Screening tests on groundwater samples

Environmental Assessment Program (EAP)

Samples collected December 13, 17 and 18, 2018

Final Report

January 16, 2018

Submitted to: **Washington Department of Ecology**
PO BOX 47600
Olympia, WA

8664 Commerce Court, Burnaby, BC V5A 4N7

SUMMARY

This is a summary of the test data for the 7-d *Ceriodaphnia dubia* survival and reproduction tests conducted on three samples of ground water submitted by the Washington State Department of Ecology. The purpose of these tests was to determine if one or more of the groundwater samples could be used as a negative site control for further testing on groundwater samples contaminated with weathered diesel. The three samples were tested at 100% (v/v) concentration. The sample information and test type are presented in Table 1.

Table 1. Sample Information and Test Type

Sample ID	KING-MW11, CNG-MW2, BFT-MW8
Sample collection date	December 13, 17 and 18, 2018
Sample receipt date	December 19, 2018
Sample receipt temperature	5.3°C
Test types	*Ceriodaphnia dubia* survival and reproduction

Table 2 provides a summary of the toxicity test results.

Table 2. Summary of Results

Endpoint	Control	CNG-MW2[1]	BFT-MW8[1]	KING-MW11
Survival (%)	100	100	100	100
Reproduction (Mean ± SD)	24.4 ± 2.8	19.0 ± 2.5*	15.0 ± 7.4*	18.7 ± 6.6*

SD = Standard Deviation, LC = Lethal Concentration, IC = Inhibition Concentration
* = indicates that reproduction is significantly lower than control, [1]= sample had hydrocarbon odour

Two of the samples submitted, CNG-MW2 and BFT-MW8 had strong hydrocarbon odours that were readily apparent to the biologists conducing the tests. None of the samples showed any acute toxicity to the *Ceriodaphnia dubia*. Reproduction was statistically significantly lower in all of the negative control groundwater samples relative to the laboratory control. However, the reproduction mean in all three samples met Environment Canada-specified passing control health criteria of ≥15 young per surviving female with three broods.

Report By:
Kania Lywe, B.Sc.
Laboratory Biologist

Reviewed By:
Curtis Eickhoff , Ph.D.
Senior Reviewer

1 – Emma Marus, B.Sc. on behalf of Kania Lywe

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.

Ceriodaphnia dubia Summary Sheet

Client: WDOE
Work Order No.: 18251

Start Date/Time: Dec 21/18 @ 1400h
Set up by: KL/JB

Sample Information:

Sample ID: CNG-MW2, BFT-MW8, KNG-MW11 ~~various (see below)~~ ^K
Sample Date: Dec 17, Dec 18, Dec 17, 2018
Date Received: Dec 19/18
Sample Volume: 1 x 1 gal

Test Validity Criteria:

1) Mean survival of first generation controls is ≥80 %
2) At least 60% of controls have produced three broods within 8 days
3) An average of ≥15 live young produced per surviving female in the control solutions during the first three broods.
4) Invalid if ephippia observed in any control solution at any time.

WQ Ranges:

T (°C) = 25 ± 1; DO (mg/L) = 3.3 to 8.4 ; pH = 6.0 to 8.5

Test Organism Information:

Broodstock No.: BB121218A & BB121118
Age of young (Day 0): <24-h (within ~~12-h~~ ^K 8-h)
Avg No. young in first 3 broods of previous 7 d: 31
Mortality (%) in previous 7 d: 13
Individual female # used ≥8 young on test day: 21-28, 30 & 10.

NaCl Reference Toxicant Results:

Reference Toxicant ID: Cd204
Stock Solution ID: ~~Dec~~ 18NaCl (100 g/L NaCl)
Date Initiated: Dec 12/18

7-d LC50 (95% CL): 2.0 (1.9 - 2.3) g/L NaCL
7-d IC50 (95% CL): 1.5 (1.1 - 1.8) g/L NaCL

7-d LC50 Reference Toxicant Mean and Historical Range: 2.0 (1.8 - 2.2) g/L NaCL CV (%): 5
7-d IC50 Reference Toxicant Mean and Historical Range: 1.3 (0.9 - 1.9) g/L NaCL CV (%): 18

Test Results:

	Survival (%)	Reproduction (Mean ± SD)
Negative Control	100	24.4 ± 2.8
CNG-MW2	100	19.0* ± 2.5
BFT-MW8	100	15.0* ± 7.4
KNG-MW11	100	18.7* ± 6.6
		±
		±
		±
		±
		±

*Indicates that reproduction is significantly lower than control.

Reviewed by: [signature]
Date reviewed: Jan. 14, 2019

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: WSDE
Sample ID: see below (various)
Work Order #: 18225)

Start Date & Time: Dec 21/18 @ 1400h
Stop Date & Time: Dec 27/18 @ 1600h
CER #: 4
Test Species: *Ceriodaphnia dubia*

% (v/v) Concentration (control)	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	25.0	25.0	24.0	25.0	24.0	25.0	25.0	24.0	24.0	25.0	25.0	25.0		
DO (mg/L)	8.0	7.9	8.0	7.4	8.0	7.9	8.0	7.6	8.1	7.0	7.9	6.9		
pH	8.2	7.8	8.2	7.8	8.1	7.8	8.1	7.8	8.1	8.1	8.3	8.1		
Cond. (µS/cm)	211	218		219		221		220		223	~~225~~	217		
Initials	VL	A		A		JB		EMM		VL		JB		

① KING MW-2 100 ~~MW-8~~

~~LSC MW-8~~ Concentration	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.6	25.0	24.0	25.0	24.0	24.0	24.0	25.0	24.0	25.0		
DO (mg/L)	7.9	7.3	8.0	7.4	8.0	7.9	7.5	7.6	7.3	6.6	7.2	7.1		
pH	8.0	8.0	7.9	8.2	8.2	8.4	7.8	7.9	7.8	~~8.5~~	8.0	8.4		
Cond. (µS/cm)	1477	1498		1500		1508		1510		1502		1439		
Initials	VL	A		A		JB		EMM		VL		JB		

8.5 VL

② ~~BSL MW-8~~ 100 ~~LSC MW-8~~

Concentration	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.2	24.6	25.0	24.0	25.0	24.0	24.0	24.0	25.0	24.0	25.0		
DO (mg/L)	8.1	7.9	8.0	7.2	7.9	7.9	7.7	7.6	7.6	6.6	7.3	6.9		
pH	7.9	8.2	7.9	8.1	7.8	8.1	7.8	8.1	7.8	8.2	7.9	8.1		
Cond. (µS/cm)	960	982		990		991		990		984		944		
Initials	VL	A		A		JB		EMM		VL		JB		

③ KING MW-11 100 ~~100 MW-11~~

Concentration	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.5	25.0	24.0	25.0	24.0	24.0	24.0	25.0	24.0	25.0		
DO (mg/L)	7.9	7.4	8.0	7.3	8.2	7.9	7.6	7.8	7.6	6.6	7.3	6.9		
pH	8.1	8.0	8.4	8.1	7.9	8.5	8.1	8.4	8.0	8.3	8.2	8.3		
Cond. (µS/cm)	314	325		326		335		336		321		315		
Initials	VL	A		A		JB		EMM		VL		JB		

Thermometer: 4 **DO meter/probe:** 1 (MW-2) / 1 **pH meter/probe:** 1 (MW-8) / 1 (MW-11) **Conductivity meter/probe:** 1 / 1

	Control	(100%)	(100%)	~~(100%)~~ (100%)
Hardness*	100	520	384	148
Alkalinity*	93	282	~~216~~ 216	144

Analysts: VL, AWD, EMM, JB

Reviewed by: [signature]

Date reviewed: Jan. 11, 2019

* mg/L as $CaCO_3$

Sample Description: ③ light brown, slightly turbid, odourless, some particulates. ① light yellow, ~~colour~~ clear, hydrocarbon smell, some particulates. ② clear, colourless, hydrocarbon odour, some particulates

Comments: **Broodboard Used:** BS121218A (#21-28, 30) & BS121118 (#10) test initiated using blocking method ~~and~~ VL

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: WDSE
Sample ID: See below (various)
Work Order: 18225

Start Date & Time: Dec 21/18 @ 1400h
Stop Date & Time: Dec 27/18 @ 1600h.
Set up by: KL/JB

06/06 CNG-MW2

Concentration: control

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
3	3	4	3	2	4	3	4	3	X[3]	4	JB
4	6	✓	✓	6	✓	✓	6	✓	6	✓	JM
5	✓	8	10	8	9	10	✓	10	✓	11	K
6	11	10	12	13	12	14	14	14	11	14	JB
7											
8								K			
Total	20	22	25	23	25	27	24	~~24~~ 27 K	22	29	K

Concentration: (100) ~~6.25 MW-11~~ KING-MW11

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
3	✓	2	4	✓	2	2	2	4	✓	2	JB
4	2	✓	✓	5	✓	✓	✓	✓	6	✓	JM
5	✓	10	9	8	2	9	✓	7	6	8	K
6	13	14	12	5	11	12	5	✓	14	11	JB
7											
8											
Total	15	26	25	18	15	23	7	11	26	21	K

Concentration: (100) ~~3.12 MW-8~~ CPT-MW8

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
3	3	✓	2	2	✓	✓	✓	✓	✓	✓	JB
4	6	2	✓	✓	✓	4	4	3	3	2	JM
5	4	7	7	5	8	✓	✓	6	7	3	K
6	✓	11	13	✓	10	+JB	✓	11	13	14	JB
7											
8		K								K	
Total	13	~~9~~ 20 K	22	7	18	4	4	20	23	~~2~~ 19 K	K

Concentration: 12.5

Days	A	B	C	D	E	F	G	H	I	J	Init
1											
2											
3											
4											
5											
6											
7											
8											
Total											

Concentration: 25

Days	A	B	C	D	E	F	G	H	I	J	Init
1											
2											
3											
4											
5											
6											
7											
8											
Total											

Concentration: 50

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
3											
4											
5											
6											
7											
8											
Total											

Concentration: 100

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
3											
4											
5											
6											
7											
8											
Total											

Concentration:

Days	A	B	C	D	E	F	G	H	I	J	Init
1											
2											
3											
4											
5											
6											
7											
8											
Total											

Notes: X = mortality.

Comments: 1. Total # Young only based on the first 3 Broods. Fourth and subsequent broods not included in total count.

2. Ephippia present in Controls (Y) (N)?

Reviewed by: [signature]

Date reviewed: Jan. 11, 2019

CETIS Summary Report

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

Batch ID:	03-7602-2964	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Kania Lywe		
Start Date:	21 Dec-18 14:00	**Protocol:**	Washington DOE (2016)	**Diluent:**			
Ending Date:	27 Dec-18 16:00	**Species:**	Ceriodaphnia dubia	**Brine:**			
Test Length:	6d 2h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:**	<24

Sample Code	Sample ID	Sample Date	Receipt Date	Sample Age	Client Name	Project
Control	08-0445-7004	21 Dec-18	21 Dec-18	14h	WDOE	
CNG-MW2	19-9747-9816	17 Dec-18 12:30	19 Dec-18 11:30	4d 2h (5.3 °C)		
BFT-MW8	08-7433-8747	18 Dec-18	19 Dec-18 11:30	86h (5.3 °C)		
KING-MW11	01-8042-6889	13 Dec-18 12:00	19 Dec-18 11:30	8d 2h (5.3 °C)		

Sample Code	Material Type	Sample Source	Station Location	Lat/Long
Control	control	WDOE	Control	
CNG-MW2	Water Sample	WDOE	CNG-MW2	
BFT-MW8	Water Sample	WDOE	BFT-MW8	
KING-MW11	Water Sample	WDOE	KING-MW11	

SIngle Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	BFT-MW8 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	CNG-MW2 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	Control passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	KING-MW11 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	Control passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	KING-MW11 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	BFT-MW8 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	CNG-MW2 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	KING-MW11 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	BFT-MW8 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	CNG-MW2 passed 6d survival rate	1
07-2523-3397	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	1.0000	Control passed 6d survival rate	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	1.1E-04	BFT-MW8 failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	1.1E-04	CNG-MW2 failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	1.1E-04	Control failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	1.1E-04	KING-MW11 failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	7.4E-04	KING-MW11 failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	7.4E-04	BFT-MW8 failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	7.4E-04	CNG-MW2 failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	7.4E-04	Control failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	0.0108	Control failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	0.0108	KING-MW11 failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	0.0108	BFT-MW8 failed reproduction	1
04-8232-6638	Reproduction	Equal Variance t Two-Sample Test	0.0108	CNG-MW2 failed reproduction	1

6d Survival Rate Summary

Sample	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
Control	LC	10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
CNG-MW2		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
BFT-MW8		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
KING-MW11		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

Reproduction Summary

Sample	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
Control	LC	10	24.4	22.43	26.37	20	29	0.8718	2.757	11.30%	0.00%
CNG-MW2		10	19	17.22	20.78	15	22	0.7888	2.494	13.13%	22.13%
BFT-MW8		10	15	9.679	20.32	4	23	2.352	7.439	49.59%	38.52%
KING-MW11		10	18.7	13.97	23.43	7	26	2.093	6.617	35.39%	23.36%

Analyst: _____ QA: JM-14/19

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

6d Survival Rate Detail

Sample	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
Control	LC	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
CNG-MW2		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
BFT-MW8		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
KING-MW11		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Reproduction Detail

Sample	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
Control	LC	20	22	25	23	25	27	24	27	22	29
CNG-MW2		21	21	17	16	18	21	15	18	21	22
BFT-MW8		13	20	22	7	18	4	4	20	23	19
KING-MW11		15	26	25	18	15	23	7	11	26	21

6d Survival Rate Binomials

Sample	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
Control	LC	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
CNG-MW2		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
BFT-MW8		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
KING-MW11		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

CETIS Analytical Report

Test Code/ID: 182251 / 13-6046-6519

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

Analysis ID:	07-2523-3397	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.9.4
Analyzed:	04 Jan-19 17:11	**Analysis:**	STP 2xK Contingency Tables	**Status Level:**	1
Batch ID:	03-7602-2964	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Kania Lywe
Start Date:	21 Dec-18 14:00	**Protocol:**	Washington DOE (2016)	**Diluent:**	
Ending Date:	27 Dec-18 16:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Test Length:	6d 2h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture **Age:** <24

Sample Code	Sample ID	Sample Date	Receipt Date	Sample Age	Client Name	Project
Control	08-0445-7004	21 Dec-18	21 Dec-18	14h	WDOE	
CNG-MW2	19-9747-9816	17 Dec-18 12:30	19 Dec-18 11:30	4d 2h (5.3 °C)		
BFT-MW8	08-7433-8747	18 Dec-18	19 Dec-18 11:30	86h (5.3 °C)		
KING-MW11	01-8042-6889	13 Dec-18 12:00	19 Dec-18 11:30	8d 2h (5.3 °C)		

Sample Code	Material Type	Sample Source	Station Location	Lat/Long
Control	control	WDOE	Control	
CNG-MW2	Water Sample	WDOE	CNG-MW2	
BFT-MW8	Water Sample	WDOE	BFT-MW8	
KING-MW11	Water Sample	WDOE	KING-MW11	

Fisher Exact/Bonferroni-Holm Test

Sample I	vs	Sample II	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		CNG-MW2	1.0000	Exact	1.0000	Non-Significant Effect
		BFT-MW8	1.0000	Exact	1.0000	Non-Significant Effect
		KING-MW11	1.0000	Exact	1.0000	Non-Significant Effect

Data Summary

Conc-%	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
Control	LC	10	0	10	1	0	0.0%
CNG-MW2		10	0	10	1	0	0.0%
BFT-MW8		10	0	10	1	0	0.0%
KING-MW11		10	0	10	1	0	0.0%

6d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
Control	LC	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
CNG-MW2		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
BFT-MW8		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
KING-MW11		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

6d Survival Rate Binomials

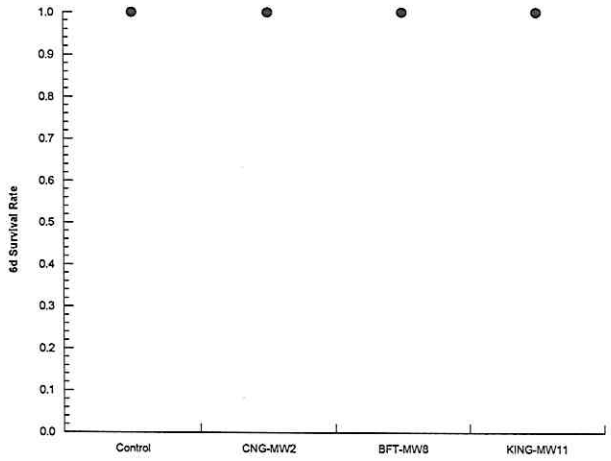
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
Control	LC	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
CNG-MW2		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
BFT-MW8		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
KING-MW11		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

Analyst: QA: 1/19

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

Analysis ID:	07-2523-3397	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.9.4
Analyzed:	04 Jan-19 17:11	**Analysis:**	STP 2xK Contingency Tables	**Status Level:**	1

Graphics



CETIS Analytical Report

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

Analysis ID:	04-8232-6638	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.4
Analyzed:	14 Jan-19 15:11	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	03-7602-2964	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Kania Lywe
Start Date:	21 Dec-18 14:00	**Protocol:**	Washington DOE (2016)	**Diluent:**	
Ending Date:	27 Dec-18 16:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Test Length:	6d 2h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture **Age:** <24

Sample Code	Sample ID	Sample Date	Receipt Date	Sample Age	Client Name	Project
Control	08-0445-7004	21 Dec-18	21 Dec-18	14h	WDOE	
CNG-MW2	19-9747-9816	17 Dec-18 12:30	19 Dec-18 11:30	4d 2h (5.3 °C)		
BFT-MW8	08-7433-8747	18 Dec-18	19 Dec-18 11:30	86h (5.3 °C)		
KING-MW11	01-8042-6889	13 Dec-18 12:00	19 Dec-18 11:30	8d 2h (5.3 °C)		

Sample Code	Material Type	Sample Source	Station Location	Lat/Long
Control	control	WDOE	Control	
CNG-MW2	Water Sample	WDOE	CNG-MW2	
BFT-MW8	Water Sample	WDOE	BFT-MW8	
KING-MW11	Water Sample	WDOE	KING-MW11	

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	CNG-MW2 failed reproduction	16.11%
		BFT-MW8 failed reproduction	16.11%
		KING-MW11 failed reproduction	16.11%

Equal Variance t Two-Sample Test

Sample I	vs	Sample II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Lab Control		CNG-MW2*	4.593	1.734	2.039	18	CDF	1.1E-04	Significant Effect
		BFT-MW8*	3.747	1.734	4.35	18	CDF	7.4E-04	Significant Effect
		KING-MW11*	2.514	1.734	3.931	18	CDF	0.0108	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	449.475	149.825	3	5.306	0.0039	Significant Effect
Error	1016.5	28.2361	36			
Total	1465.98		39			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	14.74	11.34	0.0021	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9512	0.9236	0.0834	Normal Distribution

Reproduction Summary

Sample	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	LC	10	24.4	22.43	26.37	24.5	20	29	0.8718	11.30%	0.00%
CNG-MW2		10	19	17.22	20.78	19.5	15	22	0.7888	13.13%	22.13%
BFT-MW8		10	15	9.679	20.32	18.5	4	23	2.352	49.59%	38.52%
KING-MW11		10	18.7	13.97	23.43	19.5	7	26	2.093	35.39%	23.36%

Reproduction Detail

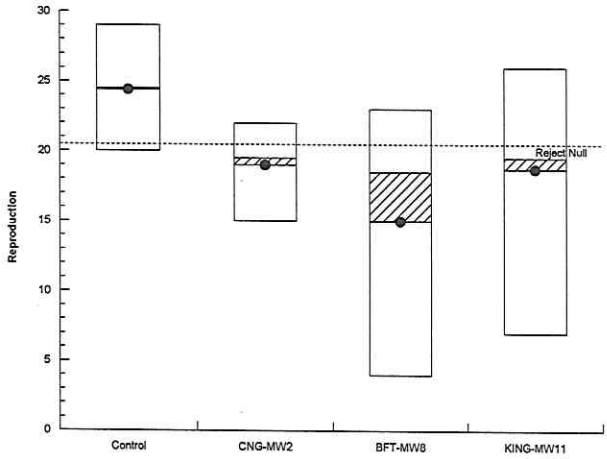
Sample	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
Control	LC	20	22	25	23	25	27	24	27	22	29
CNG-MW2		21	21	17	16	18	21	15	18	21	22
BFT-MW8		13	20	22	7	18	4	4	20	23	19
KING-MW11		15	26	25	18	15	23	7	11	26	21

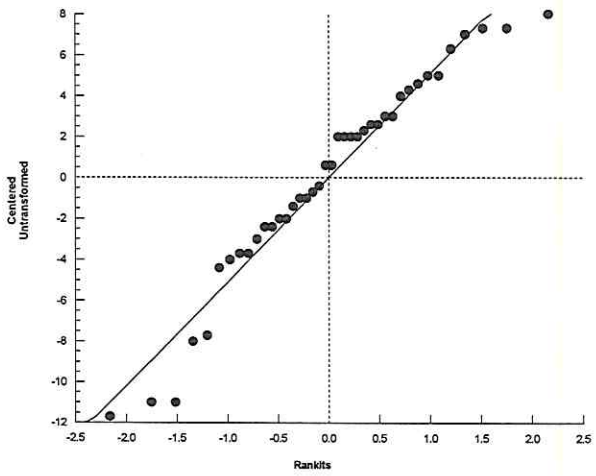
Analyst: _____ QA: 1/14/19

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

Analysis ID:	04-8232-6638	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.4
Analyzed:	14 Jan-19 15:11	**Analysis:**	Parametric-Two Sample	**Status Level:**	1

Graphics





Analyst: _____ QA: 1m 1/4/19

Client: WDOE

W.O.#: 182251

Hardness and Alkalinity Datasheet

	Alkalinity						Hardness			
Sample ID	Subsample Date	Date Measured	Sample Volume (mL)	(mL) 0.02N HCL/H_2SO_4 used to pH 4.5	(mL) of 0.02N HCL/H_2SO_4 used to pH 4.2	Total Alkalinity ($mg/LCaCO_3$)	Sample Volume (mL)	Volume of 0.01M EDTA Used (mL)	Total Hardness ($mg/L\ CaCO_3$)	Technician
CNG-MW-2	Dec21/18	~~Dec 27/18~~ 12/18	50	14.3	14.5	282	50	26.0	520	BW
BFT-MW-8	Dec21/18	↓	50	10.9	11.0	216	50	19.2	384	BW
KING-MW-11	Dec21/18	↓	50	7.3	7.4	144	50	7.4	148	BW
20% pemb	Dec21/18	Dec21/18	~~50~~ 100	9.5	9.7	93	50	5.0	100	VL

Notes:

Reviewed by: [signature]

Date Reviewed: Jan. 11, 2019

TESTING LOCATION (Please Circle)

NAUTILUS ENVIRONMENTAL

Burnaby
8664 Commerce Court
Burnaby, British Columbia, Canada
V5A 4N7
Phone 604.420.8773

Calgary
#4, 6125 12 Street SE
Calgary, Alberta, Canada
T2H 2K1
Phone 403.253.7121

Chain of Custody

Date 12/18 Page 1 of 1

Report to:

Company	WA DEPT OF ECOLOGY
Address	PO BOX 47600
City/State/Zip	OLYMPIA WA 98502
Contact	WILL HEBBS
Phone	360-407-7512
Email	whob461@ecy.wa.gov

Invoice To:

Company	ECOLOGY - MANCHESTER LAB
Address	7411 BEACH DR E
City/State/Zip	PORT ORCHARD WA 98366
Contact	NANCY ROSENBOWER
Phone	360-871-8827
Email	nros461@ecy.wa.gov
PO No.	

Sample Collection By:

Sample Type: Grab ○ OR Composite ○

	SAMPLE ID	DATE	TIME	MATRIX	# OF CONTAINERS AND VOLUME (e.g. 1 x 20 L)	COMMENTS	ANALYSES REQUIRED: CERODAPHNIA TRIAL	ANALYSES REQUIRED: FOOT OF COLUMBIA	Receipt Temperature (°C)
1	KING-MW11	12/13	12:00	water	1 - 1gal		✓		5.3
2	CNG-MW2	12/17	12:30	"	"		✓		↓
3	BFT-MW8	12/18	N/A	"	"		✓		
4									
5									
6									
7									
8							22		
9							51		
10							18		

SPECIAL INSTRUCTIONS/COMMENTS (CLIENT)

WILL SEND INFO ON
PH, COND, ORP AND DO

SAMPLE RECEIPT DETAILS (LABORATORY)

1. Total No. of Containers	3	4. Ice Present in Cooler?	(Y) / N
2. Courier	FedEx	5. Seal Present?	Y / (N)
3. Good Condition?	(Y) / N	6. Initials Present on Seal?	Y / (N)

SAMPLE DESCRIPTION AND COMMENTS (LABORATORY)

RELINQUISHED BY (CLIENT)

WILL HEBBS (Printed Name) (Signature)

ECY (Company) 12/18/18 13:00 (Date and Time)

RECEIVED BY (LABORATORY)

Tyrone Hamilton (Printed Name) (Signature)

NorthLus (Company) 19/12/18 @ 11:30 (Date and Time)

Our liability is limited to the cost of the test requested. The test results only relate to the sample as received. No liability in whole or in part is assumed for the collection, handling, or transport of the sample, application or interpretation of the test data or results in part or in whole.

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