	0		0		0		7	14		20						0.0%	31.8	8.1
Control	6	0		0		0		7	15		14						0.070	31.0	0.1
	7	0		0		0		5	9		12								
	8	0		0		0		8	12		15								
	9	0		0		0		10	12		18								
	10	0		0		0		5	13		13								
	1	0		0		0		6	13		15								
	2	0		0		0		4	15		16								
	3	0		0		0		6	11		20								
	4	0		0		0		8	19		0								
100%	5	0		0		0		7	14		10						0.0%	33.9	3.9
100/0	6	0		0		0		8	13		13						0.070	33.3	3.3
	7	0		0		0		7	12		10								
	8	0		0		0		7	12		17								
	9	0		0		0		8	15		13								
	10	0		0		0	1	9	10		21	l			I				

#### **Average Values for Chemical Data of Test Concentrations**

		Before Efflue	ent Renew	al				After Ef	fluent Renewal	
Test Concentration (% vol/vol)	Date	Temperature (°C)	рН	Conductivity (µS/cm)	DO (mg/L)	Date	Temperature (°C)	рН	Conductivity (μS/cm)	DO (mg/L)
Control	2018/12/12	25	7.9	180	7.0	2018/12/13	25	7.6	190	5.4
100	2018/12/12	26	7.3	537	8.3	2018/12/13	25	7.5	537	6.1
Control	2018/12/13	25	7.9	180	7.0	2018/12/14	25	7.8	181	6.4
100	2018/12/13	25	7.3	537	7.8	2018/12/14	25	7.5	518	6.5
Control	2018/12/14	25	7.9	176	7.1	2018/12/15	25	7.7	178	6.5
100	2018/12/14	26	7.3	537	8.5	2018/12/13	25	7.5	519	6.5
Control	2018/12/15	25	7.8	176	7.3	2018/12/16	26	7.6	183	6.6
100	2010/12/13	25	7.4	529	7.8	2010/12/10	26	7.5	531	6.7
Control	2018/12/16	24	7.9	175	7.4	2018/12/17	25	7.7	179	6.5
100	2016/12/10	24	7.4	526	8.4	2010/12/17	25	7.5	516	6.7
Control	2018/12/17	24	8.1	173	7.4	2018/12/18	25	7.5	180	5.9
100	2016/12/17	25	7.3	525	8.7	2010/12/10	25	7.3	519	5.9
Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A



Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B8A8117 UY0944-04 Client Project Name & Number: Quarterly Tox SNP-A Sample Number:

Test Result:

48 hrs Mortality % 0% Statistical Method:

0 Control 0 Mean percent mortality: Sample

Sample Name: 1645-18 Sample Matrix: **Grab Water** 

Description: **CLEAR COLOURLESS** Sample Prior to Analysis:

Sampling Method: Sample Collected: Dec 11, 2018 12:19 AM N/A pH: 7.1 Sample Collected By: ΑН Site Collection: N/A Temperature: 18 °C

Sample Received: Dec 11, 2018 02:06 PM Volume Received: 1 L Dissolved Oxygen: 11.0 mg/L Analysis Start: Dec 12, 2018 11:40 AM Temp.Upon Arrival: -1 °C Sample Conductance: 459 µS/cm

End: Dec 14, 2018 10:48 AM Storage: 2-6°C Hardness: 120 mg CaCO <sub>3</sub>/L

					U						U	
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hr	48 hrs	48 hrs
0	20	8.1	360	7.9	0	0	0	0	19	8.4	371	8.1
0	20	8.2	358	8.0	0	0	0	0	19	8.2	368	8.1
0	20	8.2	361	8.0	0	0	0	0	20	8.2	363	8.0
100	19	7.3	466	10.3	0	0	0	0	19	7.8	497	8.1
100	19	7.3	466	10.5	0	0	0	0	20	7.8	480	8.1
100	19	7.3	467	10.5	0	0	0	0	20	7.8	482	8.1

Concentration	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)
% vol/vol	48 hrs	48 hrs	48 hrs	48 hrs
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
100	0	0	0	0
100	0	0	0	0
100	0	0	0	0

**Comments:** Many daphnia in sample concentration test vessels floating on water surface.

**Culture/Control/Dilution Water:** City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO<sub>3</sub> Other parameters available on request.

**Test Conditions** Test concentration: 0,0,0,100,100,100 (% vol/vol)

10 Organisms per Vessel: Pre-aeration Time: 30 min Rate of Pre-aeration: 25-50 mL/min/L

60 Total # of Organisms Used: Test Temperature: 20 ± 2 °C Test Hardness Adjusted: No Test Volume: 150 mL Vessel Volume: 225 mL Test pH Adjusted: No

Loading Density: 15.0 mL/Daphnia Photoperiod: 16:8 (light: dark)

In House Culture Daphnia magna Source: **Test Organism:** 

Average Brood Size: 24.8 Age at Test Initiation: <24 hrs Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 1.6 Culture Temperature: 20 ± 2 °C Time To First Brood: 8 Days **Culture Diet** 

Pseudokirchnriella and YTC at a ratio of 2 mL/L of culture daily. New cultures weekly, 63 daphnids

distributed into 6 culture vessels and 3 reproductive vessels.





Dec 22, 2018 02:46 PM

Date:

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B8A8117Client Project Name & Number:Quarterly Tox SNP-ASample Number:UY0944-04

Reference chemical:Sodium ChlorideTest Date:Dec 05, 2018Test Endpoint 48 hrs LC50 (95% confidence interval):6.96 (5.70, 8.50)g/LStatistical Method:Binomial

Historical Mean LC50 (warning limits): 5.80 (4.37, 7.71) g/L Concentration: 0,1.71,2.56,3.82,5.7,8.5 g/L

<u>Test Method</u> EPS 1/RM/14

Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Dustin Banks, Natasha Lloyd

Verified By: Chelsea Tessier, Sample Logistics Supervisor

Maxxam Analytics





Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B8A8117

Client Project Name & Number: Quarterly Tox SNP-A

**Test Result:** 

96 hrs Mortality % 0% Statistical Method: Visual

Sample Name: 1645-18 Sample Matrix: Grab Water

Description: Clear, colourless Sample Number: UY0944-05
Sample Collected: Dec 11, 2018 12:19 AM Sampling Method: N/A Site Collection: N/A

Sample Collected By: AH Volume Received: 20 L Temp. Upon Arrival: -1 °C Storage: 2-6°C

Sample Received: Dec 11, 2018 02:06 PM pH: 7.3 Dissolved Oxygen: 11.3 mg/L
Analysis Start: Dec 12, 2018 11:17 AM Temperature: 12 °C Sample Conductance: 390 μS/cm

Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
0	15	8.0	291	9.1	0	0	0	0	0	0	0	0
100	14	7.4	411	9.2	0	0	0	0	0	0	0	0

Concentration	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	72 hrs	72 hrs	72 hrs	72 hrs	96 hrs	96 hr	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs
0	0	0	0	0	15	7.9	301	9.2	0	0	0	0
100	0	0	0	0	15	7.7	431	9.3	0	0	0	0

<u>Comments:</u> The control chart result for this reference toxicant test was outside of 2SD limits. A check of all acclimation and test conditions was performed, and all requirements were met.

<u>Culture/Control/Dilution Water</u> City of Edmonton dechlorinated tap water

Hardness: 170 mg/L CaCO<sub>3</sub> Other parameters available on request.

<u>Test Conditions</u> Test concentration : 0,100 (% vol/vol)

Organisms per Vessel : 10 Test Temperature :  $15 \pm 1$  °C Solution Depth : >15 cm

Total # of Organisms Used: 20 Pre-aeration Time: 120 min. Rate of Aeration 6.5±1 mL/min/L

Test Volume : 20 L Vessel Volume : 38L Test pH Adjusted: No

Loading Density: 0.4 g/L Photoperiod: 16:8 (light: dark)

<u>Test Organism :</u> Rainbow Trout (Oncorhynchus mykiss) Source : Spring Valley Trout Hatchery

Culture Temperature :  $15 \pm 2$  °C Weight (Mean) +- SD :  $0.7 \pm 0.1$  g Length (Mean) +- SD :  $4.36 \pm 0.23$  cm Culture Water Renewal :  $\geq 1.0$  L/min/kg fish Weight (Range) : 0.6 - 0.9 g Length (Range) : 4.00 - 4.80 cm

Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 0.3% Feeding rate and frequency: daily: 1-5% biomass of trout. Acclimation Time: >14 days

Reference chemical: Phenol Test Date: Nov 14, 2018

Test Endpoint 96 hrs LC50 (95% confidence interval): 12.2 (10.9, 13.8)mg/L Statistical Method: Probit Historical Mean LC50 (warning limits): 10.2 (8.60, 12.1) mg/L Concentration: 0,8,10,12,15,20 mg/L

Test Method EPS 1/RM/13
Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Dustin Banks, Natasha Lloyd

Verified By: Natasha Lloyd, Analyst 2 Date: Dec 21, 2018 08:32 AM

Maxxam Analytics 9331 - 48th Street, Edmonton, Alberta T6B 2R4 Tel: (780) 577-7100 Fax: (780) 450-4187

www.maxxam.ca



## Toxicity testing on samples UY0944-1645-18 and UY0945-1645-18B

Collected December 11, 2018

**Final Report** 

February 15, 2019

Submitted to: **Maxxam Analytics** 

Burnaby, BC



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APPENDIX A – Oncorhynchus mykiss Toxicity Test Data

APPENDIX B – Chain-of-Custody Form



## **SIGNATURE PAGE**

Report By:

Yvonne Lam, B.Sc. Laboratory Biologist Reviewed By: Armando Tang, R.P.Bio

A. Tong

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	UY0944-1645-18
Sample 1D	UY0945-1645-18B
Sample collection date	December 11, 2018
Sample receipt date	December 12, 2018
Sample receipt temperature	4.0 – 5.0°C
Test type	7-d rainbow trout (Oncorhynchus mykiss) embryo viability

## **Summary of Results**

	Mean ± SD							
Endpoint	Control	UY0944-1645-18	Control	UY0945-1645-18B				
Embryo viability (%)	73.3 ± 13.0	77.5 ± 12.6	72.5 ± 9.6	75.1 ± 10.5				

SD = Standard Deviation



#### 1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability toxicity tests for Maxxam Analytics on two samples identified as UY0944-1645-18 and UY0945-1645-18B. The samples were collected on December 11, 2018 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on December 12, 2018. The samples were each transported in four 10-L or two 20-L plastic containers and received at temperatures of 4.0 to 5.0°C. The samples were stored in the dark at  $4 \pm 2$ °C prior to testing.

This report describes the results of the toxicity tests. Copies of raw laboratory data sheets and statistical analysis 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 toxicity test is summarized in Table 1, and followed procedures described by Environment Canada (1998) and modified by Canaria *et al.* (1999). Statistical analyses were performed using CETIS (Tidepool Scientific Software, 2013).



## Table 1. Summary of test conditions: 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability single concentration 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 100% (undiluted) sample, 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  $14 \pm 1^{\circ}$ C Feeding None Light intensity Dark

Photoperiod 24 hours dark

Aeration Continuous gentle aeration

Temperature, dissolved oxygen, pH and conductivity measured

Test measurements 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 endpoints Embryo viability

Reference toxicant Sodium dodecyl sulphate (SDS)



## 3.0 RESULTS

Results of the rainbow trout embryo viability toxicity tests conducted on samples UY0944-1645-18 and UY0945-1645-18B are summarized in Table 2. There were no statistically significant differences relative to the laboratory controls for either sample, with embryo viability in both samples and all test treatments  $\geq$ 72% (v/v).

Table 2. Results: 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability single concentration test.

Concentration	•	iability (%) n ± SD)
(% v/v)	UY0944-1645-18	UY0945-1645-18B
Laboratory Control	73.3 ± 13.0	72.5 ± 9.6
100	77.5 ± 12.6	75.1 ± 10.5

SD = Standard Deviation

The samples were not statistically significantly different relative to the Laboratory Control



#### 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 this test is best described by the standard deviations around the means and/or confidence limits around the point estimates.

There was a deviation from the test methodology. 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.

Results of the reference toxicant test conducted during the testing program are summarized in Table 3. Results for this test fell within the acceptable range for organism performance of 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 was performed under the same conditions as those used for the samples.

Table 3. Reference toxicant test results.

Test Species	Endpoint	Historical Mean (2 SD Range)	CV (%)	Test Date
O. mykiss	Viability (EC50): 6.6 mg/L SDS	4.2 (2.2 – 8.1) mg/L SDS	34	December 12, 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 1.9.4 Tidepool Scientific Software, McKinleyville, CA. 255 pp.



**APPENDIX A – Oncorhynchus mykiss** Toxicity Test Data

## Rainbow Trout Early Life Stage Summary Sheet

Client:	Maxxam	Start Date/I	ime: LXUM	Par 15, 5018 6
Work Order No.:	182202	Test Specie	s: Oncorhyr	nchus mykiss
Sample Informa	ition:			
Sample ID: Sample Date: Date Received: Sample Volume:	040944-1645-18 December 11, 2018 December 12, 2018			
Dilution Water:				
Type: Hardness (mg/L Alkalinity (mg/L (	T T T T 400	ap Water		
Test Organism	Information:			
	121218		2.00/10/5	
Batch No.: Source: Loading Density:	Voncour Island Tro	ut Hatchey,	DOVCEN, BC	
Source: Loading Density: Number of male Number of femal	broodstock used:	1		
Source: Loading Density: Number of male Number of femal Sperm motility cl	broodstock used:	1		
Source: Loading Density: Number of male Number of femal Sperm motility cl  SDS Reference Reference Toxic Stock Solution ID Date Initiated:	broodstock used: broods	motility using a c	ompound micr	
Source: Loading Density: Number of male Number of femal Sperm motility cl  SDS Reference Reference Toxic Stock Solution ID Date Initiated: 7-d EC50 (95% (	broodstock used: le broods	motility using a c	ompound micr	
Source: Loading Density: Number of male Number of femal Sperm motility ch  SDS Reference Reference Toxic Stock Solution ID Date Initiated: 7-d EC50 (95% (  Reference Toxic	broodstock used: le broods	olb (2.2-8.1)	ompound micr	

Client:		Kyttin.		645		Start Date & Time: DeanWF 12, W/8 @ 16451								
Sample ID: Nork Order #:	18	Stop Date & Time: CER #:				- 15-								
								Test Sp	secles:	Oncorhynchus mykiss				
Control							Da	ys.						
Concentration	0		1	5-1	2	- 4	3		4	13- 03	5		6	7
(°6 ul-)	init.	new	old	new	old	new	old	new	old	new	old	now	old	final
Temperature (°C)	14.0	BK	14.0	135	140	14.0	MA	14.0	140	No	CYL	140	140	140
DO (mg/L)	197	10.2	10.0	10/2	LAI	12/2	9.9	10,0	99	101	(0.0)	(40	100	(00
pH	68	7.0	1.5	609	79	700	6.9	30	20	11	7.2	71	11	69
Cond. (µS/cm)	3>	3	6		3 5	3	5		5	30		35		37
Initials	uw-	44	_	W	^		A-	-	3	W	W.	4	44	Will
100		-	Days											
Concentration	0		1		2	3		4		5		6		7
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (*C)	135	135	M.O	136	190	143	142	140	140	190	140	140	140	140
DO (mg/L)	10.3	10.	10.0	192	191	10.3	91	142		10.0	9.9	9.9	100	99
pH	6.7	73	7.8	69	7.7	69	716	70	3.3	12	70	7.7	7.8	7,60
Cond. (µS/cm)	534	53	6	5	21	- 4	532	5	34	5	41	2	98	544
Initials	ulu	V	w	- 0	VA, I		~	8		W	·	W	<b>U</b>	Whi
							Da	ys						
Concentration	0 1		= 20	2			1			5		6	7	
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)	14.1	1000												
DO (mg/L)	6-6		55										0	

							- 27	ay a						
Concentration	0 1		2		3		4		5		6		7	
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)	75	and the same					100000		248.73					
DO (mg/L)	8 3 6		55											
pН					7-4					15.5	E			
Cond. (µS/cm)	JE.					he -				10 11 11				
Initials	THE T													

	Days													
Concentration	0	0 1		2		3		4		5		6		7
	-init	new	old	new	old	new	old	new	old	new.	old	new	old	final
Temperature (°C)	100	2122				WILLIAM.	y contra	1200		PALIE DE	750-9	1	1,000	P. West
DO (mg/L)											1 =			CLT.
pH								B- 13						
Cond. (µS/cm)														
Initials														

Thermometer: (25 43 DO meter/probe: 213 / 213 Conductivity meter/probe: 213 / 213

	Control	100%		Analy
Hardness*	11	136		1000
Alkalinity*	10	48		Revie
mg/L as CaCO3		7. 30336		Date re

rwed by: VOL

Date reviewed:

Sample Description:

Comments:

## Embryo Toxicity Test Daily Mortality

Client:	Maygan	Start Date & Time: Deanber 12, 201	8
Sample ID:	UY0944-1645-18	Stop Date & Time: Deunbar 19, 2018	5
Work Order #:	18202 182202	Test Species: Oncorhynchus mykiss	

Concentration	Rep		Day of	Test	- No.	of Mo	rtalitic	rs	Total	Total	Total No.	Total
	177328	1	2	3	4	5	6	7	Dead Eggs	Undeveloped	Embryo	Exposed
Lenhol	1	0	5	0	0	0	0	0	0	- 3	2.7	30
	2			1	1		0	6	6	6	18	30
	3					1		2	3	7	20	30
	4						0	3	3	- 4	23	30
(0.5	1			1873		1163	0	V	0	9	26	30
	2			t		8 9		3	4	9	18	30
	3	200	-	1	0.0		C	1	2	5	23	30
	4	上		0	1	V	0	1	v	2	26	30
	1										F 27	
	2			1								
	3									1		
	4		100					200				
	1		0	0.00								
	2											
	3											
	4											
	1	()		0.75						5		
	2											
	3			la de						,		
	4			0.3								
	1											
	2								I			
	3							9 15 1				
	4	-							A. 163			
	1											
	2						1					
	3	8 -	100	47		1.5						
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ech Initials		A	1	A	0	William	13/44	W	ww-	WIL	NW	week

Comments:			
	- Ku		0
Reviewed by:	Our	Date reviewed:	Teb-4/19

Version 1.0 Issued June 26, 2006

Nautilus Environmental Company Inc.

## **CETIS Summary Report**

Report Date: Test Code/ID: 31 Jan-19 11:07 (p 1 of 1) 182202a / 09-1419-7120

							Test	Code/ID:		82202a / 05	F1419-7120
Salmonid Em	bryo Survival a	nd Devel	opment T	est					Na	utillus Envi	ronmental
Batch ID:	11-2501-8055	- 63	est Type:	Development			Anal	yst: Yv	onne Lam		
Start Date:	12 Dec-18 16:4	5 F	rotocol:	col: EC/EPS 1/RM/28			Dilu	ent: De	chlorinated T	ap Water	
Ending Date:	19 Dec-18 11;3	0 5	pecies:	Oncorhynchus mykiss			Brin	e:			
Test Length:	6d 19h	1	axon:	Actinopterygii			Sou	roe: Va	ncouver Islan	nd Trout Hat	Age:
Sample ID:	03-8887-7508	- 0	ode:	UY0944-1645-	18		Proj	ect:			
Sample Date:	11 Dec-18 00:1	9 9	Naterial:				Sour	roe: Ma	pocam		
Receipt Date:	12 Dec-18 13:0	3 (	AS (PC):				Stati	ion: UY	0944-1645-1	8	
Sample Age:	40h (4 °C)		Client:	Maxxam							
Single Compa	arison Summary	t .									
Analysis ID	Endpoint		Comp	parison Method	P-Value	Compan	son Result		S		
09-3823-3781	Proportion Norm	ted.	Equal	al Variance t Two-Sample Test			0.6618	100% pa	used proport	ion normal	1
Proportion No	ormal Summary										
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.733	3 0.5256	0.9410	0.6000	0,9000	0.0653	0.1305	17.80%	0.00%
100		4	0.775	0.5748	0.9752	0.6000	0.8887	0.0629	0.1258	16.24%	-5.68%
Proportion No	ormal Detail										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	0.9000	0.600	0 0.6667	0.7667						
100	7811	0.8667	0.600	0 0.7667	0.8667						
Proportion No	ormal Binomials										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	27/30	18/30	20/30	23/30						

JGh. Feb. 4/10

100

26/30

18/30

23/30

26/30

## **CETIS Analytical Report**

Report Date: Test Code/ID: 31 Jan-19 11:07 (p 1 of 2) 182202a / 09-1419-7120

Salmonid Em	bryo Survival	and Devel	opment Tes	t					N	autilus Env	rironmenta
Analysis ID: Analyzed:	09-3823-3781 31 Jan-19 11:	S 40 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		roportion Nor	10.00			IS Version us Level:	CETISV	1.9.4	
0.000	200000000000000000000000000000000000000	CO			o campio		0.740.000	CONTRACTOR OF THE		_	
Batch ID:	11-2501-8055			Vevelopment	5.e.		Anal		onne Lam	no constitute	
Start Date:	12 Dec-18 16			C/EPS 1/RM/	THE STATE OF		Dilu	DESCRIPTION OF THE PARTY OF THE	chlorinated 1	Tap Water	
Ending Date:		200 1203	The second second second	ncorhynchus	mykiss		Brin				
Test Length:	6d 19h	1	axona /	ctinopterygli			Sour	rce: Va	ncouver Isla	nd Trout Ha	it Age:
Sample ID:	03-8887-7508		ode: (	JY0944-1645-	18		Proj	ect:			
Sample Date:			tateriál: i	Muent			Sou	roe: Ma	xxxam		
DUBLISHED BY THE	12 Dec-18 13	977	AS (PC):				Stati	ion: UY	0944-1645-1	18	
Sample Age:	40h (4 °C)	C	lient: A	faxxam							
Data Transfor		Alt Hy	p .				Comparis	son Result			PMSD
Angular (Corre	octed)	C>T					100% pas	sed propor	tion normal		25.85%
Equal Variance	e t Two-Samp	ole Test									
Control	vs Conc-1	6	Test St	at Critical	MSD DE	P-Type	P-Value	Decision	(a:5%)		
Negative Contr	rol 100		-0,4385	1.943	0.210 6	CDF	0.6618		ificant Effec	1	
Auxiliary Test	5										
Attribute	Test		*		Total Proc	distribution of	m have	Bestelle	Constitution of the last of th		
Control Trend	1000	Gendall Tre	nd Test	_	Test Stat	Critical	P-Value 1 0000	Decision	i(a:5%) ificant Trent	d in Control	
ANOVA Table	7.400,700	serious rre	IN THE				1,0000	reun-orga	INCASK ITHIK	a in Compos	•
		000000	100000000000000000000000000000000000000		22724	2200	9250000000	12010000000	000000		
Source	Sum Sq		Mean S	-	DF	F Stat	P-Value	Decision			
Between	0.00449		0,00448	73.00	1	0.1923	0.6763	Non-Sign	ificant Effec	4	
Error	0.14025		0.02337	63	6	_					
Total	0.14475	3			7						
Distributional	Tests										
Attribute	Test				Test Stat	Critical	P-Value	Decision	(a:1%)		
Variances	Variance	Ratio F T	est .		1.158	47.47	0.9067	Equal Va	riances		
Distribution	Shapiro	Wilk W No	rmality Test		0.9674	0.6451	0.8768	Normal C	Stribution		
Proportion No	rmal Summar	ry									
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.7333	0.5256	0.9410	0.7167	0.6000	0.9000	0.0653	17.80%	0.00%
100		4	0.7750	0.5748	0.9752	0.8167	0.6000	0.8667	0.0629	16.24%	-5.68%
Angular (Com	ected) Transfe	ormed Sun	mary								
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	1.039	0.7872	1.291	1.011	0.8861	1.249	0.0792	15.24%	0.00%
100	-27	4	1.087	0.8525	1.321	1.132	0.8861	1.197	0.07359	13.54%	4.56%
Proportion No	rmal Detail			11/2/200	200	700000	25050000				
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	0.9000	0.6000	0.6667	0.7667				-		
		0.8667	0.6000	0.7667	0.7667						
100			-17000000000	0.7007	0,0001						
			No.								
Angular (Corn				100000000000000000000000000000000000000							
Angular (Corre	Code	Rep 1	Rep 2	Rep 3	Rep 4						
Angular (Corre Conc-%		Rep 1	Rep 2 0.8861	0.9553	1.067						
Angular (Corre Conc-%	Code	Rep 1	Rep 2	The second second							
Angular (Corn Conc-% 0 100 Proportion No	Code N	Rep 1 1.249 1.197	Rep 2 0.8861	0.9553	1.067						
Angular (Corn Conc-% 0 100	Code N	Rep 1 1.249 1.197	Rep 2 0.8861	0.9553	1.067						
Angular (Corn Conc-% 0 100 Proportion No	N Primal Binomia	Rep 1 1,249 1,197	Rep 2 0.8861 0.8861	0.9553 1.067	1.067						

Analyst: WW QA: Fels.4/m

## **CETIS Analytical Report**

Report Date: Test Code/ID: 31 Jan-19 11:07 (p 2 of 2) 182202a / 09-1419-7120

Salmonid Embryo Survival and Development Test

Nautilus Environmental

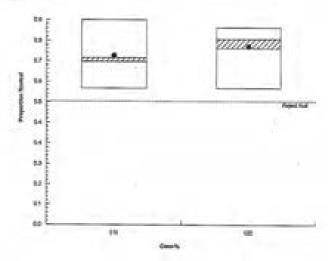
Analysis ID: 09-3823-3781 Analyzed:

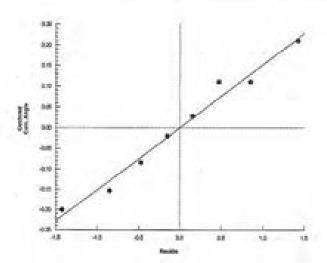
31 Jan-19 11:07

Endpoint: Proportion Normal Analysis: Parametric-Two Sample **CETIS Version:** Status Level:

CETISVI.9.4

## Graphics





## Rainbow Trout Early Life Stage Summary Sheet

Client:	Margran	Start Date/Time	e: Occapar 12, 2018 @1695L
Work Order No.:	1820202	Test Species:	Oncorhynchus mykiss
Sample Informa	tion:		
Sample ID: Sample Date: Date Received: Sample Volume:	December 11, 2018 December 12, 2018 December 12, 2018		
Dilution Water:			
Type: Hardness (mg/L ( Alkalinity (mg/L C		p Water	
Test Organism I	nformation:		
Batch No.: Source: Loading Density:	121218 Vancount Island T	rout Hatchery	, Duncan, BC
Number of male I Number of female Sperm motility ch	e broodstock used: 4	notility using a con	npound microscope
SDS Reference	Toxicant Results:		
Reference Toxica Stock Solution ID Date Initiated: 7-d EC50 (95% C	18503 Permon 12,201		
Reference Toxica Reference Toxica	ant Mean and Range: 4.2 ant CV (%): 3.5	(2.2-8.1) m	NIL SOS
Test Results:	Embryo vzebility ("1 vh") (mon ± 250) EC25 % (v/v) (95% CL) 7 EC50 % (v/v) (95% CL)	Control UXO	ample ID 645-1645-185 1 ± 10.5
Reviewed by:	Jou	Date re	viewed: Feb - 4/19

Date reviewed:

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

lient: ample ID:		NPAN POYU		1645	-183		Star	t Date &	Time:	DEC	anber anber	12,2	01B 6	1130
Vork Order #:	- 1:	17.20	2				100		CER#:	3		mykiss		
C # 1		-					Da	iys	=					
Contro   Concentration	0	100	1	2 - 5	2				4		5		6	7
(4/2 u/u)	init	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (*C)	11-	135	14.2	135	140	140	14,0	14.2	140	CHI	140	140	No-	140
DO (mg/L)	10/3	10.2	100	10.2	101	10,2	9,9	19:2	100	1.07	100	19.0	(90	99
pH	GX	7.1	11	69	20	3,0	71	20	34	15	セン	7.1	7.1	7.0
Cond. (µS/cm)	37	3	0	3	3	3	5		35	35		3		37
Initials	12.WE	Vu	~	W	w		2		4	W	4	W	et.	Uw
							-							
160	4						Da	lys						
Concentration	0	- 22	1	3	2	3	3		4	3	5	1 3	6	7
alle districts	Init	new	old	пем	old	now	old	DOW	old	now	old	new	old	fina
Temperature (°C)	135	140	140	134	140	140	14.0	140	14,0	140	No	14.0	Nio	195
DO (mg/L)	103	101	100	193	VO.A	10/2	9.8	1012	10,1	[0.1	100	10.0	101	49
pH	6.8	7.3	35	6.9	11	大つ	2,7	40	36	7.3	78	1.3	7.8	26
Cond. (µS/cm)	577	3	32	5	M	53	1	-5	37	5	7.1	53		510
Initials	WWA		Mm	- W	NA.		<i>~</i>	1	-	Mi	44.	1000		VARCE.
Concentration	0		1		2		Da 3	rys .	4		5	i en	6	7
	init	new	old	now	old	new	blo	new	old	new	old	new	old	final
Temperature (°C)	72.20	111111111111111111111111111111111111111			10000	1		1440		Wale A		150000		
DO (mg/L)														
pН			1	100					200					
Cond. (µS/cm)														
Initials				-							-			
							Da	iys .						
Concentration	0		1		2		3		4		5	1	6	7
505000000000000000000000000000000000000	inst.	new	old	пем	old	new	old	new	old	new	old	new.	old	fina
Temperature (°C)	4-5			7719		1		-	1997 15		947		1	
DO (mg/L)					F 7	三日		1/47			2			1
pH	9	- 2	1	25.67		8 =								1
Cond. (µS/cm)														1
Initials														1
hermometer: (IPA)		er/probe		213	pH met	en/probe:	213	213	Conduc	tivity me		H: 213	3.000	
Hardness*		1	1	30		-/				0.0000000				
Alkalinity*	15			50		1				Ravio	wed by	J6	L	
MINABILITY	10.00	100	100								terminal section	Fel		

## Embryo Toxicity Test Daily Mortality

Client:	Margan	Start Date & Time:	December	12, 2018 @ 16456
Sample ID:	UY0945-1645-18B	The state of the s		19, 2018 @ 1180h
Work Order #:	182202	Test Species:	Oncorhynchus i	mykiss

3 4 1 2 3 3 4 4 1 1 2 3 3 4 4 1 1 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 4 4 1 1 2 2 3 3 3 4 4 1 1 2 2 3 3 3 4 4 1 1 2 2 3 3 3 4 4 1 1 2 2 3 3 3 4 4 1 1 2 2 3 3 3 4 4 1 1 2 2 3 3 3 4 4 1 1 2 2 3 3 3 4 4 1 1 1 2 2 3 3 3 4 4 1 1 1 2 2 3 3 3 4 4 1 1 1 2 2 3 3 3 4 4 1 1 1 2 2 3 3 3 4 4 1 1 1 1 2 2 3 3 3 4 4 1 1 1 1 2 2 3 3 3 4 4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 0	2	3	4	5	0 1000	7 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Dead Eggs	Total Undeveloped	Total No. Embryo	Total Exposed 30 30 30 30 30 30 30 30
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3 4 1 2 3 4 1 2 3 4 1 1 2 3 4 1											
4 1 2 3 4 1 2 3 4 1 1 2 3 4 1											
1 2 3 4 1 2 3 4 1 2 3 4 1 2											
2 3 4 1 2 3 4 1 2 3 4 1											
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3	_										
ech Initials				~	ww		8	vun	lun	VIIIL	uni

Tech Initials	1	IN	0-1	\	AND WALL	05	www	hun	MAL	uni
Comments:										
Reviewed by:		Ja	-			Date	reviewed:	Felo	. 4/19	
Version 1.0 Issued Jun	e 26, 2006							Macelline	Endonmental Co	energy by

## **CETIS Summary Report**

Report Date:

31 Jan-19 11:09 (p 1 of 1)

						Test	Code/ID:		182202b / 16	3-8024-91	128	
Salmonid Em	bryo Survival ar	nd Deve	elopment T	est					N	utilus Envi	ironment	cal
Batch ID:	19-9100-1679	18	Test Type:	Development			Ana	vst: Yv	onne Lam			
Start Date:	12 Dec-18 16:45	5	Protocol:	EC/EPS 1/RM/	28		Dilu	ent: De	chiorinated T	ap Water		
Ending Date:	19 Dec-18 11:30		Species:	Oncorhynchus	mykiss		Brin	7.77		25.76-33		
Test Length:	6d 19h		Taxon:	Actinopterygii			Sou	rce: Va	ncouver Islan	nd Trout Hat	Age:	
Sample ID:	09-8206-0820	10	Code:	UY0945-1645-1	188		Proj	ect:				
Sample Date:	11 Dec-18 01:14		Material:	Effluent			Sou		podam .			
Receipt Date:	12 Dec-18 13:03		CAS (PC):				Stat	ion: UY	0945-1645-1	88		
Sample Age:	40h (5 °C)		Client:	Maxxam				0000 1000 100		370		
Single Compa	rison Summary	4										
Analysis ID	Endpoint		Comp	parison Method			P-Value	Compar	son Result			8
16-1432-2040	Proportion Norm	ral .	Equal	Variance t Two-	Sample Ter	st	0.6455	The second second	ssed proport			1
Proportion No	ormal Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effec	
0	N	4	0.725	0 0.5727	0.8773	0.6000	0.8333	0.0479	0.0957	13.21%	0.00%	
100		4	0.751	1 0.5836	0.9186	0.6333	0.8710	0.0526	0.1053	14.01%	-3.60%	
Proportion No	ormal Detail								240-03-00		101.000.000	
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4							
0	N	0.7333	0.600	0 0.8333	0.7333							_
100		0.8000	0.633	3 0.7000	0.8710							
Proportion No	rmal Binomials											_
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4							
0	N	22/30	18/30	25/30	22/30							=
100		24/30	19/30									

## **CETIS Analytical Report**

Report Date: Test Code/ID: 31 Jan-19 11:09 (p 1 of 2) 182202b / 16-8024-9128

Salmonid Em	bryo Survival	and Develo	pment To	est					N:	autilus Env	ironment
Analysis ID:	16-1432-2040	E	ndpoint	Proportion Nor	mail		CET	IS Version:	CETISY	1.9.4	
Analyzed:	31 Jan-19 11:		nalysis:	Parametric-Tw			Stati	us Level:	1	0505	
Batch ID:	19-9100-1679	To	est Type:	Development			Anal	vst: Yvor	ne Lam		
Start Date:	12 Dec-18 16:		rotocol:	EC/EPS 1/RM	28		Dilu		niorinated 1	Tap Water	
Ending Date:	19 Dec-18 11:	30 S	pecies:	Oncorhynchus	mykiss		Brin	100	Call Control	Borner St.	
Test Length:	6d 19h	200	xxon:	Actinopterygli	1,004,000,000		Sour	The second	couver Islan	nd Trout Ha	f Age:
Sample ID:	09-8206-0820	C	ode:	UY0945-1645-	18B		Proj	ect			
	11 Dec-18 01:		aterial:	Efficient			Sou		ram		
	12 Dec-18 13:	193	AS (PC):	2000			Stati	Contract Con	945-1645-1	188	
Sample Age:		200	lient:	Maxxam							
Data Transfor	m	Alt Hyp					Comparis	son Result			PMSD
Angular (Come	icted)	C>T						sed proporti	on normal		20.43%
Equal Variance	e t Two-Samp	ole Test		1							
Control	vs Conc-1	6	Test 5	Stat Critical	MSD DE	P-Type	P-Value	Decision(	an5%)		
Negative Contr	rol 100	***	-0.391	5 1.943	0.161 6	CDF	0.6455	Non-Signit	icant Effec	t	
Auxiliary Test	la .										
Attribute	Test				Test Stat	Critical	P-Value	Decision(	0:5%)		
Control Trend		Gendall Tren	d Test				1.0000	The second secon	and the same of th	in Controls	8
ANOVA Table											
Source	Sum Sq	uares	Mean	Square	DF	F Stat	P-Value	Decision	a:6%)		
Between	0.00209	27	0.002	0927	1	0.1533	0.7090		cant Effec		
Error	0.08193	27	0.013	6554	6	777					
Total	0.08402	54			7						
Distributional	Tests										
Attribute	Test				Test Stat	Critical	P-Value	Decision/	or 4563		
Variances	Variance	Ratio F Te	et.	_	1.341	47.47	0.8151	Equal Vari			
Distribution	Shapiro	Wilk W No	mality Ter	st .	0.9275	0.6451	0.4939	Normal Di			
Proportion No	ormal Summar	ry									
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.725	0.5727	0.8773	0.7333	0.6000	0.8333	0.0479	13.21%	0.00%
100		4	0.751	0.5836	0.9186	0.7500	0.6333	0.8710	0.0526	14,01%	-3.60%
Angular (Corr	ected) Transfo	ormed Sum	mary								
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	1.023	0.8513	1.195	1.028	0.8861	1.15	0.054	10.56%	0.00%
100		4	1.056	0.8565	1.255	1.049	0.9204	1.203	0.05254	11.85%	-3.16%
		4	1.056	0.8565				1.203	0.06254	11.85%	-3.16%
Proportion No		4 Rep 1	1.056 Rep 2		1.255			1.203	0.06254	11.85%	-3.16%
Proportion No Conc-%	ormal Detail	De si		Rep 3	1.255 Rep 4			1.203	0.06254	11.85%	-3.16%
Proportion No Conc-%	ormal Detail	Rep 1	Rep 2	Rep 3	1.255			1.203	0.06254	11.85%	3.16%
Proportion No Conc-% 0 100	ormal Detail	Rep 1 0.7333 0.8000	Rep 2 0.6000 0.6333	Rep 3	1.255 Rep 4 0.7333			1.203	0.05254	11.85%	-3.10%
Proportion No Conc-% 0 100 Angular (Corn	ormal Detail Code N	Rep 1 0.7333 0.8000	Rep 2 0.6000 0.6333	Rep 3 0 0.8333 0.7000	1.255 Rep 4 0.7333			1.203	0.06254	11.85%	3.16%
Proportion No Conc-% 0 100 Angular (Corn Conc-%	Code N ected) Transfo	Rep 1 0.7333 0.8000 ormed Deta	Rep 2 0.6000 0.6333	Rep 3 0.8333 0.7000	1.255 Rep 4 0.7333 0.8710			1.203	0.06254	11.85%	3.16%
Proportion No Conc-% 0 100 Angular (Corn Conc-%	Code N ected) Transfo	Rep 1 0,7333 0,8000 ormed Deta Rep 1	Rep 2 0.6000 0.6333 II Rep 2	Rep 3 0 0.8333 3 0.7000 Rep 3	1.255 Rep 4 0.7333 0.8710			1.203	0.05254	11.85%	3.16%
Proportion No Conc-% 0 100 Angular (Corn Conc-% 0	Code N ected) Transfo	Rep 1 0.7333 0.8000 ormed Deta Rep 1 1.028 1.107	Rep 2 0.6000 0.6333 II Rep 2 0.886	Rep 3 0 0.8333 0 0.7000 Rep 3	1.255 Rep 4 0.7333 0.8710 Rep 4 1.028			1.203	0.06254	11.85%	-3.16%
Proportion No Conc-% 0 100 Angular (Corre Conc-% 0 100	Code N ected) Transfo	Rep 1 0.7333 0.8000 ormed Deta Rep 1 1.028 1.107	Rep 2 0.6000 0.6333 II Rep 2 0.886	Rep 3 0 0.8333 0 0.7000 Rep 3 1 1.15 0.9912	1.255 Rep 4 0.7333 0.8710 Rep 4 1.028			1.203	0.06254	11.89%	3.16%
Conc-% 0 100	Code N ected) Transfo Code N	Rep 1 0.7333 0.8000 ormed Deta Rep 1 1.028 1.107	Rep 2 0.6000 0.6333 II Rep 2 0.8861 0.9204	Rep 3 0 0.8333 0 0.7000 Rep 3 1 1.15 0.9912	Rep 4 0.7333 0.8710 Rep 4 1.028 1.203			1.203	0.06254	11.85%	3.16%

Ananose WW DA. Feb. 4/10

## **CETIS Analytical Report**

Report Date: Test Code/ID: 31 Jan-19 11:09 (p 2 of 2) 1822026 / 16-8024-9128

Salmonid Embryo Survival and Development Test

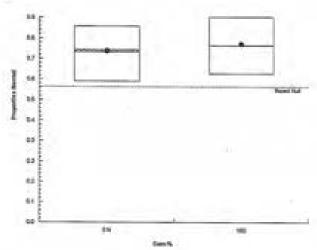
Nautilus Environmental

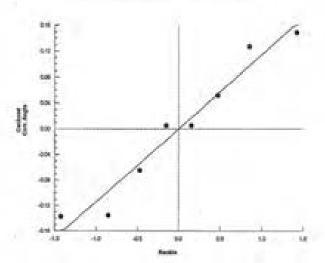
Analyzed:

Analysis ID: 16-1432-2040 31 Jan-19 11:09 Endpoint: Proportion Normal Analysis: Parametric-Two Sample **CETIS Version:** Status Level:

**CETISV1.9.4** đ

Graphics







**APPENDIX B – Chain-of-Custody Form** 

CHAIN OF CUSTODY RECORD FOR SUBCONTRACTED WORK

Page 01 of 01

COC# 88A8117-VNAU-01-01

Sent To: Nautilus Environmental - Burnaby 8664 Commerce Court Burnaby, BC, VSA 4N7 Tel: (604) 420-8773

REF	ORT INFORMAT	ION							A	NALYSIS RE	QUESTED	701 170				
Cor	mpany:	Maxxam						JL		TT						
Adi	dress:	4606 Canada Way, Burnab	y, British Colu	mbia, VSG 1K	5			1/4 m		Н						
Cor	ntact Name:	Veronica De Guzman						100								
Em	all:	VDeGuzman@maxxam.ca,	edmenvirocs	@maxamana	lytics.com			Day Embryo Subco			1 1					
Pho	one:							8		1 1	-1-1		-			
Ma	xxam Project I	: 88A8117						8					(%)			
	SAMPLE ID		MATRIX	DATE SAMPLED (YYYY/MM/DD)	TIME SAMPLED (HIR MAN)	SAMPLER INITIALS	# CONT.	Aubbow Tribur 7					Temp		ADDITIONAL SA	MPLE INFORMATION
1	UY0944-1645	-18	GRAS	2018/12/11	00:19	AH	41	X				4	1.0	(P: 03)		
2	UY0945-1645	188	GRAB	2018/12/11	01:14	AH	27	P <sup>k</sup>					Territoria de la constanta de	(Pt 03)		
3																
4																
5																
6								24.0								
7.								-								
*								202								
9						_	_	24		-						
	-							20								
-	IULATORY CRITE vik 1645-18 Ave 8			SPECIAL INSTR				-10					- 47			TURNAROUND TIME
	NA 2010 10 ME 6	· Collabo		**Please return	n a copy of t	nediately if his form wit	you the	ane no e repo	ot accredited for the ort.**	requested	test(s).					Rush Required
Cust	OLER ID: ody Seal Present ody Seal Intact ing Modia Present	YES NO Temp:		COOLER ID: Custody Seal Pre Custody Seal Into Cooling Mediu Pr	et.	YES NO	Ten (°C		1 1	Custody S	eal Present		Temp: (°C)	9.	7 (4)	Date Required  Please inform us if rush charges will be incurred.
keu L	NQUISHED BY: (	ENW (MOUL		8712/12	TIME: 0	The second second		IVED />c	BY: (SIGN & PRINT)  Alban/Peri	#/		2018/12			тіме (немы) 13:03	



**END OF REPORT** 



## Ceriodaphnia dubia Bioassay Report

Job and Sample ID: B8A8117 - UY0945

Test Performed By: NM9, DBA

Date sample collected: 2018/12/11 Test Initiation Date: 2018/12/12 Test End Date: 2018/12/18

Dates when subsamples used: 2018/12/12 2018/12/13 2018/12/16 2018/12/17 2018/12/18

2018/12/14 2018/12/15

Dissolved Oxygen prior to test (mg/L): 8.3

Temperature (°C): 25 pH prior to test (pH units): 7.2 Conductivity (µS/cm): 531

	Test Results		Signif	icant or non-signit	ficant	Method	Data Transforms	Outliers* ( concentration-replicate)
Sample Test Results (%	7 day Survival Result	Effect		Non-Significant		Fisher Exact Test	Log X	None
vol/vol)	7 day Biomass Result	Effect		Non-Significant		Wilcoxon Rank Sum Two- Sample Test	Log X	None
Refere	nce Toxicant Resu	lts	Endpoint	95% LCL	95% UCL	Method	Data Transforms	Outliers* ( concentration-replicate)
Reference	7 day Survival Result	LC50	1.87	1.51	2.31	Spearman-Karber	Log X	None
Toxicant Test Results (g/L)	7 day Biomass Result	IC50	1.18	1.03	1.38	Regression: 4P Log- Logistic+Hormesis	Log X	None
Control Chart	7 day Survival Result	LC50	1.51	0.977	2.33	Shewart	n/a	n/a
Data (g/L)	7 day Biomass Result	IC50	1.20	0.855	1.69	Silewait	II/a	liya

<sup>\*</sup> If outliers were removed, describe in comments.

#### Comments

Reference toxicant analyzed 2018/12/12

## **Test Organisms**

Species: Ceriodaphnia dubia Source of test organisms: Aquatic Biosystems, Colorado Age of organisms at test initiation: ≤ 24 hours, within 12 hours

Unusual Appearance, behaviour or treatment prior to use in test: None Mean % mortality of brood organisms during 7-day period proceding test: Number of neonates produced by each organism in its third or fourth brood: ≥8 neonates Mean number of neonates per adult during first 3 broods in 7  $\,$ 37 Observations of ephippia: None

All test organisms used to initiate this test were taken from a series of individual cultures, that originated from the same mass culture.

The 4th brood or subsequent broods produced during the test are not included in the final statistical analysis.

#### **Test Facilities and Apparatus**

Name address of test laboratory: Maxxam Analytics. 9331 48st NW, Edmonton, AB Fisherbrand 20 x 150 mm lime glass test tubes Test vessels used:

Control/Dilution Water

Consists of: 16 L RODI from in-house system, to which the following are added:

4 L Perrier Brand carbonated spring water 1 mL cyanocobalamin (Vitamin B-12) 1 mL Sodium Selenate Decahydrate

Test Method

Reference method used for testing:  $Biological \ Test \ Method: Test \ of \ Reproduction \ and \ Survival \ Using \ the \ Cladoceran \ \textit{Ceriodaphnia dubia} \ . \ Environment \ Canada, EPS \ 1/RM/21$ 

Second Edition - February 2007

Did the following occur during sample preparations:

Filtered: No
Adjusted for hardness: No
Adjusted for pH: No
Frequency of observations: 24 ± 1 hour
Frequency of water quality measurements: Daily
Design and description of any specialized procedure: N/A

Program used for statistical calculations: Comprehensive Environmental Toxicity Information System (CETIS). Tidepool Scientific Software. Version 1.9.3.0

Reference method used for statistical calculations: Guidance Document on Statistical Methods for Environmental Toxicity Tests. Environment Canada, EPS 1/RM/46 - March 2005.

#### **Test Conditions and Procedures**

Number of test solutions: 20

Number of test concentrations: 1 and a negative control

 Concentrations tested (%):
 100

 Units of tested concentrations:
 % vol/vol

 Number of replicates:
 10

 Volume of test solutions:
 ≥ 15 mL

 Depth of test solutions:
 ≥ 5 cm

 Individuals per test vessel:
 1

 Was pre-aeration performed:
 Yes

Procedure: Oil-free compressed air is dispensed through airline tubing and a disposable pipette

Rate: ≤ 100 bubbles / minute

Duration: 20 minutes

Dates where pre-aeration occurred: 2018/12/12 2018/12/16

2018/12/13 2018/12/17

2018/12/14 2018/12/15

Aeration during testing: None

Refer to comments section for any deviations.

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

Refer to comments section for any deviations regarding reference toxicity testing.

#### Ceriodaphnia dubia Survival and Reproduction Observations

Note: Mortalities are indicated by an "X" in the column and row of the concentration, replicate and day of occurance. If organism was not used for testing, this will be indicated by "N/A" in mortality and neonate columns for the replicate.

Concentration	Donliesto								Day of	Testing								Cumulative	Cumulative	
(%/%vol)	number		1		2		3		4	5		6		7			8	Mean %	Mean	SD of mean reproduction
(76/76V01)	number	neonates	Mortality	neonate	s Mortality	neonates	Mortality	neonates	Mortality	Mortality	Reproduction									
	1	0		0		0		7		11		20								
	2	0		0		0		6		10		5								
	3	0		0		0		5		12		14								
	4	0		0		0		6		13		15								
Control	5	0		0		0		6		15		11						0.0%	31.3	6.7
Control	6	0		0		0		4		14		0						0.070	31.3	0.7
	7	0		0		0		7		10		15								
	8	0		0		0		8		14		16								
	9	0		0		0		7		10		18								
	10	0		0		0		6		12		16								
	1	0		0		0		5		17		16								
	2	0		0		0		6		12		12								
	3	0		0		0		4		12		15								
	4	0		0		0		5		12		12								
100%	5	0		0		0		7		10		0						0.0%	29.1	6.1
100/0	6	0		0		0		7		12		12						0.070	23.1	0.1
	7	0		0		0		9		13		0								
	8	0		0		0		5		16		6				I				
	9	0		0		0		7		11		12				I				
	10	0		0		0		7		13		16								

#### **Average Values for Chemical Data of Test Concentrations**

		Before Efflu	ent Renew	al				After E	ffluent Renewal	
Test Concentration (% vol/vol)	Date	Temperature (°C)	рН	Conductivity (µS/cm)	DO (mg/L)	Date	Temperature (°C)	рН	Conductivity (µS/cm)	DO (mg/L)
Control	2018/12/12	25	7.8	180	7.1	2018/12/13	25	7.7	192	6.1
100	2016/12/12	26	7.2	536	8.1	2016/12/13	25	7.5	531	6.1
Control	2018/12/13	25	7.9	182	7.3	2018/12/14	25	7.8	183	6.4
100	2016/12/15	25	7.3	535	7.8	2016/12/14	25	7.6	517	6.5
Control	2018/12/14	25	8.0	176	7.1	2018/12/15	25	7.7	178	6.6
100	2010/12/14	26	7.4	537	8.1	2016/12/13	25	7.4	510	6.6
Control	2018/12/15	25	7.9	176	7.3	2018/12/16	26	7.7	183	6.6
100	2010/12/13	25	7.5	527	7.6	2016/12/10	24	7.6	511	7.1
Control	2018/12/16	25	8.0	175	7.4	2018/12/17	25	7.7	178	6.8
100	2016/12/10	24	7.4	525	8.1	2016/12/17	25	7.5	514	6.8
Control	2018/12/17	24	8.0	174	7.6	2018/12/18	25	7.6	180	6.1
100	2010/12/17	25	7.3	526	8.2	2016/12/16	25	7.3	517	5.9
Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
100	/^	N/A	N/A	N/A	N/A	,^	N/A	N/A	N/A	N/A







Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B8A8117

Client Project Name & Number: Quarterly Tox SNP-A

**Test Result:** 

96 hrs Mortality % 0% Statistical Method: Visual

Sample Name: 1645-18B Sample Matrix: **Grab Water** 

Description: Clear, colourless Sample Number: UY0945-05

Site Collection: Dec 11, 2018 01:14 AM Sample Collected: Sampling Method: N/A N/A

Sample Collected By: Volume Received: 20 L Temp.Upon Arrival: -1 °C Storage: 2-6°C

Sample Received: Dec 11, 2018 02:06 PM pH: 7.1 Dissolved Oxygen: 11.3 mg/L Analysis Start: Dec 12, 2018 11:17 AM Temperature: 12 °C Sample Conductance: 392 µS/cm

,			•		•				•		• •	
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
0	15	8.0	292	9.0	0	0	0	0	0	0	0	0
100	14	7.3	404	9.5	0	0	0	0	0	0	0	0

Concentration	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	72 hrs	72 hrs	72 hrs	72 hrs	96 hrs	96 hr	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs
0	0	0	0	0	15	7.8	302	9.1	0	0	0	0
100	0	0	0	0	14	7.5	422	9.0	0	0	0	0

The control chart result for this reference toxicant test was outside of 2SD limits. A check of all acclimation and test conditions was Comments: performed, and all requirements were met.

**Culture/Control/Dilution Water** City of Edmonton dechlorinated tap water

Hardness: 170 mg/L CaCO<sub>3</sub> Other parameters available on request.

**Test Conditions** Test concentration: 0,100 (% vol/vol)

Organisms per Vessel: 10 Test Temperature: 15 ± 1 °C Solution Depth: >15 cm

20 6.5±1 mL/min/L Total # of Organisms Used: Pre-aeration Time: 120 min. Rate of Aeration

Test Volume: 20 L Vessel Volume: 38L Test pH Adjusted: No

Loading Density:  $0.3 \, g/L$ Photoperiod: 16:8 (light: dark)

Rainbow Trout (Oncorhynchus mykiss) **Spring Valley Trout Hatchery Test Organism:** Source:

15 ± 2 °C Weight (Mean) +- SD: Length (Mean) +- SD: 4.15 ± 0.36 cm Culture Temperature:  $0.6 \pm 0.2 \,\mathrm{g}$ ≥ 1.0 L/min/kg fish Weight (Range): 0.4 - 0.9 gLength (Range): 3.70 - 4.70 cm Culture Water Renewal:

% Mortality within 7 days: 0.3% Culture Photoperiod: 16:8 (light: dark) Feeding rate and frequency: daily: 1-5% biomass of trout. Acclimation Time: >14 days

Nov 14, 2018 **Reference chemical:** Phenol Test Date: Test Endpoint 96 hrs LC50 (95% confidence interval): 12.2 (10.9, 13.8)mg/L Statistical Method: Probit

Historical Mean LC50 (warning limits): 10.2 (8.60, 12.1) mg/L Concentration: 0,8,10,12,15,20 mg/L

**EPS 1/RM/13 Test Method** Method Deviations: None

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entirety, without the written approval of the laboratory.

Analyst: Dustin Banks, Natasha Lloyd

Date: Dec 21, 2018 08:33 AM Verified By: Natasha Lloyd, Analyst 2

Maxxam Analytics 9331 - 48th Street, Edmonton, Alberta T6B 2R4 Tel: (780) 577-7100 Fax: (780) 450-4187 Page 1 of 1

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Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B8A8117 UY0945-04 Client Project Name & Number: Quarterly Tox SNP-A Sample Number:

Test Result:

48 hrs Mortality % 0% Statistical Method:

0 Control 0 Mean percent mortality: Sample

Sample Name: 1645-18B Sample Matrix: **Grab Water** 

Description: **CLEAR COLOURLESS** Sample Prior to Analysis:

Dec 11, 2018 01:14 AM Sampling Method: Sample Collected: N/A pH: 7.2 Sample Collected By: ΑН Site Collection: N/A Temperature: 19°C

Sample Received: Dec 11, 2018 02:06 PM Volume Received: 1 L Dissolved Oxygen: 10.7 mg/L Analysis Start: Dec 12, 2018 11:42 AM Temp.Upon Arrival: -1 °C Sample Conductance: 462 μS/cm

End: Dec 14, 2018 10:51 AM Storage: 2-6°C Hardness: 100 mg CaCO <sub>3</sub>/L

Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hr	48 hrs	48 hrs
0	20	8.2	359	8.0	0	0	0	0	19	8.2	363	8.1
0	20	8.2	361	8.0	0	0	0	0	20	8.3	369	8.0
0	20	8.2	361	8.0	0	0	0	0	20	8.2	365	8.0
100	19	7.3	467	10.3	0	0	0	0	20	7.8	477	8.0
100	19	7.3	469	10.4	0	0	0	0	20	7.8	478	8.1
100	19	7.3	469	10.3	0	0	0	0	20	7.8	479	8.1

Concentration	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	
% vol/vol	48 hrs	48 hrs	48 hrs	48 hrs	
0	0	0	0	0	
0	0	0	0	0	
0	0	0	0	0	
100	0	0	0	0	
100	0	0	0	0	
100	0	0	0	0	

**Comments:** Many daphnia in sample concentration test vessels floating on water surface.

**Culture/Control/Dilution Water:** City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO<sub>3</sub> Other parameters available on request.

**Test Conditions** Test concentration: 0,0,0,100,100,100 (% vol/vol)

10 25-50 mL/min/L Organisms per Vessel: Pre-aeration Time: 30 min Rate of Pre-aeration:

60 Total # of Organisms Used: Test Temperature: 20 ± 2 °C Test Hardness Adjusted: No Test Volume: 150 mL Vessel Volume: 225 mL Test pH Adjusted: No

Loading Density: 15.0 mL/Daphnia Photoperiod: 16:8 (light: dark)

In House Culture Daphnia magna Source: **Test Organism:** 

Average Brood Size: 24.8 Age at Test Initiation: <24 hrs Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 1.6 20 ± 2 °C Time To First Brood: Culture Temperature: 8 Days **Culture Diet** 

Pseudokirchnriella and YTC at a ratio of 2 mL/L of culture daily. New cultures weekly, 63 daphnids

distributed into 6 culture vessels and 3 reproductive vessels.





Dec 22, 2018 02:46 PM

Date:

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B8A8117Client Project Name & Number:Quarterly Tox SNP-ASample Number:UY0945-04

Reference chemical:Sodium ChlorideTest Date:Dec 05, 2018Test Endpoint 48 hrs LC50 (95% confidence interval):6.96 (5.70, 8.50)g/LStatistical Method:BinomialHistorical Mean LC50 (warning limits):5.80 (4.37, 7.71) g/LConcentration: 0,1.71,2.56,3.82,5.7,8.5 g/L

<u>Test Method</u> EPS 1/RM/14

Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Dustin Banks, Natasha Lloyd

Verified By: Chelsea Tessier, Sample Logistics Supervisor

Maxxam Analytics





### Ceriodaphnia dubia Bioassay Report

Client Name and Sample ID: DDMI - 1645-18

Job and Sample ID: B921715- VK6758

Test Performed By: DBA, NM9, CSH

Date sample collected: 2019/03/26 Test Initiation Date: 2019/03/27

Test End Date: 2019/04/03

Dates when subsamples used: 2019/03/27 2019/03/31 2019/04/01 2019/03/28

2019/03/29 2019/04/02

2019/03/30

Dissolved Oxygen prior to test (mg/L): 9.4 pH prior to test (pH units): 7.0

Temperature (°C): 25 Conductivity (µS/cm): 507

	Test Results		Sign	ificant or non-sign	ificant	Method	Data Transforms	Outliers* ( concentration-replicate)
Sample Test	Sample Test Results (%			Non-Significant		Fisher Exact Test	Untransfomed	N/A
vol/vol)	' Talan Banan dan Man		Wilcoxon Rank Sum Two- Sample Test	Untransformed	100 - 5			
Refere	ence Toxicant Resu	lts	Endpoint	95% LCL	95% UCL	Method	Data Transforms	Outliers* ( concentration-replicate)
Reference Toxicant Test	7 day Survival Result	LC50	1.38	1.27	1.50	Spearman-Karber	Log- X	0.5- 10
Results (g/L)	7 day Reproduction Result	IC50	1.29	1.13	1.36	Linear Interpolation	Log- X	N/A
Control Chart	7 day Survival Result	LC50	1.55	1.02	2.36	Shewart	n/a	n/a
Data (g/L)	7 day Reproduction Result	IC50	1.20	0.867	1.67	Snewart	11/ d	11/ a

<sup>\*</sup> If outliers were removed, describe in comments.

Comments			
		•	

#### **Test Organisms**

Species: Ceriodaphnia dubia Source of test organisms: Aquatic Biosystems, Colorado

≤ 24 hours, within 12 hours Age of organisms at test initiation:

Unusual Appearance, behaviour or treatment prior to use in test: None Mean % mortality of brood organisms during 7-day period proceding test: 6.9 Number of neonates produced by each organism in its third or fourth brood: ≥8 neonates Mean number of neonates per adult during first 3 broods in 7 days: Observations of ephippia: None

All test organisms used to initiate this test were taken from a series of individual cultures, that originated from the same mass culture.

The 4th brood or subsequent broods produced during the test are not included in the final statistical analysis.

**Test Facilities and Apparatus** 

Name address of test laboratory: Maxxam Analytics. 9331 48st NW, Edmonton, AB Test vessels used: Fisherbrand 20 x 150 mm lime glass test tubes

Control/Dilution Water

Consists of: 16 L RODI from in-house system, to which the following are added:

4 L Perrier Brand carbonated spring water 1 mL cyanocobalamin (Vitamin B-12) 1 mL Sodium Selenate Decahydrate

**Test Method** 

Reference method used for testing:

Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dubia. Environment Canada, EPS

1/RM/21 Second Edition - February 2007

Did the following occur during sample preparations:

Filtered: No
Adjusted for hardness: No
Adjusted for pH: No
Frequency of observations: 24 ± 1 hour
Frequency of water quality measurements: Daily
Design and description of any specialized procedure: N/A

Design and description of any specialized procedure: N/A
Program used for statistical calculations: Comprehensive Environmental Toxicity Information System (CETIS). Tidepool Scientific Software. Version 1.9.3.0

Reference method used for statistical calculations: Guidance Document on Statistical Methods for Environmental Toxicity Tests. Environment Canada, EPS 1/RM/46 - March 2005.

#### Test Conditions and Procedures

Number of test solutions: 20

Number of test concentrations: 1 and a negative control

 Concentrations tested (%):
 100

 Units of tested concentrations:
 % vol/vol

 Number of replicates:
 10

 Volume of test solutions:
 ≥ 15 mL

 Depth of test solutions:
 ≥ 5 cm

 Individuals per test vessel:
 1

 Was pre-aeration performed:
 Yes

Procedure: Oil-free compressed air is dispensed through airline tubing and a disposable pipette

Rate: ≤ 100 bubbles / minute

Duration: 20 minutes

Dates where pre-aeration occurred: 2019/03/27 2019/03/31 2019/03/28 2019/04/01

2019/03/29 2019/04/02

2019/03/30

Aeration during testing: None

Refer to comments section for any deviations.

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

Refer to comments section for any deviations regarding reference toxicity testing.

#### Ceriodaphnia dubia Survival and Reproduction Observations

Note: Mortalities are indicated by an "X" in the column and row of the concentration, replicate and day of occurance. If organism was not used for testing, this will be indicated by "N/A" in mortality and neonate columns for the replicate.

Concentration	Poplicato					Day of Testing							Cumulative	Cumulative	SD of mean					
(%/%vol)			1		2		3	4		5		6		7			8	Mean %	Mean	
(%/%VOI)	number	neonates	Mortality	neonates	Mortality	neonates	Mortality	neonates	Mortality	neonates	Mortality	neonates	Mortality	neonates	Mortality	neonates	Mortality	Mortality	Reproduction	reproduction
	1	0		0		0		7		10		0		17						
	2	0		0		0		4		10		0		14						
	3	0		0		0		5		12		19								
	4	0		0		0		7		11		0		17						
Control	5	0		0		0		3		13		0		16				10.0%	31	4.5
Control	6	0		0		0		8		12		3						10.0%	31	4.5
	7	0		0		0		7		9		0		9	х					
	8	0		0		0		6		9		15								
	9	0		0		0		7		10		19								
	10	0		0		0		4		12		15								
	1	0		0		0		5		10		0		11						
	2	0		0		0		5		9		0		9						
	3	0		0		0		5		12		8								
	4	0		0		0		5		10		0		11						
100%	5	0		0		0		5		2	Х							20.0%	24.1	6.2
100%	6	0		0		0		5		11		0		11				20.070	24.1	0.2
	7	0		0		0		5		9		0		13						
	8	0		0		0		6		10		0		9						
	9	0		0		0		5		8		13			X					
	10	0		0		0		7		13		9								

**Average Values for Chemical Data of Test Concentrations** 

		Before Efflu	ent Rene	wal			After E	ffluent Re	newal	
Test Concentration (% vol/vol)	Date	Temperature (°C)	pН	Conductivity (μS/cm)	DO (mg/L)	Date	Temperature (°C)	pН	Conductivity (µS/cm)	DO (mg/L)
Control	2019/03/27	26	7.7	179	7.3	2019/03/28	26	7.7	191	6.5
100	2015/05/27	26	7.0	513	8.7	2015/05/28	26	7.5	516	6.8
Control	2019/03/28	25	7.9	174	7.4	2019/03/29	25	7.6	176	6.2
100	2019/03/28	25	7.2	505	8.8	2015/05/25	25	7.3	487	6.4
Control	2019/03/29	25	7.9	174	7.2	2019/03/30	25	7.6	178	6.3
100	2019/03/29	25	7.2	496	8.8	2019/03/30	25	7.5	496	6.4
Control	2019/03/30	25	7.8	176	7.0	2019/03/31	26	7.8	184	7.2
100	2015/05/50	25	7.2	502	8.1	2015/05/51	26	7.5	500	7.4
Control	2019/03/31	24	8.1	171	7.8	2019/04/01	26	7.8	179	6.6
100	2015/05/51	25	7.3	499	8.8	2013/04/01	26	7.6	503	6.8
Control	2019/04/01	25	7.9	131	7.3	2019/04/02	25	7.4	140	6.6
100	2013/04/01	25	7.3	500	9.1	2013/04/02	25	7.2	495	6.7
Control	2019/04/02	26	7.7	130	7.4	2019/04/03	25	7.1	132	6.7
100	2013/04/02	26	7.1	515	8.4	2013/04/03	25	7.1	502	6.6
Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
100	IV/A	N/A	N/A	N/A	N/A		N/A	N/A	N/A	N/A



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Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B921715Client Project Name & Number:Quarterly Tox SNP-ASample Number:VK6758-01

Test Result:

48 hrs Mortality % 0 Statistical Method:

Mean percent mortality: Sample 0 Control 0

Sample Name: 1645-18 Sample Matrix: Grab Water

Description: Clear and colourless Sample Prior to Analysis:

Mar 26, 2019 04:19 AM Sampling Method: 7.0 Sample Collected: N/A pH: Sample Collected By: AΗ Site Collection: N/A Temperature: 18 °C Sample Received: Mar 26, 2019 02:42 PM Volume Received: 1 L Dissolved Oxygen: 11.1 mg/L Analysis Start: Mar 27, 2019 03:16 PM Temp.Upon Arrival: -2 °C Sample Conductance: 439 µS/cm

End: Mar 29, 2019 03:22 PM Storage: 2-6°C Hardness: 120 mg CaCO <sub>3</sub>/L

			•		U						U	
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hr	48 hrs	48 hrs
0	20	8.2	391	8.4	0	0	0	0	20	8.0	393	8.3
0	20	8.0	391	8.4	0	0	0	0	20	8.0	395	8.4
0	20	8.0	390	8.4	0	0	0	0	20	8.0	395	8.4
100	20	7.2	451	10.8	0	0	0	0	20	7.6	457	8.5
100	20	7.1	452	10.9	0	0	0	0	20	7.6	457	8.5
100	20	7.0	452	10.7	0	0	0	0	20	7.6	455	8.5

Concentration	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)
% vol/vol	48 hrs	48 hrs	48 hrs	48 hrs
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
100	0	0	0	0
100	0	0	0	0
100	0	0	0	0

Comments: None

Culture/Control/Dilution Water: City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO<sub>3</sub> Other parameters available on request.

Test Conditions Test concentration: 0,0,0,100,100,100 (% vol/vol)

Organisms per Vessel: 10 Pre-aeration Time: 30 min Rate of Pre-aeration: 25-50 mL/min/L

Total # of Organisms Used : 60 Test Temperature :  $20 \pm 2$  °C Test Hardness Adjusted : No Test Volume : 150 mL Vessel Volume : 225 mL Test pH Adjusted: No

Loading Density: 15.0 mL/Daphnia Photoperiod: 16:8 (light: dark)

<u>Test Organism :</u> Daphnia magna Source : In House Culture

Age at Test Initiation :<24 hrs</th>Average Brood Size :26.2Culture Photoperiod :16:8 (light: dark)% Mortality within 7 days :1.6Culture Temperature : $20 \pm 2$  °CTime To First Brood :8 Days

Culture Diet Pseudokirchnriella and YTC at a ratio of 2 mL/L of culture daily. New cultures weekly, 63 daphnids

distributed into 6 culture vessels and 3 reproductive vessels.





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Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B921715Client Project Name & Number:Quarterly Tox SNP-ASample Number:VK6758-01

Reference chemical: Sodium Chloride Test Date: Mar 27, 2019

Test Endpoint 48 hrs LC50 (95% confidence interval) : 6.17 (5.50, 6.93)g/L Statistical Method : Untrimmed

Spearman-Kärber

Historical Mean LC50 (warning limits) : 6.10 (4.57, 8.14) g/L Concentration : 0,1.71,2.56,3.82,5.7,8.5 g/L

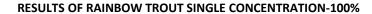
Test Method EPS 1/RM/14
Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Analyst 2 Date: Apr 02, 2019 04:09 PM







Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B921715

Client Project Name & Number: Quarterly Tox SNP-A

**Test Result:** 

96 hrs Mortality % 0 Statistical Method: Visual

Sample Name: Sample Matrix: Grab Water

Description: Clear and colourless. Sample Number: VK6758-03

Sample Collected: Mar 26, 2019 04:19 AM Sampling Method: N/A Site Collection: N/A

Sample Collected By: AH Volume Received: 20L Temp.Upon Arrival: -2 °C Storage: 2-6°C

Sample Received: Mar 26, 2019 02:42 PM pH: 7.1 Dissolved Oxygen: 10.7 mg/L
Analysis Start: Mar 27, 2019 01:20 PM Temperature: 13 °C Sample Conductance: 383 μS/cm

•					•				•		•	
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
0	15	7.7	347	9.0	0	0	0	0	0	0	0	0
100	14	7.2	388	9.3	0	0	0	0	0	0	0	0

Concentration	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	72 hrs	72 hrs	72 hrs	72 hrs	96 hrs	96 hr	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs
0	0	0	0	0	15	7.9	351	9.2	1	10.0	0	0
100	0	0	0	0	15	7.6	413	9.1	0	0	0	0

Comments: None

**Culture/Control/Dilution Water**City of Edmonton dechlorinated tap water

Hardness: 190 mg/L CaCO<sub>3</sub> Other parameters available on request.

Test Conditions Test concentration: 0,100 (% vol/vol)

Organisms per Vessel : 10 Test Temperature :  $15 \pm 1$  °C Solution Depth : >15 cm

Total # of Organisms Used: 20 Pre-aeration Time: 120 min. Rate of Aeration 6.5±1 mL/min/L

Test Volume : 20 L Vessel Volume : 38L Test pH Adjusted: No

Loading Density: 0.3 g/L Photoperiod: 16:8 (light: dark)

<u>Test Organism</u>: Rainbow Trout (Oncorhynchus mykiss) Source: Spring Valley Trout Hatchery

Culture Temperature :  $15 \pm 2$  °C Weight (Mean) +- SD :  $0.5 \pm 0.2$  g Length (Mean) +- SD :  $3.96 \pm 0.38$  cm Culture Water Renewal :  $\geq 1.0$  L/min/kg fish Weight (Range) : 0.4 - 0.8 g Length (Range) : 3.40 - 4.40 cm

Culture Photoperiod : 16:8 (light: dark) % Mortality within 7 days : 0.2% Feeding rate and frequency : daily: 1-5% biomass of trout. Acclimation Time: >14 days

Reference chemical:PhenolTest Date:Mar 07, 2019Test Endpoint 96 hrs LC50 (95% confidence interval):9.50 (8.64, 10.3)mg/LStatistical Method:Probit

Test Endpoint 96 hrs LC50 (95% confidence interval): 9.50 (8.64, 10.3)mg/L Statistical Method: Probit Historical Mean LC50 (warning limits): 10.3 (8.65, 12.3) mg/L Concentration: 0,8,10,12,15,20 mg/L

Test Method EPS 1/RM/13
Method Deviations: None

<u>Note:</u> The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Natasha Lloyd, Analyst 2 Date: Apr 09, 2019 09:57 AM

Maxxam Analytics

9331 - 48th Street, Edmonton, Alberta T6B 2R4 Tel: (780) 577-7100 Fax: (780) 450-4187

www.maxxam.ca



# Toxicity testing on samples VK6758-1645-18 and VK6759-1645-18B

Collected March 26, 2019

**Final Report** 

April 24, 2019

Submitted to: Maxxam Analytics

Burnaby, BC



i

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APPENDIX A – Oncorhynchus mykiss Toxicity Test Data

APPENDIX B – Chain-of-Custody Form



### **SIGNATURE PAGE**

Report By:

Yvonne Lam, B.Sc. Laboratory Biologist Reviewed By: Armando Tang, R.P.Bio

1. Tag

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	VK6758-1645-18
Sumple 12	VK6759-1645-18B
Sample collection date	March 26, 2019
Sample receipt date	March 27, 2019
Sample receipt temperature	2.7°C and 3.4°C
Test type	7-d rainbow trout (Oncorhynchus mykiss) embryo viability

## **Summary of Results**

	Mean ± SD								
Endpoint	Control	VK6758-1645-18	Control	VK6759-1645-18B					
Embryo viability (%)	86.7 ± 23.1	86.7 ± 20.3	82.8 ± 27.0	81.1 ± 32.7					

SD = Standard Deviation



### 1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability toxicity tests for Maxxam Analytics on two samples identified as VK6758-1645-18 and VK6759-1645-18B. The samples were collected on March 26, 2019 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on March 27, 2019. The samples were each transported in four 10-L plastic containers and received at temperatures of 2.7 and 3.4°C. The samples were stored in the dark at  $4 \pm 2$ °C prior to testing.

This report describes the results of the toxicity tests. Copies of raw laboratory data sheets and statistical analysis 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 toxicity test is summarized in Table 1, and followed procedures described by Environment Canada (1998) and modified by Canaria *et al.* (1999). Statistical analyses were performed using CETIS (Tidepool Scientific Software, 2013).



# Table 1. Summary of test conditions: 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability single concentration 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 100% (undiluted) sample, 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  $14 \pm 1^{\circ}$ C Feeding None Light intensity Dark

Photoperiod 24 hours dark

Aeration Continuous gentle aeration

Temperature, dissolved oxygen, pH and conductivity measured

Test measurements 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 endpoints Embryo viability

Test acceptability criteria for controls Embryo viability ≥70%

Reference toxicant Sodium dodecyl sulphate (SDS)



### 3.0 RESULTS

Results of the rainbow trout embryo viability toxicity tests conducted on samples VK6758-1645-18 and VK6759-1645-18B are summarized in Table 2. There were no statistically significant differences relative to the laboratory controls for either sample, with embryo viability in both samples and all test treatments  $\geq 81\%$  (v/v).

Table 2. Results: 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability single concentration test.

Concentration	Embryo Viability (%) (Mean ± SD)						
(% v/v)	VK6758-1645-18	VK6759-1645-18B					
Laboratory Control	86.7 ± 23.1	82.8 ± 27.0					
100	86.7 ± 20.3	81.1 ± 32.7					

SD = Standard Deviation

The samples were not statistically significantly different relative to their respective Laboratory Control



### 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 this test is best described by the standard deviations around the means and/or confidence limits around the point estimates.

There were deviations from the test methodology. 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 method. 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. While the method specifies that a minimum of four female egg sources must be used, at test termination one egg source (Replicate A) produced results that were inconsistent with the other three. Thus, this replicate was removed from the final statistical analyses. The deviations did not seem to affect the results of the test and control criterion was met at the end of the exposure.

Results of the reference toxicant test conducted during the testing program are summarized in Table 3. Results for this test fell within the acceptable range for organism performance of 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 was performed under the same conditions as those used for the samples.

Table 3. Reference toxicant test results.

Test Species	Endpoint	Historical Mean (2 SD Range)	CV (%)	Test Date
O. mykiss	Viability (EC50): 2.2 mg/L SDS	4.1 (2.2 – 7.7) mg/L SDS	32	March 27, 2019

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 1.9.4 Tidepool Scientific Software, McKinleyville, CA. 255 pp.



**APPENDIX A – Oncorhynchus mykiss** Toxicity Test Data

# Rainbow Trout Early Life Stage Summary Sheet

Client	Margan	Start Date/	Time: Ma	rch 27, 20	11 @ 17454
Work Order No.:	190577	Test Speci	es: Onco	orhynchus myk	iss
Sample Informat	ion:				
Sample ID: Sample Date: Date Received: Sample Volume:	NK6758-1645-18 March 26, 2019 March 27, 2019 4 × 10L	-			
Dilution Water:					
Type: Hardness (mg/L C Alkalinity (mg/L Ca		Tap Water			
Test Organism In	formation:				
Batch No.: Source: Loading Density:	032719 Trout Ladge, Sun 1.14 SIL	ver, wh			
Number of male b Number of female Sperm motility che		3 m motility using a	compound	microscope	
SDS Reference T	oxicant Results:				
Reference Toxical Stock Solution ID: Date Initiated: 7-d EC50 (95% C	18303 March 27, 20				
Reference Toxical Reference Toxical	nt Mean and Range: 4.0 mt CV (%): 3	(22-7.7)	ngil Si	25	
Test Results:	Embro viewliky (twb)		Sample I	D	
	(mean = 250) EC25 % (v/v) (96% CL) EC50 % (v/v) (95% CL)		86,7-1 20.	15-18	
Reviewed by:	W	Date	reviewed:	Agu	118,2019

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

Client: Sample ID: Nork Order #:			- 164	5-18				t Date 8 p Date 8		A-pol		7,20		745 15 15
								Test Sp	ecies:	Oncort	hyrnchus	mykiss		
Control							Da	rys						
Concentration	0		1	-	2		3	4		5		6		7
( b v(v)	init	new	old	new	old	new	old	new	old	new	old	new	old	fina
Temperature (°C)	135	140	135	140	13.5	13.0	13.5	140	135	140	135	190	135	13:5
DO (mg/L)	10.2	10.3	10.2	100	9.8	10.5	14.2	10/2	123	10,0	10.0	192	10.2	101
pH	605	7.1	7.1	6.3	6.5	71	7.2	6.9	7.1	66	6.4	68	65	70
Cond. (µS/cm)	32	3	2		2	7	4	- 5	4	-	4	54	Access to the second	34
Initials	un	- 4	w	csi	yu .		'n	_	w-		ML	Min	-	mil
100														
Concentration	0	-	1	H 3	2			ys	4					
	init	new	old	C		Bridge Company	3	The same of		No. of Concession,	5		6	7
Temperature (°C)	14.0	140	R.	13.S	old 13.5	13.5	old (3.5	new 142	old (3.5	new	oid	new	old	fina
The state of the s			100	100			A company of the comp			140	13.5	140	135	13.5
DO (mg/L)	10,2	10.2	19.2	CO	9.9	10-2	102	10.1	10.3	102		49	10.2	122
рН	17	13	175	7.4	73	74	26	7.7	7.5	13	7,4	13	15	16
Cond. (µS/cm)	200	S		502			27	50	5	50	-		2	509
Initials	Wh	U	M	CS.	yuu	5	ρ	(M)	V^	W.	ساره	W	-	WW
	4						Da	IV'S					_	
Concentration	0	100	1 2		3 4				5		8	7		
	init	new	old	new	old	new	old	new	old	new	old	DOW	old	fina
Temperature (°C)					- 010	100.44	200		- CHU	1,00,00	COLL	LILLYN	- UIU	THEFT
DO (mg/L)		-					1 - 0							
pH														_
Cond. (µS/cm)														
		_												
Initials					-							_		
CHANGE TO THE MEDICAL							Da	ys .						
Concentration	0		1		2			4			5	- 36	8	7
	init	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)			- 0.00	0.000	2000			40.00			A			
DO (mg/L)														
pH	1	E.S												
Cond. (µS/cm)														
Initials								7						-
ermometer:(£2#3)	DO mete	mprobe:	<u> 243</u> /	213	pH mete	r/probe:	<u>43</u> /	213	Conduc	tivity me	tenlprobe	<u> 43</u>	1_115	
- 1	Con	2000000		01-			/			Analys	ts:	50, C	S WW	4
Hardness* Alkalinity*	12		111	4		/					-		1	-
			36			200							200	

Comments:

# Embryo Toxicity Test Daily Mortality

Client:	Maxxan	Start Date & Time: March 27 2018 @ 1745h
Sample ID:	VK6-758-1645-1B	Stop Date & Time: April 3, 2019 @ 1515h
Work Order #:	190577	Test Species: Oncorhynchus mykiss

Concentration	Rep		Day of	Test	- No.	of Mo	rtaliti	es	Total	Total	Total No.	Total
(% vlv)		1	2	3	4	5	6	7	Dead Eggs	Undeveloped	Embryo	Exposed
Control	1	0	0	0	0	0	5	0	-0	251	0	29 30
	2	1	1						0	0	30	357
	3		11	136	13/10				0	0	10	30
	4							742	0	12	19	30 30
00	1			190	11.12			2	7	28	0	
	2					100	П	0	0	1	28	30 30 30
	3	13/2		14 5.5				0	.0	0	30	30
	4	4	V	4	19	U	U	0	0	11	19	30
	1			(E)		14	100		-000			-
	2			1								
	3										Jan Barry	
	4		45-3	(5.3)				1.17				
	1	1 7										
	2											
	3							-				
	4		Ties.									
	1											
	2							-				
	3											
	4		_						-			
	1							4				
	2											
	3	1	1									
	4											
	1 2											
	3											
	4	-	-									
	1	-										
	2											
	3	-	-					9.94				
	4											
ech Initials		200.00	VWI	700				WW.	WWW	Aug -	· uu	MM

	3											
Fech Initials	4	VMV.	VWV.	70	un	الملالا	w	AWA	ww	NW	WW	NW
Comments:			VI - II O		VALUE OF THE PARTY							
Reviewed by:			W					Date re	viewed:	April	118,3	819
Version 1.0 Issued J	une 26.	2006										water of

CETIS Sur	IS Summary Report							ort Date: Code/ID:			44 (p 1 of 1) 1-2177-9031
Salmonid Em	ibryo Survival a	nd Develo	pment T	est					N	autilus Env	ironmental
Batch ID:	12-5813-6512	Te	st Type:	Development			Ana	lyst: Yv	onne Lam		
Start Date:	27 Mar-19 17:4	5 Pr	otocol:	EC/EPS 1/RM/	28		Diligent: Dechlorinated Tap Water			Tap Water	
Ending Date:	03 Apr-19 15:15	Sp	ecies:	Oncorhynchus	Oncorhynchus mykiss			e:			
Test Length:	6d 21h	Ta	xon:	Actinopterygii	ctinopterygii			roe: Tr	out Lodge Fi	sh Farm	Age:
Sample ID:	19-9428-3318	Co	ede:	76DE5936			Proj	ect			
Sample Date:	26 Mar-19 04:11	M <sub>c</sub>	eterial:	Effluent			Sou	roe: Ma	Docum		
Receipt Date:	: 27 Mar-19 13:3	C/	IS (PC):				Stat	ion: Vi	6758-1645-	18	
Sample Age:	37h (2.7 °C)	CI	ient:	Maxiam							
Single Comp	arison Summary	e									
Analysis ID	Endpoint		Comp	parison Method			P-Value	Compar	ison Resul		s
15-6649-1090	Proportion Nom	nal	Equa	Variance t Two-	-Sample Tes	iA.	0.4725	100% pa	issed propor	tion normal	1
Proportion N	ormal Summary										
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	3	0.866	7 0.2930	1.0000	0.6000	1.0000	0.1333	0.2309	26.65%	0.00%
100		3	0.866	7 0.3830	1.0000	0.6333	1,0000	0.1171	0.2028	23.40%	0.00%
Proportion N	ormal Detail										
Conc-%	Code	Rep 1	Rep 2	Rep 3							
0	N	1.0000	1.000	0.6000							
100000											

Only 3 replicates used in statistical analysis (exp A excluded due to possible poor ess further)

Analyst VIW OA PULLIS / 7

100

0

100

Conc-%

**Proportion Normal Binomials** 

Code

0.9667

Rep 1

30/30

29/30

1.0000

Rep 2

30/30

30/30

0.6333

Rep 3

18/30

19/30

# **CETIS Analytical Report**

Report Date: Test Code/ID: 09 Apr-19 13:44 (p 1 of 2) 190577a / 11-2177-9031

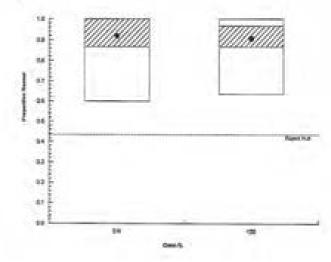
bryo Survival										
PARTY OF THE PROPERTY OF	and Develo	pment Ter	st.					N	autilus Env	ironment
15-6849-1090	Er	idpoint:	Proportion Non	mal		CET	S Version:	CETISY	1.9.4	
09 Apr-19 13:4	14 An	alysis:	Parametric-Tw	o Sample		Stati	us Level:	1		
12-5813-6512	Te	st Type: 1	Development			Anal	yst: Yvor	ne Lam		
27 Mar-19 17:	45 Pr	otocol:	EC/EPS 1/RM/	/28		Dilu	ent: Deci	hiorinated '	Tap Water	
03 Apr-19 15:1	15 Sp	ecies:	Oncorhynchus	mykiss		Brin	00			
6d 21h	Ta	xon:	Actinopterygli			Sour	roe: Trou	t Lodge Fi	sh Farm	Age:
19-9428-3318	Co	de:	76DE5936			Proj	ect			
26 Mar-19 04:	19 Ma	iterial:	Effluent			Sour	roe: Max	kām.		
	30 C/	IS (PC):				Stati	on: VK6	758-1645-	18	
37h (2.7 °C)	CI	ient:	Maxxam							
						Comparis	ion Result			PMSD
cted)	C>T					100% pas	sed proporti	on normal		49.67%
e t Two-Samp	le Test									
vs Conc-%		Test St	tat Critical	MSD DE	P-Type	P-Value	Decision(	005%)		
rol 100		0.0734	5 2.132	0.560 4	CDF	0.4725	Non-Signif	cant Effec	t	
Sum Sq	uares	Mean S	Square	DF	F Stat	P-Value	Decisioné	a:5%)		
0.00055	88	0.0005	588	1 0.005395		0.9450	Non-Signif	cant Effec	t	
		0.1035	79	4						
0.41487	5			5						
Tests										
Test				<b>Test Stat</b>	Critical	P-Value	Decision(	0:1%)		
				1.306	199	0.8672				
Shapiro-	Wilk W Non	nality Test		0.735	0.43	0.0141	Normal Dis	stribution		
rmal Summar	у									
Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
N	3	0.8667	0.2930	1.0000	1.0000	0.6000	1,0000	0.1333	26.65%	0,00%
	3	0.8667	0.3630	1.0000	0.9667	0.6333	1.0000	0.1171	23.40%	0.00%
ected) Transfo	rmed Sum	mary								
Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
N	3	1.282	0.4307	2.133	1.479	0.8861	1.479	0.1978	26.73%	0.00%
	3	0.000		PRODUCTION OF THE PROPERTY OF				0.173	23.74%	1.51%
		1.262	0.5178	2.007	1,387	0.9204	1,479	0.150	44.14.16	212411
rmal Detail		1.262	0.5178	2.007	1,387	0.9204	1,479	0.173	20.1470	03000
rmal Detail Code	Rep 1	1.262 Rep 2	0.5178 Rep 3	2.007	1.387	0.9254	1,479	0.173	20,1438	13.01.
				2.007	1.387	0.9204	1.479	9.173	20.143	08497.
Code	Rep 1	Rep 2	Rep 3	2.007	1.387	0.9204	1,479	0.173	20.143	
Code	Rep 1 1,0000 0,9667	Rep 2 1.0000 1.0000	Rep 3 0.6000	2.007	1.387	0.9204	1,479	0.173	23.14%	
Code N	Rep 1 1,0000 0,9667	Rep 2 1.0000 1.0000	Rep 3 0.6000	2.007	1.387	0.9204	1.479	0.173	20,143	03417.
Code N ected) Transfo	Rep 1 1.0000 0.9667 rmed Detail	Rep 2 1.0000 1.0000	Rep 3 0.6000 0.6333	2.007	1.387	0.9204	1.479	0.173	20,140	03417.
Code N ected) Transfo Code	Rep 1 1,0000 0,9667 rmed Detail	Rep 2 1,0000 1,0000	Rep 3 0.6000 0.6333 Rep 3	2.007	1.387	0.9204	1.479	0.173	20,143	03411.
Code N ected) Transfo Code	Rep 1 1,0000 0,9667 rmed Detail Rep 1 1,479 1,387	Rep 2 1,0000 1,0000 Rep 2 1,479	Rep 3 0.6000 0.6333 Rep 3 0.8861	2.007	1.387	0.9204	1.479	0.173	20,143	
Code N ected) Transfo Code N rmal Binomial	Rep 1 1,0000 0,9667 rmed Detail Rep 1 1,479 1,387	Rep 2 1.0000 1.0000 Rep 2 1.479 1.479	Rep 3 0.6000 0.6333 Rep 3 0.8861 0.9204	2.007	1.387	0.9204	1.479	0.173	20,143	03415.
Code N ected) Transfo Code N	Rep 1 1,0000 0,9667 rmed Detail Rep 1 1,479 1,387	Rep 2 1,0000 1,0000 Rep 2 1,479	Rep 3 0.6000 0.6333 Rep 3 0.8861	2.007	1.387	0.9204	1.479	0.173	20,143	
	12-5813-6512 27 Mar-19 17: 03 Apr-19 15: 6d 21h 19-9428-3318 26 Mar-19 04: 27 Mar-19 13: 37h (2.7 °C) m cted) ce t Two-Samp vs Conc-9 rol 100  Sum Sq 0.00055 0.414318 0.414879  Tests  Test Variance Shapiro- ormal Summar Code N  ected) Transfo	12-5813-6512 Te 27 Mar-19 17:45 Pr 03 Apr-19 15:15 Sp 6d 21h Ta 19-9428-3318 Cc 26 Mar-19 04:19 Mar 27 Mar-19 13:30 CJ 37h (2.7 °C) Cl m Alt Hyp cted) C > T be t Two-Sample Test vs Conc-% fol 100  Sum Squares 0.0005585 0.414316 0.414875  Tests Test Variance Ratio F Ter Shapiro-Wilk W Non ormal Summary Code Count N 3 3 ected) Transformed Summary Code Count	12-5813-6512 Test Type: 27 Mar-19 17:45 Protocol: 03 Apr-19 15:15 Species: 6d 21h Taxon:  19-9428-3318 Code: 25 Mar-19 04:19 Material: 27 Mar-19 13:30 CAS (PC): 37h (2.7 °C) Client:  m Alt Hyp cted) C > T  be t Two-Sample Test vs Conc-% Test Shol 100 0.0734  Sum Squares Mean 3 0.0005585 0.0005 0.414316 0.1035 0.414875  Tests  Test Variance Ratio F Test Shapiro-Wilk W Normality Test ormal Summary Code Count Mean N 3 0.8667 3 0.8667 ected) Transformed Summary Code Count Mean N 3 0.8667	12-5813-6512 Test Type: Development 12-5813-6512 Test Type: Development 12-7 Mar-19 17:45 Protocol: EC/EPS 1/RW/ 03 Apr-19 15:15 Species: Oncorhynchus 6d 21h Taxon: Actinopterygii 19-9428-3318 Code: 76DE5936 26 Mar-19 04:19 Material: Effluent 27 Mar-19 13:30 CAS (PC): 37h (2.7 °C) Client: Maxoam  M Alt Hyp  cted) C > T  be t Two-Sample Test ws Conc-% Test Stat Critical fol 100 0.07345 2.132  Sum Squares Mean Square 0.0005588 0.414316 0.103579 0.414875  Tests  Test Variance Ratio F Test Shapiro-Wilk W Normality Test  ormal Summary  Code Count Mean 95% LCL N 3 0.8667 0.2930 3 0.8667 0.3630  ected) Transformed Summary  Code Count Mean 95% LCL N 3 0.8667 0.3630	12-5813-6512   Test Type: Development	12-5813-6512   Test Type: Development	12-5813-6512   Test Type:   Development	19	12-8813-8512   Test Type: Development   Analyst: Yvonne Lam	12-6813-6512

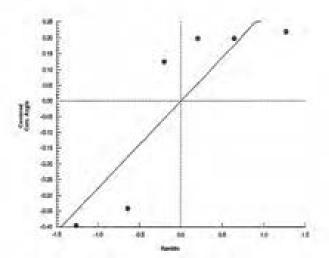
### **CETIS Analytical Report**

Report Date: Test Code/ID: 09 Apr-19 13:44 (p 2 of 2) 190577a / 11-2177-9031

Salmonid En	nbryo Survival and I		Nautilus Environmental		
Analysis ID:	15-6649-1090	Endpoint	Proportion Normal	CETIS Version:	CETISy1.9.4
Analyzed:	09 Apr-19 13:44	Analysis:	Parametric-Two Sample	Status Level:	1







Analyst New OA Frul 18/15

# Rainbow Trout Early Life Stage Summary Sheet

Client:	MARKAM	Start Date/Time	: MARCH 27, 2019@1745h
Work Order No.:	190577	Test Species:	Oncorhynchus mykiss
Sample Informat	ion:		
Sample ID: Sample Date: Date Received: Sample Volume:	March 26, 2019 March 27, 2019 March 27, 2019 4 × 101		
Dilution Water:			
Type: Hardness (mg/L C Alkalinity (mg/L C		Water	
Test Organism Ir	nformation:		
Batch No.: Source: Loading Density:	Trout Lodge, Susper	, war	
Number of male b Number of female Sperm motility che	broodstock used:	5	pound microscope
SDS Reference 1	oxicant Results:		
Reference Toxica Stock Solution ID: Date Initiated: 7-d EC50 (95% C	18503 March 27, 2019	ALC SDS	
Reference Toxica Reference Toxica	nt Mean and Range: 4\ nt CV (%): 32	(22-7,7) m	BIL SOS
Test Results:		entro / VK67	ample ID 59-1645-188 ( ± 32,7
Reviewed by:	W	Date rev	viewed: Aquil 18, 2019

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

Client: Sample ID; Nork Order #:	190 190	MAXAM 1667569-1645-18B					Stop	Date 8	L Time: CER #:	3	ch 2: 11 ° nymohus	5, 20	19 @	1745
Control							De	ys.						
Concentration	0	LL. S	1		2		3		4		5	8		7
( > L U   )	init	new	old .	new	old	new	old	new	old	new	old	new	old	final
Temperature (*C)	13.5	(40	135	No	13.5	130	135	140	135	190	13.5	140	\$100 market and the second	135
DO (mg/L)	10.2	10.1	10.2	10.0	9.9	125	103	10.2		Col	9.9	102	103	10.2
pH	69	7.1	11	60	6.5	7.1	7.2	6.9		46	6.3	68		7.0
Cond. (µS/cm)	32	3	2	37		3	4		34		3 y	3	φ	35
Initials	m w			1444	7	50	1X		4	lar-		44	uni	
							-							
100		7				-		ys .		_				
Concentration	0	1 33	1	-	2		3		4	1	5		6	7
	Init.	new	old	now	old	new	old	пеуу	old	new	old	new	old	final
Temperature (°C)	140		13.5	13.5	13,5	135	13.5	14,0	12.5	140	13.5	140	135	13.5
DO (mg/L)	193	10,1	10.2		10.0	63		10.2	1500, 000	10.1	10.0	10.0	And the Control of the	10,2
pH	7.1	72		7.2	7.3	7.4	76		15	7.3	7.3	1.3	15	16
Cond. (µS/cm)	50 Z	The second second	75	50		5	09		506		Bee	5	56	513
Initials	HILL MILL			cshu To			tur - Mr			44		WW.		
		_					-							
Concentration	0	0 1 2					3	ys .	4		5		6	7
	init	new	old	new	old	new	old	new	old	new	old	new	old	fing
Temperature (°C)	THE S	Name of		Sec. 1995	3.00		3000	10.11077	74.2	10000			-	
DO (mg/L)								-						
pH				1/2	-3	ti ur			7 =					
Cond. (µS/cm)														
Initials														
							Da	ys						
Concentration	0	_	1		2		3	-	4		5		6	7
	init	пем	old	new	blo	new	old	new	old	new	old	new	old	funal
Temperature (°C)								The rest				100		
DO (mg/L)														
pH														
Cond. (µS/cm)														
Initials			- 4											
hermometer: CERE3	DO met	eriprobe:	<u>내</u> 3	213	pH met	niprobe:	213/	243	Conduc	tivity me	teriprob	e: <u>45</u>	1213	
	-	itrol		1°			/			Analys	ts:	50,0	5,44	4
Hardness*		2-		6		/								
Alkalinity*			- 4	10	1		Mar-				ved by:		4	47.00
mg/L as CaCO3										Date res	riewed:	A	que	187
ample Description:		وماي	F. A.	2 (0)	our, A		docur	0	par	Heule	tes			10
Comments: Version 1.2 Issued July 19.									100					

# Embryo Toxicity Test Daily Mortality

Client:	Maryon	Start Date & Time: Morch 27, 2018 @ 17454
Sample ID:	VK6759-1645-18B	Stop Date & Time: April 3, 2019 @ 15154
Work Order #:	190577	Test Species: Oncorhynchus mykiss

Concentration	Rep		Day of	Test	- No.	of Mo	rtaliti	0S	Total	Total	Total No.	Total
(% U(V)		1	2	3	4	5	6	7	Dead Eggs	Undeveloped	Embryo	Exposed
control	1	0	3	0	0	0	0	1	1	29	0	30
	2	T i	Mile		11		1	0	1	0	30	31
	3				M R		0		0	0	30	30
	4	17/11				II			0	IS	16	31
100	1		146	640					10	30	0	30
	2		Mari						0	0	30	30
	3	1	H. S.	11 8	11			W	0	0	30	30
	4	W	Y	4	V	V	V		1	16	13	30
	1		N-L	300	T-S		-					1977
	2											
	3			H=3								
	4		16.4	ME S	1	1	1				4 0	MEET.
	1											
	2			250							3 - 5	
	3	O. I		(C. )								
	4											
	1	7 3			113						3	
	2									A CONTRACTOR OF THE PARTY OF TH		
	3											
	4											
	1	1	(3.21)			-					\$ == £	
	2											
	3										17	
	4											
	1	7	13 = 3								6	
	2											
	3								1	14-7-3	4 3	
	4											
	1				-							
	2					-						
	3				-							
ech Initials	4	000	<b>≥</b> 0	Day.	1000				-tim_			

Tech Initials	W 30 5	p ur um ur	untum	IIW	lun	WW.
Comments:	w.	2000 - NAVESS	- LONG WEST			15 - 3aV10:
=	4.			1	À 10	
Reviewed by:	841		Date reviewed:	App	ul 18, 2	2019

CETIS	Summary	Report
The second second second	Contract of the Contract of th	Company of the Company

Report Date: Test Code/ID: 09 Apr-19 13:46 (p 1 of 1) 190577b / 03-5139-8410

							1695	COCION	IMS .	MODITO LO	SHOT I SIDNON IN
Salmonid Em	bryo Survival an	d Devel	opment T	est					N	utilus Env	ironmental
Batch ID:	06-6452-2148	T	est Type:	Development	7		Anal	yst:	Yvonne Lam		
Start Date:	27 Mar-19 17:45	P	rotocol:	EC/EPS 1/RM/2	28		Dillu	ent:	Dechlorinated 1	ap Water	
Ending Date:	03 Apr-19 15:15	S	pecies:	Oncorhynchus r	mykiss		Brin	e:			
Test Length:	6d 21h	T	axon:	Actinopterygli			Sou	rce:	Trout Lodge Fis	h Farm	Age:
Sample ID:	02-2850-9630	C	ode:	D9EC78E			Proj	ect:			
Sample Date:	26 Mar-19 04:15	M	lateriat:	Effluent			Sou	rce:	Maxxam		
Receipt Date:	27 Mar-19 13:30	C	AS (PC):				Stati	ion:	VK6759-1654-1	88	
Sample Age:	38h (3,4 °C)	C	lient:	Maxxam							
Single Compa	arison Summary										
Analysis ID	Endpoint		Comp	parison Method			P-Value	Com	parison Result		
16-9121-8660	Proportion Norm	al	Fishe	r Exact Test			0.4721	100%	passed proport	ion normal	
Proportion No	ormal Summary										
Cone-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std E	er Std Dev	CV%	%Effect
0	N	3	0.828	0.1559	1.0000	0.5161	1.0000	0.156	0.2705	32.67%	0.00%
100		3	0,811	1 0.0000	1.0000	0.4333	1.0000	0,188	9 0.3272	40.34%	2.03%
Proportion No	ormal Detail										
Conc-%	Code	Rep 1	Rep :	Rep 3							
0	N	0.9677	1.000	0 0.5161							
100		1.0000	1.000	0 0.4333							
Proportion No	ormal Binomials										
Conc-%	Code	Rep 1	Rep 2	Rep 3							
0	N	30/31	30/30	16/31							
100											

Orly 3 replicates used to Statistical analysis (Rep A excluded the to possible poor ess quality)

Analysis New GA:

### **CETIS Analytical Report**

Report Date: Test Code&D: 09 Apr-19 13:46 (p 1 of 1) 190577b / 03-5139-8410

		-							Test Code	MID:	1905775 / 0	3-5139-8410
Salmonid Em	bryo Sur	vival an	d Deve	lopment T	est						Nautilus Env	ironmental
Analysis ID:	16-9121	-8660	1	Endpoint	Pro	portion Nor	mal		CETIS Ve	rsion:	CETISv1.9.4	
Analyzed:	09 Apr-1	9 13:46	- 1	Analysis:	Sing	gle 2x2 Cor	ntingency Ta	ble	Status Le	vet:	1	
Batch ID:	06-6452	-2148	- 3	Test Type:	Dev	relopment			Analyst:	Yvos	ne Lam	
Start Date:	27 Mar-1	19 17:45	- 1	Protocol:	EC	EPS 1/RM	/28		Diluent:	Dech	nlorinated Tap Water	
Ending Date:	03 Apr-1	9 15:15	- 1	Species:	One	corhynchus	mykiss		Brine:			
Test Length:	6d 21h			Faxon:	Acti	nopterygii			Source:	Trou	t Lodge Fish Farm	Age:
Sample ID:	02-2850	-9630	- (	Code:	Des	C7BE			Project			
Sample Date:	26 Mar-1	9 04:15	1	Material:	Emi	uent			Source:	Maro	cam	
Receipt Date:	27 Mar-1	19 13:30	- (	CAS (PC):					Station:	VK6	759-1654-18B	
Sample Age:	38h (3.4	°C)	- (	Client:	Mas	ocam						
Data Transfor	m	7	Alt Hy	(P					Comparison R	esult		
Untransformed			C>T						100% passed p	roportic	on normal	
Fisher Exact	Test											
Control	vs G	roup		Test	Stat	P-Type	P-Value	Decision	(a:5%)			
Negative Cont	rol 14	00		0.472	1	Exact	0.4721	Non-Sign	ificant Effect			
Data Summar	у											
Conc-%	Ce	ode	NR	R		NR + R	Prop NR	Prop R	%Effect			
0	N		76	16		92	0.8261	0.1739	-1.85%			
100			73	17		90	0.8111	0.1889	0.0%			
Proportion No	ermal De	tail										
Conc-%	Co	de	Rep 1	Rep 2		Rep 3						
0	N	7	0.9877	1.000	0	0.5161						
100			1.0000	1,000	0	0.4333						

### Graphics

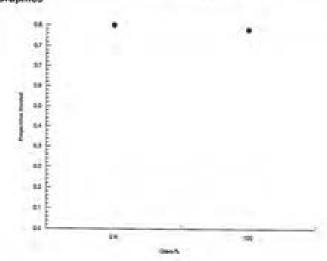
Conc-%

100

**Proportion Normal Binomials** 

Code

N



Rep 1

30/31

30/30

Rep 2

30/30

30/30

Rep 3

16/31

13/30

Analyst: Www OA: April 18/19



**APPENDIX B – Chain-of-Custody Form** 



Sent To: Nautilus Environmental - Burnaby

8664 Commerce Court Burnaby, BC, VSA 4N7 Tel: (604) 420-8773

### CHAIN OF CUSTODY RECORD FOR SUBCONTRACTED WORK

Page 01 of 01

COC # B921715-VNAU-01-01

REPORT IN	FORMATION									ANALYSI	S REQUES	TED					
Company	2 Maxxam						,U										
Address:	9331 - 48th Street, Edmon	ton, Alberta,	768 2R4				- 砂										
Contact N	lame: Geraldlyn Gouthro						Embryo Subcontract										
Email:	GGouthro@maxxam.ca, er	Imenvirocs@	naxxamanaly	tics.com			25.0										
Phone:	(403) 735-2230		Carrie District				Merca										
Maxxam F	Project #: 8921715						E										
# SAMP	LE ID	MATRIX	DATE SAMPLED (YTYY/MM/CO)	TIME SAMPLED (DEMM)	SAMPLER INITIALS	# CONT.	Rainbow Troop 7								,	ODITIONAL SA	MPLE INFORMATION
1 VK6	758-1645-18	W	2019/03/26	64:19	AM	4	Х						279	- 0	P: 040		
2 VI05	759-1645-188	W	2019/03/26	04:15	AM	4	Х						34.0		P: 04)		
3																	
4					- 134		1			THE		(E)					
5												41.					
6																	
7														123			
1							P.	#									
10							20	10.5				430					
	RY CRITERIA		amendary income				25-	- 19				53					
	-18 Ave & Grab		SPECIAL INSTR		madiates.	and the same			March Server								TURNAROUND TIME
			Please inform **Please retur	n a copy of I	his form w	th the	e repor	F.**	dited for	the reque	sted test;	0.					Rush Required
COOLER ID: Custody Seal Custody Seal Cooling Medi	Present Temp: SE	EXR	COOLER ID: Custody Seal Pre Custody Seal Inc. Cooling Media P	ect vesent	YES NO	Ten (°C	)			Custo Custo Cooli	LER ID: ody Seal Po ody Seal Inc ng Media I	ect	YES NO	Temp:			Date Required  Please inform us if right charges will be incurred.
1. David Tidm	HED BANCHON & PRINT)	2019/00	YYY/MM/DO	TIME: 04	HAME	RECE	NEO B	M: pag	N & PRINT				DATE: (Y)			TIME: (HH: MM	
. /	. (5/15)	4 700	100	1649	A	- D	ZL	BR	ATTE	INY B	URLO	WE_	2019	103	27	09:55	
	No HEDRO TOOK	1906	11282	:11 =	70	4							y.n.2.1		100		

Record Mar 27/19 C 13304 YML 4x10c per surpre



**END OF REPORT** 





### Ceriodaphnia dubia Bioassay Report

Client Name and Sample ID: DDMI - 1645-18B

Job and Sample ID: B921715- VK6759 Date sample collected: 2019/03/26

2019/03/31

Test Initiation Date: 2019/03/27 Test End Date: 2019/04/03

Dates when subsamples used: 2019/03/27

2019/04/01 2019/03/28 2019/03/29 2019/04/02

2019/03/30

Dissolved Oxygen prior to test (mg/L): 9.4 pH prior to test (pH units): 6.7

Temperature (°C): 25

Conductivity (µS/cm): 513

Test Performed By: DBA, NM9, CSH

	Test Results		Signit	icant or non-signif	ficant	Method	Data Transforms	Outliers* ( concentration-replicate)
Sample Test Results (%	7 day Survival Result	Effect		Non-Significant		Fisher Exact Test	Untransformed	N/A
vol/vol)	7 day Reproduction Result	Non-Significant			Wilcoxon Rank Sum Two- Sample Test	Untransformed	0 - 7	
Refere	Reference Toxicant Results		Endpoint 95% LCL 95% UC		95% UCL	Method	Data Transforms	Outliers* ( concentration-replicate)
Reference Toxicant Test	7 day Survival Result	LC50	1.38	1.27	1.50	Spearman-Karber	Log- X	0.5- 10
Results (g/L)	7 day Reproduction Result	IC50	1.29	1.13	1.36	Linear Interpolation	Log- X	N/A
Control Chart	7 day Survival Result	LC50	1.55 1.02 2.36 Shewart		Shewart	n/a	n/a	
Data (g/L)	7 day Reproduction Result	IC50	1.20	0.867	1.67	Shewart	.,, u	, u

<sup>\*</sup> If outliers were removed, describe in comments.

Comments			

#### **Test Organisms**

Species: Ceriodaphnia dubia Source of test organisms: Aquatic Biosystems, Colorado

Age of organisms at test initiation: ≤ 24 hours, within 12 hours Unusual Appearance, behaviour or treatment prior to use in test : None Mean % mortality of brood organisms during 7-day period proceding test: 6.9 Number of neonates produced by each organism in its third or fourth brood: ≥8 neonates

Mean number of neonates per adult during first 3 broods in 7 days: 24 Observations of ephippia: None

All test organisms used to initiate this test were taken from a series of individual cultures, that originated from the same mass culture.

The 4th brood or subsequent broods produced during the test are not included in the final statistical analysis.

**Test Facilities and Apparatus** 

Name address of test laboratory: Maxxam Analytics. 9331 48st NW, Edmonton, AB Test vessels used: Fisherbrand 20 x 150 mm lime glass test tubes

Control/Dilution Water

16 L RODI from in-house system, to which the following are added: Consists of:

> 4 L Perrier Brand carbonated spring water 1 mL cyanocobalamin (Vitamin B-12) 1 mL Sodium Selenate Decahydrate

**Test Method** 

Reference method used for testing: Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dubia. Environment Canada,

EPS 1/RM/21 Second Edition - February 2007

Did the following occur during sample preparations:

Nο Filtered: Adjusted for hardness: No Adjusted for pH: No Frequency of observations: 24 ± 1 hour Frequency of water quality measurements: Daily Design and description of any specialized procedure: N/A

Program used for statistical calculations:

Comprehensive Environmental Toxicity Information System (CETIS). Tidepool Scientific Software. Version 1.9.3.0

Guidance Document on Statistical Methods for Environmental Toxicity Tests. Environment Canada, EPS 1/RM/46 - March Reference method used for statistical calculations:

#### **Test Conditions and Procedures**

Number of test solutions: 20

Number of test concentrations: 1 and a negative control

Concentrations tested (%): 100 Units of tested concentrations: % vol/vol Number of replicates: 10 Volume of test solutions: ≥ 15 mL Depth of test solutions: ≥ 5 cm Individuals per test vessel: 1 Was pre-aeration performed: Yes Procedure: Oil-free

≤ 100 bubbles / minute Rate:

Duration: 20 minutes

2019/03/27 2019/03/31 Dates where pre-aeration occurred: 2019/03/28 2019/04/01

2019/04/02 2019/03/29 2019/03/30

Aeration during testing: None

Refer to comments section for any deviations.

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

Refer to comments section for any deviations regarding reference toxicity testing.

### Ceriodaphnia dubia Survival and Reproduction Observations

Note: Mortalities are indicated by an "X" in the column and row of the concentration, replicate and day of occurance. If organism was not used for testing, this will be indicated by "N/A" in mortality and neonate columns for the replicate.

Concentration	Ponlicato								Day of	Testing								Cumulative	Cumulative	SD of mean
			1		2		3		4	5		6		7			8	Mean %	Mean	
(%/%vol)	number	neonates	Mortality	Mortality	Reproduction	reproduction														
	1	0		0		0		5		8		0		15						
	2	0		0		0		5		10		13								
	3	0		0		0		0		10		18								
	4	0		0		0		4		11		0		16						
Control	5	0		0		0		5		10		0		15				10.0%	27	6.0
Control	6	0		0		0		7		10		16						10.0%	27	0.0
	7	0		0		0		4		11		0	Х							
	8	0		0		0		7		10		0		16						
	9	0		0		0		5		9		12								
	10	0		0		3		0		8		7								
	1	0		0		0		6		9		0		11						
	2	0		0		0		4		9		0		11						
	3	0		0		0		5		13		8								
	4	0		0		0		6		8		0		13						
100%	5	0		0		0		6		11		0		13				0.0%	27.2	2.0
100%	6	0		0		0		7		10		0		14				0.0%	27.2	2.0
	7	0		0		0		5		10		0		13						
	8	0		0		0		6		7		0		14						
	9	0		0		0		0		14		12								
	10	0		0	1	0		6		10		11				I				

Average Values for Chemical Data of Test Concentrations

		Before Efflu	ent Renew	al			After	Effluent Re	newal	
est Concentration (% vol/vol)	Date	Temperature (°C)	рН	Conductivity (μS/cm)	DO (mg/L)	Date	Temperature (°C)	рН	Conductivity (μS/cm)	DO (mg/L)
Control	2019/03/27	26	7.8	180	7.5	2019/03/28	25	7.7	189	6.7
100	2019/03/27	26	7.0	518	8.8	2015/05/28	25	7.4	515	6.9
Control	2019/03/28	25	7.9	175	7.5	2019/03/29	24	7.5	173	6.4
100	2019/03/28	25	7.0	509	9.1	2019/03/29	24	7.3	480	6.6
Control	2019/03/29	25	7.9	173	7.3	2019/03/30	25	7.7	178	6.4
100	2019/03/29	25	7.0	501	8.9	2019/03/30	24	7.3	493	6.6
Control	2019/03/30	25	7.9	176	7.1	2019/03/31	26	7.8	180	7.3
100	2015/05/50	25	7.0	507	8.6	2013/03/31	26	7.5	502	7.4
Control	2019/03/31	24	8.1	172	7.7	2019/04/01	26	7.8	178	6.7
100	2015/05/51	25	7.1	503	9.2	2015/04/01	26	7.5	507	6.7
Control	2019/04/01	25	8.0	127	7.4	2019/04/02	25	7.4	136	6.8
100	2013/04/01	25	7.1	502	8.7	2013/04/02	24	7.3	453	7.4
Control	2019/04/02	26	7.7	130	7.6	2019/04/03	25	7.4	133	6.6
100	2013/04/02	26	7.0	517	8.7	2015/04/05	25	7.2	501	6.5
Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
100	IV/A	N/A	N/A	N/A	N/A	7 19/4	N/A	N/A	N/A	N/A



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Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B921715 Client Project Name & Number: Quarterly Tox SNP-A Sample Number: VK6759-01

Test Result:

48 hrs Mortality % 0 Statistical Method:

0 Control 0 Mean percent mortality: Sample

Sample Name: 1645-18B Sample Matrix: **Grab Water** 

Description: Clear and colourless. Sample Prior to Analysis:

Mar 26, 2019 04:15 AM Sampling Method: 6.9 Sample Collected: N/A pH: Sample Collected By: AΗ Site Collection: N/A Temperature: 19°C

Sample Received: Mar 26, 2019 02:42 PM Volume Received: 1 L Dissolved Oxygen: 10.9 mg/L Analysis Start: Mar 27, 2019 03:19 PM Temp.Upon Arrival: -2 °C Sample Conductance: 442 μS/cm

End: Mar 29, 2019 03:29 PM Storage: 2-6°C Hardness: 120 mg CaCO <sub>3</sub>/L

			-,		0 -						- 0 -	5,
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hr	48 hrs	48 hrs
0	20	8.0	389	8.5	0	0	0	0	20	8.0	395	8.4
0	20	7.9	391	8.4	0	0	0	0	20	8.0	398	8.3
0	20	8.1	391	8.4	0	0	0	0	20	8.0	392	8.4
100	20	7.0	456	10.7	0	0	0	0	20	7.6	463	8.5
100	20	7.0	456	10.8	0	0	0	0	20	7.6	460	8.5
100	20	6.9	455	10.7	0	0	0	0	20	7.5	466	8.4

Concentration	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)
% vol/vol	48 hrs	48 hrs	48 hrs	48 hrs
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
100	0	0	0	0
100	0	0	0	0
100	0	0	0	0

**Comments:** None

**Culture/Control/Dilution Water:** City of Edmonton dechlorinated tap water

180 mg/L CaCO<sub>3</sub> Hardness: Other parameters available on request.

**Test Conditions** Test concentration: 0,0,0,100,100,100 (% vol/vol)

10 25-50 mL/min/L Organisms per Vessel: Pre-aeration Time: 30 min Rate of Pre-aeration:

60 Total # of Organisms Used: Test Temperature: 20 ± 2 °C Test Hardness Adjusted: No Test Volume: 150 mL Vessel Volume: 225 mL Test pH Adjusted: No

Loading Density: 15.0 mL/Daphnia Photoperiod: 16:8 (light: dark)

Source : In House Culture Daphnia magna **Test Organism:** 

Average Brood Size: 19.9 Age at Test Initiation: <24 hrs Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 3.2 20 ± 2 °C Time To First Brood: Culture Temperature: 8 Days **Culture Diet** 

Pseudokirchnriella and YTC at a ratio of 2 mL/L of culture daily. New cultures weekly, 63 daphnids

distributed into 6 culture vessels and 3 reproductive vessels.





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Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B921715Client Project Name & Number:Quarterly Tox SNP-ASample Number:VK6759-01

Reference chemical: Sodium Chloride Test Date: Mar 27, 2019

Test Endpoint 48 hrs LC50 (95% confidence interval) : 6.17 (5.50, 6.93)g/L Statistical Method : Untrimmed

Spearman-Kärber

Historical Mean LC50 (warning limits): 6.10 (4.57, 8.14) g/L Concentration: 0,1.71,2.56,3.82,5.7,8.5 g/L

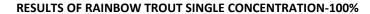
Test Method EPS 1/RM/14
Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Analyst 2 Date: Apr 02, 2019 04:11 PM







Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B921715

Client Project Name & Number: Quarterly Tox SNP-A

**Test Result:** 

96 hrs Mortality % 0 Statistical Method: Visual

Sample Name: 1645-18B Sample Matrix: Grab Water

Description: Clear and colourless Sample Number: VK6759-03

Sample Collected: Mar 26, 2019 04:15 AM Sampling Method: N/A Site Collection: N/A

Sample Collected By: AH Volume Received: 20 L Temp.Upon Arrival: -2 °C Storage: 2-6°C

Sample Received: Mar 26, 2019 02:42 PM pH: 6.8 Dissolved Oxygen: 11.6 mg/L Analysis Start: Mar 27, 2019 01:20 PM Temperature: 13 °C Sample Conductance: 387 μS/cm

•												
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
0	15	7.8	347	9.0	0	0	0	0	0	0	0	0
100	14	6.9	374	10.6	0	0	0	0	0	0	0	0

Concentration	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	72 hrs	72 hrs	72 hrs	72 hrs	96 hrs	96 hr	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs
0	0	0	0	0	15	8.0	352	9.0	0	0	0	0
100	0	0	0	0	15	7.5	416	8.8	0	0	0	0

Comments: None

Culture/Control/Dilution Water City of Edmonton dechlorinated tap water

Hardness: 190 mg/L CaCO<sub>3</sub> Other parameters available on request.

Test Conditions Test concentration: 0,100 (% vol/vol)

Organisms per Vessel : 10 Test Temperature :  $15 \pm 1$  °C Solution Depth : >15 cm

Total # of Organisms Used: 20 Pre-aeration Time: 120 min. Rate of Aeration 6.5±1 mL/min/L

Test Volume: 20 L Vessel Volume: 38L Test pH Adjusted: No

Loading Density: 0.2 g/L Photoperiod: 16:8 (light: dark)

<u>Test Organism</u>: Rainbow Trout (Oncorhynchus mykiss) Source: Spring Valley Trout Hatchery

Culture Temperature :  $15 \pm 2$  °C Weight (Mean) +- SD :  $0.4 \pm 0.1$  g Length (Mean) +- SD :  $3.73 \pm 0.13$  cm Culture Water Renewal :  $\geq 1.0$  L/min/kg fish Weight (Range) : 0.4 - 0.5 g Length (Range) : 3.40 - 3.90 cm

Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 0.2% Feeding rate and frequency: daily: 1-5% biomass of trout. Acclimation Time: >14 days

Reference chemical:PhenolTest Date:Mar 07, 2019

Test Endpoint 96 hrs LC50 (95% confidence interval): 9.50 (8.64, 10.3)mg/L Statistical Method: Probit Historical Mean LC50 (warning limits): 10.3 (8.65, 12.3) mg/L Concentration: 0,8,10,12,15,20 mg/L

Test Method EPS 1/RM/13
Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Natasha Lloyd, Analyst 2 Date: Apr 09, 2019 10:00 AM

Maxxam Analytics

9331 - 48th Street, Edmonton, Alberta T6B 2R4 Tel: (780) 577-7100 Fax: (780) 450-4187

www.maxxam.ca



# Toxicity Testing on VM7449-1645-18 and VM7450-1645-18B

(collected April 8, 2019)

**Final Report** 

May 8, 2019

Submitted to: **Maxxam Analytics**Burnaby, BC



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APPENDIX A – *Pseudokirchneriella subcapitata* Toxicity Test Data APPENDIX B – Chain-of-Custody Form



#### **SIGNATURE PAGE**

Report By: Jeslin Wijaya, B.Sc.

Laboratory Biologist

Reviewed By:

Edmund Canaria, 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	VM7449-1645-18 and VM7450-1645-18B
Sample collection date	April 8, 2019
Sample receipt date	April 11, 2019
Sample receipt temperature	4.8 – 4.9°C
Test types	72-h Pseudokirchneriella subcapitata growth inhibition

## **Summary of Results**

% (v/v)	Cell Yield (Mean ± SD)					
(4)	VM7449-1645-18	VM7450-1645-18B				
Laboratory Control	38.6 ± 3.1	38.2 ± 2.7				
95.2	90.0 ± 5.1*	86.8 ± 5.4*				

SD = Standard Deviation

<sup>\* =</sup> indicates cell yield that was significantly greater than the laboratory control



#### 1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted a 72-h *Pseudokirchneriella subcapitata* growth inhibition toxicity test for Maxxam Analytics on samples identified as VM7449-1645-18 and VM7450-1645-18B. The samples were collected on April 8, 2019 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on April 11, 2019. Samples were transported in two 1-L plastic containers each and were received at temperatures of 4.9 and 4.8°C. The sample was stored in the dark at  $4 \pm 2$ °C prior to testing.

This report describes the results of the toxicity tests. 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

Methods for the toxicity tests are summarized in Table 1. Testing was conducted according to procedures described by Environment Canada (2007). Statistical analyses were performed using CETIS (Tidepool Scientific Software, 2013).



# Table 1. Summary of test conditions: 72-h *Pseudokirchneriella subcapitata* growth inhibition test.

Test species Pseudokirchneriella subcapitata, strain CPCC #37

In-house axenic culture, obtained from Canadian Phycological

Organism source 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 Full strength sample diluted to 95.2% (v/v) with nutrients, 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  $24 \pm 2^{\circ}C$ 

Feeding None

Light intensity 3600 to 4400 lux Photoperiod 24 hours light

Aeration None

Test area temperature measured daily; temperature and pH

Test measurements measured at test initiation; pH of two control wells measured

at test termination

Test protocol Environment Canada (2007), EPS 1/RM/25

Statistical software CETIS Version 1.9.4

Test endpoint Algal cell growth inhibition

>16-fold increase in number of algal cells; CV ≤ 20%; no trend

when analyzed using Mann-Kendall test

Reference toxicant Zinc (added as ZnSO<sub>4</sub>)



#### 3.0 RESULTS

The results of the toxicity test on samples VM7449-1645-18 and VM7450-1645-18B are summarized in Table 2. Significant stimulatory effects on cell yield were observed in both samples. Percent stimulation was 133% for sample VM7449-1645-18 and 126.8% for sample VM7450-1645-18B.

Table 2. Results: 72-h *Pseudokirchneriella subcapitata* growth inhibition test.

	Mean ± SD									
Concentration	VM7449-16	45-18	VM7450-1645-18B							
(% v/v)	Cell Yield	Stimulation	Cell Yield	Stimulation						
	(x 10 <sup>4</sup> cells/mL)	(%)	$(x 10^4 cells/mL)$	(%)						
Laboratory Control	38.6 ± 3.1		38.2 ± 2.7							
95.2	90.0 ± 5.1*	133.0	86.8 ± 5.4*	126.8						

SD = Standard Deviation

#### 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 tests. There were no deviations from the test methodology. Uncertainty associated with the test is best described by the standard deviation around the mean and/or the confidence intervals around the point estimates.

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 the test was appropriate. The reference toxicant test was performed under the same conditions as those used for the sample.

Table 3. Reference toxicant results.

Test Species	est Species Endpoint		CV (%)	Test Date
P. subcapitata	Growth (IC50): 32.5 μg/L Zn	31.0 (25.0 – 38.5)	11	March 29, 2019

SD = Standard Deviation, CV = Coefficient of Variation, IC = Inhibition Concentration

<sup>\* =</sup> indicates cell yield that was significantly greater than the laboratory control



#### 5.0 REFERENCES

Environment Canada. 2007. 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 – Pseudokirchneriella subcapitata** Toxicity Test Data

# Pseudokirchneriella subcapitata Summary Sheet

Client Work Order No.:	M3XX6M 19063>	Start Date: Set up by:	April 11/19	
Sample Information:				
Sample ID: Sample Date: Date Received: Sample Volume:	MATHAR-1645-18 ADDJ 8/19 BROJ 11/19 BXIL			
Test Organism Informa	ation:			
Culture Date: Age of culture (Day 0):	Apá)	5/18		
Zinc Reference Toxica	nt Results:			10
Reference Toxicant ID: Stock Solution ID: Date Initiated:	SC181 19702 March 28,	48_		
72-h IC50 (95% CL):	325(26,4-37,2	) NG/LZn_		
72-h IC50 Reference To	oxicant Mean and Range: 31,0 (25.	5/LZh	v (%): 11	
Test Results:			ield (Mean± SD)	
	Negative Control タウン(イルイル)		b ± 3,1 .0 ± 5,1 #	
			± ±	
			1	2
			*	
	+ inducates that all yield	is significant	ty histor than the l	ab control
Reviewed by:	Jon	Date reviewed:	May Hig	

Issued May 10, 2014; Ver. 1.0

Nautitus Environmental Company Inc.

# 72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client :		Maxve	isil			Setup by:					
Sample ID:	V+131	149-164	5-18			Test Date	Time:	- April 11/19@ 1200th			
Work Order No.:		190687		CER #:			4				
						Test Spec	ies:	Pseudokirohnerialia subcapitata			
Culture Date:	Ap.	75/18		Age of Cu	ilture:	60	Culture He	alth:	- 6	)00d	
Culture Count:	1 345	2 365		Average:	355	Culture C	ell Density (	ct):	355 X J	64 cell/ml	
	22.2	220,000 co					- » t				
	100	220,000 co (c1)				cells/mi					
Time Zero Counts		1 2		2 87		Average:		29.5			
io. of Cells/mL:		23.51	101		Initial Dec	nsity:	# cells/mL	• 220 µL x 1	10 µL =	10087 (6/10)	
Concentration	Water	Quality		Incubator	Temperatu	ture Microplates r			ated 2X per	day?	
%(v/v)	pH	Temp (°C)		-	°C)				CHICARIST .	30.70	
Control	0 h	0 h	0 h	24 h	45 h	72 h	0 h	24 h	46 h	72 h	
	7.3	24,0	29-	252	26.0	14.0	V	1	-/	/_	
95.2	7.6	240	L	L	1	1	-	0			
						10					
							1 31				
Initials		1	MO	MLY	4	0	мЧ	#NL")	0		
Hecute	MO	MO		IMC1	14	0				I C	
nitial control pH:	Well 1		7,3		_	Well 2		3			
Final control pH:	Well 1	-	7.1		_	Well 2	7.	1			
ight intensity (lu	x)c	4100	)			Date mea	isured:	P	الرسط	18	
hermometer: _	4	Light me	ter:	F .	iH meter/pr	obe:	11_				
Sample Description	onc		dear ,	10/01	w (285	, oda	y kis	IND P	suricu	lates_	
Comments:											
Reviewed:		de	1			Da	te reviewed		May	7/19	

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

Client:		Maxx	W.	Start D	ate/Time:	April	MERDON	
Work Order #:	- 1	90682		Terminat	tion Date:	April 11/2@ 1200h		
Work Order #: Sample ID: V	Mayy	1-16-6-19		Test	set up by:	MC		
%(v/v)						-		Initials
Concentration	Rep	Count 1	Count 2	Count 3	Count 4	U	omments	M/L)
Control	B	38 41						
	C	37						
	D	44						
	E	39						
	F	36						
	G	44		4 - 3				
	Н	38						100
	A	93						
40.7	В	84						
99.7	C	91						100
	D	96						1
	A							
	В				NET THE			
	С							-
	D							
	A							
	В							
	С							
	D							
	Α							
	В							
	C							
	D	7			6			
	Α							
	В							
	С				7 - 7			
	D							
	A		_					
	В		_					
	C							
	A							
	В	Jan. 1						
	C							- 7
	D							
Comments:					W. 184			
Reviewed by:	9	JG.		Date I	Reviewed:	μ	an Hig	

# Pseudokirchneriella subcapitata Algal Counts

Client: WO#:	Maxxiam 190682			Start Date/ Termination	Time: n Date/Time		9 @ 1200h 9 @ 1200h		
Sample ID:	VM7449-1	645-18		Initial Cell I	Janeihr	1068	2 cell/mL		235000
				HIIDEI COIL	zenany.	100			0.22 0.01
Concentration (v/v)	n Rep	Count 1 (x 10 <sup>4</sup> )	Count 2 (x 10 <sup>4</sup> )	Count 3 (x 10 <sup>4</sup> )	Count 4 (x 10 <sup>4</sup> )	Mean (x 10 <sup>4</sup> )	Cell Yield (x 10*) cell/mL		10681.82
Control	ABCDEFG	38 41 37 44 39 36 44				38 41 37 44 39 36 44	36.9 39.9 35.9 42.9 37.9 34.9 42.9	mean SD CV	38.6 3.067689 7.956281
95.2	HABCDABCDABCDA	38 93 84 91 96				38 93 84 91 96 #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0!	36.9 91.9 82.9 89.9 94.9 #DIV/0!		
	BCDABCDABCD					#DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0!	#DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0! #DIV/0!		

Reviewed by:	J64	Date reviewed:	Hay 7/19	
		Bed street waters		

# **CETIS Summary Report**

Report Date: Test Code/ID: 07 May-19 14:38 (p 1 of 1) ... 190682a / 15-1978-1798

EC Alga Grow	vth Inhibition Te	64						Na	utilus Em	ironmental
Batch ID:	11-3520-3886	Test Typ	e: Cell Growth			Anal	yst: Mir	ni Tran		
Start Date:	11 Apr-19 12:00	Protoco	E EC/EPS 1/RM/	25		Dillur	ent: De	onized Wate	r + nutrien	ts
Ending Date:	14 Apr-19 12:00	Species	Pseudokirchne	riella subcap	itata	Brine	ic .			
Test Length:	72h	Taxon:	Chlorophyta	TO COLOR DE SA		Sour	ce: In-l	House Cultur	0	Age: 6d
Sample ID:	08-2891-8816	Code:	31684C20			Proje	oct:			
Sample Date:	08 Apr-19 15:09	Material	Water Sample			Sour	eec Ma	xxam		
Receipt Date:	11 Apr-19 07:45	CAS (PC	j:			Stati	on: VM	7449-1645-1	8	
Sample Age:	69h (4.9 °C)	Client	Maoosam							
Single Compa	arison Summary	6								
Analysis ID	Endpoint	Co	mparison Method			P-Value	Compari	son Result		
03-4059-8565	Cell Yield	Eq	ual Variance t Two-	Sample Tes	st.	<1.0E-37	<1.0E-37 95.2% failed cell yield			
Cell Yield Sur	mmary									
Conc-%	Code	Count Me	am 95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	8 38	62 36.06	41,19	35	43	1.085	3.068	7.94%	0.00%
95.2	200	4 90	81,89	98.11	83	95	2.55	5.099	5.67%	-133.019
Cell Yield Det	tall									
Conc-%	Code	Rep 1 Re	p 2 Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8		
0	N	37 40	36	43	38	35	43	37		
95.2		92 83	90	95						

## **CETIS Analytical Report**

Report Date: Test Code/ID: 07 May-19 14:38 (p 1 of 2) 190682a / 15-1978-1798

EC Alga Grow	vth Ini	hibition Te	st									Ni Ni	utilus En	rironmental
Analysis ID:	03-40	059-8565	En	dpoint	Cell	Yield				CET	S Version:	CETISV	9.4	
Analyzed:	07 M	ay-19 14:37	7 An	alysis:	Pan	ametric-Tw	o Sampli	8		State	is Level:	1		
Batch ID:	11-38	520-3886	Te	st Type:	Cell	Growth				Anal	yst: Mim	Tran		
Start Date:	11 A	pr-19 12:00	Pr	otocol:	EC/	EPS 1/RM	25			Dilu	ent: Delo	nized Wate	er + nutrier	ts:
Ending Date:	14 A	pr-19 12:00	Sp	ecies:	Pse	udokirchne	riella sub	жар	data	Brin	DC .			
Test Length:	72h		Ta	ocon:	Chi	orophyta				Sour	ce: In-H	ouse Cultu	rei	Age: 6d
Sample ID:	08-2	891-8816	Co	de:	316	84020				Proj	ect			
Sample Date:	08 A	pr-19 15:09	M:	iterial:	Wat	ter Sample				Sour	roe: Max	xam		
Receipt Date:			C/	US (PC):						Stati	on: VM7	449-1645-	18	
Sample Age:	69h (	(4.9 °C)	Cli	ient:	Mag	oxam								
Data Transfor			Alt Hyp								on Result			PMSD
Untransformed	4		C < T							95.2% fail	ed cell yield			10.90%
Equal Variano	ce t T	wo-Sample	Test					Т						
Control	VS	Control I		Test	Stat	Critical	MSD	DF	P-Type	P-Value	Decision(	a:5%)		
Negative Cont	rol	95.2*		22.12		1,812	4.21	10	CDF	<1.0E-37	Significant	Effect		
Auxiliary Test	s													
Attribute		Test					Test S	tat	Critical	P-Value	Decision(	0:5%)		
Control Trend		Mann-Ke	ndall Tren	d Test				-		0.9061	Non-Signit	icant Treni	in Contro	8
ANOVA Table														
Source		Sum Squ	ares	Mean	Squ	are	DF		F Stat	P-Value	Decision(	a:5%)		
Between		7038,38		7038.	38		1		489.2	<1.0E-37	Significant	Effect		
Error		143.875		14.38	75		10							
Total		7182.25					- 11							
Distributional	Test	s												
Attribute		Test					Test S	tet	Critical	P-Value	Decision(	a:1%)		
Variances:		Variance F	Ratio F Te	st			2.763		10.88	0.2422	Equal Vari	ances		
Distribution		Shapiro-W	/ilk W Non	mality Te	st		0.9586		0.8025	0.7633	Normal Di	stribution		
Cell Yield Sur	mmar	у												
Conc-%		Code	Count	Mean		95% LCL	95% U	ICL.	Median	Min	Max	Std Err	CV%	%Effect
		N	8	38.62		36.06	41.19		37.5	35	43	1.085	7.94%	0.00%
95.2			4	90		81.89	98,11		91	83	95	2,56	5.67%	-133.01%
Cell Yield Det	ail													
Conc-%		Code	Rep 1	Rep 2	2	Rep 3	Rep 4		Rep 5	Rep 6	Rep 7	Rep 8		
0		N	37	40		36	43		38	35	43	37		
95.2			92	83		90	95							

## **CETIS Analytical Report**

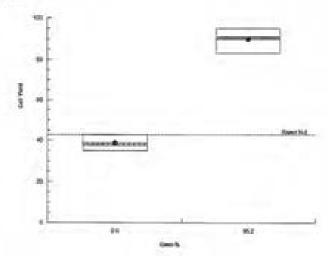
Report Date: Test Code/ID: 07 May-19 14:38 (p 2 of 2) 190682a / 15-1978-1798

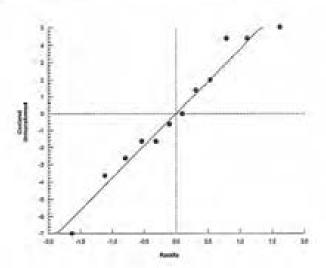
EC Alga Growth Inhibition Test

Analysis ID: 03-4059-8565 Endpoint: Cell Yield CETIS Version: CETISv1.9.4

Analyzed: 07 May-19 14:37 Analysis: Parametric-Two Sample Status Level: 1







# Pseudokirchneriella subcapitata Summary Sheet

Client Work Order No.:	MCXXGM 190637	Start Date: Set up by:	ADVI IVIS	
Sample Information:				3.
Sample ID: Sample Date: Date Received: Sample Volume:	VM7450-1645-188 April 11/19 April 11/19 2XIL			
Test Organism Informa	rtion:			
Culture Date: Age of culture (Day 0):	- Ap	W 5/19		
Zinc Reference Toxica	nt Results:			
Reference Toxicant ID: Stock Solution ID: Date Initiated:	SC181 19300 March 29,	19		
72-h IC50 (95% CL):	33.5 (26.4-37.2)	ug/Lim_		-
72-h IC50 Reference To	oxicant Mean and Range: 31.0 (35	1.0-38,5) cv	(%):	
Test Results:		Cell Yie	d (Mean± SD)	
	Negative Control	96.8		
			1	- 1
			±	
-				
			±	
			± ±	
			* *	
	# indicates that rell -	Tield 11 signific	* *	u leb contro

# 72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client:	Maxxau				Setup by:		MLT					
Sample ID:	VIVIO	<b>3</b> 3-164	7-120			Test Date	Time	A	261 11/	9010	weh	
	1.40.72	10901	N 30				A Development of the Land	- "	4			
Work Order No.:	_	121-2	000			CER #:						
		1				Test Spec		Pseudokirchneriella subcapitata  fealth: Gluco A				
Culture Date:	Apr	15/19		Age of Cu	liture:	60	Culture He	alth:	G	1000	Villa:	
Culture Count:	1 345	2 345		Average:		Culture C	ell Density (	c1):	322×10	st Cells	/m-	
		220,000 ce (c1)	Bs/ml x	bo mi			- 1	magi				
						cells/mi						
Time Zero Counts		1 25	ŧ	2 23		Average:		23.5				
No. of Cells/mL:		23.5 X	104		Initial Den	sity:	# cells/mL	+ 220 pL x 1	10 pL =	142301	cells/ml	
Concentration	Water	Quality	1 = 1	Incubator 1	Temperatur	0	Mic	roplates rot	ated 2X per	day?		
%(v/v)	pH	Temp (°C)			(C)					21.50%		
Control	0 h	0 h	0 h	24 h	46 h	72 h	0 h	24 h	48 h	72 h		
Contact	7.3	24.0	X26	252	26.0	26,2	L-	U				
95:2	7.6	940	1	1	1	T	~					
							30					
		-										
Initials	10				p	سم	r No.		P	*		
	MVI	IM/S)	Wich	ME	l P	1.00	Mg	MO	1	1	1	
Initial control pH:	Well 1	<u> </u>	7.3	_	-	Well 2	- 9	3				
Final control pH:	Well 1	·	7.1		-0	Well 2		7.1	-			
Light intensity (lu	x):	4	040			Date mea	sured:		pril 11	119		
Thermometer: _	4	Light me	ter:	p	H meter/pr	obec	1					
Sample Description	ONC		char	, rolle	wen	, today	wlest,	ساياة .	parti	culati	EJ.	
Comments:												
Reviewed:		J	Gu			De	te reviewed		Man	17/19		

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

Client:		Marxan		Start D	ate/Time:	ADM 11/19/01	Joseph
Work Order #:		190682	-	Terminal	ion Date:	Provid 14/19 @	Orbh
Sample ID: YMP	450-1	645 -	186	Test	set up by:	NO.	
%(v/v)					-		Initials
Concentration	Rep	A CONTRACTOR OF THE PARTY OF TH	Count 2	Count 3	Count 4	Comments	Initials M/C)
Control	A	40					- MACI
	В	36					
	С	41					
	D	49					
	E						
	F	38					
	G	43					
	Н	36					
22 - 5	A	9/8					
95.8	В	A CONTRACTOR OF THE PARTY OF TH					
1.00	C	95					1
	D	87					
	A						
	В	-					
	C						
	D	-					
	A						
	B						
	D						
	A						
	B						
	C						
	D				75 T \$ 1.5		
	A						
	В						
	C						
	D				18 8		
	A						
	В				The Control		
	C						
	D						
	A						
	В				3 3 4		
	C	-					
	1 0		-				
Comments:							
2000-2009-2009		404		restron	40520,00020	May 7/19	
Reviewed by:		J84		Date	Reviewed:	May 1/19	

## Pseudokirchneriella subcapitata Algal Counts

Client: WOW: Sample ID:	Maxxam 190682 VM7450-1	645.10D		Start Date/ Termination	Time: n Date/Time		9 @ 1200h 9 @ 1200h		
Sample ID.	VM/400-1	040-100		Initial Cell I	Density:	1068	2 cell/mL		235000 0.22 0.01
Concentration %(v/V)	1 Rep	Count 1 (x 10 <sup>4</sup> )	Count 2 (x 10 <sup>4</sup> )	Count 3 (x 10 <sup>4</sup> )	Count 4 (x 10 <sup>4</sup> )	Mean (x 10 <sup>4</sup> )	Cell Yield (x 10 <sup>4</sup> ) cell/mL		10681.82
Control	A B C D E F G	40 35 41 40 41 38 43				40 35 41 40 41 38 43	38.9 33.9 39.9 38.9 39.9 36.9 41.9	mean SD CV	38.2 2.712405 7.103919
95.2	HABCDABCDABCDABCDABCDA	36 88 86 95 82				36 88 86 95 82 #DIV/0!	34.9 86.9 84.9 93.9 80.9 #DIV/0!		
	C					#DIV/0! #DIV/0!	#DIV/0! #DIV/0!		

	-Po .
Reviewed by:	700

Date reviewed: Key 7/15

CETIS:	Summary	Report
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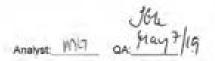
Report Date: Test Code/ID: 07 May-19 14:40 (p 1 of 1) 190682b / 06-2042-6889

									A CONTRACTOR OF THE PARTY OF TH	the state of the s
EC Alga Grow	th Inhibition Te	st						Na	utilus Env	rironmental
Batch ID:	04-7047-9893	Test Type:	Cell Growth			Anal	rst: Min	ni Tran		
Start Date:	11 Apr-19 12:00	Protocol:	EC/EPS 1/RM/2	25		Ditue	nt: Del	ionized Wate	r + nutrien	ts
Ending Date:	14 Apr-19 12:00	Species:	Pseudokirchner	iella subcap	itata.	Brine	K			
Test Length:	72h	Taxon:	Chlorophyta	080-0016-040		Sour	cec (n-i	House Cultur		Age: 6d
Sample ID:	09-3303-2160	Code:	379CF0E0			Proje	et			
Sample Date:	08 Apr-19 15:11	Material:	Water Sample			Sour	ce: Ma	xxam		
Receipt Date:	11 Apr-19 07:45	CAS (PC):				Stati	on: VM	7450-1645-1	8B	
Sample Age:	69h (4.8 °C)	Client	Maxxam							
Single Comp.	arison Summary							Company Company		
Analysis ID	Endpoint	Com	parison Method			P-Value	Compari	son Result		
14-0268-3040	Cell Yield	Equa	l Variance t Two-	Sample Tes	t	<1.0E-37	95,2% fa	iled cell yield		
Cell Yield Su	nmary									
Conc-%	Code	Count Mean	n 95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	8 38.2	5 35.98	40.52	34	42	0.959	2.712	7.09%	0.00%
95.2		4 86.79	5 78.1	95.4	81	94	2.72	5.439	6.27%	-126.80%
Cell Yield De	tail									
Conc-%	Code	Rep 1 Rep	2 Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8		
0	N	39 34	40	39	40	37	42	35		
95.2		87 85	94	81						

# **CETIS Analytical Report**

Report Date: Test Code/ID: 07 May-19 14:40 (p 1 of 2) 190682b / 06-2042-6889

EC Alga Grow	rth Inhib	ition Test										No	utilus Em	rironmental
Analysis ID:	14-0268	F3040	En	dpoint:		Yield .					S Version:		.9.4	
Analyzed:	07 May-	19 14:40	And	allysis:	Pan	ametric-Tw	o Sampi	le:		Statu	is Level:	1		
Batch ID:	04-7047	-9893	Tes	st Type:	Cell	Growth				Anal	yst: Min	ni Tran		
Start Date:	11 Apr-1	19 12:00	Pro	stocol:	EC/	EPS 1/RM/	25			Dillus	nt: Dei	onized Wate	r + nutrien	ts
Ending Date:	14 Apr-1	19 12:00	Sp	ecles:	Pse	udokirchne	riella su	beap	stata	Brim	ic .			
Test Length:	72h		Tag	con:	Chi	orophyta				Sour	ce: In-l-	louse Cultu	re e	Age: 6d
Sample ID:	09-3303	-2160	Co	de:	379	CF0E0				Proje	et:	2010-2		
Sample Date:	08 Apr-	19 15:11	Ma	torial:	Wa	ter Sample				Sour	ce: Max	ocam		
Receipt Date:	11 April	19 07:45	CA	S (PC):						Stati	on: VM	7450-1645-1	188	
Sample Age:	69h (4.8	(0° (	CII	ent:	Mac	ocam								
Data Transfor	m		Alt Hyp							The second second	on Result			PMSD
Untransformed	1		CKT		Т					95.2% fail	ed cell yield			10.87%
Equal Variance	e t Two	Sample 1	fest		П						-			
Control	vs C	Control II		Test 8	Stat	Critical	MSD	DF	P-Type	P-Value	Decision	(a:5%)		
Negative Cont	rol 9	5.2*		21.15		1.812	4,157	10	CDF	<1.0E-37	Significan	it Effect		
Auxiliary Test	is				Т									
Attribute	1	est					Test :	Stat	Critical	P-Value	Decision	(a:5%)	ve comme	
Control Trend		Aann-Kend	fall Trend	Test				-		1.0000	Non-Sign	ificant Trend	i in Control	15
ANOVA Table														
Source	S	um Squar	es	Mean	Squ	are	DF		F Stat	P-Value	Decision	(a:5%)		
Between	62	272.67		6272)	67		1		447.2	<1.0E-37	Significan	t Effect		
Error	14	10.25		14.02	5		10							
Total	6-	112.92					11							
Distributional	Tests													
Attribute	To	est					Test:	Stat	Critical	P-Value	Decision	(a:1%)		
Variances	V	ariance Ra	tio F Tes	st			4.021		10.88	0.1180	Equal Va	riances		
Distribution	S	hapiro-Wil	k W Norr	nality Te	st		0.977		0.8025	0.9690	Normal D	istribution		
Cell Yield Sur	mmary	-												
Conc-%	C	ode	Count	Mean		95% LCL	95%	UCL	Median	Min	Macc	Std Err	CV%	%Effect
0	N		8	38.25		35.98	40.52	37	39	34	42	0.959	7.09%	0.00%
95.2			4	86.75		78.1	95.4		86	.81	94	2.72	6.27%	-126.80%
Cell Yield Det	ail													
Conc-%	C	ode	Rep 1	Rep 2	2	Rep 3	Rep 4		Rep 5	Rep 6	Rep 7	Rep 8		
0	N		39	34		40	39		40	37	42	35		
95.2			87	85		94	81							

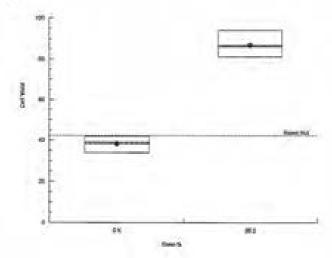


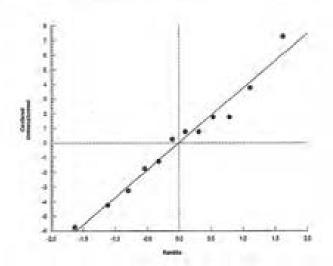
# **CETIS Analytical Report**

Report Date: Test Code/ID: 07 May-19 14:40 (p 2 of 2) 190582b / 06-2042-6889

EC Alga Gro	wth Inhibition Test				Nautilus Environmental
Analysis ID:	14-0268-3040	Endpoint	Cell Yield	CETIS Version:	CETISV1.9.4
Analyzed:	07 May-19 14:40	Analysis:	Parametric-Two Sample	Status Level:	1









**APPENDIX B – Chain-of-Custody Form** 

Sent To: Nautilus Environmental - Burnaby

8664 Commerce Court Burnaby, BC, VSA 4N7 Tel: (604) 420-8773

#### CHAIN OF CUSTODY RECORD FOR SUBCONTRACTED WORK

Page 01 of 01

COC# 8926190-VNAU-01-01

REI	PORT INFORMAT	ION							Т	T		1	MALYSIS RO	QUESTED			T		
Co	mpany:	Maosar											TT			1	1		
Ad	dress:	4606 C	snada Way, Burn	aby, British	Colun	nbia, VSG 1K	5												
Co	ntact Name:	Geraldi	yn Gouthro																
Em	ait	GGouth	го@пкакхат.са,	customers	vervicel	bc@maxxam	analytics.c	om		13	6		1.1			1			
Ph	one:	(403) 7:	35-2230							1 5									
Ma	uxam Project i	B92619	0							Carre	5		11						
	SAMPLE ID			MA	ATRIX	DATE SAMPLED (YYYY/MM/DD)	TIME SAMPLEO DREMMO	SAMPLER INITIALS	30.00	A Abres						Long oc		ADDITIONAL:	SAMPLE INFORMATION
1	VM7449-164		9		W	2019/04/08	15:09	582	-	(R.)						4.9	(P: 01)		
2	VM7450-164	5-188	0	17	W	2019/04/08	15:11	582	25	(U)						9.8	(P1 01)		
3				- 8					38										
4				-							(tall)						@ 30	mple des	engline place, adoubless, centates.
5																	éo	bulles.	adountes.
6				- 1														no part	auddel.
7 8				- 15	-					1		-							
9					-				-		3								
10					-		-		Н	. 9	a.	-				-			
-	ULATORY CRITE	PIA			-	PECIAL INSTR	I I CONTRACTOR		-	1 -2			3,734						
	dk 1645-18 Ave I					Sease Inform I		man Sirebake ii	Laster	- 74.00									TURNAROUND TIME
						*Please return	n a copy of	this form w	ith th	hie ne	sort.**	SI FOR STR	r roquestes :	enally					Rush Required 2019/05/08
coc	OLER ID:				T) [c	COLER ID:			=			- 7	COOLER	D:					Date Required
Cust Cust	ody Seal Persent ody Seal Intact ing Media Present	YES /	Temp:		0	untody Seal Pre- intody Seal Inta coling Media Pr	ci .	YES NO		mp: (c)	F 3		Custody Se Custody Se	of Present of Intact	YES MO	Temp (°C)	100	2 1	Please inform us if resh charges will be incurred.
_	NOUISHED BY:	SIGN & PRIN	I)	TD.		YYY/MM/00)	TIME: 0	HIMMS I	REC	LIVE	BY: BIGN &	PERMIT	Coowell ave	dia Present	DATE: (V	Salar Party	1000	Control of the control	1
1/	N	BILL	PUWL			1/4/11	150	0		YW.		rowl	llus		249			6745	-
2.			Constitution Const		00.000	370.75 #USS			2.	45.11.2									14-7-7



**END OF REPORT** 



#### **RESULTS OF DAPHNIA MAGNA SINGLE CONCENTRATION-100%**

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B950328Client Project Name & Number:Quarterly Tox SNP-ASample Number:VY7818-01

**Test Result:** 

Analysis Start:

48 hrs Mortality % 0 Statistical Method:

Mean percent mortality: Sample 0 Control 0

Jun 27, 2019 01:35 PM

<u>Sample Name:</u> 1645-18 Sample Matrix: Water

Description: Clear, Colourless Sample Prior to Analysis:

Sample Collected: Jun 25, 2019 12:18 AM Sampling Method: N/A pH: 6.9

Sample Collected By: AH Site Collection: N/A Temperature: 20 °C Sample Received: Jun 25, 2019 01:41 PM Volume Received: 1 L Dissolved Oxygen: 9.9 mg/L

Temp.Upon Arrival:

End: Jun 29, 2019 02:19 PM Storage: 2-6°C Hardness: 100 mg CaCO <sub>3</sub>/L

7°C

443 μS/cm

Sample Conductance:

Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hr	48 hrs	48 hrs
0	21	8.1	355	8.2	0	0	0	0	20	8.2	336	8.2
0	21	8.1	358	8.2	0	0	0	0	20	8.2	339	8.2
0	21	8.0	358	8.2	0	0	0	0	20	8.2	342	8.2
100	20	7.2	452	10.0	0	0	0	0	20	7.6	428	8.2
100	20	7.0	451	9.4	0	0	0	0	19	7.5	430	8.3
100	20	6.9	452	10.0	0	0	0	0	20	7.6	428	8.2

Concentration	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)
% vol/vol	48 hrs	48 hrs	48 hrs	48 hrs
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
100	0	0	0	0
100	0	0	0	0
100	0	0	0	0

**Comments:** None

<u>Culture/Control/Dilution Water:</u> City of Edmonton dechlorinated tap water

Hardness: 160 mg/L CaCO<sub>3</sub> Other parameters available on request.

Test Conditions Test concentration: 0,0,0,100,100,100 (% vol/vol)

Organisms per Vessel: 10 Pre-aeration Time: 30 min Rate of Pre-aeration: 25-50 mL/min/L

Total # of Organisms Used : 60 Test Temperature :  $20 \pm 2$  °C Test Hardness Adjusted : No Test Volume : 150 mL Vessel Volume : 225 mL Test pH Adjusted : No

Loading Density: 15.0 mL/Daphnia Photoperiod: 16:8 (light: dark)

<u>Test Organism :</u> Daphnia magna Source : In House Culture

Age at Test Initiation :<24 hrs</th>Average Brood Size :28.1Culture Photoperiod :16:8 (light: dark)% Mortality within 7 days :4.8Culture Temperature : $20 \pm 2$  °CTime To First Brood :8 Days

Culture Diet Pseudokirchnriella and YTC at a ratio of 2 mL/L of culture daily. New cultures weekly, 63 daphnids

distributed into 6 culture vessels and 3 reproductive vessels.



#### **RESULTS OF DAPHNIA MAGNA SINGLE CONCENTRATION-100%**

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B950328Client Project Name & Number:Quarterly Tox SNP-ASample Number:VY7818-01

Reference chemical:Sodium ChlorideTest Date:Jun 25, 2019Test Endpoint 48 hrs LC50 (95% confidence interval):5.26 (4.69, 5.91)g/LStatistical Method:Untrimmed

Spearman-Kärber

Historical Mean LC50 (warning limits) : 5.99 (4.39, 8.18) g/L Concentration : 0,1.71,2.56,3.82,5.7,8.5 g/L

Test Method EPS 1/RM/14 Method Deviations: None

<u>Note:</u> The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Team Lead, Bioassay Date: Jul 03, 2019 04:27 PM



#### **RESULTS OF RAINBOW TROUT SINGLE CONCENTRATION-100%**

Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B950328

Client Project Name & Number: Quarterly Tox SNP-A

**Test Result:** 

96 hrs Mortality % 0 Statistical Method: Visual

Sample Name: 1645-18 Sample Matrix: Water

Description: CLEAR, COLOURLESS Sample Number: VY7818-02

Sample Collected: Jun 25, 2019 12:18 AM Sampling Method: N/A Site Collection: N/A

Sample Collected By: AH Volume Received: 20 L Temp.Upon Arrival: 7 °C Storage: 2-6°C

Sample Received: Jun 25, 2019 01:41 PM pH: 6.9 Dissolved Oxygen: 10.3 mg/L Analysis Start: Jun 27, 2019 10:56 AM Temperature: 14  $^{\circ}$ C Sample Conductance: 388  $\mu$ S/cm

Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
0	15	7.9	367	9.1	0	0	0	0	0	0	0	0
100	14	7.1	392	9.8	0	0	0	0	0	0	0	0

Concentration	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	72 hrs	72 hrs	72 hrs	72 hrs	96 hrs	96 hr	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs
0	0	0	0	0	15	7.7	379	8.6	0	0	0	0
100	0	0	0	0	15	7.4	413	8.8	0	0	0	0

Comments: None

**Culture/Control/Dilution Water**City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO₃ Other parameters available on request.

Test Conditions Test concentration: 0,100 (% vol/vol)

Organisms per Vessel : 10 Test Temperature :  $15 \pm 1$  °C Solution Depth : >15 cm

Total # of Organisms Used: 20 Pre-aeration Time: 120 min. Rate of Aeration 6.5±1 mL/min/L

Test Volume : 20 L Vessel Volume : 38L Test pH Adjusted: No

Loading Density: 0.2 g/L Photoperiod: 16:8 (light: dark)

<u>Test Organism</u>: Rainbow Trout (Oncorhynchus mykiss) Source: Spring Valley Trout Hatchery

Culture Temperature :  $15 \pm 2$  °C Weight (Mean) +- SD :  $0.4 \pm 0.1$  g Length (Mean) +- SD :  $3.75 \pm 0.26$  cm Culture Water Renewal :  $\geq 1.0$  L/min/kg fish Weight (Range) : 0.3 - 0.5 g Length (Range) : 3.40 - 4.20 cm

Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 0%

Feeding rate and frequency: daily: 1-5% biomass of trout. Acclimation Time: >14 days

Reference chemical:PhenolTest Date:Jun 20, 2019Test Endpoint 96 hrs LC50 (95% confidence interval):10.0 (9.12, 10.9)mg/LStatistical Method:Probit

Historical Mean LC50 (warning limits): 10.5 (8.73, 12.6) mg/L Concentration: 0,8,10,12,15,20 mg/L

Test Method EPS 1/RM/13
Method Deviations: None

<u>Note:</u> The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst : Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Team Lead, Bioassay Date: Jul 15, 2019 01:33 PM



# Toxicity testing on samples VY7818-1645-18 and VY7819-1645-18B

Collected June 25, 2019

**Final Report** 

August 2, 2019

Submitted to: **Bureau Veritas Laboratories**Burnaby, BC



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APPENDIX C – Oncorhynchus mykiss Toxicity Test Data

APPENDIX D – Chain-of-Custody Form



#### **SIGNATURE PAGE**

Report By:

Yvonne Lam, 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	VY7818-1645-18				
Sample 15	VY7819-1645-18B				
Sample collection date	June 25, 2019				
Sample receipt date	June 27, 2019				
Sample receipt temperature	3.6°C and 2.7°C				
	72-h Pseudokirchneriella subcapitata growth inhibition				
Test type	Ceriodaphnia dubia survival and reproduction				
	7-d rainbow trout (Oncorhynchus mykiss) embryo viability				

# **Summary of Results**

	Mean ± SD						
Endpoint	Control	VY7818-1645-18	Control	VY7819-1645-18B			
P. subcapitata							
Cell Yield (x10 <sup>4</sup> cells/mL)	39.8 ± 3.5	118.2 ± 4.6*	39.6 ± 3.8	107.2 ± 7.7*			
Ceriodaphnia dubia							
Survival (%)	100	100	90	100			
Reproduction (mean ± SD)	17.5 ± 2.8	20.1 ± 4.6	19.4 ± 5.7	18.5 ± 4.7			
Oncorhynchus mykiss							
Embryo viability (%)	74.4 ± 9.8	67.5 ± 5.0	70.7 ± 9.0	80.7 ± 4.2			

SD = Standard Deviation

<sup>\* =</sup> Indicates cell yield was significantly greater than the control



#### 1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted sub-lethal toxicity tests for Bureau Veritas Laboratories on two samples identified as VY7818-1645-18 and VY7819-1645-18B. The samples were collected on June 25, 2019 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on June 27, 2019. The samples were each transported in nine 1-L and four 10-L plastic containers and received at temperatures of 3.6 and 2.7 $^{\circ}$ C. The samples were stored in the dark at 4 ± 2 $^{\circ}$ C prior to testing. The following toxicity tests were performed on the samples:

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

Testing for *C. dubia* and *O. mykiss* was initiated on June 27, 2019, and *P. subcapitata* testing was initiated on June 28, 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 C. The chain-of-custody form is provided in Appendix D.

#### 2.0 METHODS

The samples were tested at 100% single-concentration only, and the methods are summarized in Tables 1 to 3. Testing for *C. dubia* and *P. subcapitata* was conducted according to procedures described by Environment Canada (2007a and 2007b). The *O. mykiss* test followed procedures described by Environment Canada (1998) and modified by Canaria *et al.* (1999). Statistical analyses were performed using CETIS (Tidepool Scientific Software, 2013).



# Table 1. Summary of test conditions: *Pseudokirchneriella subcapitata* growth inhibition single concentration test.

Test species Pseudokirchneriella subcapitata, strain CPCC# 37

In-house axenic culture, obtained from Canadian Phycological

Organism source 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 Full strength sample diluted to 95.2% (v/v) with nutrients, 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  $24 \pm 2^{\circ}$ C
Feeding None

Light intensity 3600 to 4400 lux Photoperiod 24 hours light

Aeration None

Test area temperature measured daily; temperature and pH

Test measurements measured at test initiation; pH of two control wells measured

at test termination

Test protocol Environment Canada (2007a), EPS 1/RM/25

Statistical software CETIS Version 1.9.4

Test endpoints Algal cell growth inhibition

>16-fold increase in number of algal cells; CV ≤ 20%; no trend

when analyzed using Mann-Kendall test

Reference toxicant Zinc (added as ZnSO<sub>4</sub>)



# Table 2. Summary of test conditions: *Ceriodaphnia dubia* survival and reproduction single concentration 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  $7 \pm 1 \, day$ 

Test vessel 20-mL glass test tube

Test volume 15 mL
Test solution depth 10 cm

Test concentrations 100% (undiluted) sample, plus laboratory control

Test replicates 10 per treatment

Number of organisms 1 per replicate

20% Perrier water and 80% deionized water + 5 μg/L Se and 2

Control/dilution water 

µg/L vitamin B12

Test solution renewal Daily (100% renewal)

Test temperature  $25 \pm 1^{\circ}C$ 

Feeding Daily with Pseudokirchneriella subcapitata and TCC<sup>1</sup> (3:1 ratio)

Light intensity 100 to 600 lux at water surface Photoperiod 16 hours light / 8 hours dark

Aeration None

Temperature, dissolved oxygen, pH and conductivity measured

Test measurements daily; hardness and alkalinity of undiluted sample measured at

test initiation; survival and reproduction checked daily

Test protocol Environment Canada (2007b), EPS 1/RM/21

Statistical software CETIS Version 1.9.4

Test endpoints Survival and reproduction

≥80% survival; ≥15 young per surviving control producing

Test acceptability criteria for controls three broods; ≥60% of controls producing three or more

broods; no ephippia present

Reference toxicant Sodium chloride (NaCl)

<sup>&</sup>lt;sup>1</sup> TCC = Trout chow and Cerophyl



# Table 3. Summary of test conditions: 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability single concentration 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 100% (undiluted) sample, 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  $14 \pm 1^{\circ}$ C Feeding None Light intensity Dark

Photoperiod 24 hours dark

Aeration Continuous gentle aeration

Temperature, dissolved oxygen, pH and conductivity measured

Test measurements 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 endpoints Embryo viability

Test acceptability criteria for controls Embryo viability ≥70%

Reference toxicant Sodium dodecyl sulphate (SDS)



#### 3.0 RESULTS

Results of the single-concentration toxicity tests on samples VY7818-1645-18 and VY7819-1645-18B are summarized in Tables 4 to 6.

No adverse effects were observed on *P. subcapitata* cell yield (Table 4) for either sample; however, enhanced algal growth was observed in both samples and the percent stimulation were 198% and 171%. For *C. dubia* (Table 5), there were no significant differences observed in either sample relative to their respective laboratory controls in either survival or reproduction. There were also no statistically significant differences in either sample for *O. mykiss* (Table 6) relative to their respective laboratory controls, with embryo viability in both samples  $\geq$ 67% (v/v).

Table 4. Results: *Pseudokirchneriella subcapitata* growth inhibition single concentration test.

	Sample ID					
	VY7818	-1645-18	VY7819-1645-18B			
Concentration	Cell Yield (x 10 <sup>4</sup>		Cell Yield (x 10 <sup>4</sup>	Stimulation (%)		
(% v/v)	cells/mL)	Stimulation (%)	cells/mL)			
	(Mean ± SD)		(Mean ± SD)			
Laboratory Control	39.8 ± 3.5		39.6 ± 3.8			
100	118.2 ± 4.6*	197.5	107.2 ± 7.7*	170.7		

SD = Standard Deviation

<sup>\* =</sup> Indicates cell yield was significantly greater than the laboratory control



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

_	Sample ID									
Concentration (% v/v)	VY7818	3-1645-18	VY7819-1645-18B							
	Survival (%)	Reproduction (Mean ± SD)	Survival (%)	Reproduction (Mean ± SD)						
Laboratory Control	100	17.5 ± 2.8	90	19.4 ± 5.7						
100	100	20.1 ± 4.6	100	18.5 ± 4.7						

SD = Standard Deviation

Table 6. Results: 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability single concentration test.

_	Sample ID						
Concentration	VY7818-1645-18	VY7819-1645-18B					
(% v/v)	Embryo viability (%)	Embryo viability (%)					
	(Mean ± SD)	(Mean ± SD)					
Laboratory Control	74.4 ± 9.8	70.7 ± 9.0					
100	67.5 ± 5.0	80.7 ± 4.2					

SD = Standard Deviation

The samples were not statistically significantly different relative to their respective Laboratory Control

The samples were not statistically significantly different relative to their respective Laboratory Control



#### 4.0 QA/QC

The health history of the test organisms used in the exposures were acceptable and met the requirements of the Environment Canada protocols. The tests met all control acceptability criteria and water quality parameters remained within ranges specified in the protocols throughout the tests. Uncertainty associated with these tests is best described by the standard deviations around the means and/or confidence limits around the point estimates.

There was a deviation from the test methodology in the *O. mykiss* 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 method. 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. The deviation did not seem to affect the results of the test and control criterion was met at the end of the exposure.

Results of the reference toxicant tests conducted during the testing program are summarized in Table 7. Results for these tests fell within the acceptable range for organism performance of mean and two standard deviations, based on historical results obtained by the laboratory with these tests. Thus, the sensitivities of the organisms used in the tests were appropriate. The reference toxicants were performed under the same conditions as those used for the samples.

Table 7. Reference toxicant test results.

Test Species	Endpoint	Historical Mean (2 SD Range)	CV (%)	Test Date
P. subcapitata	Growth (IC50): 29.6 µg/L Zn	31.0 (25.2 – 38.3) µg/L Zn	10	June 28, 2019
C dubia	Survival (LC50): 2.1 g/L NaCl	2.0 (1.8 – 2.2) g/L NaCl	5	luna 10, 2010
C. dubia	Reproduction (IC50): 1.9 g/L NaCl	1.5 (1.0 – 2.3) g/L NaCl	20	June 19, 2019
O. mykiss	Viability (EC50): 4.1 mg/L SDS	3.8 (2.3 – 6.0) mg/L SDS	24	June 27, 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: 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.
- Environment Canada. 2007b. 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.
- Tidepool Scientific Software. 2013. CETIS comprehensive environmental toxicity information system, version 1.9.4 Tidepool Scientific Software, McKinleyville, CA. 255 pp.



APPENDIX A – Pseudok	kirchneriella subc	<i>apitata</i> Toxicity T	est Data	

### Pseudokirchneriella subcapitata Summary Sheet

Client	Bureau Ventas Laboratrias.	Start Date: dune 28/19	
Work Order No.:	191291	Start Date: ひょんこう3/19 Set up by:	
Sample Information			
Sample ID:	Vu7818-1645-18		
Sample Date:	June 25/19		
Date Received:	dune 27/19		
Sample Volume:	2×11		
Test Organism Info	rmation:		
Culture Date:	due	11/19	
Age of culture (Day 0	7.	<u>d</u>	
Zinc Reference Tox	icant Results:		
Reference Toxicant I	D: Se184		
Stock Solution ID:	19m0>		
Date Initiated:	gum 22 10		
72-h IC50 (95% CL):	29,6 (26,0-33.6) ug/L=	7n	
	0		
72-h IC50 Reference	Toxicant Mean and Range: 31.0 (34, 2 -	383) uall cv(%): 10	
	<u> </u>	20	
Test Results:		Cell Yield (Mean± SD)	
	Negative Control	39.8 ± 3.5	
	V47818-1645+18 (9521.)	118,2 ± 4,6 *	
		±	
		±	
		±	
		±	
		±	
	6 11 1 4 4 4 11	alo Con Harris de la	la cache
	* (MCLICATE) THAT CENT GIRLS IS	Significantly greater than the la	www.
Reviewed by:	_ A - TONO,	Date reviewed: Joly 30/19	
	X	1 1	

Nautilus Environmental Company Inc.

Issued May 10, 2014; Ver. 1.0

### 72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client :	Bur	eau ve	intal 1	aborct	mel	Setup by:		MA				
Sample ID:		47818-	1645-1	8		Test Date/	Time:	ne 28/1	9@1130			
Work Order No.:		19	11291			CER#:		4				
						Test Spec	ies:	hneriella sui	ocapitate			
Culture Date:	Jun	21/19		Age of Co	ulture:	70 Culture Health: Glocal						
Culture Count:	1460	2 490		Average:	475	Culture Ce	ell Density (	c1):	479 XI	04 celli/r		
	v1 =	220,000 co	elis/mi x	to mi			- 16	63mL				
		(c1)	45	FILKID,	f	cells/ml						
Time Zero Counts		1 2		224	-	Average:		20.5				
No. of Cells/mL:		22,5	KID4		_Initial Dec	nsity:	# cells/mL	+ 220 µL x	10 µL = ]	0227 rel		
Concentration		Quality	4		Temperatu	re	Micr	oplates rot	tated 2X per	day?		
%(v/v)	pH 0 h	Temp (°C)	0 h	24 h	*C) 48 h	72 h	0 h	24 h	48 h	72 h		
Control	73	23.0	25.0	26.0	250	25/2	٧.,	2411	4011	1211		
95.8	7.6	23,0	i.	L	1	1	~	/	/	/		
							i.					
		-	- 2			-						
							-	1 -				
		1										
			1									
					-							
Initials	MO	MIS	.417		06				ă-	/5		
	1410	MG	טוין	B	N	MU	MG	N	N	mg		
initial control pH:	Well 1		7.3		-	Well 2:	7.	3				
Final control pH:	Well 1		7.0			Well 2:	7	ć,				
Light intensity (Iuo	d:	4140				Date meas	unrod:	L	no 28,	/19		
Thermometer:	4			1	-			- Ju	unu pa j	1		
						obe:						
Sample Description	on: (	elear,	colour	rless,	odour	lest, M	> part	iculai	tes.			
Comments:												
			2.0									
		all and										

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

Nork Order #:		24 Vent		Terminat	tion Date:		une 28/19@1130h	
Sample ID:	YUF	1818-160	45-18	Test	set up by:		WIC	
%(v/v)								
Concentration	Rep	Count 1	Count 2	Count 3	Count 4		Comments	Initial
Control	A	37	-					MD
	В	43						1
Y 5	С	46						
1.0	D	44						
3 15.	E	4	1					
	F	39				_ 11		
14.	G	26						
	H	40			1	. 1		
	A	2		1				
95.2.	В	115	V-					
10.2.	С	125			4 7 7 7			
	D	116						J
	A							
	В							
	С							
	D							
	Α							
	В							
	С							
	D							
	A							
	В							
	C			-				
	D							
	A							
	В							
	c							
	D							
	A							
	В	2 1						
	C							
	Ď							
	A							
	В							
	C							

### Pseudokirchneriella subcapitata Algal Counts

Client: WO#: Sample ID:	Bureau Ve 191291 VY7818-1		tories	Start Date/ Termination	n Date/Time	1-Jul-1	9 @ 1130h 9 @ 1130h 7 cell/mL		225000
					zenony.	IVEE	Commit		0.22
Concentration (v/v)	on Rep	Count 1 (x 10 <sup>4</sup> )	Count 2 (x 10 <sup>4</sup> )	Count 3 (x 10 <sup>4</sup> )	Count 4 (x 10 <sup>4</sup> )	Mean (x 10 <sup>4</sup> )	Cell Yield (x 10 <sup>4</sup> ) cell/mL		10227.27
Control	A B C D	37 43 46 44				37 43 46 44	36.0 42.0 45.0 43.0	mean SD CV	39.7 3.453776 8.693716
	E F G	41 39 36				41 39 36	40.0 38.0 35.0		
95.2	A B	40 121 115				40 121 115	39.0 120.0 114.0		
	D A	125 116				125 116 #DIV/0!	124.0 115.0 #DIV/0!		
	C D					#DIV/0! #DIV/0! #DIV/0! #DIV/0!	#DIV/0! #DIV/0! #DIV/0!		
	B C D					#DIV/0! #DIV/0! #DIV/0!	#DIV/0! #DIV/0! #DIV/0!		
	A B C					#DIV/0! #DIV/0! #DIV/0!	#DIV/0! #DIV/0! #DIV/0!		
	D A B					#DIV/0! #DIV/0! #DIV/0!	#DIV/0! #DIV/0! #DIV/0!		
	C D A					#DIV/0! #DIV/0! #DIV/0!	#DIV/0! #DIV/0! #DIV/0!		
	B C D					#DIV/0! #DIV/0! #DIV/0!	#DIV/0! #DIV/0! #DIV/0!		
	B					#DIV/0! #DIV/0! #DIV/0!	#DIV/0! #DIV/0! #DIV/0!		
	D					#DIV/0!	#DIV/0!		

Date reviewed: July 30, 2019

### **CETIS Summary Report**

Report Date: Test Code/ID: 18 Jul-19 10:18 (p 1 of 1) 191291a / 07-9282-6386

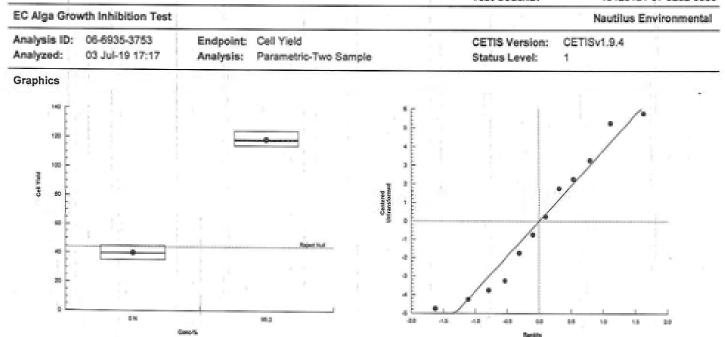
						Test	Code/ID:	1	91291a / 07	-9282	-6386
EC Alga Grow	th Inhibition Ter	st						Na	utilus Envi	ronme	ental
Batch ID:	08-0180-7424	Test Type	e: Cell Growth			Anah	yst: Min	ni Tran			
Start Date:	28 Jun-19 11:30	Protocol	EC/EPS 1/RM	/25		Dilue	nt: Dei	onized Wate	r + nutrients		
Ending Date:	01 Jul-19 11:30	Species:	Pseudokirchne	riella subcac	itata	Brine	HC.	70/W. = 4-5/II (*) 5.45	2		
Test Length:	72h	Taxon:	Chlorophyta			Sour	ce: In-l	louse Cultur	е	Age.	7d
Sample ID:	15-0705-8829	Code:	59D3E48D			Proje	ect:				
Sample Date:	25 Jun-19 00:18	Material:	Water Sample			Sour		eau Veritas	Laboratories	5	
Receipt Date:	27 Jun-19 12:05	CAS (PC)	E			Static		7818-1645-1	into such sous		
Sample Age:	83h (3.6 °C)	Client:	Bureau Veritas	Laboratorie	\$						
Single Compa	arison Summary	1 1									
Analysis ID	Endpoint	Cor	nparison Method	1		P-Value	Compari	son Result			S
06-6935-3753	Cell Yield	Equ	al Variance t Two	-Sample Tea	st	<1.0E-37		led cell yield			1
Cell Yield Sur	nmary										
Cupc-%	4 ode	Count Wes	arr 95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Ef	fect
C 94	9	8 39.	75 36.86	42.64	35	45	1.221	3.454	8.69%	0.00	96
95.2	,1.4	4 118	2 110.9	125.6	114	124	2.323	4.646	3.93%	-197	.48%
Cell Yield Det	ail										
Conc-%	Code	Rep 1 Reg	2 Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8			
0	N.	36 42	45	43	40	38	35	39			
95.2		120 114	124	115							

Report Date: Test Code/ID: 18 Jul-19 10:18 (p 1 of 2) 191291a / 07-9282-6386

					lest	Code/ID:		191291a/C	7-9282-638
EC Alga Growth Inhibition Te	st					20,200	Na	autilus Env	rironmental
Analysis ID: 06-6935-3753 Analyzed: 03 Jul-19 17:17	Endpoint: Analysis:	Cell Yield Parametric-Tw	o Sample			S Version: is Level:	CETISV1	.9.4	
Batch ID: 08-0180-7424 Start Date: 28 Jun-19 11:30 Ending Date: 01 Jul-19 11:30 Test Length: 72h		Cell Growth EC/EPS 1/RM/ Pseudokirchne Chlorophyta		oitata	Anal Dilue Brine Sour	ent: Del	ni Tran onized Wate louse Cultu		ts Age: 7d
Sample ID: 15-0705-8829 Sample Date: 25 Jun-19 00:18 Receipt Date: 27 Jun-19 12:05 Sample Age: 83h (3.6 °C)		59D3E48D Water Sample Bureau Veritas	Laboratorie	5	Proje Sour Stati	roe: Bur	eau Veritas 7818-1645-1		rs.
Data Transform	Alt Hyp		1.6		Comparis	on Result			PMSD
Untransformed	C < T				95.2% fail	ed cell yield	1		10.75%
Equal Variance t Two-Sample Control vs Control I Negative Control 95.2*				P-Type CDF	P-Value <1.0E-37	Decision	F		1
Auxiliary Tests  Attribute Test  Control Trend Mann-Kei	ndall Trend Test		Test Stat	Critical	P-Value 0.3987	Decision	(α:5%) ificant Trend	l in Control	
ANOVA Table Source Sum Squ:		Square	DF	F Stat	P-Value	Decision		in Control	•
Between 16432.7 Error 148.25 Total 16580.9	16432 14.82		1 10 11	1108	<1.0E-37	Significan	t		
Distributional Tests Attribute Test			Test Stat		P-Value	Decision	(a:1%)		
Distribution Shapiro-W	Ratio F Test filk W Normality Te	st	1.809 0.9355	10.88 9.2025	0.4663 0.4459	70.4	strisation.		
Cell Yield Summary	·				<u> </u>				
Conc-% Code	Count Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0 N 95.2	8 39.75 4 118.2	36.86 110.9	42.64 125.6	39.5 117.5	35 114	45 124	1.221	8.69% 3.93%	0.00%
Cell Yield Detail	*								
Conc-% Code	Rep 1 Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8		
0 N 95.2	36 42 120 114	45 124	43	40	38	35	39		

Analyst: 10 0A:- 14 30/19

Report Date: Test Code/ID: 18 Jul-19 10:18 (p 2 of 2) 191291a / 07-9282-6386



### Pseudokirchneriella subcapitata Summary Sheet

Client:	Bureau Vental Laboratories	Start Date:	dune 28/19
Work Order No.:	19291	Set up by:	MO,
Sample Information	nc		
Sample ID;	V47819-1645-18B		
Sample Date:	ULINE25/19		
Date Received:	clure 27/19		
Sample Volume:	2×1C		
Test Organism Info	rmation:		
Culture Date:	June 21/	18	
Age of culture (Day			
Zinc Reference To:	cicant Results:		
Reference Toxicant	10: Sc184		
Stock Solution ID:	50nfp1	_	
Date Initiated:	Jun 28/19		
	2.1	5	
72-h IC50 (95% CL)	99.6(26.0-33.6) uglL	ZN	
72-h IC50 Reference	Toxicant Mean and Range: 31.0(25.2-38	3,3) wall eve	%)·   <u>&gt;</u>
		3n	
Test Results:		Cell Yield	(Mean± SD)
	Negative Control	39.4	± 7,8
	V47818-1645-18B (95.21.)		± 7.7 *
			±
			±
			±
			±
			±
			±
	the code as the first state of the	A	± 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
	* indicates that call yold i	s significant	y migher than the lat
Reviewed by:	4.00	Date reviewed:	July 30/19
			1

Nautilus Environmental Company Inc.

Issued May 10, 2014; Ver. 1.0

### 72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client:	Bure	au Vei	ntos L	aborati	mes	Setup by:			MU			
Sample ID:		7819-		D		Test Date	/Time:	June 28/19@1140				
Work Order No.:			19/29			CER#:		+				
						Test Spec	ies:	Pseudokiro	hneriella sub	capitata		
Culture Date:	June	21/19	. 1	Age of C	ulture:	70	Culture He	alth;	Gio	d		
Culture Count:	1 460	2 490	1 1	Average:	495	Culture C	ell Density (	c1): 4	नेत्र ४१०	t cells/ml		
	v1 =	220,000 ce	ells/ml x	D) ml				t, banl				
		(c1)		75 XIO'		cells/ml						
Time Zero Counts:	2	1 2.5 X10	21	2 24		Average:	-	ي.دو			7	
No. of Cells/mL:	0.	4.9 X 10			_Initial De	nsity:	# cells/mL	÷ 220 μL x 1	10 μL = 1 <sup>3</sup>	0227 cellu	m	
Concentration %(v/v)	Water	Quality Temp (°C)	. 1		Temperatu (°C)	re	Micr	roplates rot	ated 2X per	day?		
Control	0 h	0 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h		
	7,3	23.0	25/0	25,0	25.0	25.0	V	-	1	. ~		
95.2.	7-600	23.0	レ	1	1	1	~	/	/	~		
	7.5											
								1				
				-	_							
	I								1			
Initials	MO	MIS	MQ	1	N	MO	WO	n	1	NO		
Initial control pH:	Well 1:		7.3			Well 2:	7	3				
Final control pH;	Well 1:		7.0	61			-	6.				
					-	Well 2:						
Light intensity (lux)	k	4	080		- "	Date mea	sured:	tur	4 28/1	9		
Thermometer:	4_	Light met	er:	) p	H meter/pn	obe:						
Sample Description		راء	car, c	don	<i>-</i> (1 =	1 1	Ct	0- 1		6.4		
		C	41,0	OLOUGY I	18 , 01	danve	S), no	> years	(Mal	es.		
Comments:												
		1-							,	-610		
Reviewed:	- 7	<del>/</del> 4 . ((	24		-	Dat	e reviewed:	200	4 30	2019		
			()						(			

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

Client:	Bureau	· Vento	s hab	Start D	ate/Time:	June 28/19@ 1140h	
Work Order #:		19129	(	Termina	tion Date:	dely 1/19 @ 1145h	
Sample ID:	8FVV	319-1645	-18B	Test	set up by:	June 28/19@ 1140h duly 1/19@ 1140h	
%(V/V)	,						
Concentration Control	Rep	Count 1	Count 2	Count 3	Count 4	Comments	Initials
Control	В						uG
	C	39				111	-+-
	D	43					
		37					
	F	47	-				+
	G	4(				<del></del>	
	Н						
	A	97					
05.24	В	117					
95.2	С	114					
	D	110					1
	A	1.0					1
	В.						
	C						
	D						
	A						
	В				-		
	C						
	D						
	A						
	В						
11	C	-					- 1
	D						
	Ā						
	В						
	c						
	D						
	A						
	В						
	C						
	D						
	Α						
	I B	27.15	in the second	7			
	C						
	D						
Comments:							
www.minelites.		1 _					
Reviewed by:		1.0	9	Date R	teviewed:	July 30,2019	
			X			7 00 100(1	
			U			h.	

Version 1.0 Modified May 8, 2008

Nautilus Environmental

### Pseudokirchneriella subcapitata Algal Counts

WO#:	Bureau V 191291 VY7819-1		atories	Start Date/ Termination	Time: n Date/Time		9 @ 1140h 9 @ 1140h		
				Initial Cell [	Density:	1022	7 cell/mL		225000 0.22
Concentration	Rep	Count 1	Count 2	Count 3	Count 4	Manu	0-11/1/2-1-1		0.01
%(v/v)	Keb	(x 10 <sup>4</sup> )	(x 10 <sup>4</sup> )	(x 10 <sup>4</sup> )	(x 10 <sup>4</sup> )	Mean (x 10 <sup>4</sup> )	Cell Yield (x 10 <sup>4</sup> ) cell/mL		10227.27
Control	A	44				44	43.0	mean	39.6
	В	39				39	38.0	SD	3.814914
10.00	C	43		1 . 6		43	42.0	CV	9.633069
100	D	37				37	36.0		
6/1	E	47				47	46.0		
	F	38		1		38	37.0		
	. G	41				41	40.0	2	
	H	36				36	35.0		
95.2	A	97				97	96.0		
	В	112				112	111.0		
	C	114				114	113.0		
	D	110				110	109.0		
	A					#DIV/0!	#DIV/0!		
	В					#DIV/0!	#DIV/0!		
	C					#DIV/0!	#DIV/0!		
	D A					#DIV/0!	#DIV/0!		
	В					#DIV/0!	#DIV/0!		
	C					#DIV/0!	#DIV/0!		
	D					#DIV/0!	#DIV/0!		
	Ă					#DIV/0!	#DIV/0!		
	B					#DIV/0!	#DIV/0!		
	c					#DIV/0!	#DIV/0!		
	D					#DIV/0!	#DIV/0!		
-	Ã					#DIV/0!	#DIV/0!		
	В					#DIV/0! #DIV/0!	#DIV/0!		
14.	C					#DIV/0!	#DIV/0! #DIV/0!		
	D					#DIV/0!	#DIV/0!		
	A					#DIV/0!	#DIV/0!		
	В					#DIV/01	#DIV/0!		
	C					#DIV/0!	#DIV/0!		
ät	0					#DIV/0!	#DIV/0!		
T	A					#DIV/0!	#DIV/0!		
	В					#DIV/0!	#DIV/0!		
	C					#DIV/0!	#DIV/0!		
	D					#DIV/01	#DIV/0!		

Date reviewed: July 30, 2019

### **CETIS Summary Report**

Report Date: Test Code/ID: 18 Jul-19 10:18 (p 1 of 1) 191291b / 07-8059-6487

			- 1				Test	Coderiu:	1	91291070	1-9009-0491
EC Alga Grow	vth inhibition Tes	st .							Na	utilus Env	ironmental,
Batch ID:	16-1445-2520	T	est Type:	Cell Growth			Analy	yst: Min	ni Tran		7
Start Date:	28 Jun-19 11:40	P	rotocol:	EC/EPS 1/RM/2	25		Dilue		ionized Wate	r + nutrien	ts
Ending Date:	01 Jul-19 11:40	s	pecies:	Pseudokirchner	iella subcap	itata	Brine	e:			
Test Length:	72h	T	axon:	Chlorophyta			Sour	ce: In-l	House Cultur	e	Age: 7d
Sample ID:	21-1420-7830	С	ode:	7E044056			Proje	ect			
Sample Date:	25 Jun-19 00:10	M	laterial:	Water Sample			Sour	ce: Bu	reau Veritas	Laboratorie	15
Receipt Date:	27 Jun-19 12:05	C	AS (PC):				Stati	on: VY	7819-1645-1	8B	
Sample Age:	83h (2.7 °C)	C	lient:	Bureau Veritas	Laboratories	5					
Single Compa	arison Summary										
Analysis ID	Endpoint		Comp	parison Method			P-Value	Compari	son Result		s
04-1578-7606	Cell Yield		Equal	Variance t Two-	Sample Tes	4.	<1.0E-37	95.2% fa	iled cell yield		1
Cell Yield Sur	mmary										-
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	8	39.62	36.44	42.81	35	46	1.349	3.815	9.63%	0.00%
95.2		4	107.2	95.04	119.5	96	113	3.838	7.676	7.16%	-170.66%
Cell Yield Det	tail										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8		
	A.C.	40									
٥	N	43	38	42	36	46	37	40	35		

Analyst: M7 0A. JULY 30/19

Report Date: Test Code/ID: 18 Jul-19 10:18 (p 1 of 2) 191291b / 07-8059-6487

							1995	Podewn:		191291071	37-8U59-648
EC Alga Growth I	nhibition Te	st							N	autilus Em	vironmenta
	1578-7606 Jul-13 7769			l Yield newoorlo-Than	Sample	10		IS Version us Level:	: CETISV	1.9.4	
		Pro	cies: Ps	II Growith /EPS 1/RM/ sudokirchner lorophyta		pitata	Anal Dilu Brin Sour	ent: De	mi Tran ionized Wab House Cultu		rts Age: 7d
Sample ID: 21- Sample Date: 25 Receipt Date: 27 Sample Age: 83h	Jun-19 12:05		terial: Wa S (PC):	044056 iter Sample reau Veritas	Laboratoša	\$	Proje Sour Stati	ect: rce: Bu	reau Veritas 7819-1645-1	Laboratori	
Data Transform	1 1 1	Alt Hyp	1				Comparis	on Result			PMSD
Untransformed	19.53	C < T	):			_		led cell yiel			14.79%
Equal Variance t  Control vs  Negative Control	Two-Sample Control II 95.2*		Test Stat	Critical	MSD DF 5.859 10	P-Type CDF	P-Value <1.0E-37	Decision Significa	A		
Auxiliary Tests Attribute Control Trend	Test Mann-Ker	ndall Trend	Test		Test Stat	Critical	P-Value 0.2751	Decision Non-Sign	n(α:5%) nificant Trend	d in Contro	ls
ANOVA Table Source	Sum Squa	ires	Mean Squ	uare	DF	F Stat	P-Value	Decision	n(a:5%)		
Between Error Total	12195 278,625 12473,7		12195 27.8625		1 10	437.7	<1.0E-37	Significar	nt Effect		
Distributional Tes Attribute	Test				Test Stat	Critical	P-Value	Decision	Swiffs 1		
Variances Distribution	Variance R Shapiro-W				4.048 0.9411	10.88	0.1163 0.5129	Equal Va			
Cell Yield Summa	ту										
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0 95.2	N	8 4	39.62 107.2	36.44 95.04	42.81 119.5	39 110	35 96	46 113	1.349 3.838	9.63% 7.16%	0.00%
Cell Yield Detail											
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8		
0	N	43	38	42 .	36	46	37	40	35		
95.2		96	111	113	109		.000				

Report Date: Test Code/ID: 18 Jul-19 10:18 (p 2 of 2) 191291b / 07-8059-6487

Analyst: MC QA: Schy 30/19



**APPENDIX B – Ceriodaphnia dubia Toxicity Test Data** 

### Ceriodaphnia dubia Summary Sheet

Client:	GNATERIA VENTOR	Start Date/Time:	June 27 line 1500h
Work Order No.:	191292	Set up by:	OC/ 428
Sample Information	on:	Test Validity Criteria:	
		Mean survival of first genera	tion controls is >80 %
Sample ID:	suchinal color set)	Control of the contro	produced three broods within 8 days
Sample Date:	Juvezsig		produced per surviving female in the
Date Received:	Jule 27/19	control solutions during the first	
Sample Volume:	7 x 1L	The state of the s	n any control solution at any time.
		WQ Ranges:	
IK		$T (^{\circ}C) = 25 \pm 1$ ; DO (mg/L) = 3.	3 to 8.4; pH = 6.0 to 8.5
est Organism In	formation:		
Proodstock No.:		00-06/34/07/1-20/	
Age of young (Day	m.	06061719(1-20)	
	irst 3 broods of previous 7 d:	<24-h (within 12-h) 2.2	<del></del>
Mortality (%) in pre		0	
	used ≥8 young on test day	1-6,8-12,14,16-	-19
13			
Pate Initiated:	June 19 (19)		
'-d LC50 (95% CL		g/L NaCL	
'-d IC50 (95% CL)	(19(17-20)	g/L NaCL	
7-d LC50 Reference	e Toxicant Mean and Historical	Range: 2.0(J.\$-2.2) g/	L NaCL CV (%): 5
-d IC50 Reference	e Toxicant Mean and Historical	Range: [-5 (1-0 - 2-5) gr	
			V. (///).
est Results:			
		Survival (%)	Reproduction (Mean ± SD
	Negative Control	igo	12.5 ± 2.8
	WAS18-1045-18	[00	20-1 ± 4-6
	Negative contail	૧૦	19.4 ± 5.3
	VY7819-1645-188.	loo	18.5 ± 4.7
			±
			±
			±
			±
1.0			

Jan 26, 2011; Ver. 2.0

Reviewed by:

Jou

Nautilus Environmental

Date reviewed:

# Chronic Freshwater Toxicity Test Initial and Final Water Quality Measurements

DO (mg/L) pH	0	1911.97				-	Stop	Date	& Time:		43119	10 1	tooh	
Concentration CONTROL Temperature (°C) DO (mg/L) pH									CER#:	_				
Temperature (°C)  DO (mg/L)  pH								Test Sp	pecles:	Ceriod	aphnia c	Subia		
Temperature (°C)  DO (mg/L)  pH							D.	ly's						
Temperature (*C) DO (mg/L) pH			1		2		3		4		5		6	7
DO (mg/L) pH	init.	old	Dow	old	new	old	new	old	new	old	new	old	The same	
DO (mg/L) pH	0-140	X-0	24.0		NA	1670	14.0	1673	14/2	29/0	24.5	22.0	new	fina
рН	5.0	7-8	8.2	74	Fiz	7.4	5.1	9.0	8.1	6.5	8.2	6.9	<del>\</del>	-
	8-2	8.3	8.3	7.8	FL	7.8	AZ	7.8	8-1	7.8	3.4	7.8	1	
Cond. (us/cm)	215		14		45		A G	26	-				<u> </u>	1
	SK		B								2/7	27		1
	1.3.1 (mm)	-	77		Alertin .			Ji	VI		וכוע	J	3	
100 1/.							D-				-			
Concentration	0	5 - 10c Y			2		_	ys						
1 = -	init	old	new	old	CALLES SHOW	Section Cont. 5	3	GI OUNG IN	4		5	A CONTRACTOR	6	7
	35.0	25.0	24 D		new	old	new	old	new	old	new	old	new	fina
	8.2	7.2	8.1	7-3	50	7-3	57	257		25.0	24.5	35 3	1	
	7.2	7.4	7.5	天生	73			0.F	7.9	6.7	8.3	6-9	1	
	500	1.9		- /-	92	7.4	7.3		7.3	7.72	7.1	7.5		1
	SS¥	J	1	7	40				92		196		86	1
iniciais	- ACC	V	9		/w	p		Ji	V		<u> </u>	- 3	3	7
Concentration							Da							
A Price American	0	6.55.P2m3	Tradition !	1000	2				4		5		6	7
19 7 353	init.	old	new	old	new	old	new	old	DOW	old	new	old	new	final
Temperature (°C)	-													
DO (mg/L)	~	No.												
pH		1												
Cond. (µS/cm)			-											
Initials				The state of the s	_									
					- Carlonnian	San								•
		-					Da	ys						
	0	1	W-000	- 2	2	3			1		5		6	7
Concentration			new	old	new	old	new	old	new	old	new	old	DOW	
100	init.	old						Olu					TICAA	final
100 Temperature (°C)		old					30.0	olu		\			new	final
Temperature (°C)		old						Olu					1164	final
Temperature (°C)  DO (mg/L) pH		old						Join					TIC VV	final
Temperature (°C)		old						Join			,		13.0	final

#### Chronic Freshwater Toxicity Test C. dubia Reproduction Data

1	onc.	entra			3-	1455	B	ugr								66	(0)				S	itart D itop D	ate &	Time:	10	15/2	128			14	100			
1	A	entra	tion.	_		100	lost	_			_	Con	entra	tion:	_	-	1,1-5	b- 11	17)		-	-	Conc	entrat	ion:		3	-12	-				1	SCH
1		В	C	D	E	F		Н	1	J	Init	A	_		D	E		G	Н	1	J	Init		В	С	D	E	F	G	Н	1	J	Init	> 1
		1	/	1	/	1	1	1	-	-	4	/		/	/	-	/	/		/												/		
	/	1	/	V,	1.0	IN	1/	1	/	1	1A	/	/	/	1	/	/	/	/	1	/	A									/			
3	1	3	4	13	15	3V	3	3	3	13V	~	3	2	3	1	1	3	3	/	3	2	1								1				
1 3	+	7	V	7	7	4	7	1	1	2	241	1	Lo	1	4	1	1	0	5	6		JW					V				-			
5	1	V	牙	V	1	m/c	1	6	6	10	WC	6	Q.	8	0	124	7	2	/	V		Mich						_	1	_	_		_	
1	0	H	17	U	10		12	12	1/	12.	MU	11	45	19	14	12.	8	13	15	9	17	10/87		_		_			_	_	_	_	-	
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tat 17	<u>t</u>	2	22	2	22	44	22	2	20	24	略為	20	1.4	少十	27	119	10	18	0	10	17	MIC			1					_				
Ice		entral	Slow:	- (		6-25		_				Conc	entrat	llonc			12.5						Conc	entrat	ion:			15						
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	1	_					200	2.77			/		-			- 70	1.22	75	- 12-7	1 1	1		100		1121	C								
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viewe	d b	y: .			•	(	161	1												•		Date	e revie	wed:			Ju	ly	17	19				1

### **CETIS Summary Report**

Report Date: Test Code/ID: 17 Jul-19 18:38 (p 1 of 1) 191292b / 06-2576-8471

							0.000			the property of the	the second contract of
Ceriodaphnia	7-d Survival and	d Reprodu	ction T	ist					Na	utilus Env	ironmental
Batch ID:	18-6640-7080	Tes	st Type:	Reproduction-S	Survival (7d)		Anal	lyst: Ka	nia Lywe		
Start Date:	27 Jun-19 13:00	Pro	tocol:	EC/EPS 1/RM/	21		Dillu		-		
Ending Date:	03 Jul-19 14:00	Spe	ecies:	Ceriodaphnia d	ubia		Brin	e:			
Test Length:	6d 1h	Tax	con:	Branchiopoda			Sou		House Cultur	e	Age: <24
Sample ID:	08-3720-8412	Co	de:	31E6C95C		1	Proj	ect:			
	25 Jun-19 00:10	Mar	terial:	Water Sample			Sour		reau Veritas	Laboratorie	6
Receipt Date: :	27 Jun-19 12:05	CA	S (PC):			5	Stati		7819-1645-1		
Sample Age:	61h (2.7 °C)	Clie	ent	Bureau Veritas	Laboratorie	s					
Single Compa	rison Summary		17			7	- 1				
	Endpoint		Comp	arison Method			P-Value	Compar	ison Result		5
15-7117-9767	6d Survival Rate			r Exact Test			1.0000		ssed 6d surv	ival rate	1
14-5656-3824	Reproduction		Equal	Variance t Two-	Sample Tes	ut .	0.3525		ssed reprodu		1
6d Survival Ra	te Summary			· ·							
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	0.900	0 .0.6738	1,0000	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
100		10	1.000	0 1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%
Reproduction	Summary						Ti.				
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	19.4	15.32	23.48	4	24	1.802	5.7	29.38%	0.00%
100		10	18.5	15.12	21.88	10	27	1.493	4.72	25.51%	4.64%
6d Survival Ra	te Detail		7								
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N.	1.0000	1,000	0 1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
100		1.0000	1.000	0 1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Reproduction	Detail			1							
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	17	21	22	21	22	4	22	21	20	24
100		20	14	24	27	19	18	18	10	18	17
6d Survival Ra	te Binomials										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
100											

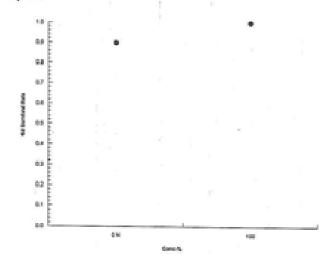
John 18/19

Analyst: N\_

Report Date: Test Code/ID: 17 Jul-19 18:38 (p 1 of 1) 191292b / 06-2576-8471

7-d Survi	val and R	eprodu	ction Te	est					-	- N	autilus Envi	ironmenta
15-7117-6	767	Enc	point:	6d Survi	val Rat	te		CETI	S Version:	CETISV	1.9.4	
17 Jul-19	18:38	Ama	lysis:	Single 2	2 Con	dingency Tab	ole .	Statu	s Level:	1		
18-6640-7	080	Tes	t Type:	Reprodu	ction-8	Survival (7d)		Anah	vst: Kar	ria Lywe		
27 Jun-19	13:00	Pro	tocol:	EC/EPS	1/RM/	21	1 3	Dilue	ent:			
03 Jul-19	14:00	Spe	cles:	Ceriodas	ihnia d	lubia		Brine	E			
6d 1h		Tax	om:	Branchic	poda			Sour	ce: In-H	louse Cultu	re	Age: <24
08-3720-6	3412	Coc	Se:	31E6C9	5C			Prois	et:	10		
: 25 Jun-19	00:10	Mat	orial:	Water S	ample					eau Veritas	Laboratorie	s
: 27 Jun-19	12:05	CAS	(PC):		1			Stati	on: VY	7819-1645-1	188	
61h (2.7	(C)	Clie	int:	Bureau '	/eritas	Laboratories			11			
rm	A	It Hyp			11 4			Comparis	on Result			
d	С	> T								/ival rate		
Test												
vs Gr	oup		Test 5	Stat P-T	voe:	P.Value	Decision	(a=5%)				
trol 10	0		1.000			1.0000		1				
ry												
Cor	de N	R	R	NR	+ R	Prop NR						
N							Pron R	% Effort				
179	9		1	10		0.9	Prop R 0.1	%Effect 10.0%				
N	9		1									
Rate Detail	_			10		0.9	0.1	10.0%				
	1			10 10		0.9	0.1	10.0%	Rep 7	Ren 8	Rep 9	Ren 10
Rate Detail	de R	0	0	10 10		0.9	0.1	10.0%	Rep 7	Rep 8	Rep 9	Rep 10
Rate Detail	de R	0 tep 1	0 Rep 2	10 10 2 Rep 0 1.0	o 3	0.9 1 Rep 4	0.1 0	10.0% 0.0% Rep 6				-
Rate Detail	de R	ep 1	0 Rep 2	10 10 2 Rep 0 1.0	3 000	0.9 1 Rep 4	0.1 0 Rep 5	10.0% 0.0% Rep 6 0.0000	1.0000	1.0000	1.0000	1.0000
Rate Detail Cor N	de R	ep 1	Rep 2	10 10 2 Rep 0 1.0 0 1.0	3 000 000	0.9 1 Rep 4 1.0000 1.0000	0.1 0 Rep 5 1.0000 1.0000	10.0% 0.0% Rep 6 0.0000 1.0000	1.0000	1.0000	1.0000	1.0000
Rate Detail Cor N Rate Binor	de R	ep 1 .0000	0 Rep 2	10 10 2 Rep 0 1.0 0 1.0	3 000 000	0.9 1 Rep 4	0.1 0 Rep 5	10.0% 0.0% Rep 6 0.0000	1.0000	1.0000	1.0000	1.0000
	15-7117-4 17 Jul-19 18-6640-; 27 Jun-19 6d 1h 08-3720-4 25 Jun-19 61h (2.7 ' rm d Test vs Gr trol 10 ry Coe	15-7117-9767 17 Jul-19 18:38  18-6640-7080 27 Jun-19 13:00 03 Jul-19 14:00 6d 1h  08-3720-8412 25 Jun-19 00:10 27 Jun-19 12:05 61h (2.7 °C)  rm A d C  Test vs Group trol 100 ry  Code N	15-7117-9767 End 17 Jul-19 18:38 Ana  18-6640-7080 Tes 27 Jun-19 13:00 Pro 03 Jul-19 14:00 Spe 6d 1h Tax  08-3720-8412 Cod 25 Jun-19 00:10 Mat 27 Jun-19 12:05 CAS 61h (2.7 °C) Clie rm Alt Hyp d C > T  Test vs Group trol 100	15-7117-9767 Endpoint: 17 Jul-19 18:38 Analysis:  18-6640-7080 Test Type: 27 Jun-19 13:00 Protocol: 03 Jul-19 14:00 Species: 6d 1h Taxon:  08-3720-8412 Code: 25 Jun-19 00:10 Material: 27 Jun-19 12:05 CAS (PC): 61h (2.7 °C) Client:  rm Alt Hyp d C > T  Test vs Group Test 5 trol 100 1.000	17 Jul-19 18:38 Analysis: Single 2s  18-6640-7080 Test Type: Reproduct 27 Jun-19 13:00 Protocol: EC/EPS 03 Jul-19 14:00 Species: Ceriodap 6d 1h Taxon: Branchio  08-3720-8412 Code: 31E6C98 25 Jun-19 00:10 Material: Water Se 27 Jun-19 12:05 CAS (PC): 61h (2.7 °C) Client: Bureau \  Test  VS Group Test Stat P-T  trol 100 1.0000 Exa	15-7117-9767 Endpoint: 6d Survival Rat 17 Jul-19 18:38 Analysis: Single 2x2 Con 18-6640-7080 Test Type: Reproduction-3 27 Jun-19 13:00 Protocol: EC/EPS 1/RW 03 Jul-19 14:00 Species: Ceriodaphnia d 6d 1h Taxon: Branchiopoda  08-3720-8412 Code: 31E6C95C 25 Jun-19 00:10 Material: Water Sample 27 Jun-19 12:05 CAS (PC): 61h (2.7 °C) Client: Bureau Veritas  rm Alt Hyp d C > T  Test  vs Group Test Stat P-Type trol 100 1.0000 Exact	15-7117-9767 Endpoint: 6d Survival Rate 17 Jul-19 18:38 Analysis: Single 2x2 Contingency Tat 18-6640-7080 Test Type: Reproduction-Survival (7d) 27 Jun-19 13:00 Protocol: EC/EPS 1/RM/21 03 Jul-19 14:00 Species: Ceriodaphnia dubia 6d 1h Taxon: Branchiopoda  08-3720-8412 Code: 31E6C95C 25 Jun-19 00:10 Material: Water Sample 27 Jun-19 12:05 CAS (PC): 61h (2.7 °C) Client: Bureau Veritas Laboratories  m Alt Hyp  d C > T  Test  vs Group Test Stat P-Type P-Value frel 100 1.0000 Exact 1.0000	15-7117-9767	15-7117-9767	15-7117-9767	15-7117-9767	15-7117-9767

Graphics



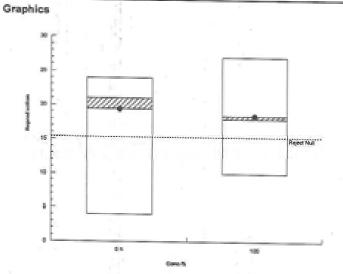
Analyst:\_\_\_\_ QA: July (8)

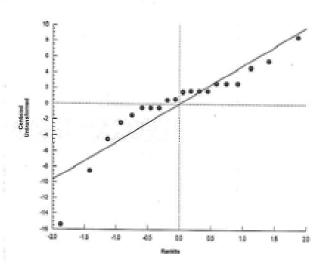
000-184-496-8

CETIS™ v1.9.4.11

Report Date: Test Code/ID: 11 Jul-19 17:26 (p 1 of 1) 191292b / 06-2576-8471

		A December	1 -								
Ceriodaphnia	a 7-d Survival as	na meprodu	ction Tes						Na	utilus Env	ironmenta
Analysis ID:	14-5656-3824		ipoint: F	Reproduction			CET	IS Version:	CETISv1	.9.4	
Analyzed:	11 Jul-19 17:26	Ana	ilysis: F	arametric-Tw	o Sample		State	us Level:	1		
Batch ID:	18-6640-7080	Tes	t Type: F	Reproduction-S	Survival (7d)		Anal	vst: Kan	ia Lywe		
Start Date:	27 Jun-19 13:0			C/EPS 1/RM/			Dilu	The second secon	,		
Ending Date:	03 Jul-19 14:00	Spe	cles: C	Seriodaphnia d	lubia		Brin				
Test Length:	6d 1h	Tax	on: E	Branchiopoda			Sour	X 5.	louse Cultur	e	Age: <2
Sample ID:	08-3720-8412	Coo	ie: 3	1E6C95C			Proj	ect			
Sample Date:	: 25 Jun-19 00:1	0 Mat	erial: V	Nater Sample			Sour		sau Veritas	Laboratoria	et.
Receipt Date:	: 27 Jun-19 12:0	5 CAS	3 (PC):	1 2			Stati		819-1645-1		10
Sample Age:		Clie		Bureau Veritas	Laboratorie	s	Stati	on. vii	018-1045-1	ОВ	
Data Transfor	em	Alt Hyp	1				Comparis	son Result			PMSD
Untransformed	d	C>T	1					sed reprodu	iction		20.92%
Equal Variance	ce t Two-Sampl	e Test									
			T1 01								
			Test St	at Critical	MSD DF	P-Type	P-Value	Decision(	a:5%)		
	vs Conc-%			4.754		0.04	*****	41 44 4			
Negative Cont	trol 100		0.3846	1.734		CDF	0.3525	Non-Signi	ficant Effect	t	
Negative Cont	trol 100			1.734		CDF	0.3525	Non-Signit	ficant Effect		
Negative Cont ANOVA Table	trol 100					CDF F Stat	0.3525 P-Value			,,,,	
	trol 100		0.3846		4.058 18		P-Value	Decision(	(a:5%)		
Negative Cont ANOVA Table Source Between Error	trol 100 B Sum Squ		0.3846 Mean S	quare	4.058 18 DF	F Stat	Linna	Decision(			
Negative Cont ANOVA Table Source Between Error	trol 100  Sum Squ 4.05		0.3846 Mean S 4.05	quare	4.058 18 DF	F Stat	P-Value	Decision(	(a:5%)		-
Negative Cont ANOVA Table Source Between Error Total	Sum Squ 4.05 492.9 496.95		0.3846 Mean S 4.05	quare	4.058 18 DF 1 18	F Stat	P-Value	Decision(	(a:5%)		
Negative Cont ANOVA Table Source Between Error Total Distributional	Sum Squ 4.05 492.9 496.95		0.3846 Mean S 4.05	quare	4.058 18 DF 1 18	F Stat 0.1479	P-Value 0.7051	Decision( Non-Signit	(α:5%) ficant Effect		
Negative Cont ANOVA Table Source Between Error Total Distributional	Sum Squ 4.05 492.9 496.95		0.3846 Mean S 4.05 27.3833	quare	4.058 18  DF 1 18 19  Test Stat	F Stat 0.1479 Critical	P-Value 0.7051	Decision( Non-Signit	(a:5%) ficant Effect		11
Negative Cont ANOVA Table Source Between Error Total Distributional Attribute Variances	Sum Squ 4.05 492.9 496.95 Il Tests Test Variance	ares	Mean S 4.05 27.3833	quare	4.058 18 DF 1 18 19	F Stat 0.1479	P-Value 0.7051	Decision( Non-Signit	(a:5%) ficant Effect (a:1%)		
Negative Cont ANOVA Table Source Between Error Total Distributional Attribute Variances Distribution	Sum Squ 4.05 492.9 496.95 Il Tests Test Variance Shapiro-V	rares	Mean S 4.05 27.3833	quare	4.058 18  DF 1 18 19  Test Stat 1.458	F Stat 0.1479 Critical 6.541	P-Value 0.7051 P-Value 0.5831	Decision( Non-Signit	(a:5%) ficant Effect (a:1%)		
Negative Cont ANOVA Table Source Between Error Total Distributional Attribute Variances Distribution	Sum Squ 4.05 492.9 496.95 Il Tests Test Variance Shapiro-V	rares	Mean S 4.05 27.3833	quare	4.058 18  DF 1 18 19  Test Stat 1.458 0.8705	F Stat 0.1479 Critical 6.541 0.866	P-Value 0.7051 P-Value 0.5831 0.0120	Decision( Non-Signit Decision( Equal Vari Normal Di	(a:5%) ficant Effect a:1%) iances stribution		0/555-1
Negative Cont ANOVA Table Source Between Error Total Distributional Attribute Variances Distribution Reproduction Conc-%	Sum Squ 4.05 492.9 496.95 A Tests Test Variance Shapiro-V	Ratio F Tesi Vilk W Norm	0.3846  Mean S 4.05 27.3833	quare	4.058 18  DF 1 18 19  Test Stat 1.458 0.8705	F Stat 0.1479 Critical 6.541 0.866	P-Value 0.7051 P-Value 0.5831 0.0120 Min	Decision( Non-Signit  Decision( Equal Vari Normal Di	(a:5%) ficant Effect a:1%) iances stribution Std Err	CV%	%Effect
Negative Cont ANOVA Table Source Between Error Total Distributional Attribute Variances Distribution Reproduction Conc-%	Sum Squ 4.05 492.9 496.95  I Tests Test Variance Shapiro-V n Summary Code	Ratio F Tesi Vilk W Norm	Mean S 4.05 27.3833	quare	4.058 18  DF 1 18 19  Test Stat 1.458 0.8705	F Stat 0.1479 Critical 6.541 0.866	P-Value 0.7051 P-Value 0.5831 0.0120	Decision( Non-Signit Decision( Equal Vari Normal Di	(a:5%) ficant Effect a:1%) iances stribution		%Effect 0.00% 4.64%
Negative Cont ANOVA Table Source Between Error Total Distributional Attribute Variances Distribution Reproduction Conc-% 0 100	Sum Squ 4.05 492.9 496.95  I Tests Test Variance Shapiro-V n Summary Code N	Ratio F Tesi Vilk W Norm Count	0.3846  Mean S 4.05 27.3833	95% LCL 15.32	4.058 18  DF 1 18 19  Test Stat 1.458 0.8705	F Stat 0.1479 Critical 6.541 0.866 Median 21	P-Value 0.7051 P-Value 0.5831 0.0120 Min	Decision( Non-Signit Decision( Equal Vari Normal Di	(a:5%) ficant Effect (a:1%) lances stribution  Std Err 1.802	CV%	0.00%
Negative Cont ANOVA Table Source Between Error Total Distributional Attribute Variances Distribution Reproduction Conc-% 0 100	Sum Squ 4.05 492.9 496.95  I Tests Test Variance Shapiro-V n Summary Code N	Ratio F Tesi Vilk W Norm Count	0.3846  Mean S 4.05 27.3833  Mean 19.4 18.5	95% LCL 15.32 15.12	4.058 18  DF 1 18 19  Test Stat 1.458 0.8705  95% UCL 23.48 21.88	F Stat 0.1479 Critical 6.541 0.866 Median 21 18	P-Value 0.7051 P-Value 0.5831 0.0120 Min 4	Decision( Non-Signit Decision( Equal Vari Normal Di Max 24 27	(a:5%) ficant Effect (a:1%) iances stribution  Std Err 1.802 1.493	CV% 29.38% 25.51%	0.00% 4.64%
Negative Cont ANOVA Table Source Between	Sum Squ 4.05 492.9 496.95 Il Tests Test Variance Shapiro-V n Summary Code N	Ratio F Tesi Vilk W Norm Count 10	0.3846  Mean S 4.05 27.3833	95% LCL 15.32	4.058 18  DF 1 18 19  Test Stat 1.458 0.8705	F Stat 0.1479 Critical 6.541 0.866 Median 21	P-Value 0.7051 P-Value 0.5831 0.0120 Min	Decision( Non-Signit Decision( Equal Vari Normal Di	(a:5%) ficant Effect (a:1%) lances stribution  Std Err 1.802	CV%	0.00%





analyse u on July (7/19

# Chronic Freshwater Toxicity Test Initial and Final Water Quality Measurements

(%V/V)								1001.01	pecies:	UGI IUUK	aprimina u	UUND		
							De	iys						
Concentration	0		1		2		3		4		5		6	7
CONTROL	init.	old	new.	old	new	old	new	old	new	old	new	old	mew	final
Temperature (°C)	24.0	25-0	24-5		240	1670	24,0	257.0	WA	अहर	245	25-9	1	
DO (mg/L)	8-0	6-8	8.7-	74	22	子光	Li	7.0	8-1	5.5	8.2	60		
pH	8.1	4.0	8.3	78	1-30	3/9	5,2	9.3	8-1	7.3	8.4	7.3		
Cond. (µS/cm)	015	2	15	1	145	2	46	2	l'S	2	17	21	.5	/
Initials	SIK	J	3	1		1		3	VV.		MUT	3	B	1
100%						_	Di	ry's			į.			
Concentration	0		1		2		3		4		5		6	7
456 m	init.	old	лем	old	new	old	new	old	new	old	Terrano Sa	old	Artes Pourse	ALCOHOL: NAME OF THE PARTY OF T
Temperature (*C)	25.0	25-0	240	260.0	1H D	250	140	245	340	35%	new 24.0	25.0	new	final
DO (mg/L)	82	7.2	S-1	33	20	7.4	in	6.9	80	6.7	8.3	6.0	1	
pH	7.2	7.4	7.6	7.3				7.5	7.1	7.5	7.2	7-0	<u> </u>	
Cond. (µS/cm)	500	-	00	49			-		491	-	1	1.	97	1
Initials	22K		90			49	1		W		97			1
initials	031		00		0	0-			NO.		MET	U	3	- 1
\							Da	rys						
Concentration	0		1		2		3		4		5		6	7
18.5	init.	old	new	old	DOW	old	DOW	old	new	old	new	old	new	final
Temperature (*C)	-	_										- William	1000000	THE STATE OF THE S
DO (mg/L)		-					-							
pH			-	Carried States										
Cond. (µS/cm)					No. of Concession, Name of Street, or other Persons, Name of Street, or ot								-	
Initials						1								
							Salar Sa	_						
Consessation	Tes.							ys \	The same of the sa					
Concentration	0	WORTHWAT - THE	ESC. 28640 No.	- Office -	2	W1000000	3		4		5		6	7
100	init.	old	new	old	new	old	new	old	new	òld	new	old	new	final
Temperature (°C)											1			
DO (mg/L)												Salar Sa		
pH													Salar Black Concession	
Cond. (µS/cm)														The same of the last
Initials														
rermometer: 4	DO mete	niprobe;		_	pH mete	n'probe:			Conduct	dvity met	len/probe	: 1	1.1	
	Cor	ntrol	· in	04.			1		1	Analys	tar	LI	JR.	MUN
Hardness*			158				_	1	t	AllalyS	is,	5614	-0)	<del>two</del>
Alkalinity*	- 4	No	3		_				1	Davie	wed by:	7	W.	Mile!
ng/L as CaCO3									ı o	Date rev		- A	uly (=	Mia

#### Chronic Freshwater Toxicity Test C. dubia Reproduction Data

Clie	nt:		5	311	yau	LV(	wit	as				-									S	tart D	ate &	Time:	ブ	M 2	7/1	10	13	100h			-	
Sam Wor	ple IL k Ord	er:	1788	100	1917	192						-				CW	10)				3	toh D	Set u	ip by:	- VL	ISSK	136	7			10			· M ·
2000	Con	confi	ration	VI -		1.0	(entro)	de la	1000		31015	Con	ceintra	tion:			1-5	6	00 2	_			Conc	ontra	lion:		3	-12					and the second	10
Days 1	A	В			-		-	H	1	1	Init.	A DESCRIPTION	8	0	0	E	-	G	H'	1	1	Init	A	B	С	0	E	F	G	н	1	*	Init	
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3	3	3	1	2	3	13.	13	4	3	3	1	1	1	4	1	3	3	3	3	3	3_	100		-					1		-	_	-	
4	7	6	13	5	1	30	V	1	17	4	200		4	5	4	8	V	5	5	v	12	CVC	-		-			1		-				
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ıe	wed b	y:	-	-																		Date	. 10410											
	er 2.2 lie	sound 5	lop. 13,	2015																1										9.		territory)	Contrionne	antid Com

### **CETIS Summary Report**

Report Date: Test Code/ID: 11 Jul-19 17:22 (p 1 of 1) 191292a / 02-8345-8739

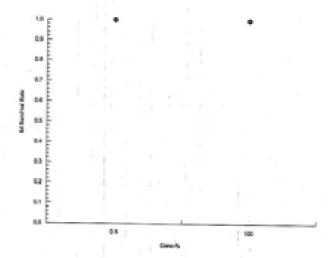
							1650	Code/ID.		191292810	Z-0343-0139
Ceriodaphnia	a 7-d Survival and	d Reprodu	uction To	est							ironmental
Batch ID: Start Date: Ending Date: Test Length:	tanom			Reproduction-S EC/EPS 1/RM/ Ceriodaphnia d Branchiopoda	Anal Dilu Brin Sou	ent ie:	nia Lywe -House Cultu	re	Age: <24		
	09-8732-8648 : 25 Jun-19 00:18 : 27 Jun-19 12:05 61h (3.6 °C)	Ma	de: terial: S (PC): ent:	3AD97088 Water Sample Bureau Veritas	Laboratorie	s	Proj Sour Stati	rce: Bu	reau Veritas (7818-1645-1		\$
Analysis ID	erison Summary Endpoint 6d Survival Rate			parison Method			P-Value		ison Result		s
	Reproduction	1100		Variance t Two-	Sample Tes	đ	0.9285		issed 6d sun issed reprodi		1
6d Survival R Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	. NEWs.
100	N	10	1,000	0 1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	%Effect 0.00% 0.00%
Reproduction Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0 100	N	10 10	17.5 20.1	15.53 16.81	19.47 23.39	13 15	21 29	0.8724	2.759 4.606	15.76%	0.00%
6d Survival R											
Conc-%	Code	Rep 1	Rep 2		Rep 4 1,0000	Rep 5	Rep 6 1.0000	Rep 7	Rep 8	Rep 9	Rep 10
100		1.0000	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Reproduction											
Conc-%	Code	Rep 1	Rep 2		Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
	N	19	17	19	16	19	20	18	21	13	13
100		29	26	18	21	23	17	16	15	17	19
	ate Binomials										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
J	N	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					10.0	100 11	10.1

malyat in as July 17/19

Report Date:

11 Jul-19 17:22 (p 1 of 1)

					1937		1111	Test	Code/ID:		191292a / 0	2-8345-8739
Ceriodaphnia 7-d	Survival an	d Reprodu	ction Te	est	7171	1			1	N	lautilus Em	rironmental
,	7458-4694 Jul-19 17:22		lpoint: lysis:		Survival Ra de 2x2 Cor	te ntingency Ta	ble		IS Version us Level:	CETISV	1.9.4	
State of the same of the same		Pro	t Type: tocol: cies:	EC/I	roduction-S EPS 1/RM/ odaphnia c			Ana Dilu Brin Sou	ent: e:	nia Lywe	ine	Age: <24
Sample ID: 09- Sample Date: 25 Receipt Date: 27 Sample Age: 61h	Jun-19 12:05		erial: 3 (PC):	Wat	97088 er Sample eau Veritas	Laboratorie	5	Proj Sou Stat	roe: Bu	reau Veritas 7818-1645-	Laboratorie	
Data Transform	3	Alt Hyp		p-				Comparis	son Result			
Untransformed		C>T		.10	19.50				sed 6d sur			
Fisher Exact Test Control vs Negative Control	Group 100		Test 5		P-Type Exact	P-Value	Decision					
Data Summary			Lanna	v	CARCI	1.0000	Non-Sign	ificant Effect				
Conc-%	Code	NR	R		NR + R	Prop NR	Prop R	%Effect				
0 100	N	10 10	0		10 10	1	0	0.0%				
6d Survival Rate I	Detail											
Conc-%	Code	Rep 1	Rep 2		Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
100	N	1.0000	1.0000		1.0000 1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
6d Survival Rate B	Binomials									201230		k
Conc-%	Code	Rep 1	Rep 2		Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1/1	1/1		1/1	1/1	1/1	1/1	1/1	1/1 ·	1/1	1/1
100		1/1	1/1		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Graphies	. 11											25.0

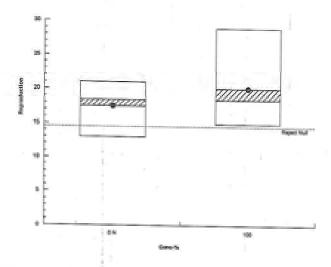


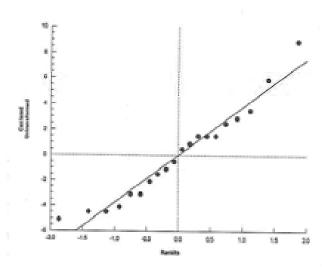
Analyst: 🛰

Report Date: Test Code/ID: 11 Jul-19 17:22 (p 1 of 1) 191292a / 02-8345-8739

							Tes	t Code/ID:		191292a / (	12-8345-87
Ceriodaphnia	7-d Survival a	and Repr	oduction T	est					. N	autilus Em	vironmenta
Analysis ID:	08-0577-6558		Endpoint:	Reproduction			CET	1S Version	n: CETISy	194	
Analyzed:	11 Jul-19 17:2	2	Analysis:	Parametric-Tw	o Sample			us Level:	1	130.41	
Batch ID:	15-1560-5987		Test Type:	Reproduction-	Survival (7d)		Ann	lyst: K	ania Lywe		
Start Date:	27 Jun-19 13:		Protocol:	EC/EPS 1/RM				ent:	ariia Lywe		
Ending Date:	03 Jul-19 14:0		Species:	Ceriodaphnia			Brin				
Test Length:	6d 1h		Taxon:	Branchiopoda					-House Cultu	IN-	Age: <2
Sample ID:	09-8732-8648		Code:	3AD97088					- I News Count		Age. 16
	25 Jun-19 00:		Material:	Water Sample			Proj		70.00		
	27 Jun-19 12:		CAS (PC):	Travel Galilpie					ureau Veritas		es
Sample Age:			Client:	Bureau Veritas	Laboratorio		Stat	ion: V	Y7818-1645-	18	
Data Transfor				OCIONO VELICAS	Laboratorie			100			
Untransformed		Alt H						son Resul			PMSD
viilualisioimed		C≯T					100% par	ssed repro	duction		16.82%
Equal Variance	e t Two-Samp	le Test	49								
Control	rs Conc-1	6	Test:	Stat Critical	MSD DI	F P-Type	P-Value	Daniela	n(a:5%)		
Vegative Contr	ol 100		-1.53			CDF	0.9285		nificant Effec		
ANOVA Table						001	0.0200	Invitroity	IIIIGant Enet	a .	
	411										
Source	Sum Sq	uares	Mean	Square	DF	F Stat	P-Value	Decisio	n(a:5%)		
Between	33.8		33.8		1	2.345	0.1430		nificant Effec	4	
Error	259.4		14,41	11	18						
Total	293.2				19	_					
Distributional	Tests										
Attribute	Test				Test Stat	Critical	P-Value	Decisio	-1400		
/ariances	Variance	Ratio F T	Test		2.787	6.541	0.1428	Equal V	and a second		
Distribution	Shapiro-	WIK W N	ormality Tes	st	0.9519	0.866	0.3961		Distribution		
Reproduction	Summary						V.0001	rvonnan	Distribution		
Conc-%	Code	Count	Mean	0.000 1.00	APRIL TOWN						
)	N	10	17.5	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
100		10	20.1	15.53	19.47	18.5	13	21	0.8724	15.76%	0.00%
	P	10	20.1	16.81	23.39	18.5	15	29	1.456	22.91%	-14.86%
Reproduction											
one-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
L	N	19	17	19	16	19	20	18	21	13	13
00		29	26	18	21	23	17	16	15	17	19
Braphics									190	- 12	10







Jigh Analyst: QA: Fully



**APPENDIX C** – *Oncorhynchus mykiss* Toxicity Test Data

## Rainbow Trout Early Life Stage Summary Sheet

Client:	Bureau Varitas	Start Date/Time	: Jule 27, 2019@1245h
Work Order No.:	191293	Test Species:	Oncorhynchus mykiss
Sample Informati	on:		,
Sample ID: V Sample Date: Date Received: Sample Volume:	7018-1645-18 JUNE 25, 2019 JUNE 27, 2019 TYNOL		
Dilution Water:			
Type: Hardness (mg/L C Alkalinity (mg/L Ca	40	Vater	
Test Organism In	formation:		
Batch No.: Source: Loading Density:	562619 Trout Lodge, Smile, W C.66 gil	Pr.	
Number of male be Number of female Sperm motility che	broodstock used: 9	tility using a com	pound microscope
SDS Reference T	oxicant Results:		
Reference Toxical Stock Solution ID: Date Initiated: 7-d EC50 (95% CI	19501 Jun 27, 2019	کارت ۶۵۶	
Reference Toxicar Reference Toxicar	nt Mean and Range: 3.8 (2.1)	3-60) mgi	C 303
Test Results:	Entropo viastrity (124) (mesn ± 230) (con- EC25 % (w/v) (95%-CL) (Con- EC50-26 (W/v) (95% CL)	hol Wit	ample ID 818-164518 5 = 5.0
Reviewed by:	John	Date rev	viewed:

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

lient:			remite			-						2019		2454
ample ID: VY		- 164	15-15	ò		_	Sto		& Time:		44,	2019	@11	154
Vork Order #:	191	283							CER#:					
								Test Sp	secles:	Oncort	lynchus	mykiss		
Control						Days								
Concentration	0		1		2		3		4	5		6		7.
(4 4 W)	init.	DOM	old	new	old	new	old	new	old	new	old	DOW	old	fina
Temperature (°C)	142	140	125	140	135	140	13.5	14.0	135	14.0	35	140	135	140
DO (mg/L)	19.2	122	10.3	10,2	25	101	10.1	10.0	10,2	10.1	10.2	10.2	101	101
pH	6.9	7.0	6.9	69	71	6.9	67	63	68	7.0	7.0	7.1	15	71
Cond. (µS/cm)	33	28	)	25	3	2	8		26	2	B	26	3	29
Initials	0-									w	W	W_	ww	
	_													
100	100000000000000000000000000000000000000					_		iys						
Concentration	0		1		2	2	3		4		5		6	7
	init	new	old	new	old	new	old	new	old	new	old	now	old	final
Temperature (°C)	13,5	14.0	135		13-5	140	13.5	140	135	140	135	CYD	135	140
DO (mg/L)	10.3	121	10.2	10-1	9.8	10,0	1,01	98	10.1	9.8	10.1	10.0	10-1	(22
pH	6.9	7.2	7.4	7.0	16	7.0	3.5	69	75	6.6	1.3	6.6	7.3	74
Cond. (µS/cm)	480 49		90 ·	44	65	4	87	48		48		44	96	184
Initials	0	W	~	Uw	<u> </u>		14-	la.	-	W	~	W	W	
							Da	ıys						
Concentration	0 1			2		3		4		5		6	7	
	init.	menv -	old	DOW	old	new	old	new	old	mew	old	mew	old	final
Temperature (°C)	1													
DO (mg/L)														
рН														
Cond. (µS/cm)														
Initials														
		4												
							Da	IVS.						
Concentration	0		1		2		3		4		5		6	7
	init	new	old	new	old	new	old	now	old	new	old	new	old	final
Temperature (°C)	-1111-1-11		1000	E CONTRACT		11000000								
DO (mg/L)														
pH														
Cond. (µS/cm)														
Initials									5.6					-
		-	2	-		-	-	_					-	
hermometer (533)	DO mes	egiprobe:	2		pH met	eniprobe:	31	3	Conduc	tivity me	ter/prob	e: <u>3</u>	1_3_	
	Cor	ntrol	10	ool.	1				ı	A 2			×10 × 1	
Hardness*	(			8		-		_		Analys	is:	AWD	ا المر	-
Alkalinity*	8			4	1					Povin	and her	76	11	
mg/L as CaCO3			1 3	2						Reviev Date rev				16/10
				colou							AND THE SECOND	=	1	1907116
ample Description:														

## Embryo Toxicity Test Daily Mortality

Client: Burgo varitas	Start Date & Time: June 27, 2019 @1245h
Sample ID: VY 7318-1645-18	Stop Date & Time: July 4, 2019 @ 1115h
Work Order #: 191293	Test Species: Oncorhynchus mykiss

Concentration	Rep		Day of	Test	- No.	of Mo	rtaliti	es	Total	Total Undeveloped	Total No. Embryo	Total Exposed
(% VL)		1	2	3	4	5	6	7	Dead Eggs			
Control	1	0	0	0	0	11.	5	2	-3	1	-27	26
	2	-	TI		1.1	0	2	11	3	4	23	30
	3				1-1-	12	2	0	4	1	15	30
	4				1	0	1	3	4	0	26	-
100	1					5	1	3	10	2	18	30 
	2				0	1	0	0	1	8	21	-425 3
	3				12	1	.1	0	4	5	21	30
	4	1	V	1	0	0	2	2	4	5	21	30
	1											30
	2											
	3											
	4											
	1											
	2											
	3									1 - 0 1-1		
	4											
	1											
	2											
	3											
	4											
	1				200							
	2											
	3											
	4											
	1											
	2											
	3											
	4											
	1											
	2											
	3											
	4											
ech Initials		im	in	8	in	m	Vin	in	nw	ww	um	nm

Comments:			
Reviewed by:	づい	Date reviewed:	July 16/19
			0 1-1-1

Version 1,0 Issued June 28, 2006

Nautilus Environmental Company Inc.

### **CETIS Summary Report**

Report Date: Test Code/ID: 12 Jul-19 17:38 (p 1 of 1) 191293a / 00-8921-6166

							A SALESTA	NOT NOT NOT THE OWNER.			
Salmonid Em	bryo Survival an	d Develo	pment T	est					Na	utilus Envi	ronmental
Batch ID:	02-1260-0826	Te	est Type:	Development			Anal	yst: Yv	onne Lam		
Start Date:	27 Jun-19 12:45	P	rotocol:	EC/EPS 1/RM/	Diluent: Dec		echlorinated T	ap Water			
Ending Date:	04 Jul-19 11:15	S	pecies:	Oncorhynchus	mykiss		Brine	e:			
Test Length:	6d 22h	Ta	axon:	Actinopterygii			Sour	roe: Tr	out Lodge Fis	h Farm	Age:
Sample ID:	09-8732-8648	C	ode:	3AD97088			Proje	ect:			
Sample Date:	25 Jun-19 00:18	M	aterial:	Water Sample			Sour	roe: Bu	reau Veritas	Laboratorie	5
Receipt Date:	: 27 Jun-19 12:05	C	AS (PC):				Stati	on: V	7818-1645-1	8	
Sample Age:	60h (3.6 °C)	C	lient:	Bureau Veritas	Laboratorie	8					
Single Comp	arison Summary										
Analysis ID	Endpoint		Comp	parison Method			P-Value :	Compa	rison Result		5
21-1488-3114	Proportion Norm	al	Equa	Variance t Two-	Sample Tes	id.	0.1250		assed proport		-
Proportion N	ormal Summary	1 3	11				-				
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N I	4.	0.744	1 0.5877	0.9005	0.6333	0.8667	0.0492	0.0983	13.21%	0.00%
100		4	0.675	0 0.5954	0.7546	0.6000	0.7000	0.0250	0.0500	7.41%	9.28%
Proportion N	ormal Detail										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	0.7097	0.766	7 0.6333	0.8667						
100		0.6000	0.700	0 0.7000	0.7000						
Proportion N	ormal Binomials										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0 .	- N	22/31	23/30	19/30	26/30						
100		18/30	21/30	21/30	21/30						

Analyst: War QA: July 16/10

Report Date: Test Code/ID: 12 Jul-19 17:38 (p 1 of 2) 191293a / 00-8921-6166

							a ware	NAME AND ADDRESS OF THE PARTY.			
Salmonid Emi	bryo Survival a	and Develo	pment Test						Na	utilus Env	ironment
Analysis ID:	21-1488-3114	Fe	dooint: Pr	oportion Nom	mail		CETI	S Version:	CETISv1	9.4	
Analyzed:	12 Jul-19 17:3			rametric-Two				is Level:	1		
Batch ID:	02-1260-0826	Te	st Type: De	evelopment			Anal	vst: Yvo	nne Lam		
Start Date:	27 Jun-19 12:4		5/3/	DEPS 1/RM/	28		Dilu	,	chlorinated T	an Water	
	04 Jul-19 11:1:	7		ncorhynchus			Brin		ATTION IT TO SECURE	all traini	
Test Length:				tinoptervali	iii y niao		Sour		ut Lodge Fis	h Earm	Age:
									or coole Lie	HILF-BILLII	rige.
Sample ID:	09-8732-8648			D97088			Proje				
	25 Jun-19 00:1			ater Sample			Sour		eau Veritas		6
	27 Jun-19 12:0		IS (PC):				Stati	on: VY	7818-1645-1	8	
Sample Age:	60h (3.6 °C)	Gli	ient: Bu	ureau Veritas	Laboratorie	5					
Data Transfor		Alt Hyp						son Result			PMSD
Angular (Corre	cted)	C>T	2				100% pas	ised proport	tion normal		14.689
Equal Variance	e t Two-Samp	le Test		1.01			1				
Control	vs Conc-%	Ó	Test Sta	t Critical	MSD DF	P-Type	P-Value	Decision	(a:5%)		
Negative Cont	rol 100		1.273	1.943	0.125 6	CDF	0.1250	Non-Sign	ificant Effec	t	
Auxiliary Test	5										
Attribute	Test				Test Stat	Critical	P-Value	Decision	(a.5%)		
Extreme Value		Extreme Va	alue Test		1.795	2_127	0.3341	3-163-1116-16	rs Detected		
ANOVA Table	7.184			1							
Source	Sum Sq	uares	Mean Sq	quare	DF	F Stat	P-Value	Decision	(a:5%)		
Between	0.01330	52	0.013306	52	1	1.621	0.2501	Non-Sign	ificant Effec	t	
Error	0.04925	08	0.008208	35	6						
Total	0.06255	59			7	_					
Distributional	Tests					-					
Attribute	Test				Test Stat	Critical	P-Value	Decision	(m:1%)		
Variances		Ratio F Te	ef		4.947	47.47	0.2219	Equal Va	the same of the sa		
Distribution		Wilk W Non			0.9245	0.6451	0.4678		istribution		
Proportion No	ormal Summar	ν									
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CUTY	N DM-
0	N	4	0.7441	0.5877	0.9005					CV%	%Effect
100	-1	4	0.6750	0.5954	0.7546	0.7382	0.6333	0.8867	0.0492	13.21%	0.00%
				0.5954	0.7546	0.7000	0.6000	0.7000	0.0250	7.41%	9.28%
	ected) Transfe		mary								
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Macc	Std Err	CV%	%Effect
0	N	4	1.046	0.8605	1.232	1.034	0.9204	1.197	0.05843	11.17%	0.00%
100		4	0.9649	0.8813	1.048	0.9912	0.8861	0.9912	0.02627	5.45%	7.79%
Proportion No	ormal Detail										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N ·	0.7097	0.7667	0.6333	0.8667						
100		0.6000	0.7000	0.7000	0.7000						
Angular (Corr	ected) Transfe										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	1.002	1.067	0.9204							
100		0.8861	0.9912	0.9204	1.197 0.9912						
	ormal Binomia		- Service Date	- FF 18	W. 0'0' 1 K						
Conc-%	Code		B		B- 1						
0		Rep 1	Rep 2	Rep 3	Rep 4						
	N	22/31	23/30	19/30	26/30						
100		18/30	21/30	21/30	21/30						3.2
											N 0

Analyst New DA: July 16/19

Report Date: Test Code/ID: 12 Jul-19 17:38 (p 2 of 2) 191293a / 00-8921-6166

Salmonid Embryo Survival and Development Test

Nautilus Environmental

Analyzed:

Analysis ID: 21-1488-3114 12 Jul-19 17:37 Endpoint: Proportion Normal Analysis: Parametric-Two Sample

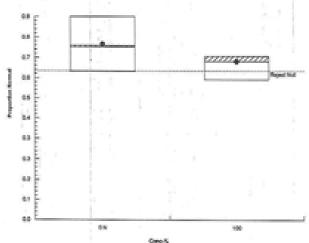
CETIS Version:

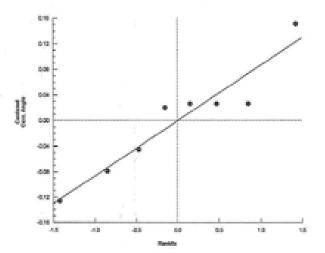
**CETISV1.9.4** 

Status Level:

1







# Rainbow Trout Early Life Stage Summary Sheet

Client:	Bureau veritas	Start Date/Time	Jun 27,2019@1245h
Work Order No.:	191293	Test Species:	Oncorhynchus mykiss
Sample Informat	ion:		
Sample ID: Sample Date: Date Received: Sample Volume:	JUNE 25,2019 JUNE 25,2019 JUNE 27, 2019 4 × 10C		
Dilution Water:			
Type: Hardness (mg/L C Alkalinity (mg/L C		Water_	
Test Organism Ir	nformation:		
Batch No.: Source: Loading Density:	Troot Lodge Summer, WA 0,66518		
Number of male b Number of female Sperm motility che	broodstock used: 4	tility using a com	pound microscope
SDS Reference T	oxicant Results:		
Reference Toxica Stock Solution iD: Date Initiated: 7-d EC50 (95% C	July 27, 2018	<u>~</u>	
Reference Toxica Reference Toxica		3-60) mg/	- 575
Test Results:	EC25 % (v/v) (95% CL)	STYV LOND	mple ID 113-1645188 1± 4.2
Reviewed by:	JOL	Date rev	iewed: July (6/19

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

Vork Order #:	300 1319 191	293				-			CER #:	3 Oncort	,			115h
0.00	1						Dr	ays						
Control Concentration	0		1		2		3		4		5		6	7
(% 4/u)	init	new	old	DOW	old	пеж	old	now	old	new	old	new	old	final
Temperature (°C)	14,5	ito	135	140	135	140	13,5	CPI	134	[40	13.5	14.0	A CONTRACTOR OF THE PARTY OF TH	140
DO (mg/L)	10.3	192	10.3	102	99	10,1	10,0	10.0	10/	121	120	102	101	122
pH	6.9	7.0	6.5	69	7.1	6.9	6.8	6.9	60	7,0	7.1	71	70	7.1
Cond. (µS/cm)	33	24		24	8	28	1	2		26		2	8	30
Initials	2	W	~	V	-	1	<b>-</b>	w	_	w	u	W	٨	mi
			_											
100							Da	lys						
Concentration	0		1		2		3	20	4		5		6	7
G2000000000000000000000000000000000000	init	now	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)	13.5	14.0	138	14.0	135	142	13,5	140	135	14.0	73.4	140	135	19,0
DO (mg/L)	103	10-1	122	10.1	29	10/2	19,1	100	10.1	9.5	1,0,1	23	(a.1	122
pH	6,9	15	7.4	7.0	7.0	70	7.5	69	7.5	6.8	1.3	6.7	73	7.4
Cond. (µS/cm)	483					40	71	490		488		५६६		4990
Initials	a m in a							W	-	V	N	W	<b>~</b>	and
	_													
								lys.				,		
Concentration	0		1	and the second	2		3	No. of Concession, Name of Street, or other Designation, Name of Street, Name	4		5	The second	6	7
Temperature (°C)	init	new	old	new	old	menv	old	new	old	new	old	D0W	old	final
DO (mg/L)	1							_	-					-
pH														+-
Cond. (µS/cm)			1		-	1				-				
Initials				<u> </u>		1								+
11-11-12														
							Do	IVS						
Concentration	0		1		2	1	3		4		5	1	6	7
	Init.	new	old	new	old	now	old	new	old	new	old	new	old	final
Temperature (°C)				CHIE		150000	T-6	CINCO.		0.000	20.000	3000		
DO (mg/L)														
pH														
Cond. (µS/cm)				1								1		
Initials										-				
hermometer:(5093)		er/probe		3	pH met	er/probe:	3_1	3	Conduc	stivity me			13	
Hardness*	1	1	4n 8	0 154		/			1	Analys		AWD	1171	
Alkalinity*	81	Sign Way	3		-					Review	wed by:	16	le	,
mg/L as CaCO3			_						1	Date re				olla

# Embryo Toxicity Test Daily Mortality

Client: Bureau Wertlas	Start Date & Time: June 27, 2019 @ 12454
Sample ID: 147819-1645-1818	Stop Date & Time: July 4, 2019 @ 11156
Work Order #: 191293	Test Species: Oncorhynchus mykiss

Concentration	Rep	Day of Test - No. of Mortalities							Total	Total	Total No.	Total
(% V/U)		1	2	3	4	5	6	7	Dead Eggs	Undeveloped	Embryo	Exposed
Contro	1	0	0	0	6)	0 3	14	3	10	2 5	18	30
	2						٥	Ó		5	243	- 2-7 30 340
	3	2.90	10/1-				0	0	1	9	23	30
	4				11	0	2	3	5	2	23	310
100	1			$\Box$		1-	2_	1 -	4	3	24	30 -
	2	11		1-1		13	0	0	3	3	23	28
	3		1			1	0	0	1	6	23	30
	4	V	P	1	14	1.1	0	2	3		26	30
	1											
	2											
	3											
	4											
	1											
	2											
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	1		<b>-</b>	$\vdash$			-					
	2			-			-	-				
	3											
	4	-		-								4
ech Initials	_	المالا	w	A-	I.A	w	te.	2.1	ww	(ML)	um	ww

Tech Initials	m m	8 W	www w	WW	(ML)	nin	ww
Comments:			-				
Reviewed by:	Joh		Date i	reviewed:	Thely	16/19	
Version 1.0 Issued Jun	e 26, 2006				Nautilion P	Environmental Co	empany inc

# **CETIS Summary Report**

12 Jul-19 17:38 (p. 1 of 1)

Test Code/ID:	1912935 / 18-4118-0101
report bate.	12 001-10 11:00 (p 1 01 1)

							3.40004	www.		or takenoon 1 15	ALTERNATION IN
Salmonid Emi	bryo Survival an	d Develop	oment T	est					Na	utilus Envi	ronmental
Batch ID:	07-2052-4791	Tes	st Type:	Development			Anal	yst: Y	vonne Lam		
Start Date:	27 Jun-19 12:45	Pro	stocol:	EC/EPS 1/RM/2	28		Diluc	ent: D	echlorinated T	ap Water	
Ending Date:	04 Jul-19 11:15	Sp	ecies:	Oncorhynchus	mykiss		Brin	e:			
Test Length:	6d 22h	Ta	con:	Actinopterygii			Sour	ce: T	rout Lodge Fis	h Farm	Age:
Sample ID:	08-3720-8412	Co	de:	31E6C95C			Proje	ect:			
Sample Date:	25 Jun-19 00:10	Ma	terial:	Water Sample			Sour	rce: B	lureau Veritas	Laboratorie	8
Receipt Date:	27 Jun-19 12:05	CA	S (PC):				Stati	on: V	Y7819-1645-1	88	
Sample Age:	61h (2.7 °C)	CII	ent:	Bureau Veritas	Laboratories	S					
Single Compa	arison Summary										
Analysis ID	Endpoint		Comp	parison Method			P-Value	Compa	rison Result		8
10-5310-7670	Proportion Norm	al	Equal	Variance t Two-	Sample Tes	4.	0.9564	100% p	passed proport	ion normal	1
Proportion No	ormal Summary				1						
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Em	Std Dev	CV%	%Effect
0	N	4	0.706	6 0.5641	0.8491	0.6000	0.7931	0.0448	0.0895	12.67%	0.00%
100		4	0.806	6 0.7389	0.8743	0.7667	0.8667	0.0213	0.0425	5.27%	-14.15%
Proportion No	ormal Detail				:						
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N ;	0.6000	0.793	1 0.6667	0.7667						
100		0.8000	0.793	1 0.7667	0.8667						
Proportion No	ormal Binomials										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	18/30	23/29	20/30	23/30						
100		24/30	23/29	23/30	26/30						

000-184-496-8

CETIS™ v1.9.4.11

Report Date: Test Code/ID: 12 Jul-19 17:37 (p 1 of 2) 191293b / 18-4118-0101

			6	4 3 4			1620	COGENID:		81283071	0-4110-01
Salmonid Em	bryo Survival a	and Develop	pment Test	1 1					Na	utilus Env	ironment
Analysis ID:	10-5310-7670	En	dpoint: P	roportion Nor	nal		CETI	S Version:	CETISv1	9.4	
Analyzed:	12 Jul-19 17:3			arametric-Two				ıs Level:	1		
Batch ID:	07-2052-4791	Tes	st Type: D	evelopment			Anal	yst: Yvo	nne Lam		
Start Date:	27 Jun-19 12:4			C/EPS 1/RM/	28		Dilu	ent: Dec	hlorinated T	ap Water	
Ending Date:	04 Jul-19 11:1:	5 Sp	ecles: 0	ncorhynchus	mykiss		Brin	e:			
Test Length:	6d 22h	Tao	xom: A	ctinopterygli			Sour	roe: Trou	ut Lodge Fis	h Farm	Age:
Sample ID:	08-3720-8412	Co	de: 3	1E6C95C			Proj	ect:			
Sample Date:	25 Jun-19 00:1	0 Ma	iterial: W	/ater Sample			Sour	roe: Bur	eau Veritas	Laboratorie	is.
Receipt Date:	27 Jun-19 12:0	25 CA	S (PC):				Stati	on: VY7	819-1645-1	88	
Sample Age:	61h (2.7 °C)	CII	ent: B	ureau Veritas	Laboratorie	8					
Data Transfo		Alt Hyp					Comparis	son Result			PMSD
Angular (Corre	ected)	C > T					100% pas	sed proport	ion normal		14.329
Equal Varians	ce t Two-Samp	le Test	1.1	1							
Control	vs Conc-%	ó	Test Sta	t Critical	MSD DF	P-Type	P-Value	Decision	(a:5%)		
Negative Cont	rol 100		-2.043	1.943	0.110 6	CDF	0.9564		ficant Effec	t	
Auxiliary Test	ts										
Attribute	Test				Test Stat	Critical	P-Value	Decision	(ec:5%)		
Extreme Value		Extreme Va	lue Test		1.559	2.127	0.7540		s Detected		
ANOVA Table				7							
Source	Sum Sq	uares	Mean Se	quare	DF	F Stat	P-Value	Decision	(a:5%)		
Between	0.02676	83	0.02676	83	1	4.172	0.0871	Non-Signi	ficant Effect	t	
Error	0.038496	58	0.00641	61	6						
Total	0.06526	51			7						
Distributional	Tests										
Attribute	Test				Test Stat	Critical	P-Value	Decision	(or-1%)		
Variances	Variance	Ratio F Tes	st		3.109	47.47	0.3765	Equal Var			
Distribution	Shapiro-	Wilk W Non	mality Test		0.9356	0.6451	0.5688		istribution		
Proportion N	ormal Summar	У									
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.7066	0.5641	0.8491	0.7167	0.6000	0.7931	0.0448	12.67%	0.00%
100		4	0.8066	0.7389	0.8743	0.7966	0.7667	0.8667	0.0213	5.27%	-14.15%
Angular (Com	rected) Transfo	rmed Sum	mary								
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effec
0	N	4	1.002	0.8449	1.158	1.011	0.8861	1.099	0.04927	9.84%	0.00%
100		4	1.117	1.028	1.206	1.103	1.067	1.197	0.02794	5.00%	-11.559
Proportion No	ormal Detail										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	0.6000	0.7931	0.6667	0.7667						
100		0.8000	0.7931	0.7667	0.8667						
Angular (Con	rected) Transfo	rmed Detai	1								
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	0.8861	1.099	0.9553	1.067						
100		1.107	1.099	1.067	1.197						
Proportion No	ormal Binomia	ls									
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	18/30	23/29	20/30	23/30						
100		24/30	23/29	23/30	26/30						
		er Trivity	00000	23/30	20/30						3.0

Analyst Nove OA July 16/19

Report Date: Test Code/ID: 12 Jul-19 17:37 (p 2 of 2) 191293b / 18-4118-0101

Salmonid Embryo Survival and Development Test

Nautilus Environmental

Analysis ID: Analyzed:

10-5310-7670 12 Jul-19 17:37

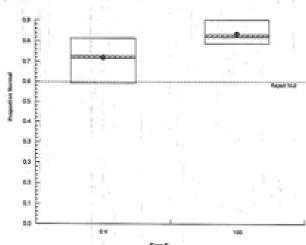
Endpoint: Proportion Normal Parametric-Two Sample **CETIS Version:** 

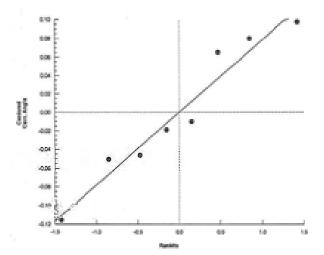
**CETISv1.9.4** 

Analysis:

Status Level:









**APPENDIX D – Chain-of-Custody Form** 

Sent To: Nautilus Environmental

8664 Commerce Court Burnaby, BC, V5A 4N7

Tel: (604) 420-8773

## CHAIN OF CUSTODY RECORD FOR SUBCONTRACTED WORK

Page 01 of 01

COC # B950328-ENAU-01-01

REP	ORT INFORMATION	ON								^	AN	ALYSIS F	EQUES	TED			1		
Cor	mpany:	Bureau Veritas Laboratories							7	(000)						Г			
Adı	dress:	9331 - 48th Street, Edmonton,	Alberta,	T68 2R4				7	1										
Con	ntact Name:	Geraldlyn Gouthro						N	Test Subc	804									
Em	all:	GGouthro@bvlabs.com, custor	nerservic	e@bvlabs.con	n			~		8									
Pho	one:	(403) 735-2230						20	95	2		ш				1			
BV I	Labs Project II:	B950328						8	8	87						Ų			
,	SAMPLE ID		MATRIX	DATE SAMPLED (YYY/MM/DD)	TIME SAMPLED (HH:MM)	SAMPLER INITIALS		PKS Algae (	Ceriodaghnia (100%) Screen	Rainbow Trout 7 Day Embrya Subcontract						Tem, o		ADDITIONAL SAN	RPLE INFORMATION
1	VY7818-1645-	18	GRAB	2019/06/25	00:18	AH	13	Х	×	X						3.6	(P: 03 <sub>4</sub> 1	12/13)	
2	VY7819-1645-	188 <sup>(1)</sup>	GRAS	2019/06/25	00:10	АН	13	Х	х	×							(P: 03.1		
3																			
4																			
5																			
6																			
7																			
8							Ų.	16	2	5.									
9								12	12	12	)				-				
10	101100000000000000000000000000000000000							Chr.	6	2									
REG	ULATORY CRITER	A		SPECIAL INSTR	00.000.000.000					S 177									TURNAROUND TIME
				Please inform I **Please return Sample	n a copy of t	his form w	ith th	e repo	rt.**										Rush Required 2019/07/30
Cust Cust Cool	ody Seal Present ody Seal Intact ing Media Present	YES NO Temp: (°C)	à	COOLER ID: Custody Seal Pre Custody Seal Inta Cooling Media Pr	nct	YES NO	Ten (°(	0.02	7	1	9	Custody	Seal Pre	ict	YES NO	Temp (°C)		, J.	Date Required  Please inform us if rush charges will be incurred.
-	INQUISHED BY-TH avid Tidman	A PRINT)	DATE: (	/25	TIME: 01 17:36	The second second	100000000000000000000000000000000000000	IVED B		N & PRIN 1-1/100					DATE: 0		Veey	12:05	
_							4												



**END OF REPORT** 



#### **RESULTS OF DAPHNIA MAGNA SINGLE CONCENTRATION-100%**

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B950328Client Project Name & Number:Quarterly Tox SNP-ASample Number:VY7819-01

**Test Result:** 

48 hrs Mortality % 0 Statistical Method:

Mean percent mortality: Sample 0 Control 0

<u>Sample Name</u>: 1645-18B Sample Matrix: Water

Description: Clear, colourless Sample Prior to Analysis:

Sample Collected: Jun 25, 2019 12:10 AM Sampling Method: N/A pH: 6.9

Sample Collected By: AH Site Collection: N/A Temperature : 20 °C

Sample Received: Jun 25, 2019 01:41 PM Volume Received: 1 L Dissolved Oxygen: 9.9 mg/L Analysis Start : Jun 27, 2019 01:38 PM Temp.Upon Arrival:  $7 \, ^{\circ}$ C Sample Conductance:  $434 \, \mu$ S/cm

End: Jun 29, 2019 02:21 PM Storage: 2-6°C Hardness: 100 mg CaCO <sub>3</sub>/L

Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hr	48 hrs	48 hrs
0	21	8.1	358	8.1	0	0	0	0	20	8.1	339	8.2
0	21	8.1	358	8.1	0	0	0	0	20	8.1	341	8.3
0	21	8.0	358	8.1	0	0	0	0	20	8.1	340	8.3
100	20	7.0	454	9.9	0	0	0	0	20	7.6	428	8.2
100	20	6.9	454	9.9	0	0	0	0	20	7.6	429	8.3
100	20	6.9	454	9.9	0	0	0	0	20	7.6	429	8.3

Concentration	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)
% vol/vol	48 hrs	48 hrs	48 hrs	48 hrs
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
100	0	0	0	0
100	0	0	0	0
100	0	0	0	0

**Comments:** None

Culture/Control/Dilution Water: City of Edmonton dechlorinated tap water

Hardness: 160 mg/L CaCO₃ Other parameters available on request.

Test Conditions Test concentration: 0,0,0,100,100,100 (% vol/vol)

Organisms per Vessel: 10 Pre-aeration Time: 30 min Rate of Pre-aeration: 25-50 mL/min/L

Total # of Organisms Used : 60 Test Temperature :  $20 \pm 2$  °C Test Hardness Adjusted : No Test Volume : 150 mL Vessel Volume : 225 mL Test pH Adjusted: No

Loading Density: 15.0 mL/Daphnia Photoperiod: 16:8 (light: dark)

<u>Test Organism :</u> Daphnia magna Source : In House Culture

Age at Test Initiation :<24 hrs</th>Average Brood Size :21.6Culture Photoperiod :16:8 (light: dark)% Mortality within 7 days :6.3Culture Temperature : $20 \pm 2$  °CTime To First Brood :8 Days

Culture Diet Pseudokirchnriella and YTC at a ratio of 2 mL/L of culture daily. New cultures weekly, 63 daphnids

distributed into 6 culture vessels and 3 reproductive vessels.



#### **RESULTS OF DAPHNIA MAGNA SINGLE CONCENTRATION-100%**

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B950328Client Project Name & Number:Quarterly Tox SNP-ASample Number:VY7819-01

Reference chemical:Sodium ChlorideTest Date:Jun 25, 2019Test Endpoint 48 hrs LC50 (95% confidence interval):5.26 (4.69, 5.91)g/LStatistical Method:Untrimmed

Spearman-Kärber

Historical Mean LC50 (warning limits): 5.99 (4.39, 8.18) g/L Concentration: 0,1.71,2.56,3.82,5.7,8.5 g/L

Test Method EPS 1/RM/14
Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Team Lead, Bioassay Date: Jul 03, 2019 04:28 PM



### **RESULTS OF RAINBOW TROUT SINGLE CONCENTRATION-100%**

Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B950328

Client Project Name & Number: Quarterly Tox SNP-A

**Test Result:** 

96 hrs Mortality % 0 Statistical Method: Visual

Sample Name: 1645-18B Sample Matrix: Water

Description: CLEAR, COLOURLESS Sample Number: VY7819-02

Sample Collected: Jun 25, 2019 12:10 AM Sampling Method: N/A Site Collection: N/A

Sample Collected By: AH Volume Received: 20 L Temp. Upon Arrival: 7 °C Storage: 2-6°C

Sample Received:Jun 25, 2019 01:41 PMpH:6.8Dissolved Oxygen:10.1 mg/LAnalysis Start :Jun 27, 2019 10:56 AMTemperature :14 °CSample Conductance: 389 μS/cm

•					•				•		•	
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
0	15	7.9	366	9.0	0	0	0	0	0	0	0	0
100	14	7.0	392	9.5	0	0	0	0	0	0	0	0

Concentration	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	72 hrs	72 hrs	72 hrs	72 hrs	96 hrs	96 hr	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs
0	0	0	0	0	15	7.8	376	8.8	0	0	0	0
100	0	0	0	0	15	7.3	412	8.7	0	0	0	0

Comments: None

Culture/Control/Dilution Water City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO₃ Other parameters available on request.

Test Conditions Test concentration: 0,100 (% vol/vol)

Organisms per Vessel : 10 Test Temperature :  $15 \pm 1$  °C Solution Depth : >15 cm

Total # of Organisms Used: 20 Pre-aeration Time: 120 min. Rate of Aeration 6.5±1 mL/min/L

Test Volume : 20 L Vessel Volume : 38L Test pH Adjusted: No

Loading Density: 0.2 g/L Photoperiod: 16:8 (light: dark)

<u>Test Organism</u>: Rainbow Trout (Oncorhynchus mykiss) Source: Spring Valley Trout Hatchery

Culture Temperature :  $15 \pm 2$  °C Weight (Mean) +- SD :  $0.5 \pm 0.1$  g Length (Mean) +- SD :  $3.83 \pm 0.19$  cm Culture Water Renewal :  $\geq 1.0$  L/min/kg fish Weight (Range) : 0.4 - 0.6 g Length (Range) : 3.50 - 4.20 cm

Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 0%

Feeding rate and frequency: daily: 1-5% biomass of trout. Acclimation Time: >14 days

Reference chemical:PhenolTest Date:Jun 20, 2019Test Endpoint 96 hrs LC50 (95% confidence interval):10.0 (9.12, 10.9)mg/LStatistical Method:ProbitHistorical Mean LC50 (warning limits):10.5 (8.73, 12.6) mg/LConcentration: 0,8,10,12,15,20 mg/L

Test Method EPS 1/RM/13
Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst : Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Team Lead, Bioassay Date: Jul 15, 2019 01:35 PM



#### **RESULTS OF DAPHNIA MAGNA SINGLE CONCENTRATION-100%**

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B979875Client Project Name & Number:Monthly (13,18,18B) SNP-ASample Number:WN6547-01

**Test Result:** 

48 hrs Mortality % 0 Statistical Method:

Mean percent mortality: Sample 0 Control 0

Sample Name: 1645-18 Sample Matrix: Grab Water

Description: Clear, colourless Sample Prior to Analysis:

Sample Collected: Sep 16, 2019 03:54 PM Sampling Method: N/A pH: 7.2
Sample Collected By: AH Site Collection: N/A Temperature: 19 °C

Sample Received: Sep 20, 2019 04:18 PM Volume Received: 1 L Dissolved Oxygen: 9.7 mg/L
Analysis Start: Sep 20, 2019 12:07 PM Temp.Upon Arrival: 13 °C Sample Conductance: 604 μS/cm

End: Sep 22, 2019 01:01 PM Storage: 2-6°C Hardness: 140 mg CaCO <sub>3</sub>/L

			,		0 -						- 0 -	5,
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hr	48 hrs	48 hrs
0	21	8.1	341	8.1	0	0	0	0	20	8.1	330	8.4
0	21	8.2	346	7.9	0	0	0	0	20	8.1	341	8.3
0	21	8.2	346	7.9	0	0	0	0	20	8.1	339	8.3
100	20	7.3	617	9.4	0	0	0	0	20	7.7	614	8.3
100	20	7.2	620	9.4	0	0	0	0	20	7.6	620	8.3
100	20	7.2	619	9.3	0	0	0	0	20	7.7	620	8.3

Concentration	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)
% vol/vol	48 hrs	48 hrs	48 hrs	48 hrs
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
100	0	0	0	0
100	0	0	0	0
100	0	0	0	0

Comments: None

Culture/Control/Dilution Water: City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO₃ Other parameters available on request.

Test Conditions Test concentration: 0,0,0,100,100,100 (% vol/vol)

Organisms per Vessel: 10 Pre-aeration Time: 30 min Rate of Pre-aeration: 25-50 mL/min/L

Total # of Organisms Used : 60 Test Temperature :  $20 \pm 2$  °C Test Hardness Adjusted : No Test Volume : 150 mL Vessel Volume : 225 mL Test pH Adjusted : No

Loading Density: 15.0 mL/Daphnia Photoperiod: 16:8 (light: dark)

<u>Test Organism :</u> Daphnia magna Source : In House Culture

Age at Test Initiation :<24 hrs</th>Average Brood Size :31.7Culture Photoperiod :16:8 (light: dark)% Mortality within 7 days :0Culture Temperature : $20 \pm 2$  °CTime To First Brood :10 Days

Culture Diet Pseudokirchnriella and YTC at a ratio of 2 mL/L of culture daily. New cultures weekly, 63 daphnids

distributed into 6 culture vessels and 3 reproductive vessels.



#### **RESULTS OF DAPHNIA MAGNA SINGLE CONCENTRATION-100%**

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B979875Client Project Name & Number:Monthly (13,18,18B) SNP-ASample Number:WN6547-01

Reference chemical:Sodium ChlorideTest Date:Sep 07, 2019Test Endpoint 48 hrs LC50 (95% confidence interval):6.69 (6.20, 7.21)g/LStatistical Method:Untrimmed

Spearman-Kärber

Historical Mean LC50 (warning limits): 6.01 (4.52, 8.00) g/L Concentration: 0,1.71,2.56,3.82,5.7,8.5 g/L

Test Method EPS 1/RM/14
Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Team Lead, Bioassay Date: Sep 26, 2019 02:43 PM



### **RESULTS OF RAINBOW TROUT SINGLE CONCENTRATION-100%**

Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B979875

Client Project Name & Number: Monthly (13,18,18B) SNP-A

Sep 16, 2019 03:54 PM

**Test Result:** 

Sample Collected:

96 hrs Mortality % 0 Statistical Method: Visual

Sample Name: 1645-18 Sample Matrix: Grab Water

Sampling Method:

Description: CLEAR, COLOURLESS Sample Number: WN6547-11

Sample Collected By: AH Volume Received: 20 L Temp.Upon Arrival: 13 °C Storage: 2-6°C

N/A

Site Collection:

N/A

Sample Received: Sep 20, 2019 04:18 PM pH: 7.0 Dissolved Oxygen: 9.4 mg/L Analysis Start: Sep 21, 2019 12:16 PM Temperature: 14 °C Sample Conductance:  $526 \mu S/cm$ 

Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
0	14	8.0	287	8.9	0	0	0	0	0	0	0	0
100	14	7.1	534	9.4	0	0	0	0	0	0	0	0

Concentration	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	72 hrs	72 hrs	72 hrs	72 hrs	96 hrs	96 hr	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs
0	0	0	0	0	14	7.8	291	8.9	0	0	0	0
100	0	0	0	0	14	7.4	547	9.0	0	0	0	0

Comments: None

**Culture/Control/Dilution Water**City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO₃ Other parameters available on request.

Test Conditions Test concentration: 0,100 (% vol/vol)

Organisms per Vessel : 10 Test Temperature :  $15 \pm 1$  °C Solution Depth : >15 cm

Total # of Organisms Used: 20 Pre-aeration Time: 30 min. Rate of Aeration 6.5±1 mL/min/L

Test Volume : 20 L Vessel Volume : 38L Test pH Adjusted: No

Loading Density: 0.2 g/L Photoperiod: 16:8 (light: dark)

<u>Test Organism</u>: Rainbow Trout (Oncorhynchus mykiss) Source: Spring Valley Trout Hatchery

Culture Temperature :  $15 \pm 2$  °C Weight (Mean) +- SD :  $0.4 \pm 0.2$  g Length (Mean) +- SD :  $3.72 \pm 0.46$  cm Culture Water Renewal :  $\geq 1.0$  L/min/kg fish Weight (Range) : 0.2 - 0.8 g Length (Range) : 3.00 - 4.50 cm

Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 0% Feeding rate and frequency: daily: 1-5% biomass of trout. Acclimation Time: >14 days

Reference chemical: Phenol Test Date: Sep 18, 2019

Test Endpoint 96 hrs LC50 (95% confidence interval) : 9.99 (9.10, 10.8)mg/L Statistical Method : Probit Historical Mean LC50 (warning limits) : 9.95 (7.10, 13.9) mg/L Concentration : 0,8,10,12,15,20 mg/L

Test Method EPS 1/RM/13
Method Deviations: None

**Note:** The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst : Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Team Lead, Bioassay Date: Oct 02, 2019 03:04 PM



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Toll Free 800 665 8566

# CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION TESTS ON: 1645-18 AND 1645-18B

SAMPLING DATE: SEPTEMBER 17, 2019

## Prepared for:

Diavik Diamond Mines Inc. PO. Box 2498  $300-5201-50^{th}$  Ave. Yellowknife, NT Canada X1A 2P8

## Prepared by:

**Ecotoxicology Group Bureau Veritas Laboratories** 

Job No.: B978381 October 2019



# Results for the *Ceriodaphnia dubia* Partial Life-Cycle Toxicity Test Collected 2019 September 17 Job B978381

Sample: 1645-18

Test		Significant Effect	
Ceriodaphnia dubia:	Survival	No	
	Reproduction	No	
Sample: 1645-18B			
Test		Significant Effect	
Ceriodaphnia dubia:	Survival	No	
•	Reproduction	No	



# Ceriodaphnia dubia Test Data Summary

Client Name/Location	Diavik Diamond Mines Inc. / Yellowknife, NT
Testing Lab/Location	Bureau Veritas Laboratories / Burnaby, BC
Collection Approach	2 samples each collected as 4 separate containers
Test Type	Static-renewal
Effluent Sample	
Sample name	1645-18 and 1645-18B
Information on labelling/coding	See Chain of Custody form
Sample collection date (y/m/d)	2019/Sep/17
Date (y/m/d)/time of sample receipt at lab	2019/Sep/17 @ 16:15
Test Organisms	
Species	Ceriodaphnia dubia
Source	In-house culture started from organisms obtained from Aquatic Research Organisms, Hampton, NH.
Age at start of test	<24 hours old; within 12 hours of each other
Unusual appearance, behaviour or treatment of test organisms, before use in test	None
Mean percent mortality of brood in 7d preceding test	The mean percent mortality was 1.45. See "Ceriodaphnia Culture Health - Mortality Records" sheet
Mean number of surviving young produced per adult in culture during 7d preceding test	The mean number of surviving young was 30.5. See "Ceriodaphnia Culture Health – Mortality and Mean Neonates"
Number of young produced in the 3 <sup>rd</sup> or subsequent brood	See "Ceriodaphnia dubia - Clearing Records"
Ephippia in the culture	No Ephippia present

Test Conditions & Facilities	
Test method	BBY2 SOP-00001 <i>Ceriodaphnia dubia</i> Chronic Survival and Reproduction Test EPS 1/RM/21, 2 <sup>nd</sup> Ed., February 2007
Dates or days when subsamples used	See "Initial Measurements and Observations" sheet
Date test started (y/m/d)	2019/Sep/19
Date (y/m/d) of test completion	2019/Sep/25
Test vessels	20 mL glass culture tubes
Persons performing test	G. Matharu, T. Wollelo, N. Shergill
Rate of preaeration	Minimal and controlled (100 bubbles/min)
Duration of preaeration	See "Initial Measurements and Observations" sheet
Rate/duration of aeration during test	No aeration
Procedure for pH adjustment	No pH adjustment of samples
Procedure for filtration	1645-18: Sample was not filtered
	1645-18B: Sample was filtered through a 60µm nytex mesh to remove native organisms on day 5 of test
Source of control/dilution water & quantity of chemicals added	Lab Control - Type I deionized water hardened to 92 mg/L CaCO <sub>3</sub> with 20% Perrier water, Vitamin B <sub>12</sub> & Selenium
Number and concentration of test solutions	100% and lab control
Volume and depth of solution in test vessels	15 mL & 9 cm
Number of replicates per conc.	10
Number of organisms per test vessel	1
DO & pH of sample just before its use	See "Initial Measurements and Observations"
Temperature, DO, & pH of test solutions and controls	See daily water quality on "Observation and Measurements"
Test solution renewal intervals	See "Initial Measurements and Observations"
Test observations and/or deviations from test method and standard practices	There was nothing unusual about the tests, no deviations from test method, and no problems with the tests.
Outliers?	1645-18: Yes, replicate 1 of the control was determined to be an outlier for reproduction; however, it was not removed from the statistical analysis.
	1645-18B: Yes, replicate 6 and 9 of the control were both determined to be outliers for reproduction; however, they were not removed from the statistical analysis.

QA	
Did the test pass the test validity criteria of:	Did Lab controls meet validity criteria?
	1645-18: Yes
	1645-18B: Yes
•Mean mortality of the control adults is ≤20%	•Mean mortality in Lab controls:
	1645-18: 0%
	1645-18B: 10%
•Average of ≥15 neonates/surviving adult in	Average live young produced/surviving adult:
the controls	1645-18: 21.3
	1645-18B: 22.3
<ul> <li>•3 broods produced by ≥60% of control organisms by the end of the 8<sup>th</sup> day.</li> </ul>	Percent of Lab control organisms producing 3 broods:
organisms by the end of the o day.	1645-18: 80%
	1645-18B: 80%
Reference toxicant test LC50 (95% CL) (mg NaCl/L) for survival	1579 (1424, 1751)
Reference toxicant test historic mean & 2SD	1826
range (mg NaCl/L) for survival	2SD (1514, 2202)
Reference toxicant test IC50 (95% CL) (mg	1305 (1220, 1408)
NaCl/L) for reproduction	
Reference toxicant test historic mean & 2SD	1454
range (mg NaCl/L) for reproduction	2SD (1110, 1904)
Invalid Reference toxicant test?	No
Date of reference toxicant test (y/m/d) and duration	2019/Sep/05 6 days
Conditions of reference toxicant test	Same as test conditions

Report Date:

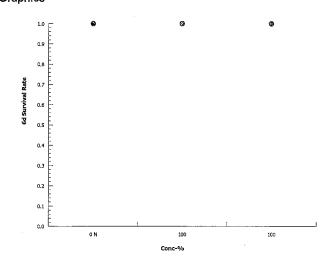
08 Oct-19 15:03 (p 1 of 1)

Test Code:

CD-4388-0119 | 05-2335-1518

							162	code.	CD-43	00-0119   00	-2333-131	
Ceriodaphnia	a 7-d Survival a	nd Reprod	uction Te	est					Burea	u Veritas La	boratories	
Analysis ID:	08-1863-5527		ndpoint:					CETIS Version: CETISv1.9.2				
Analyzed:	08 Oct-19 15	:02 <b>A</b> r	nalysis:	Single 2x2 Co	ntingency Ta	ble	Offi	cial Results	s: Yes			
Batch ID:	08-1395-2930	Te	st Type:	Reproduction-	Survival (7d)		Ana	lyst: T. \	Vollelo			
Start Date:	19 Sep-19 13:	23 <b>P</b> r	otocol:	EC/EPS 1/RM	/21		Dilu	ent: Dil	ute Perrier \	Water		
Ending Date:	25 Sep-19 10:	35 <b>S</b> p	ecies:	Ceriodaphnia	dubia		Brin	ie: Not	t Applicable			
Duration:	5d 21h	Sc	ource:	Aquatic Resea	rch Organisi	ms, NH	Age	:				
Sample ID:	03-4570-7959	Co	ode:	B978381			Clie	nt: Dia	vik Diamon	d Mines Inc		
Sample Date:	: 17 Sep-19	Ma	aterial:	Mining Discha	rge/Runoff		Proj	ect: Ge	neral Misc.	Bioassays		
Receipt Date:	: 17 Sep-19 16:	15 <b>S</b> c	ource:	Diavik			-			•		
Sample Age:	61h	St	ation:	1645-18								
Data Transfor	rm	Alt Hyp				•	Compari	son Result	-11-11-11		·	
Untransformed	d	C > T					100% pa	ssed 6d sur	vival rate			
Fisher Exact	Test								, <u></u> .			
Control	vs Group		Test S	Stat P-Type	P-Value	Decision	(α:5%)					
Negative Cont	trol 100		1.000	0 Exact	1.0000	Non-Sign	ificant Effec	t				
Data Summar	ry											
Conc-%	Code	NR	R	NR + R	Prop NR	Prop R	%Effect					
0	N	10	0	10	1	0	0.0%					
100		10	0	10	1	0	0.0%					
6d Survival R	late Detail								· ·			
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	
0	N	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	
100		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	
6d Survival R	tate Binomials						-					
		D 4	D 0	Don 2	Don 4	Don 5	Rep 6	Rep 7	Dan 0	Day 0	D 40	
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	reh o	Keb i	Rep 8	Rep 9	Rep 10	
Conc-% 0	Code N	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	

## Graphics



2019

Analyst: 100 QA: V

Report Date:

Test Code:

08 Oct-19 15:03 (p 1 of 2) CD-4388-0119 | 05-2335-1518

								1631	Couc.	OD-430	,0-0 1 10   U	7-2000-101
Ceriodaphnia	7-d Survival	and Repro	duction T	est						Bureau	Veritas La	boratorie
Analysis ID:	17-0740-891	7 <b>E</b>	indpoint:	Reproduction				CET	IS Versio	n: CETISv1	.9.2	
Analyzed:	08 Oct-19 15	5:03 A	nalysis:	Nonparametri	c-Two Sar	nple	е	Offic	ial Resul	ts: Yes		
Batch ID:	08-1395-2930	т	est Type:	Reproduction-	Survival (7	7d)		Anal	vst: T	Wollelo		
Start Date:	19 Sep-19 13		rotocol:	EC/EPS 1/RM	,	,		Dilu	•	Dilute Perrier V	Vater	
Ending Date:	•		pecies:	Ceriodaphnia	dubia			Brin		ot Applicable		
Duration:	5d 21h		ource:	Aquatic Resea		nisn	ns, NH	Age:				
Sample ID:	03-4570-7959	) (	ode:	B978381				Clie	nt: D	iavik Diamond	Mines Inc	
Sample Date:	17 Sep-19	M	laterial:	Mining Discha	rge/Runof	ff		Proj	ect: G	eneral Misc. E	Bioassays	
Receipt Date:	17 Sep-19 16	:15 <b>S</b>	ource:	Diavik				•			•	
Sample Age:	61h	s	tation:	1645-18								
Data Transfor	m	Alt Hy	р					Comparis	son Resu	it		PMSD
Untransformed		C > T						100% pas	sed repro	duction		18.78%
Wilcoxon Ran	ık Sum Two-S	ample Tes	t									
Control	vs Conc-	%	Test	Stat Critical	Ties	DF	P-Type	P-Value	Decisio	n(α:5%)		
Negative Cont	rol 100		145.5	n/a	2	18	Exact	0.9996	Non-Sig	nificant Effec	t	
Auxiliary Test	s											
Attribute	Test				Test St	tat	Critical	P-Value	Decisio	n(α:5%)		
Extreme Value	e Grubbs	Extreme V	/alue Test		3.246		2.708	0.0018	Outlier	Detected		
ANOVA Table											······································	
Source	Sum So	quares	Mean	Square	DF		F Stat	P-Value	Decisio	n(α:5%)		
Between	304.2		304.2		1		11.43	0.0033	Signific	ant Effect		
Error	479		26.61	11	18							
Total	783.2				19							
Distributional	Tests											
Attribute	Test				Test St	tat	Critical	P-Value	Decisio	n(α:1%)		
Variances	Varianc	e Ratio F T	est		4.047		6.541	0.0492	Equal \	/ariances		
Distribution	Shapiro	-Wilk W No	rmality Te	st	0.8346		0.866	0.0030	Non-No	rmal Distribut	ion	
Reproduction	Summary											
Conc-%	Code	Count	Mean	95% LCL	. 95% U	CL	Median	Min	Max	Std Err	CV%	%Effect
0	Ν.	10	21.3	16.63	25.97		22	5	28	2.066	30.67%	0.00%
100		10	29.1	26.78	31.42		28	26	35	1.027	11.16%	-36.62%
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	5	20	19	27		23	24	28	20	21	26

2019

Analyst: 1/100 QA: 14

Report Date:

08 Oct-19 15:03 (p 2 of 2)

Test Code:

CD-4388-0119 | 05-2335-1518

Ceriodaphnia 7-d Survival and Reproduction Test

**Bureau Veritas Laboratories** 

Analysis ID: Analyzed:

17-0740-8917 08 Oct-19 15:03

Analysis:

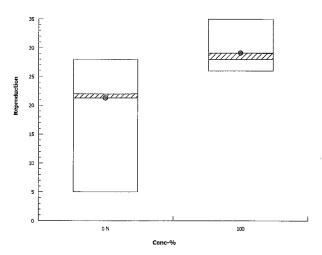
Endpoint: Reproduction

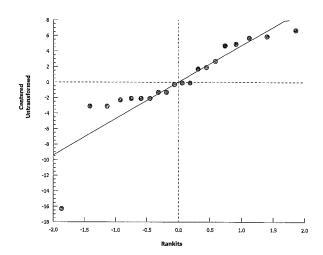
Nonparametric-Two Sample

**CETIS Version:** Official Results:

CETISv1.9.2 Yes

## Graphics





Analyst: 1000

# Ceriodaphnia dubia Survival and Reproduction Test - Initial Measurements and Observations

Max am
BBY2FCD-00149/10
Tab - Observations
Page 1 of 1

	C	Client # & Name:	8, Diavik Diamond M	lines	,	Job/Sample:	B978381/WM9244	· · · · · · · · · · · · · · · · · · ·	Page
		Sample ID:	1645-18	7	Date 8	& Time Sampled:	2019 Sep 17 @00:0	0	
	Date	e & Time Started	2019 Sep 19	@13:2	3 Date 8	& Time Received:	2019 Sep 17 @16:1	5	
			2018 Sep 2			Organism Lot #:	A80 2019	ROOR	-
			ts After Temperatur			ations - Şee BLNC		Worksheet Crea	ited: 🗌
				Initial Temp		Aeration*	Post Aeration DO	Post Aeration	
	Day	Date	Initial DO (mg/L)	(°C) 25, 3	Initial pH	(min)	(mg/L)	Temp (°C)	Analyst
	0	2019				20	8.6	24.9	<u> </u>
	1	Serso	8.6	25.7	N/A	· · · · · · · · · · · · · · · · · · ·	8.3	25.2	<u></u>
	2	30021	5.6	25,8	N/A	NO	N 9	NIE	<u></u>
	3	250477	8,8	<u> </u>	N/A	80	8.5	25.4	<i>N</i> <sub>e</sub>
	4	Sept 33		25.8	N/A	<u>\$0</u>	8,4	25.2	<u> </u>
_	5	7807 94		7b.0	N/A	20	8.8	94.5	_N2
	6				N/A	ms 2010	10C+07		
	7	<u> </u>		,	N/A		, 0		
	* Rate of	aeration must be	e ≤ 100 bubbles/min	ute Inst	rument ID's:	<u>DBY</u> .	2-5405		
		e Description	Clean L	<u>chrade</u>	کر		Initials	<u>_</u>	
	Samp	ole Hardness	16	&			Initials	CS.	
	Observat	ions during the 1	Test (e.g. aeration dura	ition, behaviour of	test organism	s, bag effluent take	en from etc.)		
	Day								Analyst Initials
	0	Date: あしん	SIS Bottle / C	arboy #:	, , , , , , , , , , , , , , , , , , ,	re - Aerated Fror	n: 117 50	-12:10	3,Th
		Feeding volume	(μL) PKS: 150 YC	T: 50	Test Seed	ed @: /3 ´	23		
	1	Date: Saz	= 2019 (Bottle))C	arboy #:	P	re - Aerated Fror	n: 11-53-	12:13	<u>م</u>
		Feeding volume	e (µL) PKS: \ 50 Y	CT: <b>5</b> >	Test Wat	er Change @:	12:40		
	2	Date: Sen	3 1 200 Bottle AC	arboy #:	Р	re - Aerated Fron	n: <b>V</b> \\	عو	ಲು
			(μL) PKS: \ 50 YC		Test Wate	er Change @:	12:34		
			V	······································	· · · · · · · · · · · · · · · · · · ·			, , , , , , , , , , , , , , , , , , , ,	
	3	Date: DOCK	630 12 B (ttl) / C	arboy #: 2	P	re - Aerated Fror	n: 1010 - 10	230	NS
			(μL) PKS: \ 🗘 YC		***************************************	er Change @: 🚺	· · · · · · · · · · · · · · · · · · ·		
					· · · · · · · · · · · · · · · · · · ·	·			
	4	Date: 2095	201 23 BEED/C	arbov #: 3	P	re - Aerated Fror	n: 0920 - C	940	NS
			(μL) PKS: <b>\5</b> Ο YC			er Change @: \\			
	5	Date: 20101	Senta 4 Bottle)/C	arbov #· H	P	re - Aerated Fron	n: 1130-11	7)	21
	Ĵ	Feeding volume		т: <b>5</b> 0		er Change @:			
		recamb volume	. (με) 1 του	<u> </u>	1CSC VVdC	criange &.	)-11		
٠	6	Date: 201/	5 Sep 25		-,,,,,,,,,		<del>, , , , , , , , , , , , , , , , , , , </del>		Thi
	Ü	1 00	t alad	@ 10:3			<del></del>		150
		1 4	- EMALEC	<u> (w) [0]</u>	( <del>)</del> ,,,	· · · · · · · · · · · · · · · · · · ·	<del>-,,,-,-,</del>		
٠	7	Date:					<del></del>	- The state of the	AND THE PROPERTY OF THE PARTY O
					۱۸	14	-0.1		
						201852	ヤイナ		
	8	Date:				<i>y.</i> • · · · · · · · · · · · · · · · · · ·	<del></del>	· · · · · · · · · · · · · · · · · · ·	
	J			<del>, , , , , , , , , , , , , , , , , , , </del>		<del>,</del>	<del>,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,</del>		<u> </u>
				<del>~~~~~~~~</del>	, <del>-</del> ,	,	· · · ·		

**ECOTOXICOLOGY** 

Ceriodaphnia dubia Survival and Reproduction Test -Observations and Measurements

Maxarm
BBYZFCD-00149/10
Tab - Test Concentrations
Page 1 of 8

(

Job/Sample # B978381/WM9244

Control

Concentration:

Date(s): 2019 Sepo9

Control Water Batch:

Analyst(s): G Dothes, Tynullels

Hardness(es):

	Reviewed by:	$[S]_{\mathcal{S}}$	ું. જ	<u> </u>	8,3	3	SSN SSN	)			
Analyst	Final	S	V	3	N	3		}		2	
Ana	Initial	S	V	S	3	2	3			R	
Hd	Final	25.3 25.3 8.3 7.8 8.3 8.3	25.225-1 8.3 8.1 83 8.3	25.1 25.58.27.9 8.3 8.3	8.3	8.2	2-8				
	Initial	\$ 3	20	8,3	400	$\infty$	8.4 8-2				
DO (mg/L)	Final	٦ م	6	6,1	25.0 24.9 8.3 8.0 8.4 8.3	180 250 251 812 8.0 8.3 8.2	7.5	)	604		
00	Initial	8.3	ام ش	8.3	8,3	8,2	25,2 25.7 8,4		9		
Temperature (°C)	Final	25.3	125-1	25.5	74.9	75.	45.5%				
Tempera	Initial	25.3	25.2	25.1	25.0	250	23.7	,			
Conductivity (µS/cm)	Initial	181	(8)	501	189	180	181	<b>&gt;</b>			
	10	0	0	0	$\mathcal{D}$	7	14			978	
	6	0	0	0	8	Q	112			12 de 8	
	8	0	0	0	2	$\omega$	IŚ		,	2	
onates)	7	0	<u>۵</u>	0	3	0	16			TX.	
Reproduction (# Live neonates)	9	0	0	0	7	1	15			74	,
duction (	5	0	0	0	7	$\infty$	13			23	
Repro	4	Q	9	0	$\frac{c}{c}$	<u></u>	7			2	
	.33	0	0	0	7	9				5	
	2	0	O	0		· N	<u> </u>			9%	
	1	C	0	0	۵	0	>			8	>
760	Cay	₩	<i>C</i> 1	æ	4	ڻ	yo .	7	∞	Total	

X = dead adult S = small adult
M = missing/lost adult P = pale adult

(#) immobilised neonates
\* dead neonates present

(B) Corrected calculation error- no 2019 oct 08

# Ceriodaphnia dubia Survival and Reproduction Test -

Observations and Measurements

Maxxam
BBY2FCD-00149/10
Tab - Test Concentrations
Page 8 of 8

100 Concentration (%v/v)

Job/Sample #:

B978381/WM9244

	Reviewed by:	ა න	v	3 I	32	32	13.	)			-
Analyst	Final	S	v	NS	SP SP	ź	-			2	
Anë	Initial	$\Im$	$\mathcal{J}$	5	5	SN	. Z	)		55	
Hd	Final	8-L	7,5	0,8	7.8	7.9	t, so				. +
d	Inițial	しし		とし	1.8	61	L, T				immobilised neonates dead neonates present
DO (mg/L)	Final	C. P. 8. L	25.2 25.2 8.2 8.0 7.7	6,7	<b>6'L</b>	7.8	7. t.		607		# immobilised neonates * dead neonates present
ı) od	Initial	م، م	8.3	5.6	٤1%	8.4	8 1	3	0 0		
ture (°C)	Final	25.5	25.2	25.7	25.1	75.7	25.4				
Temperature (°C)	Initial	24.825.5 8.6	25.2	25.4	24.7	25.2	249	ξ			
Conductivity (µS/cm)	Initial	127	2	762 25:425.75.67.97.78.0	762 24.7 25.1 8.3 7.9	754 252 8.4 7.8 7.9 7.9	754 249 254 8,5				S = small adult P = pale adult
	10	0	0	0	1	₩	17			20	In:
	6	9	0	0	$\omega$		20 17			34 20	adult ng/lost adult
	8	0	0	0	S					27	X = dead adult M = missing/lo
nates)	7	0	0	0	Ŋ		19			123	
t Live nec	9	ದಿ	9	0	60	F	17	,		7	
Reproduction (# Live neonates)	5	0	0	Ö	Ŋ	$\infty$	19			29	
Reprod	4	0	9	0	士	σ	18			3	
	3	0	0	0	0	JU	16			to	
	2	0	ଦ	0	റ	Ţ	16			9%	
	₩	0	0	Q	ゴ		` <u>`</u>			97	
)av	Ça	H	2	3	4	5	Q	7	∞	Total	

Report Date:

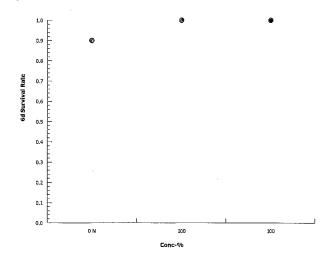
08 Oct-19 15:39 (p 1 of 1)

Test Code:

CD-4388-0219 | 05-2400-5545

									163	coue.	OD-4	000-02 19   00	7-2400-334
Ceriodaphnia	7-d Sur	vival and	d Reprod	uction Te	est						Burea	u Veritas La	boratories
Analysis ID:	00-431	6-0955	Er	ndpoint:		Survival Ra			CET	IS Versio	on: CETISV	1.9.2	
Analyzed:	08 Oct	-19 15:38	3 Ar	nalysis:	Sin	gle 2x2 Cor	ntingency Ta	ble	Offi	cial Resu	Its: Yes		
Batch ID:	15-3532	2-2097	Te	st Type:	Rep	production-S	Survival (7d)		Ana	lyst: T	. Wollelo		
Start Date:	19 Sep-	19 13:18	Pr	otocol:	EC	EPS 1/RM/	21		Dilu	ent: I	Dilute Perrier	Water	
Ending Date:	25 Sep-	19 10:40	Sp	ecies:	Cer	iodaphnia c	lubia		Brin	e: N	lot Applicable	)	
Duration:	5d 21h		Sc	ource:	Αqι	iatic Resea	rch Organisr	ms, NH	Age	:			
Sample ID:	19-7152	2-2137	Co	ode:	B97	78381			Clie	nt: D	Diavik Diamor	d Mines Inc	
Sample Date:	17 Sep-	19	Ma	ateriai:	Min	ing Dischar	ge/Runoff		Proj	ect: G	eneral Misc.	Bioassays	
Receipt Date:	: 17 Sep-	19 16:15	Sc	ource:	Dia	vik						-	
Sample Age:	61h		St	ation:	164	5-18B							
Data Transfor	rm		Alt Hyp	ı					Compari	son Resu	ılt		
Untransformed	t		C > T						100% pa	ssed 6d s	urvival rate		
Fisher Exact	Test												
Control	vs G	roup		Test S	Stat	P-Type	P-Value	Decision	(α: <b>5</b> %)				
Negative Cont	rol 1	00		1.000	0	Exact	1.0000	Non-Sign	ificant Effec	t			
Data Summar	у												
Conc-%	C	ode	NR	R		NR + R	Prop NR	Prop R	%Effect				
0	N		9	1		10	0.9	0.1	0.0%				
100			10	0		10	1	0	-11.11%				
6d Survival R	ate Deta	il											
Conc-%	C	ode	Rep 1	Rep 2	2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N		1.0000	1.000	0	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
100			1.0000	1.000	0	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
6d Survival R	ate Bino	mials					<del></del>					<del></del>	
		_		D 0	•	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
	C	ode	Rep 1	Rep 2				1 to p o					
Conc-%	Co N	ode	1/1	1/1	•	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1

## Graphics



Report Date:

Test Code:

08 Oct-19 15:39 (p 1 of 2) CD-4388-0219 | 05-2400-5545

Ceriodaphnia 7-	d Survival ar	nd Reprod	uction Te	est							Bureau	Veritas La	boratories
• •	9-6997-7886 8 Oct-19 15:3		idpoint: ialysis:		roduction parametric	-Two Sa	ımple	9		IS Version		.9.2	
Start Date: 19 Ending Date: 25	i-3532-2097 Sep-19 13:1 Sep-19 10:4 21h	8 Pr 0 <b>S</b> p	st Type: otocol: ecies: ource:	EC/E	Reproduction-Survival (7d)  EC/EPS 1/RM/21  Ceriodaphnia dubia  Aquatic Research Organisms, NH  Analyst:  Diluent:  Dilute Perrier Water  Not Applicable  Age:				Vater				
Sample ID: 19 Sample Date: 17 Receipt Date: 17 Sample Age: 61	Sep-19 16:1	<b>M</b> a	ode: aterial: ource: ation:	Diav	ng Dischar	ge/Rund	off			Client: Diavik Diamond Mines Inc Project: General Misc. Bioassays			
Data Transform		Alt Hyp							Comparis	son Resul	it	<u></u>	PMSD
Untransformed		C > T					-			sed repro			23.64%
Wilcoxon Rank	Sum Two-Sa	mple Test									<del></del>		
Control vs	Conc-%	-	Test S	Stat	Critical	Ties	DF	P-Type	P-Value	Decisio	n(α:5%)		
Negative Control	100		108		n/a	5		Exact	0.5959		nificant Effec	t	
Auxiliary Tests									-				
Attribute	Test					Test S	Stat	Critical	P-Value	Decisio	n(α:5%)		
Extreme Value	Grubbs E	Extreme Va	lue Test			3.047		2.708	0.0074		Detected		
ANOVA Table												-	
Source	Sum Squ	ıares	Mean	Squa	are	DF		F Stat	P-Value	Decisio	n(α:5%)		
Between	48.05		48.05			1		1.183	0.2910	Non-Sig	nificant Effec	t	
Error	730.9		40.60	56		18		_					
Total	778.95					19							
Distributional Te	sts												
Attribute	Test					Test S	Stat	Critical	P-Value	Decisio	n(α:1%)		
Variances	Variance	Ratio F Te	st			27.11		6.541	3.6E-05	Unequa	l Variances		
Distribution	Shapiro-V	Vilk W Nor	mality Te	st		0.796	8	0.866	7.7E-04	Non-No	rmal Distribut	ion	
Reproduction Su	ımmary												
Conc-%	Code	Count	Mean		95% LCL	95% L	JCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	10	20.9		14.57	27.23		23.5	2	29	2.799	42.34%	0.00%
100		10	24		22.78	25.22		24	21	27	0.5375	7.08%	-14.83%
Reproduction De	etail												
Conc-%	Code	Rep 1	Rep 2	!	Rep 3	Rep 4		Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	21	23		29	26		22	8	27	27	2	24
100		25	26		23	21		24	27	24	24	23	23

Report Date:

08 Oct-19 15:39 (p 2 of 2)

Test Code:

CD-4388-0219 | 05-2400-5545

**Bureau Veritas Laboratories** 

Analysis ID: Analyzed: 19-6997-7886 08 Oct-19 15:39

Endpoint: Analysis:

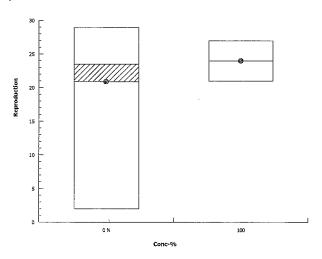
Endpoint: Reproduction

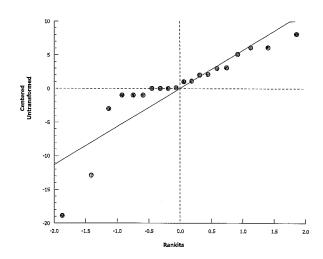
Nonparametric-Two Sample

CETIS Version: CET Official Results: Yes

CETISv1.9.2

Graphics





# Ceriodaphnia dubia Survival and Reproduction Test - Initial Measurements and Observations

Maxxam BBY2FCD-00149/10 Tab - Observations Page 1 of 1

С	lient # & Name:							
	Sample ID:	1645-188		. Date 8	& Time Sampled:	2019 Sep 17 @00:00	)	
Date	& Time Started	2019 Sep 1	9@13-	/8 Date 8	& Time Received:	2019 Sep 17 @16:15	5	
Date	& Time Ended:	2019 Sep 2	5@10:	40	Organism Lot #:	AR0 2619	Jero d	
Before Us	e Measurement	s After Temperatur	e Adjustment	Devia	ations - See BLNC		Worksheet Crea	ated: 🗆
			Initial Temp		Aeration*	Post Aeration DO	Post Aeration	
Day	Date	Initial DO (mg/L)	(°C)	Initial pld	(min)	(mg/L)	Temp (°C)	Analyst
0	20198119	10.8	26-0	7.9	20	8.7	25.7	2)
	Sen 20	8.7	256	NI/A	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	8.4	25.)	9
1	5626		~	N/A	20	1		
2	160%	5, 9	25.)	N/A	Ma	NO	NO	C)
3	56433	8,9	26.0	N/A	20,440	8,3 40	25.3	NS NS
4	50,03	0.01	95.8	N/A	90 O	8.8	25.2	777
				, , , , , ,	1	- C) 4 S-4		
5	Sept 24	9.8	25.9	N/A	50	8.9	24.6	NS.
6		***		N/A	m 2010	00100		
7				N/A		100° 00°		
		9 Scot 22 e ≤ 100 bubbles/min	ute Inst	rument ID's:	BBY	2-0408		
	e Description	11111 (ESOL) Decorated (I)	1	مريما وي			€\$	
	,	100		<del>* ,, ,</del>	<del>-5,</del>	•		
•	le Hardness	120	<del></del>		<del> </del>	_		
Observati	ions during the 1	Fest (e.g. aeration dura	tion, behaviour of	f test organism	s, bag effluent take	en from etc.)		
Day								Analyst Initials
0	Date: 2019&	619 Bottle	arbov #:	F	Pre - Aerated From	m: 11:50-12	2:10	The
•	Farding volume	e (μL) PKS: \ S ο YC	T. 50			- 1		· /- P-3
	reeding volume	(μL) PK3: <b>\ \ 3</b>	,1; , , , , , , , , , , ,	Test Seed	led @: /3^/	8		
1	Date: Sen )	- 2019 Bottle % C	arboy#: \	F	re - Aerated From	m: 11:59-	12:14	Co
		e (µL) PKS: ( 50 Y			er Change @:	12151		
	reeding volume	(μL) FN3. ( S3 1	<u> </u>	I CSL WAL	er change w.	1~2:1	,	
			<del>,</del>		<del></del>	<del></del>		T
2	Date: Se >	1,2019 (Bottie)/C	arboy #:	F	re - Aerated Fron	m: <u>√</u> 🕨		ල
		e (μL) PKS: \$55 YC		Test Wate	er Change @:	19743		
	recomb volume	(100) 1 100	<u> </u>	1000 1100			·····	
							<del></del>	By.
3	Date: 2019 S	Cot 2) Battle/C	arboy#: 2		re - Aerated Fron	m: 1010-10	930	NS
	Feeding volume	e (μL) PKS: 150 YC	T: 50	Test Wate	er Change @: 🔰	LUL		
	T CCUITS TOTAL	(1,2)	<del></del>	V	<u> </u>	) <del></del>		
		- 200 cm	<del></del>	<del></del>	<del>, , , , , , , , , , , , , , , , , , , </del>	······································	~	1.50
4	Date: 2019	rot 23 B(ttl)/C	arboy #: 乙	<u> </u>	re - Aerated Fron	m: 0920-1	<u> </u>	NE
	Feeding volume	e (μL) PKS: 150 YC	T: 50	Test Wat	er Change @: 🚺	150		
		· · · · · · · · · · · · · · · · · · ·			······································		<del></del>	
				<del></del>	<del></del>			1.30
5	Date: <b>209</b>	Ectal Bottle / C	arboy #: 💍	F	Pre - Aerated Fro	m: 1130~115	$\infty$	251
	Feeding volume	e (μL) PKS: <b>\</b>	T: CT	Test Wat	er Change @: 🚺	355		
	_			,			ماعدام	
	1	red ration	z ordo	マシンス	> 1871N	d Dim	, mostr	1
6	Date: 2018			y <del></del>	·	<u> </u>		TW
	To	st endeo	l at 10:	40	**			
		the desired of the second of t		<del></del>	**************************************		····	
			**********		<del></del>	,,,,,		
7	Date:			· · · · · · · · · · · · · · · · · · ·			-,, <u></u> -	ASSESSED AND ASSESSED AND ASSESSED AND ASSESSED AND ASSESSED ASSESSED AND ASSESSED AND ASSESSED AND ASSESSED AND ASSESSED AND ASSESSED ASSESSED AND ASSESSED ASSESSEDANCE ASSESSED ASSESSED ASSESSED ASSESSED ASSE
	1				JA			
			······································	7	010	2027		
,	·				- W 17	vy c-1		
8	Date:				<u> </u>		<del> </del>	<u>, L</u>
		,,,	<del></del>			······································		

ECOTOXICOLOGY

Ceriodaphnia dubia Survival and Reproduction Test -

Maxxam

BBY2FCD-00149/10 Tab - Test Concentrations Page 1 of 8

Observations and Measurements

Analyst(s): C Methers, T. Wollelo NSWENDIN

Control Concentration:

B978381/WM9245

Job/Sample #\_

Control Water Batch:

Hardness(es):

OW. 355.0	Reviewed by:	v.	00	z,	R R	2	J. S.				
	R Final	5	-0 -0	N. A.	2	2 2 2	12	·		٤	
Analyst	Initial	3	J		4	Z	4	<del></del>		Z	
Hd	Final	8	2 40	8,3	185 24.7 25.08.3 7.9 8.4 8.2 NS	8,3	2				
d	Initial	8.3 7.8 8.3 8.3	25,225.2 8.3 8.0 8.3 8.3	رم مه	₩. ₩.	25,2 15,2 8,3 7.8 8.4 8,3	7.5 8.4 8.2		60	Ś	#) immobilised neonates
DO (mg/L)	Final	رم رم	0,00	6,1	67	7.8	4.5		706		(#) immobil
) OO	Initial	\$\do	23	8	& &	∞ w)	183 24.8 27.8 8.1E	(	03		
Temperature (°C)	Final	25.1 25.4	25.2	25,6	25.D	15.7	35-8				
Tempera	Initial	25.)	25,2	250	24.7	25.2	24.8	) 2.			
Conductivity (µS/cm)	Initial	(8)	(%)	184 25-025,68 2 7.9 8-3 813 C	185	180	(83				S = small adult
	10	0	0	0	I	<u>ر</u> و	, 7			ho	
	6	0	0	0	7	Š	S	-		2	adult
		0	0	0	N		7			\$	X = dead adult
onates)	7	0	0	0	ユ	80	X			27	
# Live ne	و	9	D	0	ナ	<u> </u>				XX	
Reproduction (# Live neonates)	2	0	0	0	7	2	さ			2	
Repro	4	0	0	0	3	2	4			37	
	ന	0	0	0	3	80	18			29	
	2	0	9	0	2	0	300		,	23	
	H	0	0	C	S	9	<u>an</u>			76	
Dav	)	Н	2	33	. 4	Š	, e	7	∞	Total	

@www.20Aseptal

X = dead adult M = missing/lost adult

S = small adult P = pale adult

(#) immobilised neonates\* dead neonates present

Ceriodaphnia dubia Survival and Reproduction Test Observations and Measurements

Page 8 of 8 Maxxam BBY2FCD-00149/10 Tab - Test Concentrations

B978381/WM9245

Job/Sample #:

100

Concentration (%v/v)

13 J. 130 Daily WQ Reviewed v S Final 8 Analyst 5 S Initial 2 A 404 6.78 7 7.9 0/8/C·L 7.67.8 Final Hd dead neonates present 1. B #) immobilised neonates 7,8 7,8 Initial 7 7.6 8, 156 253 256 5.578 742 25,2 25,0 8,3 7,9 0.0/0.8 Final DO (mg/L) 2019 746 25,2 25.2 8.8 248 26.7 8.5 L. 06 Initial 25-5/25-7 750 25.125.3 Temperature (°C) Final NO PRO Initial Conductivity 見記 (m2/cm) S = small adult P = pale adult 551 Initial 3 23 10 0 0 DIVITIE Organisms present - NS M=missing/lost adult 0 t  $\bar{\omega}$ *₽* 0 0 9 X = dead adult 3 O 力 9 Total 25 16 25 21 25 24 24 24 24 ∞ 0 Reproduction (# Live neonates)  $\mathbb{Q}$ 1 5 0 / 0 N  $\infty$ O 9 4 O Ŋ 0 0 0 7 (h) ナ 0 0 4 0 <u>.</u> 2 0 0  $\sim$ Ó W エ 9 ユ O5 土 Day 9 4 ŕυ

5

DLE WE USOOR OCK 15

E WESGRONDSPAG WESGRONDS

348 8020 **(** 

@WETW 2015EP25

Culture Date: ARO2019 Sep 09

### **% MORTALITY**

70 WORTALITI							
							% Mortality in Previous
				Removed or	Total	% Daily	7 Days
Day	Date	Started	Deaths	Lost	Number	Mortality	
0	09-Sep	80	0	0	80	0.00%	
1	10-Sep	24 2	0	10	70	0.00%	
2	11-Sep	7.0	0	0	70	0.00%	
3	12-Sep	0.00	1	0	69	1.43%	
4	13-Sep		0	0	69	0.00%	
5	14-Sep	147 Maria	0	1	68	0.00%	
6	15-Sep		1	0	67	1.47%	
7	16-Sep	F 30 50 50 50 50 50 50 50 50 50 50 50 50 50	0	0	67	0.00%	
8	17-Sep		0	0	67	0.00%	2.86%
9	18-Sep		0	0	67	0.00%	2.90%
10	19-Sep		0	0	67	0.00%	1.45%
11					67	0.00%	1.47%
12					67	0.00%	1.49%
13					67	0.00%	0.00%
14		46.40			67	0.00%	0.00%
15					67	0.00%	0.00%

### **MEAN NEONATES**

Day	1	2	3	4	5	6	7	Total
Date	10-Sep	11-Sep	12-Sep	13-Sep	14-Sep	15-Sep	16-Sep	
Replicate 1	0	0	0	7	8	0	16	31
Replicate 2	0	0	0	5	9	13	0	27
Replicate 3	0	0	0	6	8	0	16	30
Replicate 4	0	0	0	6	12	0	17	35
Replicate 5	0	0	0	5	10	13	0	28
Replicate 6	0	0	0	8	9	14	0	31
Replicate 7	0	0	0	5	10	0	18	33
Replicate 8	0	0	0	6	8	0	18	32
Replicate 9	0	0	0	4	8	14	0	26
Replicate 10	0	0	0	5	11	16	0	32
Mean								30.5

**Health Criteria** 

An average of at least 15 young per adult must be produced in the first 3 broods (Environment Canada).

An average of at least 20 young per adult must be produced in the first 3 broods (US EPA).

**Comments:** \* Fourth or subsequent brood not counted.

Proofed: Kt 20400176

## CERIODAPHNIA DUBIA - CLEARING RECORDS

BBY2FCD-00242/5 Page 1 of 2

Clearing Records for Young Produced by Each Brood Organism Used in a Test for parent culture AB170413CD

Check once (√) if 2-7 neonates present (1 neonate ≠ a brood)

Check twice (√√) if ≥8 neonates

Check thrice (VVV) if ≥16 neonates

Test Day: Record actual number of neonates present

2019 Sep | Batch:

	-	1 -	1 0		1 -	10	T en		
Date	Sepio	Sep11	Sep/2	Sep13	1102	21092	Sep16	Sep17	Sep18
Time	08114	10:14	1128	07,95	7501	0,8:50	07200	07:29	OR1:32
Tracking/Clear	clear	Clear	Clear	Clear	Class	Clear	clear	deas	Clear
YCT (μL)	570	50	50	570	50	<u>c8</u>	50	50	So
PKS (μL)	iso	157>	150	150	CO	150	150	021	OZL
Cup#		, -			Brood size	<del></del>			
1	<b>₩</b>		0	7	8	0	16 A	17	21
2	0	0	0	3	9	13	9	16	19
3 _	0	0	0	6	8	0	16 A	23	20
4	0_	0	0	6	12	0	1+ A	17	19
5	0	0	0	2	0	13	0	19	18
6 ,	0	<u> </u>	X Repose is	70 8	Q	14	01510		16
7	P	0	0	3	16		18 A	23	2ŏ .
8	Ö	0	0	6	8		0018	21	21
9	0	0	0	4	8	14		23	19
10	0	0	0	15	1)	16		22	19
11	0	.0	0	V		0	AVVV	VI	W
12	0	0	0	レ		0	AUN	w	W
13 .	0	0	0	~	ساس	0	AVVV	w	W
14	0	0	O	V	<u> </u>	0	AUU	w	w
15	0	0	0	1	س س	W		w	wi
16		0	0	v	レー	W	0	w	w
17	W W	0	0	レ	س س	W	0	VV	w
18	٥	: ઇ	0	V		0	VV	w	w
19	0	0	O	V		W	0	un	1/1/
20	0	0	0	V.	ر · · · · · · · · · · · · · · · · · · ·	W	0	in	Wil
21	0	0	0	V	~	0	in	w	MAZ
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24	0	٥	0	V	"نسسا نسيا	0	W A	w	w
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26	0	0	0	V	، ساس	0	vul	VIV	W
27	Ŏ	_0	0	V	-		AND THE RESERVE	-	Accessive to the same of the s
28	0	Ŏ	0	V	سسية	θx	Later Annual Control of the Control		
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34	0	ð	0	V	レー	Ö	VVV	VVV	W
35	Ŏ.	Ö	Ō	V	س س	Ö	レレ	VVV	WW
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Time	08:25	10-37	1135	07:155	7:53	08:33	07:11	12 [1]	08:40
			nvironment			uirements '		W 1 164	
								00 00.0	

On test day, select appropriate neonates (i.e.>8 neonates in a brood) and indicate in the brood size box which test they will be used for by placing a letter according to this legend:

E -

F-

@werszagspt12 @pcccdw45 sorlled -co zi9 seg14

Culture Date:

Maxxam BBY2FCD-00242/5 Page 2 of 2

Clearing Records for Young Produced by Each Brood Organism Used in a Test

AR 02019 Sep 09

Analyst(s): Ful , co pro

Cup#			<u></u>		Brood size			·	
Date	Sep10	Sepl1	Sent 12	Sep 13	2019	SROIS	Septh	Sep17	Se018
Time	11:80	110.39	1135	D7:55	7:35	08,33	07/12	07:21	08:40
36	0	0	0	QL		W	0	W	W
37	0	0	0	OV	レレ	W	0	inc	w
38	0	0	0	PV	- U	0	VVV	w	W/
39	0	0	Ö	ΦV	<u> </u>	W	0	in	W
40	0	O	6)	1	2	W	0	VVV	IM
41	0	0	0	i.	レレ	W	0	VVV	1/1/
42	0	0	0		ر د د	W	0	W	w
43	O	0	0	V	0	W	0	w	W
44	Ø	0	O		0-	W	6	w	WU
45	0	Ò	Ö		-	W	0	u	W
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48	$\tilde{\rho}$	0	0	3.7		W	0	w	W
49 .	0	0	0			W	8	w	W
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57	0	2	Ö	V	ساس	N	0	w	W
58	0	Ŏ	0			W	.0	in	100/
59	0	0	Ö	V	س	0	in	W.	W
60	Ŏ	8	0	0	レー	W	D.	vir	WW
61	Ø	0	0	U	u	W	0	w	ViV
62	0	0	Ŏ.		<i></i>	W	0	w	W
63		0	Ö		レー	0	W	w	1/1/
64		0	0		<u></u>	W	0	U.	1/1/
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66		9 .	0		LL	W	0	w	VVV
67	Ď	Õ	Õ:			W	Ö	1100	VW
68	10	0	Ŏ	<u> </u>	40	W	0	w	UN
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# Missing/Lost	t Ø	0	0	0	' 0	0	0	0	0
# Dead	0	0	0	Ö	0	Ö	0	0	Ö
Analyst .	With .	TW	NS	TW.	25	mo	TW	900	wo
ime	08-35	10.46	1142	49.70	8705	08:40	07120	07:00	08:43

Environment Canada: At least 8 young must be produced by each brood organism in their third or subsequent broods, and in the brood to be used in a test.

Neonates used in tests must be < 24 hours old and within 12 hours of each other.

US EPA: Neonates used in tests must be < 24 hours old and within 8 hours of each other.

Neonates used in tests must be taken from adults that have had at least 8 or more young in the brood to be used in a test, and in their third or subsequent broods.

A WETW 2019 Sep16

Example:

Cup#	Brood size									
Date	2008Jan22	2008Jan23	2008Jan23	2008Jan24	1					
Time	10:10am	8:25am	8:15pm	8:00am	E					
1	٧	V	√√	12 A						
2	٧	V	√√	14 B						

A - Rx #1

B - Client # 123

#### CERIODAPHNIA DUBIA - CLEARING RECORDS

Maxiam BBY2FCD-00242/5 Page 1 of 2

Clearing Records for Young Produced by Each Brood Organism Used in a Test for parent culture AB170413CD

Check once (V) if 2-7 neonates present (1 neonate ≠ a brood)

Check twice (VV) if ≥8 neonates

Check thrice (VVV) if ≥16 neonates

Test Day: Record actual number of neonates present

YCT Batch: 2018 Aug 27, PKS Batch: 2018 Sept 10 Water Batch: 2018 Sept 15

Culture Date: ARO 2619 Sept 09

Analyst(s): A, Tw

Culture Date:	MO & O	11000	9			<del></del>	Analyst(s):	<u>_</u> &J,T	(L)
		.*		T	<del></del>				
Date	17:00	Septis 2116 track	Jep13		ļ <u></u>				
Time	13:00	2116	07114	ļ	ļ				
Tracking/Clear	Trecto	track	Clear						
YCT (μL)	ne	1 n/a	50						
PKS (μL)	nia	nla	50 150						
Cup#					Brood size				
11	0	0	21 A 25 A						
2	0	0	2) A 25 A 22 A 5 A 19 A				-	<del> </del>	<del> </del>
3	0	0	22 A						<del> </del>
4	0	0	5 A						<del> </del>
5	0	0	19 A			1	-	<del> </del>	
6	0 0	Ø	7-2- 1			†	<del> </del>		
7	0	0	22 A 3 A 18 A			<del> </del>	-	<del></del>	<u> </u>
8	0	00	180					<del> </del>	<del> </del>
9	0	Ö	00 10	**			<del> </del>	<del>                                     </del>	ļ
10		0	22 A 20 A 100 B 100 B			<del> </del>	<del> </del>	<u> </u>	
11	8		ZOF				ļ	ļ	
12	0	0	uch			ļ	ļ	ļ	<u> </u>
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22	0	0	7		···				
23	0	0	VVC D					<del></del>	
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25	0	0	D		·	<del> </del>	<del></del>		
26	0	Ö	0		······································				
27	-								
28								Appendix and the second	TANK BURNES
29	0	C					A. A. S. C.	A STATE OF THE PARTY OF THE PAR	-
30	6		VV C			ļ			
	0	600	mc me me						
31			vve		<del></del>				
32	0	0	me		*****				
33	0	9	we						
34	0		wo						
35	0	O	wu						
# Missing/Lost	0	0	0						
# Dead	0	0	0						
nalyst	<b>6</b> 5	Dhe	TW			*****			
me	17:03		26						
		page for En							

On test day, select appropriate neonates (i.e.>8 neonates in a brood) and indicate in the brood size box which test they will be used for by placing a letter according to this legend:

A-MEP D-1645

B- BAB E- 16:45B

C-BAG F-

Clearing Records for Young Produced by Each Brood Organism Used in a Test

for parent culture AB170413CD

Culture Date: 2019 Sen 09

Analyst(s): にりってい

Rx #1 Client # 123

Cup#			····	<del></del>	Brood size			
Date	J'en 18	Sept18	Sep18			1		
Time	17:03	2118	07126					
36		0	VVVC					
37	0	0	mo					
38	0	0	vw					
39	0	0	w.					
40	0	0	in					
41	0	0	und					
42	0	0	wo					
43	0	Ø	ino					
44	0	0	wp					
45	0	0	UUD					
46	6	0	wo					
47	0_		MI					
48	0	0	wo					
49	O	0	WW D					
50	0	0	UNP					
51	0	0	VUVE					
52	9	<u> </u>	INE					
53	0_	-0	LUE					
54	0	0	WE		····			
55	0	Ŏ	VVVE					
56	0	<u> </u>	VVVC					
57	0	8	VVV					
58	0	0_	WE					
59	0		INVE					
60	0	0	WE					
61	0	0	W					
62	0	0	VVV -					
63	0	0	VW.					
64		00	IN				 	
65	0	0	w		<u>.</u>			
66	Ø	0	vv.				 	
67	0	0	VV				·	
68	0	0	vvv				 ·	
69	0	0	VVV				 	
70	0						 	
# Missing/Lost	0	8	0				 	
# Dead	0		0					
Analyst	C-3	0W	TW					
Time	17:65	2119	07134					

Environment Canada: At least 8 young must be produced by each brood organism in their third or subsequent broods, and in the brood to be used in a test.

Neonates used in tests must be < 24 hours old and within 12 hours of each other.

US EPA: Neonates used in tests must be < 24 hours old and within 8 hours of each other.

Neonates used in tests must be taken from adults that have had at least 8 or more
young in the brood to be used in a test, and in their third or subsequent broods.

Example:

Cup #		Br	ood size		- 1
Date	2008Jan22	2008Jan23	2008Jan23	2008Jan24	Δ
Time	10:10am	8:25am	8:15pm	8:00am	В
1	٧	٧	<b>V</b> V	12 A	
2	V	V	٧v	14 B	



# Toxicity Testing on samples WM9244-1645-18 and WM9245-1645-18B

(collected September 17, 2019)

**Final Report** 

October 21, 2019

Submitted to: **Bureau Veritas Laboratories**Burnaby, BC



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Table 3. Reference toxicant results.	4

## **List of Appendices**

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#### **SIGNATURE PAGE**

m

Report By: Mimi Tran, Dipl. T. Laboratory Biologist 1. Tag

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	WM9244-1645-18 and WM9245-1645-18B
Sample collection date	September 17, 2019
Sample receipt date	September 19, 2019
Sample receipt temperature	2.3 to 2.9°C
Test types	72-h Pseudokirchneriella subcapitata growth inhibition

## **Summary of Results**

Enducint	Mean ± SD						
Endpoint	Control	WM9244-1645-18	WM9245-1645-18B				
Cell Yield (x10 <sup>4</sup> cells/mL)	30.2 ± 2.0	89.2 ± 7.6*					
	$34.0 \pm 2.4$		96.5 ± 10.8*				

SD = Standard Deviation

<sup>\* =</sup> Indicates cell yield was significantly greater than the control



#### 1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted a 72-h *Pseudokirchneriella subcapitata* growth inhibition toxicity test for Bureau Veritas Laboratories on samples identified as WM9244-1645-18 and WM9245-1645-18B. The samples were collected on September 17, 2019 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on September 19, 2019. The samples were collected in 1-L plastic containers and were received at temperatures of 2.3 and 2.9°C. The samples were stored in the dark at  $4 \pm 2$ °C prior to testing.

This report describes the results of the toxicity test. 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

Methods for the toxicity tests are summarized in Table 1. Testing was conducted according to procedures described by Environment Canada (2007). Statistical analyses were performed using CETIS (Tidepool Scientific Software, 2013).



## Table 1. Summary of test conditions: *Pseudokirchneriella subcapitata* growth inhibition single concentration test.

Test species Pseudokirchneriella subcapitata, strain CPCC# 37

In-house axenic culture, obtained from Canadian Phycological

Organism source 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 Full strength sample diluted to 95.2% (v/v) with nutrients, 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  $24 \pm 2^{\circ}C$ Feeding None

Light intensity 3600 to 4400 lux Photoperiod 24 hours light

Aeration None

Test area temperature measured daily; temperature and pH

Test measurements measured at test initiation; pH of two control wells measured

at test termination

Test protocol Environment Canada (2007), EPS 1/RM/25

Statistical software CETIS Version 1.9.4

Test endpoints Algal cell growth inhibition

>16-fold increase in number of algal cells; CV ≤ 20%; no trend

when analyzed using Mann-Kendall test

Reference toxicant Zinc (added as ZnSO<sub>4</sub>)



#### 3.0 RESULTS

The results of the toxicity tests on samples WM9244-1645-18 and WM9245-1645-18B are summarized in Table 2. Cell yield was significantly greater than the laboratory control; percent stimulation was 195.0% for WM9244-1645-18 and 183.8% for WM9245-1645-18B.

Table 2. Results: *Pseudokirchneriella subcapitata* growth inhibition single concentration test.

Sample ID	Cell Yield (x 10 <sup>4</sup> cells/mL) (Mean ± SD)	Stimulation (%)
Laboratory Control	30.2 ± 2.0	
WM9244-1645-18	89.2 ± 7.6*	195.0
Laboratory Control	$34.0 \pm 2.4$	
WM9245-1645-18B	96.5 ± 10.8*	183.8

SD = Standard Deviation

#### 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 tests met all control acceptability criteria and water quality parameters remained within ranges specified in the protocol throughout the tests. There were no deviations from the test methodology. Uncertainty associated with the tests are best described by the standard deviation around the mean and/or the confidence intervals around the point estimates.

Result of the reference toxicant test conducted during the testing program is 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 the test was appropriate. The reference toxicant test was performed under the same conditions as those used for the sample.

<sup>\* =</sup> Indicates cell yield was significantly greater than the laboratory control



Table 3. Reference toxicant results.

Test Species Endpoint		Historical Mean (2 SD Range)	CV (%)	Test Date
P. subcapitata	Growth (IC50): 31.8 μg/L Zn	31.1 (25.1 – 38.6)	11	September 20, 2019

SD = Standard Deviation, CV = Coefficient of Variation, IC = Inhibition Concentration

#### 5.0 REFERENCES

Environment Canada. 2007. 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 – Pseudok	kirchneriella subc	<i>apitata</i> Toxicity T	est Data	

## Pseudokirchneriella subcapitata Summary Sheet

Client: Work Order No.:	Burran Veritas Lab	Start Date:	SP+ 19/19	
Sample Information:	:			
Sample ID: Sample Date: Date Received: Sample Volume:	WM 9244-1645-18 Sept 17/19 Sept 19/19 1x14			
Test Organism Infor	mation:			
Culture Date: Age of culture (Day 0	): Sept 13/	/19		
Zinc Reference Toxi	icant Results:			
Reference Toxicant II Stock Solution ID: Date Initiated: 72-h IC50 (95% CL):	5C189 193202 Sept 20/19 31.8 (21.4-34.6),ug/L			
72-h IC50 Reference	Toxicant Mean and Range: 31.1 (25.1-3	3.6) <u>USIL</u> CV (	96): t	
Test Results:			(Mean± SD)	
	Negative Control NM 9244~1645-18 (95.2/, イレ)		± 2.5 ± 7.6 *	
			± ±	
			±	
			±	
			±	
	# indicates that cell yield is sign	nificantly great	er than the lab co	nto
Reviewed by:		Date reviewed:	Oct. 17/19	

## 72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client:	Burran Vental Labs				Setup by:		MC				
Sample ID:	Wi	1924-	1645-19	8		Test Date	/Time:	8	Pt 19/19	10 150	>h
Work Order No.:		77	191855			CER#:			4		
						Test Spec	les:	Pseudokirol	neriella sub	capitata	
Culture Date:	Sept	13/19					Culture He				
Culture Count:	1 220 2 340 Average:			230	Culture C	ell Density (	01):	230 X	04 (ell:	1/mL	
	v1 =	220,000 ce (c1)	lls/ml x	DO mi		callelest	- = <	1.56mL	_		
Time Zero Counts:		1 2									
No. of Cells/mL:							# cells/mL		10 pt = C	1318 <i>ce</i> 1	s/mL
Concentration		Quality		ncubator T							1
%(v/v)	pH	Temp (°C)			(C)	-	Micr	roplates rot	ated 2X per	day?	
	0 h	0 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	]
Control	G.F	22.5	35,0	এন, ১	25.0	250	1	سا	-/		
9.8	7.6	23.0	J	7	7	7	lum.	-	/		
											1
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Initials		nio.	wia	w D	<i>b</i> -	10-	ula.	ale.	5	8	-
	ME	MIS	MD	W/U	D	14	MA	Ma	ρ	1.5	_
Initial control pH:	Well 1		7.2		-	Well 2	:	8			
Final control pH:	Well 1		7.1			Well 2					
Light intensity (lu:	x):	3	90D			Date mea	sured:		Sept 19	/18	
Thermometer:	4	Light met	er:	ph	1 meter/pr	obe:			A.		
Sample Description	on:	cle	ar, c	wolo,	less, o	down	en , 80	mi Pi	evticu	stes	
Comments:								1			_
Reviewed:		de	u			Da	te reviewed:		Oct.	11/19	_

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

Client:	Bu	reau Ver	itas Lak	Start D	ate/Time:	9	0+19/19	e 1500/1		
Work Order #: 15	111924	1645-1	8191835	Terminat	ion Date:	5	Vot 22/19	P 15001		
Client: Work Order #: \frac{\fir}}}}}}{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac}\f{\frac{\fir}}}}}{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\f	Mylogic	14-1645	-18	Test	set up by:		ไทอ	C 170071		
%(v/v)										
Concentration	Rep	Count 1	Count 2	Count 3	Count 4		Comm	ents		nitials
Control	Α	32							b	nr)
	В	20								1
	С	99								
	D	31								
	E	29 30								
	F	70								
	G	35								
	Н	30								
	Α	99								
Ar ~	В	97				2				1
95.0	С	80								
	D	89								
	Α									
	В									
	C									
	D									
	A									_
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Reviewed by:		Jul		Date F	Reviewed:		0ct-11	ly		

#### Pseudokirchneriella subcapitata Algal Counts

Client: Bureau Veritas Labs Start Date/Time: 19-Sep-19 @ 1500h WO#: 191835 Termination Date/Time 22-Sep-19 @ 1500h Sample ID: WM9244-1645-18 Initial Cell Density: 9318 cell/mL 205000 0.220.01 Concentration Rep Count 1 Count 2 Count 3 Count 4 Mean Cell Yield 9318.182 %(v/v)  $(x 10^4)$  $(x 10^4)$  $(x 10^4)$  $(x 10^4)$  $(x 10^4)$  $(x 10^4)$ cell/mL Control 32 A 32 31.1 30.3 mean В 30 30 29.1 SD 1.982062 C 33 33 32.1 CV 6.537537 Ď 31 31 30.1 Ε 29 29 28.1 F 30 30 29.1 G 35 35 34.1 Н 30 30 29.1 95.2 A 95 95 94.1 В 97 97 96.1 C 80 80 80 79.1 D0 89 88.1 A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! #DIV/0! C #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0!

Reviewed by:	16h	Date reviewed:	Oct 11/19	
				_

### **CETIS Summary Report**

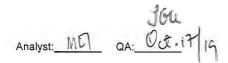
Report Date: Test Code/ID: 16 Oct-19 16:57 (p 1 of 1) 191835a / 15-5778-5830

							lest	Codeniu:		916358/1	0-0//6-0630
EC Alga Grow	th Inhibition Tes	st							Na	utilus Env	rironmental
Batch ID:	15-8744-8356	Test Ty	ype:	Cell Growth			Anal	yst: Min	ni Tran		
Start Date:	19 Sep-19 15:00	Protoc	ol:	EC/EPS 1/RM/2	25		Dilue	ent: Dei	onized Wate	r + nutrien	ts
Ending Date:	22 Sep-19 15:00	Specie	es:	Pseudokirchner	iella subcap	itata	Brine	9:			
Test Length:	72h	Taxon:		Chlorophyta			Sour	ce: In-l	House Cultur	e	Age: 6d
Sample ID:	10-5123-2224	Code:		3EA887E0			Proje	ect:			
Sample Date:	17 Sep-19	Materia	al:	Water Sample			Sour	ce: Bui	eau Veritas	Laboratorie	es
Receipt Date:	19 Sep-19	CAS (P	PC):				Stati	on: Wil	19244-1645-	18	
Sample Age:	63h (2.9 °C)	Client:		Bureau Veritas	Laboratorie:	5					
Single Compa	arison Summary										
Analysis ID	Endpoint	c	omp	arison Method			P-Value	Compari	son Result		8
11-9560-1267	Cell Yield	Ų	Jnequ	ual Variance t Tw	o-Sample 1	est	3.1E-04	95.2% fa	iled cell yield		1
Cell Yield Sur	nmary										
Conc-%	Code	Count N	lean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	8 3	0.25	28.59	31.91	28	34	0.7008	1.982	6.55%	0.00%
95.2		4 8	9.25	77.11	101.4	79	96	3.816	7.632	8.55%	-195.04%
Cell Yield Det	ail										
Conc-%	Code	Rep 1 R	tep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8		
0	N	31 2	29	32	30	28	29	34	29		
95.2		94 9	68	79	RR.						

## **CETIS Analytical Report**

Report Date: Test Code/ID: 16 Oct-19 16:57 (p 1 of 2) 191835a / 15-5778-5830

									1690	Codeniu:		3 10336 / T	2-2110-209
EC Alga Grow	rth Inhibitio	on Test									Na	utilus Env	ironmental
Analysis ID: Analyzed:	11-9560-12 01 Oct-19		Endpoint: Analysis:		Yield ametric-Two	Sample				S Version: is Level:	CETISv1	.9.4	
Batch ID: Start Date: Ending Date:		15:00	Test Type: Protocol: Species:	EC/ Pse	EPS 1/RM/2 judokirchner		cap	itata	Diluc	Brine:		er + nutrien	
Test Length:	72h		Taxon:	Chk	orophyta				Sour	ce: In-H	louse Cultur	e	Age: 6d
Sample ID: Sample Date: Receipt Date: Sample Age:	19 Sep-19		Code: Material: CAS (PC): Client:	Wat	k887E0 ter Sample eau Veritas	Laborato	ories		Proje Sour Stati	ce: Bun	eau Veritas 9244-1645-		95
Data Transfor	m	Alt	Нур						Comparis	on Result			PMSD
Untransformed	1	C <	T						95.2% fail	ed cell yield			30.18%
Unequal Varia Control Negative Cont	vs Cor	itrol II	Test		Critical 2.353	MSD 9.131	DF	P-Type CDF	P-Value 3.1E-04	Decision			
Auxiliary Test Attribute	Tes					Test S	tat	Critical	P-Value	Decision			
Control Trend	Mar	nn-Kendall	Trend Test						0.5634	Non-Signi	ficant Trend	in Control	S
ANOVA Table Source		Squares	Mean	Sau	iare	DF		F Stat	P-Value	Decision	(an5%)		
Between	9282	2.67	9282	67		1		459	<1.0E-37	Significan			
Error Total	9484		20.22	15		10	_	-		-			
Distributional													
Attribute Variances	Test					Test S	tat	Critical	P-Value	Decision			
Distribution		ance Ratio	r Test / Normality Te	ed.		14.83		10.88 0.8025	0.0041		/ariances istribution		
Cell Yield Sur		ALC-VIIIK VI	rivormality re	ist.		0.901		0.8025	0.1632	Normal D	istricution		
Conc-%	Cod	e Co	unt Mean	1	95% LCL	95% U	CI.	Median	Min	Max	Std Err	CV%	%Effect
0	N	8	30.25		28.59	31.91		29.5	28	34	0.7008	6.55%	0.00%
95.2		4	89.25		77.11	101.4		91	79	96	3.816	8.55%	-195.04%
Cell Yield Det	ail												
Conc-%	Cod	e Rej	1 Rep	2	Rep 3	Rep 4		Rep 5	Rep 6	Rep 7	Rep 8		
0	N	31	29		32	30		28	29	34	29		
95.2		94	96		79	88							

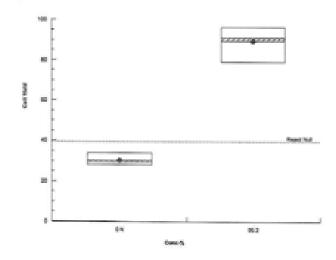


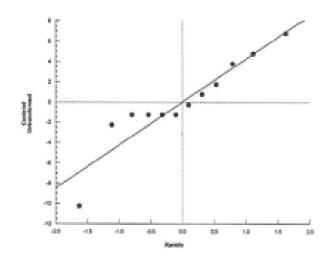
### **CETIS Analytical Report**

Report Date: Test Code/ID: 16 Oct-19 16:57 (p 2 of 2) 191835a / 15-5778-5830

EC Alga Gro	wth Inhibition Test				Nautilus Environmental
Analysis ID:	11-9560-1267	Endpoint:	Cell Yield	CETIS Version:	CETISv1.9.4
Analyzed:	01 Oct-19 13:38	Analysis:	Parametric-Two Sample	Status Level:	1

#### Graphics





## Pseudokirchneriella subcapitata Summary Sheet

Client:	Darra No Fox Labor	Ct-+ D-t C	02-10/10	
Work Order No.:	Bureau Venitas Labs	Start Date: Sc	<u>9T 17/17</u>	
WORK Officer No.:	111897	Set up by:	Mini	
Sample Information				
Sample ID:	WWG245-1645-18B			
Sample Date:	Sept 17/19			
Date Received:	\$20+ 19/19			
Sample Volume:	IXIL			
	10.10			
Test Organism Info	rmation:			
Culture Date:		1/19		
Age of culture (Day (	D):	<del>-</del>		
Zinc Reference Tox	icant Results:			
Reference Toxicant I	D: Sc189			
Stock Solution ID:	19700			
Date Initiated:	Sept 20/19		,	
	- /			
72-h IC50 (95% CL):	31,8 (21.4-34.6) UG/L	<u>2n</u>		
	9			
72-h IC50 Reference	Toxicant Mean and Range: 31.1 (25.1-	38.6)ug/LZn_cv(%):	[]	
		v	,	
Test Results:		Cell Yield (N	Mean± SD)	
	Negative Control	34.0 34.5 ±	H8- 24	
	NM9245-1645-18B (95.21.4)	96,5 ±	10.8 #	
		±		
		+		
		+		
		±		
	\$ indicates that call water	two confined	Oceano dos d	[a] - 1 m h 1
	in the contract	I was significant	A deeper mon the	INFO CALLIAR
Reviewed by:	* indicates that cell yield	Date reviewed:	Uct.17/19	
			7.7	

Nautilus Environmental Company Inc.

Issued May 10, 2014; Ver. 1.0

## 72-h Algal Growth Inhibition Toxicity Test Water Quality Measurements

Client:	Bur	eau Va	ntas	Labs		Setup by:			MA		
Sample ID:	W	19245	-1645	-18B		Test Date	e/Time:	3	ept 19/	19015	nooh
Work Order No.:		191	835			CER #:			4		
						Test Spe	cies:	Pseudokiro	hneriella sub	capitata	
Culture Date:	9-24	13/19		Age of Cu	Iture:	601	Culture He	alth:	(	Bod	
Culture Count:	1 2000	2 240		Average:	230	Culture C	cell Density (	c1):	330×1	04 (ell	s/mL
		(c1)	55	401× 06	r .		9				
Time Zero Counts:				2 2							
No. of Cells/mL:		2015 X	104		Initial Der	sity:	# cells/mL	÷ 220 μL x	10 μL = C	1318 CC	16/mL
Concentration	Water	Quality		ncubator T	emperatur	re	Min	nalster set	ated 2X per	day 0	
%(v/v)	рН	Temp (°C)			(C)		MIC	ropiates rot	ated 2X per	dayr	
Control	0 h	0 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	
Control	8.F	22.0	25.0	25,0	25.0	250	-	V		//	
<u> 95.γ</u>	7.6	22.~	7	7	1	7	V				
Initials	иb	MET	Nβ	иq	p	p-	MICI	мΩ	p-	r	
Initial control pH:	Well 1:		7.7			Well 2	= =====================================	.~			
Final control pH:	Well 1:	9	7.1			Well 2	5	7.			
Light intensity (lux	):	40	90			Date mea	asured:		S-p+ 19	119	
Thermometer:	4	Light me	ter:	pl	-l meter/pr	obe:	11				
Sample Descriptio	ec .	clea	N   (6	louw/k	ч, оф	our les	5, 45	فيرابانه	pertic	nlates	
Comments:											-
Reviewed:		(	TGL.		-	Da	te reviewed:		)ct - 11/	(9	

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

Client:	bur	eau Ven	tas Lao	Start D	ate/Time:	50+18/19 C	150h
Work Order #:					ion Date:	87722186	125CN
Sample ID:	WAG	1245-164	15-1810	Test:	set up by:	ู้หเว	
%(v/v) Concentration	Rep	Causal 1	Count 2	Court 2	Count 4	Comments	Initials
Control	A	33	Count 2	Count 3	Count 4	Comments	ALC)
OGNIDO	В	38					10,01
	С	35					
	D	76					
	E	32					
	F	21.					
	G	36 35			- 97-9		1.1
	Н	31			<u> </u>		
	Ä	103					
200	В	85					
96.9	С	106					
	D	92					
	A	90					*
	В						
	C						
	Ď						
	A						
	В						
	C						
	Ď						
	A						
	В						
	C						
	D						
	A						
	В						
	С						
	D						
	A						
	В						
	C						
	D						
	A						
	В						
	C						
Comments:							
		Joh				A+	
Reviewed by:		(10h		Date F	Reviewed:	Oct-11)	119

#### Pseudokirchneriella subcapitata Algal Counts

Client: Bureau Veritas Labs Start Date/Time: 19-Sep-19 @ 1500h WO#: 191835 Termination Date/Time 22-Sep-19 @ 1500h Sample ID: WM9245-1645-18B Initial Cell Density: 9318 cell/mL 205000 0.220.01 Concentration Rep Count 1 Count 2 Count 3 Count 4 Mean Cell Yield 9318.182 %(v/v)  $(x 10^4)$  $(x 10^4)$ (x 10°)  $(x 10^4)$ (x 10°)  $(x 10^4)$ cell/mL Control A 37 37 36.1 34.1 mean В 38 38 37.1 SD 2,390457 C 35 35 CV 34.1 7.016686 D 36 36 35.1 Ε 32 32 31.1 F 36 36 35.1 G 35 35 34.1 Н 31 31 30.1 95.2 A 107 107 106.1 В 85 85 84.1 C 106 106 105.1 D 92 92 91.1 A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/0! #DIV/0! A #DIV/0! #DIV/0! В #DIV/0! #DIV/0! C #DIV/0! #DIV/0! D #DIV/01 #DIV/0!

Reviewed by:	16h-	Date reviewed:	Oct. 17/19	

#### **CETIS Summary Report**

Report Date: Test Code/ID: 16 Oct-19 16:59 (p 1 of 1) 191835b / 19-4494-7760

					Test Code/ID: 1918356 / 19-				9-4494-7750		
EC Alga Grow	vth Inhibition Ter	st							Na	utilus Env	ironmental
Batch ID:	20-1271-0259	Te	st Type:	Cell Growth			Anal	yst: Min	ni Tran		
Start Date:	19 Sep-19 15:00	Pr	otocol:	EC/EPS 1/RM/2	25		Dillu	ent: Dei	ionized Wate	r + nutrient	S
Ending Date:	22 Sep-19 15:00	Sp	ecles:	Pseudokirchner	iella subcap	itata	Brin	0:			
Test Length:	72h	Ta	xon:	Chlorophyta	-		Sour	rce: In-l	House Cultur	e	Age: 6d
Sample ID:	11-4615-1826	Co	ode:	4450E392			Proj	ect:			
Sample Date:	17 Sep-19	M	aterial:	Water Sample			Sour	roe: Bur	reau Veritas	Laboratorie	s
Receipt Date:	19 Sep-19	C/	48 (PC):				Stati	on: Wi	A9245-1645-	188	
Sample Age:	63h (2.3 °C)	CI	ient:	Bureau Veritas	Laboratories	s					
Single Compa	arison Summary										
Analysis ID	Endpoint		Comp	parison Method			P-Value	Compari	son Result		s
17-5366-0575	Cell Yield		Uneq	ual Variance t Tw	ro-Sample T	Test	7.1E-04	95.2% fa	iled cell yield		1
Cell Yield Sur	mmary										
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	8	34	32	36	30	37	0.8452	2.39	7.03%	0.00%
95.2		4	96.5	79.34	113.7	84	106	5.393	10.79	11.18%	-183.82%
Cell Yield Det	tail										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8		
0	N	36	37	34	35	31	35	34	30		
95.2		106	84	105	91						

## **CETIS Analytical Report**

Report Date: Test Code/ID: 16 Oct-19 16:59 (p 1 of 2) 191835b / 19-4494-7760

									1001	OudenD.		13 10000 7 1	3 7737 110
EC Alga Grow	vth Inhibition	Test									Na	autilus Env	ironmenta
Analysis ID:	17-5366-0575	i E	ndpoint:	Cel	l Yield				CETI	S Version:	CETISV	1.9.4	
Analyzed:	11 Oct-19 18:	02 A	nalysis:	Par	ametric-Two	Sample			Statu	us Level:	1		
Batch ID:	20-1271-0259	Te	est Type:	Cel	Growth				Anal	yst: Min	ni Tran		
Start Date:	19 Sep-19 15	:00 P	rotocol:	EC/	EPS 1/RM/	25			Dilue	ent: Dei	onized Wate	er + nutrient	s
Ending Date:	22 Sep-19 15	:00 S	pecies:	Pse	udokirchne	riella sub	cap	itata	Brine	e:			
Test Length:	72h	Ta	accorn:	Chli	orophyta				Sour	roe: In-h	louse Cultu	ne	Age: 6d
Sample ID:	11-4615-1826	s c	ode:	445	0E392				Proje	ect:			
Sample Date:	17 Sep-19	M	aterial:	Wat	ter Sample				Sour	roe: But	eau Veritas	Laboratorie	\$
Receipt Date:		C	AS (PC):						Stati	on: Wil	19245-1645	-188	
Sample Age:	63h (2.3 °C)	C	lient	Bun	eau Veritas	Laborate	orles	s					
Data Transfor		Alt Hyp	)						Comparis	on Result			PMSD
Untransformed	1	C < T							95.2% fail	ed cell yiek	d		37.78%
Unequal Varia	ance t Two-Sa	imple Test											
Control	vs Contro	al II	Test	Stat	Critical	MSD	DIF	P-Type	P-Value	Decision	(a:5%)		
Negative Cont	rol 95.2*		11.45	5	2.353	12.85	3	CDF	7.1E-04	Significar	nt Effect		
Auxiliary Test	ts												
Attribute	Test					Test S	tat	Critical	P-Value	Decision	(a:5%)		
Extreme Value	Grubbs	s Extreme V	alue Test			2.102	T	2.412	0.2284	No Outlie	rs Detected		
ANOVA Table													
Source	Sum So	quares	Mean	Squ	are	DF		F Stat	P-Value	Decision	(a:5%)		
Between	10416.7	7	1041	6.7		1		267.8	<1.0E-37	Significar			
Error	389		38.9			10				-			
Total	10805.7	7				11							
Distributional	Tests												
Attribute	Test					Test S	tat	Critical	P-Value	Decision	(a:1%)		
Variances	Variano	e Ratio F To	est			20.36		10.88	0.0016	Unequal	Variances		
Distribution	Shapiro	-Wilk W No	mality Te	st		0.9534		0.8025	0.6877	Normal D	Histribution		
Cell Yield Sur	mmary												
Conc-%	Code	Count	Mean	1	95% LCL	95% U	CL	Median	Min	Max	Std Err	CV%	%Effect
0	N	8	34		32	36		34.5	30	37	0.8452	7.03%	0.00%
95.2		4	96.5		79.34	113.7		98	84	106	5.393	11.18%	-183.829
	bail												
Cell Yield Det													
Cell Yield Det Conc-%	Code	Rep 1	Rep :	2	Rep 3	Rep 4		Rep 5	Rep 6	Rep 7	Rep 8		
		Rep 1 36	Rep :	2	Rep 3	Rep 4	_	Rep 5	Rep 6 35	Rep 7	Rep 8		

#### **CETIS Analytical Report**

Report Date: Test Code/ID: 16 Oct-19 16:59 (p 2 of 2) 191835b / 19-4494-7760

EC Alga Growth Inhibition Test

Nautilus Environmental

Analyzed:

Analysis ID: 17-5366-0575 11 Oct-19 18:02

Analysis:

Endpoint: Cell Yield

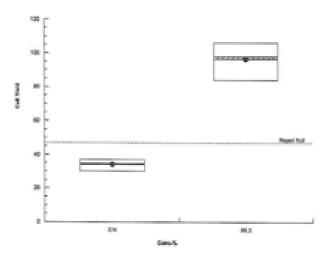
Parametric-Two Sample

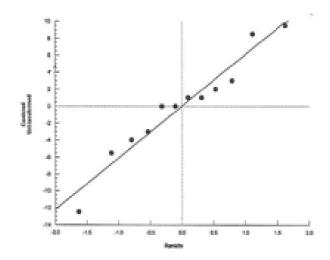
CETIS Version:

CETISv1.9.4

Status Level: 1

Graphics







**APPENDIX B – Chain-of-Custody Form** 

Page 01 of 01

COC # 8978381-ENAU-01-01

Sent To: Nautilus Environmental 8664 Commerce Court Burnaby, BC, VSA 4N7 Tel: [604] 420-8773

REF	ORT INFORMATI	DN									ANA	VLYSIS	REQUE	STED					
Cor	прапу:	Bureau Veritas Laboratorie	i .												- [				
Adi	dress:	9331 - 48th Street, Edmont	on, Alberta, 1	68 2R4															
Cor	stact Name:	Geraldlyn Gouthro						1											
Em	all:	geraldlyn.gouthro@bvlabs.	com, customi	erservice@bvl	labs.com														
Pho	one:	(780) 577-7173																	
BV I	abs Project R:	8978381														\ \subseteq			
#	SAMPLE ID		MATRIX	DATE SAMPLED (YYYY/MM/DD)	TIME SAMPLED (HH:MM)	SAMPLER INITIALS	# CONT.	PKS Alga⊪								Teres.		ADDITIONAL SA	MPLE INFORMATION
1	WM9244-1645	5-18 <b>(</b> )	W	2019/09/17	00:00	АН	1	X						2 1		2.9	(P: 02)	IXIL	
2	WM9245-1645	5-18B ()	w	2019/09/17	00:00	АН	1	Х								2.3	(P: 02)	IXIL	
3			= 12 1				= - :												
4																	Stm	ple descrip	otim: dear,
5																_	ŧ	NAMONOR	odanies, some
6															_		_	Particula	ites
7								\n							_	-	-		
			_					35						$\vdash$		_	-		
9.			_					20							+	+	-		
10	ULATORY CRITER	IA.		SPECIAL INSTR	LICTIONS			200											TURNAROUND TIME
	ik 1645-18 Ave &			Please inform I		ediately if s	weren a	ine and :	accinedi	ted for	the res	nijede	d testis	Λ.					TOURNESS OND TIME
				**Please return															Rush Required
coc	DLER ID:			COOLER ID:							71	COOL	B ID-						2019/10/15 Date Required
Cust Cust	ody Seal Present ody Seal Intact ing Media Present	YES NO Temp:	2 3	Custody Seal Pre Custody Seal Inta Cooling Media Pr	act	YES NO	Ten (°	1		ž	3	Custod Custod	y Seal Pr y Seal In y Media	tact	YES	NO Tem (°C	35.1	2 3	Please inform us if rush charges will be incurred.
HEU 1. Gi 2.	NQUISHED BY: (Sa na Antonucci	irafatonic	DATE: (	/18	TiME: 01 16:28	Contract of the Contract of th	ALC: UNKNOWN	PANA	Section Services	AND DESCRIPTION OF THE PERSON NAMED IN COLUMN 1						:pmm/34	M/00) 1/20/19	7:20	



**END OF REPORT** 



#### **RESULTS OF DAPHNIA MAGNA SINGLE CONCENTRATION-100%**

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B979875Client Project Name & Number:Monthly (13,18,18B) SNP-ASample Number:WN6548-01

**Test Result:** 

48 hrs Mortality % 0 Statistical Method:

Mean percent mortality: Sample 0 Control 0

Sample Name: 1645-18B Sample Matrix: Grab Water

Description: Clear, Colourless Sample Prior to Analysis:

Sample Collected: Sep 16, 2019 04:14 PM Sampling Method: N/A pH: 7.1
Sample Collected By: AH Site Collection: N/A Temperature: 19 °C

Sample Received: Sep 20, 2019 04:18 PM Volume Received: 1 L Dissolved Oxygen: 10.2 mg/L Analysis Start: Sep 20, 2019 12:09 PM Temp.Upon Arrival: 13 °C Sample Conductance: 608 μS/cm

End: Sep 22, 2019 01:02 PM Storage: 2-6°C Hardness: 140 mg CaCO <sub>3</sub>/L

			•		U						U	
Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hr	48 hrs	48 hrs
0	21	8.2	342	7.9	0	0	0	0	19	8.1	343	8.4
0	21	8.2	344	7.7	0	0	0	0	20	8.1	340	8.4
0	21	8.2	344	7.7	0	0	0	0	20	8.1	339	8.4
100	20	7.2	618	9.6	0	0	0	0	20	7.7	615	8.4
100	20	7.2	618	9.6	0	0	0	0	20	7.6	616	8.4
100	20	7.1	622	9.5	0	0	0	0	20	7.6	625	8.3

Concentration	Mortality (#)	Mortality (%)	Immobility (#)	Immobility (%)	
% vol/vol	48 hrs	48 hrs	48 hrs	48 hrs	
0	0	0	0	0	
0	0	0	0	0	
0	0	0	0	0	
100	0	0	0	0	
100	0	0	0	0	
100	0	0	0	0	

Comments: None

Culture/Control/Dilution Water: City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO₃ Other parameters available on request.

Test Conditions Test concentration: 0,0,0,100,100,100 (% vol/vol)

Organisms per Vessel: 10 Pre-aeration Time: 30 min Rate of Pre-aeration: 25-50 mL/min/L

Total # of Organisms Used : 60 Test Temperature :  $20 \pm 2$  °C Test Hardness Adjusted : No Test Volume : 150 mL Vessel Volume : 225 mL Test pH Adjusted: No

Loading Density: 15.0 mL/Daphnia Photoperiod: 16:8 (light: dark)

<u>Test Organism :</u> Daphnia magna Source : In House Culture

Age at Test Initiation :<24 hrs</th>Average Brood Size :31.7Culture Photoperiod :16:8 (light: dark)% Mortality within 7 days :0Culture Temperature : $20 \pm 2$  °CTime To First Brood :10 Days

Culture Diet Pseudokirchnriella and YTC at a ratio of 2 mL/L of culture daily. New cultures weekly, 63 daphnids

distributed into 6 culture vessels and 3 reproductive vessels.



#### **RESULTS OF DAPHNIA MAGNA SINGLE CONCENTRATION-100%**

Client:4388DIAVIK DIAMOND MINES INC., YELLOWKNIFEJob Number:B979875Client Project Name & Number:Monthly (13,18,18B) SNP-ASample Number:WN6548-01

Reference chemical:Sodium ChlorideTest Date:Sep 07, 2019Test Endpoint 48 hrs LC50 (95% confidence interval):6.69 (6.20, 7.21)g/LStatistical Method:Untrimmed

Spearman-Kärber

Historical Mean LC50 (warning limits): 6.01 (4.52, 8.00) g/L Concentration: 0,1.71,2.56,3.82,5.7,8.5 g/L

Test Method EPS 1/RM/14
Method Deviations: None

<u>Note:</u> The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst: Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Team Lead, Bioassay Date: Sep 26, 2019 02:44 PM



#### **RESULTS OF RAINBOW TROUT SINGLE CONCENTRATION-100%**

Client: 4388 DIAVIK DIAMOND MINES INC., YELLOWKNIFE Job Number: B979875

Client Project Name & Number: Monthly (13,18,18B) SNP-A

**Test Result:** 

96 hrs Mortality % 0 Statistical Method: Visual

Sample Name: 1645-18B Sample Matrix: Grab Water

Description: CLEAR, COLOURLESS Sample Number: WN6548-11

Sample Collected: Sep 16, 2019 04:14 PM Sampling Method: N/A Site Collection: N/A
Sample Collected By: AH Volume Received: 20 L Temp.Upon Arrival: 13 °C Storage: 2-6°C

Sample Received: Sep 20, 2019 04:18 PM pH: 6.9 Dissolved Oxygen: 9.4 mg/L Analysis Start: Sep 21, 2019 12:16 PM Temperature: 14 °C Sample Conductance: 517 μS/cm

Concentration	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	Start	Start	Start	Start	24 hrs	24 hrs	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
0	14	7.9	288	8.9	0	0	0	0	0	0	0	0
100	14	7.0	526	9.3	0	0	0	0	0	0	0	0

Concentration	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)	Temperature (°C)	pH (pH)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Mortality (#)	Mortality (%)	Atypical Behaviour (#)	Atypical Behaviour (%)
% vol/vol	72 hrs	72 hrs	72 hrs	72 hrs	96 hrs	96 hr	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs	96 hrs
0	0	0	0	0	14	7.8	290	9.1	0	0	0	0
100	0	0	0	0	14	7.3	546	8.9	0	0	0	0

Comments: None

Culture/Control/Dilution Water City of Edmonton dechlorinated tap water

Hardness: 180 mg/L CaCO₃ Other parameters available on request.

Test Conditions Test concentration: 0,100 (% vol/vol)

Organisms per Vessel : 10 Test Temperature :  $15 \pm 1$  °C Solution Depth : >15 cm

Total # of Organisms Used: 20 Pre-aeration Time: 30 min. Rate of Aeration 6.5±1 mL/min/L

Test Volume : 20 L Vessel Volume : 38L Test pH Adjusted: No

Loading Density: 0.2 g/L Photoperiod: 16:8 (light: dark)

<u>Test Organism</u>: Rainbow Trout (Oncorhynchus mykiss) Source: Spring Valley Trout Hatchery

Culture Temperature :  $15 \pm 2$  °C Weight (Mean) +- SD :  $0.3 \pm 0.1$  g Length (Mean) +- SD :  $3.37 \pm 0.24$  cm Culture Water Renewal :  $\geq 1.0$  L/min/kg fish Weight (Range) : 0.2 - 0.5 g Length (Range) : 3.00 - 3.90 cm

Culture Photoperiod: 16:8 (light: dark) % Mortality within 7 days: 0% Feeding rate and frequency: daily: 1-5% biomass of trout. Acclimation Time: >14 days

 Reference chemical:
 Phenol
 Test Date:
 Sep 18, 2019

 Test Endpoint 96 hrs LC50 (95% confidence interval):
 9.99 (9.10, 10.8)mg/L
 Statistical Method:
 Probit

Historical Mean LC50 (warning limits): 9.95 (7.10, 13.9) mg/L Concentration: 0,8,10,12,15,20 mg/L

Test Method EPS 1/RM/13
Method Deviations: None

<u>Note:</u> The results contained in this report refer only to the testing of the sample submitted. This report may not be reproduced, except in its

entirety, without the written approval of the laboratory.

Analyst : Cara Shurgot, Dustin Banks, Kyle Monaghan

Verified By: Dustin Banks, Team Lead, Bioassay Date: Oct 02, 2019 03:05 PM



## Toxicity testing on samples WT4468-1645-18 and WT4469-1645-18B

Collected October 22, 2019

**Final Report** 

November 28, 2019

Submitted to: Bureau Veritas Laboratories

Burnaby, BC



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APPENDIX B – Chain-of-Custody Form



#### **SIGNATURE PAGE**

Report By:

Yvonne Lam, B.Sc.

**Laboratory Biologist** 

Reviewed By:

Armando Tang, R.P.Bio

1. Tag

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**

	WT4468-1645-18
Sample ID	WT4469-1645-18B
Sample collection date	October 22, 2019
Sample receipt date	October 23, 2019
Sample receipt temperature	3.9°C and 2.0°C
Test type	7-d rainbow trout (Oncorhynchus mykiss) embryo viability

## **Summary of Results**

	Mean ± SD							
Endpoint	Control	WT4468-1645-18	Control	WT4469-1645-18B				
Embryo viability (%)	84.3 ± 1.9	79.1 ± 21.8	82.8 ± 12.9	82.5 ± 15.2				

SD = Standard Deviation



#### 1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability toxicity tests for Bureau Veritas Laboratories on two samples identified as WT4468-1645-18 and WT4469-1645-18B. The samples were collected on October 22, 2019 and delivered to the Nautilus Environmental laboratory in Burnaby, BC on October 23, 2019. The samples were each transported in four 10-L plastic containers and received at temperatures of 3.9 and  $2.0^{\circ}$ C. The samples were stored in the dark at  $4 \pm 2^{\circ}$ C prior to testing.

This report describes the results of the toxicity tests. Copies of raw laboratory data sheets and statistical analysis 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 toxicity test is summarized in Table 1, and followed procedures described by Environment Canada (1998) and modified by Canaria *et al.* (1999). Statistical analyses were performed using CETIS (Tidepool Scientific Software, 2013).



# Table 1. Summary of test conditions: 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability single concentration 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 100% (undiluted) sample, 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  $14 \pm 1^{\circ}$ C Feeding None Light intensity Dark

Photoperiod 24 hours dark

Aeration Continuous gentle aeration

Temperature, dissolved oxygen, pH and conductivity measured

Test measurements 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 endpoints Embryo viability

Test acceptability criteria for controls Embryo viability ≥70%

Reference toxicant Sodium dodecyl sulphate (SDS)



#### 3.0 RESULTS

Results of the rainbow trout embryo viability toxicity tests conducted on samples WT4468-1645-18 and WT4469-1645-18B are summarized in Table 2. There was no statistically significant difference relative to their respective laboratory control for either sample, with embryo viability in both samples and all test treatments  $\geq$ 79% (v/v).

Table 2. Results: 7-d rainbow trout (*Oncorhynchus mykiss*) embryo viability single concentration test.

Concentration	Embryo Viability (%) (Mean ± SD)								
(% v/v)	WT4468-1645-18	WT4469-1645-18B							
Laboratory Control	84.3 ± 1.9	82.8 ± 12.9							
100	79.1 ± 21.8	82.5 ± 15.2							

SD = Standard Deviation

The samples were not statistically significantly different relative to their respective Laboratory Control



#### 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 this test is best described by the standard deviations around the means and/or confidence limits around the point estimates.

There were deviations from the test methodology. 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 method. 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 deviation did not seem to affect the results of the tests and control criterion was met at the end of the exposure.

Results of the reference toxicant test conducted during the testing program are summarized in Table 3. Results for this test fell within the acceptable range for organism performance of 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 was performed under the same conditions as those used for the samples.

Table 3. Reference toxicant test results.

Test Species	Endpoint	Historical Mean (2 SD Range)	CV (%)	Test Date
O. mykiss	Viability (EC50): 4.5 mg/L SDS	4.2 (2.1 – 8.4) mg/L SDS	36	October 23, 2019

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 1.9.4 Tidepool Scientific Software, McKinleyville, CA. 255 pp.



**APPENDIX A – Oncorhynchus mykiss** Toxicity Test Data

### Rainbow Trout Early Life Stage Summary Sheet

Client:	Bureau Verifas	Start Date/Time	e: October 23,20	19 @ 1550k
Work Order No.:	192142	Test Species:	Oncorhynchus mykis	SS
Sample Informat	tion:			
Sample ID; Sample Date: Date Received: Sample Volume:	WT4468-1645-18 OCTOBER 22, 2019 OCTOBER 23, 2019 4 x 10L			
Dilution Water:				
Type: Hardness (193/1. C Alkalinity (mg/L C		ap Water	÷	
Test Organism I	nformation:			
Batch No.: Source: Loading Density:	Lyndon Fish Hate 0.96 gl	heries, Now Dur	as, 00	
Number of male be Number of female Sperm motility che	broodstock used:	†	pound microscope	
SDS Reference	Toxicant Results:			
Reference Toxica Stock Solution ID: Date Initiated: 7-d EC50 (95% C	19501 October 23 201	9 1 mg/L 803		
Reference Toxica Reference Toxica	int Mean and Range: 4.7	7	MC BDS	
Test Results:	ENDYO VICE (THE (THE) (MEN I ZSD)  EC25 % (W/V) (95% CL)  EC60 % (W/V) (95% CL)	COMPLO WITH	ample ID 469-1645-18 1 = 21.8	
Reviewed by:	W	Date rev	viewed: NN 21	, 219

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

Client:
Sample ID:
Work Order #:

BUNCU Neritas WT4468-1645-18 Start Date & Time: October 23, 2019@1550h Stop Date & Time: October 30, 2019@0950h CER#: 10

Test Species: Oncorhynchus mykiss

CONT	Days													
Concentration	0		1		2		3		4		5		6	7
( ob v(v)	init.	new	old	new	old	new	old	new	old	new	old	new	old	final
Temperature (°C)	140	140	14.0	140	140	140	14,2	(4)	140	145	17.0	140	140	140
DO (mg/L)	102	10,6	9.8	101	(5/5)	[01	4.9:3	10,0	94	98	9.7	90	90	(0,0)
pH	7.1	6.8	7.1	6,9	7.1	6.9	69	6/9	3º	68	7,0	68	69	6.8
Cond. (µS/cm)	30	3-	>	2	5	3 :	5	3.3		25	3	3<	>	31
Initials	المالالا		Oper-		4-	A				(u		la.v	W	1360

(00		Days													
Concentration	0		1		2		3	4		5		6		7	
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final	
Temperature (*C)	140	14,3	14/3	14,0	120	140	140	1410	140	14.0	14.0	140	140	140	
DO (mg/L)	99	10,1	9.3	10,0	19,3	10,0	9.8	120	9.8	97	10.0	98	\$ 99	[0,0	
pН	7.4	713	7.4	7.3	74	72	73	72	74	74	7.6	7.7	7.6	15	
Cond. (µS/cm)	717	7	31	70	lo	74	1	7	43	7	25	7	27	731	
Initials	inn	P	/		A-	4		pu		V	w	f.a.	MA.	Light	

	Days														
Concentration	0	1		2	2	3	3	- 4	1		5	6	5	7	
	init.	new	old	final											
Temperature (°C)															
DO (mg/L)															
pН															
Cond. (µS/cm)															
Initials															

		Days													
Concentration	0		1		2		5	4		5		6		7	
	init.	new	old	new	old	new	old	new	old	new	old	new	old	final	
Temperature (°C)															
DO (mg/L)															
рН			1 7 1		-	1		1 2 1 6		-			1 10 14		
Cond. (µS/cm)															
Initials															

Thermometer: (Sta) O DO meter/probe: 213 / 213 pH meter/probe: 215 / 2/3 Conductivity meter/probe: 2(3 / 2/3

	Control	100%-	
Hardness*	8	150	
Alkalinity*	8	38	
* mg/L as CaCO3			

Analysts: AWD, YUC

Reviewed by: No. 20, 20

Sample Description:

clear, no colour, no odour no perfluiates

Comments:

### **Embryo Toxicity Test Daily Mortality**

Client:

BURGU VES

Start Date & Time: October 23, 2019 @1550h

Sample ID: Work Order #:

WT4468-1645-18 192142

Stop Date & Time: 00% 50, 2019 @ 09504

Test Species: Oncorhynchus mykiss

Concentration	Rep	I	Day of	Test	- No.	of Mor	talitie	s	Total	Total	Total No.	Total
(% 0/0)		1	2	3	4	5	6	7	Dead Eggs	Undeveloped	Embryo	Exposed
control	1	3	0	0	0	0	1	0	1	3 2	27	31
	2	1		1	1		2		3	2	25	20
	3	0				1	0		Ô	S		30
	4					2	3		5	0	25	30
100	1			= =			0	1			28	30 30
	2						0	1	2	2	26	30
	3					0	1	1	2	14	14	30
	4	4	al al	V	-	1	2	0	3	0	26	30 29
	1											/
	2											
	3											
	4		11									
	1											
	2											
	3			_								
	4											
	1											
	2						-					
	3		-	_	-	_						
	4			_	_							
	1											20
	2											
	3											
	4			-	-							
	1		-		-	-						
	3		-		-	-	_					
	4	-	-	-	-	-						
	1				-							
	2		-	-	-	-	_					
	3		-	-	-	-	_					
	4		-	-	+							
ech Initials		Wax	Que	1	N	(ww	_	(NWA	aw	(1/4/	www	war

	4											
Tech Initials		Nav	Ow	b	14	Sw	سعالا	(NWA-	um	(IW)	im	NW
Comments:												
Reviewed by:			6	:				Date n	eviewed:	NW.Z	1, 2019	
Version 1 0 leaved	luna 26	Some										

### **CETIS Summary Report**

Report Date:

18 Nov-19 18:50 (p 1 of 1)

	MOUNT SHEET				Test	Code/ID:		92142a / 0	01-0900-9781		
Salmonid Em	bryo Survival ar	nd Deve	lopment T	est					Na	utilus Env	ironmental
Batch ID:	15-4943-2006		Test Type:	Development			Anal	vst: Yv	onne Lam		
Start Date:	23 Oct-19 15:50		Protocol:	EC/EPS 1/RM/	28		Dilu		schlorinated T		
Ending Date:	30 Oct-19 09:50		Species:	Oncorhynchus	mykiss		Brin				
Test Length:	6d 18h		Taxon:	Actinopterygii			Sou	rce: Ly	ndon Fish Ha	tcheries	Age:
Sample ID:	10-2289-6264		Code:	WT4468-1645-	18		Proj	ect:			
Sample Date:	22 Oct-19 05:02		Material:	Effluent			Sour		ireau Veritas	Laboratorio	
	23 Oct-19 09:43		CAS (PC):				Stati		T4468-1645-		
Sample Age:			Client:	Bureau Veritas	Laboratorie	5	outu	on. w	14400-1040-	10	
Single Compa	arison Summary	0									_
Analysis ID	Endpoint		Comp	parison Method			P-Value	Compa	ison Result		5
14-1544-5687	Proportion Norm	al	Fisher	r Exact Test			0.1854		assed proport		-
Proportion No	ormal Summary										
Conc-%	Code	Count	t Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.842	7 0.8128	0.8727	0.8333	0.8710	0.0094	0.0188	2.23%	0.00%
100		4	0.790	8 0.4442	1.0000	0.4667	0.9333	0.1089	0.2178	27.54%	6.16%
Proportion No	ormal Detail										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N.	0.8710	0.833	3 0.8333	0.8333						
100		0.9333	0.866	7 0,4667	0.8966						
Proportion No	ormal Binomials										
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4						
0	N	27/31	25/30	25/30	25/30						
100		28/30	26/30	14/30	26/29						

#### **CETIS Analytical Report**

Report Date: Test Code/ID: 18 Nov-19 18:50 (p 1 of 1) 192142a / 01-0900-9781

Age:

Salmonid En	nbryo Survival and D	evelopment T	est		Nautilus Environmental
Analysis ID:	14-1544-5687	Endpoint	Proportion Normal	CETIS Version:	CETISv1.9.4
Analyzed:	18 Nov-19 18:49	Analysis:	Single 2x2 Contingency Table	Status Level:	1

Batch ID: 15-4943-2008 Test Type: Development Analyst: Yvonne Lam

Start Date: 23 Oct-19 15:50 Protocol: EC/EPS 1/RM/28 Diluent: Dechlorinated Tap Water

Ending Date: 30 Oct-19 09:50 Species: Oncorhynchus mykiss Brine:

Test Length: 6d 18h Taxono Actinopterygli Source: Lyndon Fish Hatcheries

Sample ID: 10-2289-6264 Code: WT4468-1645-18 Project:

Sample Date: 22 Oct-19 05:02 Material: Effluent Source: Bureau Veritas Laboratories

Receipt Date: 23 Oct-19 09:43 CAS (PC): Station: WT4468-1645-18

Sample Age: 35h (3.9 °C) Client Saresy Veston Laboratories

Data Transform	Alt Hyp	Comparison Result
Untransformed	C > T	100% passed proportion normal

#### **Fisher Exact Test**

Negative Control 100 0.1854 Exact 0.1854 Non-Significant Effect	Control vs	Group	Test Stat	P-Type	P-Value	Decision(a:5%)
Printed Indian Street Control Street		100	0.1854	Exact	0.1854	Non-Significant Effect

#### **Data Summary**

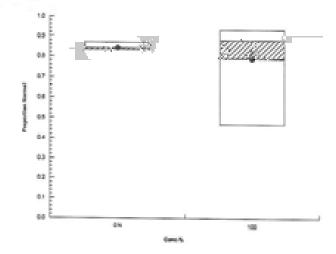
Conc-%	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	N	102	19	121	0.843	0.157	-6.72%
100		94	25	119	0.7899	0.2101	0.0%

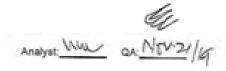
#### Proportion Normal Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	
0	N	0.8710	0.8333	0.8333	0.8333	
100		0.9333	0.8667	0.4667	0.8966	

#### Proportion Normal Binomials

#### Graphics





## Rainbow Trout Early Life Stage Summary Sheet

Client:	Bureau verites	Start Date/Tim	e: October 23, 2019
Work Order No.:	192142	Test Species:	Oncorhynchus mykiss
Sample Informat	tion:		
Sample ID: Sample Date: Date Received: Sample Volume:	00000 23, 2019 00000 23, 2019		
Dilution Water:			
Type: Hardness (mg/L 0 Alkalinity (mg/L 0		ap Water	
Test Organism I	nformation:		
Batch No.: Source: Loading Density:	LYADON FISH HATCLE 0.96 SIL	ries, New Soudi	., 610
Number of male to Number of female Sperm motility ch	broodstock used:	4	npound microscope
SDS Reference	Toxicant Results:		
Reference Toxics Stock Solution ID Date Initiated: 7-d EC50 (95% C	19801 October 23, 20	19 19 102 SDS	
Reference Toxica Reference Toxica	ant Mean and Range: 4:2 ant CV (%): 3	- (2.1-8.4) ng	<u>1L SDS</u>
Test Results:	EC25 % (V/V) (95% CL)	CONTRO / WITH	Sample ID 469-1645183 5 ± 15.2
Reviewed by:		Date re	eviewed:NN '21, 2019

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

Client:	_ Kw	REN	Ver	195	A 52		Start Date & Time: <u>00 ものない 23, 2019 € 1</u> Stop Date & Time: 0でもいと 30, 2014(0)							
Sample ID: Vork Order #:	WT4469-1645-1875						Sto				الهور	30,	20(9(	2 09
		-174						Test Sp			ynchus	mykiss	ΛĒ	
CONT							Da	ays						
Concentration			1		2		3	1	4		5		6	7
(*/. V/v)	init	new	old	new	old	new	old	new	old	new	old	new	old	fina
Temperature (°C)	140	14,2	14.2	14,5	143	140	14,0	120	14,0	195	140	(Yo	140	140
DO (mg/L)	102	101	9.9	101	100	10,0	,9,0	190	9.9	9.2	58	9	191	100
pH	71	6.8	7.1	6,9.	2-1	6.8	(49	6.9	6.7	4.8	69	68	68	68
Cond. (µS/cm)	30		3 3	3	د	7-		>	1		3		0	31
Initials	Uhu 4 a						4	4			V-	_	uw	Wien
1-0	T						D-							
(DD) Concentration	0		1		2		3	lys	4		5		6	7
	init.	new	old	new	old	new	old	new	old	new	old	new	old	fina
Temperature (°C)	13.5	14/0	14/0	140	14,0	14,0	1410	1412	14,2	140	(40	14.0	الإي	14.0
DO (mg/L)	10.1	12.0	9,9	10,0	9.9	pis		10,0		9.5	99	9.8	101	100
pH	74	24	7.5	7.1		72		3-1	22	73	76	100	679	7.6
Cond. (µS/cm)	716 236		741		745			43		24	1.5 7	25	132	
Initials	inul o			4		0	.,		and a	1	lm	ii.i-		
					4	- OF		- 5-					1100	Mari
	Days													
Concentration	0	1 1		2			3		4		5	l i	6	7
	init.	new	old	new	old	new	old	new	old	new	old	new	old	fina
Temperature (°C)								P			7 11.5			
DO (mg/L)														
pН														
Cond. (µS/cm)														
Initials														
	_													-
Company	-							ays						
Concentration	0		1		2		3		1		5	- 1	6	7
Temperature (°C)	init.	new	old	new	old	new	old	new	old	new	old	new	old	fina
DO (mg/L)														
pH														
Cond. (µS/cm)														
Initials														
hermometer: (ON_O	DO mete	en/probe:	5131	2(3	pH mete	n/probe:	70	5/2	Conduc	tivity me	ter/prob	e: <u>213</u>	13	
	Con	ntrol	ips	37-			1			Analys	ts:	AWD,	Was -	
Hardness*		3	147	2						. amang a		any	Laftera.	
Alkalinity*		8	3	9	/					Reviewed by:		t	1111	
mg/L as CaCO3	0 20 /							Date reviewed: Nin 21,001						

Version 1.2 Issued July 19, 2017

Comments:

### Embryo Toxicity Test Daily Mortality

Concentration	Rep		Day of	Test	- No.	of Mor	talitie	s	Total	Total	Total No.	Total Exposed
(% 4/4)		1	2	3	4	5	6	7	Dead Eggs	Undeveloped	Embryo	
Contro	. 1	0	0	0	0	O	0			4	25	30
	2	1	-1	1	T	1	1	1	L	1	27	30
	3					11	(	1	2	a	20	31
	4						1	0	1	1	28	30 31 30
1,06	1						D		ی	3	27	30 30
	2			-   -			0		0	o o	30	30
	3	V				Y	Q		0	10	70	30
	4	Ì	1	1		1	2	V	4	9	22	30
	1								5- 5- No			
	2											
	3											
	4											
	1											
	2											
	3											
	4											
	1											
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	2											
	3							15-34	T	1.000		
	4											
ech Initials		W	ihu	5	~	in	luc	Sans	im	(im	um	in

Comments:			
_			
Reviewed by:	<u> </u>	Date reviewed:	Nn.21,2019

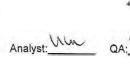
<b>CETIS Summary F</b>	Report
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Report Date:

18 Nov-19 18:51 (p 1 of 1) 192142b / 16-3318-1142

	Test Code/ID:	192
Salmonid Embryo-Alevin Survival and Development Test		Mount

				Test	Code	ID:	192142b / 16-3318-1142						
Salmonid Em	bryo-Alevin Sur	rival an	d Develop	ment Test						Nautilus Envi	ronmental		
Batch ID:	06-1126-3867	7	est Type:	Development			Anal	yst:	Yvonne Lan	ń			
Start Date:	23 Oct-19 15:50	F	rotocol:	EC/EPS 1/RM/	28		Dillu	ent	Dechlorinate	ed Tap Water			
Ending Date:	30 Oct-19 09:50	2	Species:	Oncorhynchus	mykiss		Brin	00					
Test Length:	6d 18h	7	faxon:	Actinopterygli			Sour	rce:	Lyndon Fish	Age:			
Sample ID:	10-7588-4037	(	Code:	WT4469-1645-		Proj							
Sample Date:	22 Oct-19 05:00		Material:	Effluent			Sour	rce:	Bureau Veri	5			
Receipt Date:	23 Oct-19 09:43		CAS (PC):				Stati	on:	WT4469-16	69-1645-18B			
Sample Age:	35h (2 °C)	(	Client:	Bureau Veritas	5								
Single Comp	arison Summary												
Analysis ID	Endpoint		Comp	parison Method	P-Value	alue Comparison Result							
02-3915-6312	Proportion Norm	al	Equal	Variance t Two-	Sample Tes	it.	0.5465	100% passed proportion normal					
Proportion N	ormal Summary												
Conc-%	Code Count Mean 95% LCL 95% UC		95% UCL	Min	Max	Std I	Err Std D	ev CV%	%Effect				
0	N	4	0.828	0 0.6231	1.0000	0.6452	0.9333	0.064	44 0.128	8 15.55%	0.00%		
100		41	0.825	0 0.5824	1.0000 0.6667		1.0000	0.079	62 0.152	4 18.48%	0.36%		
Proportion N	ormal Detail												
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4								
Ō	N	0.8333	0.900	0 0.6452	0.9333								
100		0.9000	1.000	0 0.5567	0.7333								
Proportion N	ormal Binomials												
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4								
0	N	25/30	27/30	20/31	28/30								
100		27/30	30/30	20/30	22/30								



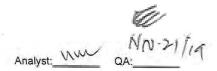
001-406-995-6

### **CETIS Analytical Report**

Report Date: Test Code/ID:

18 Nov-19 18:51 (p 1 of 2) 192142b / 16-3318-1142

Salmonid Em	bryo-Alev	rin Survival a	nd Develop	ment	Test						Na	utilus Env	ironmenta	
Analysis ID:	02-3915-	6312	Endpoint:	Prop	portion Norr	nal			CETI	S Version:	CETISv1	.9.4		
Analyzed:	18 Nov-1	9 18:51	Analysis:	Para	ametric-Two	o Sample			Statu	s Level:	1			
Batch ID:	06-1126-	3867	Test Type:	Dev	elopment				Anal	vst: Yvo	nne Lam			
Start Date:	23 Oct-1	9 15:50	Protocol:	EC/	EPS 1/RM/	28			Dilue	£ (C) (C)	chlorinated T	ap Water		
Ending Date:	30 Oct-19	9 09:50	Species:	Onc	orhynchus	mykiss			Bring		3.70,0447.800.4	- C1-3-00		
Test Length:	6d 18h		Taxon:		nopterygii				Sour	roe: Lyn	Lyndon Fish Hatcheries			
Sample ID:	10-7588-	4037	Code:	WT-	4469-1645-	18			Proje	ect				
Sample Date:	22 Oct-1	Oct-19 05:00 Material: Effluent							Sour	ce: Bur	reau Veritas Laboratories			
Receipt Date:	23 Oct-1	19 09:43 CAS (PC):							Stati	on: WT	4469-1645-	188		
Sample Age:	35h (2 °C	<b>(2)</b>	Client:	Bure	eau Veritas	Laborat	ories	-						
Data Transfo	rm	Alt I	typ						Comparis	ion Result			PMSD	
Angular (Com	ected)	C > 1	ſ						100% pas		28.26%			
Equal Varian	ce t Two-5	Sample Test												
		one-%	Test:	Stat	Critical	MSD	DF	P-Type	P-Value	Decision	(a:5%)			
Negative Cont	trol 10	10	-0.12	17	1.943	0.281	6	CDF	0.5465		ficant Effec			
ANOVA Table	)													
Source	Su	m Squares	Mean	Squ	are	DF		F Stat	P-Value	Decision	(au5%)			
Between	0.0	006173	0.000	6173		1		0.01482	0.9071		ificant Effec	t		
Error	0.2	50009	0.041	6681		6								
Total 0.250626						7								
Distributiona	Tests													
Attribute	Tes	st				Test S	tat	Critical	P-Value	Decision	(a::1%)			
Variances	ariances Variance Ratio F Test				2.046		47.47	0.5716	Equal Variances					
Distribution Shapiro-Wilk W Normality Test					0.9375	5	0.6451	0.5867	Normal D	istribution				
Proportion N	ormal Sur	mmary												
Conc-%	Co	de Cou	nt Mean		95% LCL	95% U	CL	Median	Min	Max	Std Err	CV%	%Effect	
0	N	4	0.828	0	0.6231	1.0000	)	0.8667	0.6452	0.9333	0.0644	15.55%	0.00%	
100		4	0.825	0	0.5824	1.0000	)	0.8167	0.6667	1.0000	0.0762	18.48%	0.36%	
Angular (Cor	rected) Tr	ansformed S	ummary											
Conc-%	Co	de Cou	nt Mean	ı	95% LCL	95% U	CL	Median	Min	Max	Std Err	CV%	%Effect	
O.	N	4	2.45		0.8972	4.454		1.2	0.9327	1.31	0.08271	14.26%	0.00%	
_		-	1.16		A-6617	1.424								
_		7	1.178		0.8015	1.554		1.139	0.9553	1.479	0.1183	20.08%	-1.51%	
100		4								1.479	0.1183	20.08%	-1.51%	
0 100 Proportion N Conc-%		4 tail	1.178							1.479	0.1183	20.08%	-1.51%	
100 Proportion N	ormal Det	4 tail	1.178 1 Rep 2	2	0.8015	1.554	_			1.479	0.1183	20.08%	-1.51%	
100 Proportion N Conc-%	ormal Det	4 tail de Rep	1.178 1 Rep 3 33 0.900	0	0.8015 Rep 3	1.554 Rep 4	)			1.479	0.1183	20.08%	-1.51%	
Proportion N Conc-% 0	ormal Det Co N	4 tail de Rep 0.83: 0.90	1.178 1 Rep 2 33 0.900 00 1.000	0	0.8015 Rep 3 0.6452	Rep 4 0.9333	)			1.479	0.1183	20.08%	-1.51%	
Proportion N Conc-% 0 100 Angular (Con	ormal Det Co N	4 tail de Rep 0.83: 0.90: ansformed D	1.178 1 Rep 3 33 0.900 00 1.000 etail	0	0.8015 Rep 3 0.6452	Rep 4 0.9333	)			1.479	0.1183	20.08%	-1.51%	
Proportion N Conc-% 0 100 Angular (Con Conc-%	ormal Det Co N	4 tail de Rep 0.83: 0.90: ansformed D	1.178 1 Rep 3 33 0.900 00 1.000 etail	0 0	0.8015 Rep 3 0.6452 0.6667	Rep 4 0.9333 0.7333	)			1.479	0.1183	20.08%	-1.51%	
Proportion N Conc-% 0 100 Angular (Con Conc-%	ormal Det Co N rected) Tr	4 tail de Rep 0.830 0.900 ansformed D de Rep	1.178 1 Rep 2 33 0.900 00 1.000 etail 1 Rep 2	0 0	0.8015 Rep 3 0.6452 0.6667	Rep 4 0.9333 0.7333 Rep 4	)			1.479	0.1183	20.08%	-1.51%	
Proportion N Conc-% 0 100 Angular (Con Conc-% 0	ormal Det Co N rected) Tr Co	4 tail de Rep 0.83: 0.90: ansformed D de Rep 1.15	1.178 1 Rep 2 33 0.900 00 1.000 etail 1 Rep 2	0 0	0.8015 Rep 3 0.6452 0.6667 Rep 3 0.9327	Rep 4 0.9333 0.7333 Rep 4 1.31	)			1.479	0.1183	20.08%	-1.51%	
Proportion N Conc-% 0 100 Angular (Con Conc-% 0 100 Proportion N	ormal Det Co N rected) Tr Co	4 tail de Rep 0.83: 0.90: ansformed D de Rep 1.15 1.24: comials	1.178  1 Rep 3  33 0.900  1.000  etail  1 Rep 3  1.249  1.479	0 0	0.8015  Rep 3 0.6452 0.6667  Rep 3 0.9327 0.9553	Rep 4 0.9333 0.7333 Rep 4 1.31 1.028	3			1.479	0.1183	20.08%	-1.51%	
100 Proportion N Conc-%	ormal Det Co N rected) Tr Co N	4 tail de Rep 0.83: 0.90: ansformed D de Rep 1.15 1.24: comials	1.178  1 Rep : 33 0.900 30 1.000  etail  1 Rep : 1.249 1.479	0 0	0.8015 Rep 3 0.6452 0.6667 Rep 3 0.9327	Rep 4 0.9333 0.7333 Rep 4 1.31	3			1.479	0.1183	20.08%	-1.51%	



### **CETIS Analytical Report**

18 Nov-19 18:51

Report Date: Test Code/ID: 18 Nov-19 18:51 (p 2 of 2) 1921426 / 16-3318-1142

Salmonid Embryo-Alevin Survival and Development Test Nautilus Environmental Analysis ID: 02-3915-6312 Endpoint: Proportion Normal **CETIS Version:** 

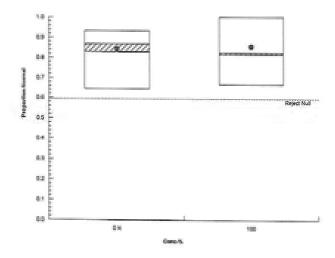
Parametric-Two Sample

Status Level:

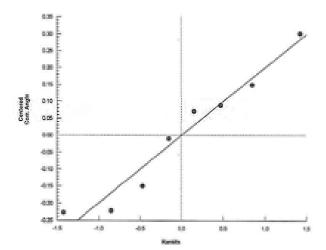
CETISv1.9.4

#### Graphics

Analyzed:



Analysis:





**APPENDIX B – Chain-of-Custody Form** 

Sent To: Nautilus Environmental

8664 Commerce Court Burnaby, BC, V5A 4N7

Tel: (604) 420-8773

### CHAIN OF CUSTODY RECORD FOR SUBCONTRACTED WORK

Page 01 of 01

COC # B990653-ENAU-01-01

REPORT INFORMATION											ANALYSI	REQUE	STED					
Company: Bureau Veritas Laboratories								ш					i i		111	1		
Address: 9331 - 48th Street, Edmonton, Alberta, T6B 2R4								2										
Contact Name: Geraldlyn Gouthro											1				1			
Ema	ail:	geraldlyn.gouthro@bv	labs.com, cus	stomerservice@b	/labs.com			Embryc Subcontract				1		1.4				
Pho	ne:	(780) 577-7173						Embr				П						
BV L	abs Project #:	B990653						Day										
#	SAMPLE ID		MA	DATE SAMPLED (YYYY/MM/DD	TIME SAMPLED ) (HH:MM)	SAMPLER INITIALS	# CONT.	Rainbow Trout 7									ADDITIONAL SAN	IPLE INFORMATION
1	WT4468-1645	18		W 2019/10/22	05:02	SS2	4	х				le-			3.9	(P: 01)		
2	WT4469-1645-	-18B		W 2019/10/22	05:00	SS2	4	Х						- 1 7	2.0	(P: 01)		
3																		
4																		
5											$\top$		$\Box$					
6																		
7								6	(4		-	1		E-0.F3				
8							(C)	42										
9								21			Y							
10								19			T			- 1				
REG	ULATORY CRITER	IA		SPECIAL INST	RUCTIONS										7			TURNAROUND TIME
1	1 1045-18 Ave	Grab ×101. Far 18 rived oct. 23111	Q 1430h	Please inform **Please retu						d for the	request	ed test(:	s).					Rush Required 2019/11/26
coo	LER ID:		COOLER ID:						COOLER ID:								Date Required	
Custo	ody Seal Present ody Seal Intact ing Media Present	YES NO Temp:	1: 3: 3	Custody Seal Pr Custody Seal In Cooling Media	tact	YES NO	Ten (°C		Ι	-1/-	Custo	dy Seal Pi dy Seal In ng Media	tact	YES N	O Tem (°C		4	Please inform us if rush charges will be incurred.
RELI	NOUISHED BY: 5	CN & PRINT)	D	ATE: (YYYY/MM/DIO)	TIME: (		-		Ys (Secien e						россум		TIME: (HEMM)	
1. Oa	wild Tidman	W	20	215/10/22	15:29		i. 79	1/20me	N.	m/1/h	Seed.			00	23/	19	9,43	
2.	Ċ						2.								- 10			



**END OF REPORT**