APPENDIX J

MEMO

Date: May 11, 2011 **HCP Ref No.:** CZN1682

From: John Wilcockson and Martin Davies

To: David Harpley, CZN

Subject: Prairie Creek Mine – Supplementary whole effluent toxicity testing (Memo 7)

1.0 INTRODUCTION

This document stems from technical discussions between regulators and CZN at Yellowknife on April 12, 2011.

In this memo, updated whole effluent toxicity testing results are presented. On April 28, 2011, new laboratory treated process water and treated mine water samples were prepared. These were submitted to Nautilus Environmental in Burnaby for additional acute *Daphnia magna* testing (Nautilus 2011c, Attachment A) and sub-lethal *Ceriodapnia duba* testing (Nautilus 2011d, Attachment B). Three mixtures of the treated mine water and treated process water were tested:

- A 4:1 (80% treated mine water: 20% treated process water) sample, representing worst case (uncommon) effluent concentrations;
- An 8:1 (71.4% mine, 12.4% process, 16.2% ditch) sample, representing more typical operating conditions; and
- Treated mine water only.

The acute (*D. magna*) and sub-lethal (*C. dubia*) results are discussed separately below. These two species were chosen because whole effluent testing using simulated effluent prepared on January 28, 2011 indicated either inconsistent results (*D. magna*) or toxicity at environmentally relevant concentrations (*C. dubia*) (Nautilus 2011a, Attachment C).

The results of a Toxicity Identification Evaluation (TIE, Nautilus 2011b, Attachment D) performed at the time indicated that the observed toxicity was likely not caused by divalent metals, non-polar organic compounds or strong anions. Instead, the results suggested that the cause of toxicity may have been attributable to the treatment of the simulated effluent.

The remaining species tested indicated no toxicity; there was no acute toxicity of 100% effluent to rainbow trout (96hr-LC50 test), and no sub-lethal toxicity to *Lemna minor (*7-day growth inhibition test, Nautilus 2011a, Attachment C).

2.0 DAPNIA MAGNA

The January 28 2011 *D. magna* test using the worst case (4:1) simulated effluent mixture yielded inconsistent results (Nautilus 2011a, Attachment C). One full-strength sample exhibited toxicity, while another did not. In addition, both the 4:1 and 8:1 mixtures diluted with Prairie Creek water showed increasing mortality at lower concentrations (Nautilus 2011b). This result was both unusual and unexplained. The 8:1 mixture and mine water-only sample did not cause any acute *Daphnia magna* toxicity at full strength.

The new treated process water and treated mine water samples were prepared to be representative of operational mine treatment conditions. The *D. magna* tests (Nautilus 2011c, attached) indicated no toxicity in any of the three mixtures tested above, suggesting that full-strength effluents discharged from the mine are unlikely to cause any acute toxicity.

3.0 CERIODAPHNIA DUBIA

The initial (January 28, 2011) *C. dubia* tests using both the 4:1 and 8:1 simulated effluent mixtures showed no mortalities, but there were effects on reproduction at all dilutions tested, down to and including 5% v/v (Nautilus 2011a, Attachment C). These results indicated that there could be sub-lethal impacts to Prairie Creek organisms downstream of the IDZ in Prairie Creek.

C. dubia toxicity testing with the new laboratory-prepared treated mine water and two simulated effluent mixtures provided the following results:

- There was no mortality seen in any of the tests;
- Treated mine water only (i.e., with no process effluent added) caused no effect on reproduction;
- In the 4:1 mixture (worst case), there was no effect on reproduction at 20% or lower, but a substantial effect at 40% and higher (an IC25 of 23.8 %v/v was calculated); and
- In the 8:1 mixture (typical case), there was no effect on reproduction at 40% and lower, and a substantial effect at 60% and higher (an IC25 of 44.5 %v/v was calculated).

These results indicate an absence of *C. dubia* mortality within the IDZ and absence of sub-lethal toxicological effect at concentrations expected to occur outside the IDZ. Since mixing in the vertical water column occurs very rapidly upon release, and no sub-lethal effects to *C. dubia* were observed below 23.8% v/v, it is likely that there would also be an absence of sub-lethal effect within much of the IDZ.

4.0 CONCLUSION

In conclusion, the compiled toxicity testing results indicate that the treated Prairie Creek mine effluent (process and mine water) will not result in any acute mortality within the IDZ and no sub-lethal effects outside the IDZ. These are prerequisites of establishing a suitable IDZ for a water discharge license. During periods of low winter flows final effluent will consist only of treated mine water, which caused no effect on *C. dubia* at even 100% treated mine water.

5.0 REFERENCES

Nautilus (Nautilus Environmental). 2011a. Toxicity Testing on Synthetic Effluent Samples, Final Toxicity Test Report, April 6, 2011. Report for Hatfield Consultants, North Vancouver, BC.

Nautilus. 2011b. Toxicity Identification Evaluation of Mill Water Sample, Final Report, April 8, 2011. Report for Hatfield Consultants, North Vancouver, BC.

Nautilus. 2011c. Re: Toxicity testing on the samples identified as Mixture 4:1, Mixture 8:1, Mine Water (Collected on April 28, 2011). Report dated May 5, 2011. Report for Hatfield Consultants, North Vancouver, BC.

Nautilus. 2011d. Re: Toxicity testing on prepared effluent samples, final toxicity test report. Report dated May 10, 2011. Report for Hatfield Consultants, North Vancouver, BC.

ATTACHMENTS

Attachment A

April 28, 2011
Whole Effluent Toxicity Testing
D. magna

Nautilus Environmental
8664 Commerce Court, Burnaby, BC V5A 4N7

WO#: 11201

Mr. John Wilcockson
Hatfield Consultants
850 Harbourside Drive
North Vancouver, BC
V7P 0A3

May 5, 2011

Dear Mr. Wilcockson:

Re: Toxicity testing on the samples identified as Mixture 4:1, Mixture 8:1, Mine Water (Collected on April 28, 2011)

Nautilus Environmental is pleased to provide you the results of the 48-h LC50 *Daphnia magna* toxicity tests on the above samples, received on April 28, 2011. Testing was conducted according to Environment Canada and 1/RM/14, (Second Edition, 2000). The results of these tests are provided in the table below and are based on the appended data. All acceptability criteria outlined in the Environment Canada protocol were met.

Table A. Results for the 48-h *D. magna* test.

Sample ID	Collection Date and Time	48-h LC50 (% v/v) [1]
Mixture 4:1	April 28, 2011 @ 1600h	>100
Mixture 8:1	April 28, 2011 @ 1600h	>100
Mine Water	April 28, 2011 @ 1600h	>100

[1] Results relate only to the sample tested.

Please feel free to contact the undersigned at 604-420-8773 should you have any questions or require any additional information.

Yours truly,

Nautilus Environmental

Krysta Banack, B.Sc.
Laboratory Biologist

Daphnia magna Summary Sheet

Client: Hatfield / Canadian Zinc
Work Order No.: 11201

Start Date/Time: April 28/11 @ 1420h
Test Species: *D.magna*
Set up by: KCB

Sample Information:

Sample ID: ~~4:1 Mixture~~ KCB Mixture 4:1
Sample Date: April 28/11
Date Received: April 28/11
Sample Volume: 20L

Test Organism Information:

Broodstock No.: 041311B
Age of young (Day 0): < 24 hours
Avg No. young per brood in previous 7 d: 25
Mortality (%) in previous 7 d: 0
Days to first brood: 10

NaCl Reference Toxicant Results:

Reference Toxicant ID: Dm69
Stock Solution ID: 10Na01
Date Initiated: April 19/11
48-h LC50 (95% CL): 4.2 (3.7-4.8) g/LNaCL

Reference Toxicant Mean (2SD Range): 4.0 (3.6-4.4) g/L NaCL
Reference Toxicant CV (%): 5

Test Results: The 48-h LC50 is >100% (v/v)

Reviewed by: A. Tang
Date reviewed: May 5, 2011

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Hatfield / Canadian Zinc
Sample ID: ~~4:1 Mixture~~ Mixture 4:1
Work Order No.: ~~WA~~ 11301 KLB

Start Date/Time: April 28/11 @ 1420h
No. Organisms/volume: 10/200mL
Test Organism: *D.magna*
Set up by: KCB

DO meter: DO-1 pH meter: pH-1 Conductivity meter: C-1

Concentration	Rep	Number of Live Organisms		No. Immobilized	Temperature (°C)			Dissolved oxygen (mg/L)			pH			Conductivity (µS/cm)	
% (v/v)		24	48	48	0	24	48	0	24	48	0	24	48	0	48
Control	A	10	10	0	20.0	20.0	19.5	8.5		8.4	7.9		7.9	352	369
	B														
	C														
	D														
6.25	A	10	10	0	20.5	20.0	19.5	8.5		8.4	8.4		8.1	512	518
	B														
	C														
	D														
12.5	A	10	10	0	20.5	20.0	19.5	8.5		8.5	8.5		8.3	679	683
	B														
	C														
	D														
25	A	10	10	0	20.5	20.0	19.5	8.5		8.4	8.8		8.5	1002	1010
	B														
	C														
	D														
50	A	10	10	0	21.0	20.0	19.5	8.5		8.4	9.0		8.8	1595	1626
	B														
	C														
	D														
100①	A	10	10	0	21.0	20.0	19.6	8.5		8.4	9.2		8.9	2690	2730
	B														
	C														
	D														
Technician Initials		KLB	~	~	KLB	KLB	~	KLB		~	KLB		~	KCB	~

	Hardness*	Alkalinity*
Conc.	*(mg/L as CaCo3)	
Control (MHW)	98	68
Highest conc.	6050	140

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	21.0		
DO (mg/L)	8.5		
pH	9.2		
Cond (µS/cm)	2690		

Sample Description: clear ① Tested a pH adjusted sample (pH 8.5) in addition to regular sample; survival @ 24h = 100%, @ 48h = 100% survival

Comments: Batch#: 041311B 7-d previous # young/brood: 25 Day of 1st Brood: 10 Previous 7-d % Mortality: 0

Reviewed by: A. Tang Date reviewed: May 5, 2011

Client: Hatfield/Canadian Zinc

W.O.#: 11201

Hardness and Alkalinity Datasheet

	Alkalinity						Hardness			
Sample ID	Sample Date	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
~~4:1 Mixture~~ 4:1 Mixture	Apr 28/11	10①	1.5	1.6	140		10①	6.5	650	KLB

Notes: ①Diluted to 100mL with DI water

Reviewed by: A. Tang

Date Reviewed: May 5, 2011

Daphnia magna Summary Sheet

Client: Hatfield / Canadian Zinc
Work Order No.: 11201

Start Date/Time: April 28/11 @ 1425h
Test Species: *D.magna*
Set up by: KCB

Sample Information:

Sample ID: ~~8:1 Mixture~~ KCB Mixture 8:1
Sample Date: April 28/11
Date Received: April 28/11
Sample Volume: 20L

Test Organism Information:

Broodstock No.: 041311B
Age of young (Day 0): < 24 hours
Avg No. young per brood in previous 7 d: 25
Mortality (%) in previous 7 d: 0
Days to first brood: 10

NaCl Reference Toxicant Results:

Reference Toxicant ID: Dm69
Stock Solution ID: 10NaCl
Date Initiated: April 19/11
48-h LC50 (95% CL): 4.2 (3.7-4.8) g/LNaCL

Reference Toxicant Mean (2SD Range): 4.0 (3.6-4.4) g/L NaCL
Reference Toxicant CV (%): 5

Test Results: The 48-h LC50 is >100% (v/v)

Reviewed by: A. Tong
Date reviewed: May 5, 2011

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Hatfield / Canadian Zinc
Sample ID: 8:1 Mixture
Work Order No.: ~~NHA~~ 11201 KLB

Start Date/Time: April 28/11 @ 1425h
No. Organisms/volume: 10/200mL
Test Organism: *D.magna*
Set up by: KLB

DO meter: DO-1 pH meter: pH-1 Conductivity meter: C-1

Concentration	Rep	Number of Live Organisms		No. Immobilized	Temperature (°C)			Dissolved oxygen (mg/L)			pH			Conductivity (µS/cm)	
% (v/v)		24	48	48	0	24	48	0	24	48	0	24	48	0	48
Control	A	10	10	0	20.0	20.0	19.5	8.5		8.4	7.9		7.9	352	365
	B														
	C														
	D														
6.25	A	10	10	0	20.5	20.0	19.5	8.5		8.5	8.3		8.1	469	480
	B														
	C														
	D														
12.5	A	10	10	0	20.5	20.0	19.5	8.5		8.4	8.5		8.3	586	594
	B														
	C														
	D														
25	A	10	10	0	20.5	20.0	19.5	8.5		8.4	8.7		8.4	817	819
	B														
	C														
	D														
50	A	10	10	0	20.5	20.0	19.5	8.5		8.4	8.9		8.6	1232	1238
	B														
	C														
	D														
100①	A	10	10	0	20.5	20.0	19.6	8.5		8.5	9.1		8.8	1994	2010
	B														
	C														
	D														
Technician Initials		KLB	~	~	KLB	KLB	~	KLB		~	KLB		~	KLB	~

	Hardness*	Alkalinity*
Conc.	*(mg/L as CaCo3)	
Control (MHW)	98	68
Highest conc.	560	130

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	20.5		
DO (mg/L)	8.5		
pH	9.1		
Cond (µS/cm)	1994		

Sample Description: clear ①Tested a pH adjusted sample (pH 8.5) in addition to regular sample; survival @ 24-h = 100% @ 48-h = 100% All survived

Comments: Batch#: 041311B 7-d previous # young/brood: 25 Day of 1st Brood: 10 Previous 7-d % Mortality: 0

Reviewed by: A. Tang Date reviewed: May 5, 2011

Client: Hatfield / Canadian Zinc

W.O.#: 11201

Hardness and Alkalinity Datasheet

	Alkalinity						Hardness			
Sample ID	Sample Date	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
8:1 Mixture	Apr 28/11	10①	1.4	1.5	130		10①	5.6	560	KLB

Notes: ① Diluted to 100mL with DI water

Reviewed by: A. Tang

Date Reviewed: May 5, 2011

Daphnia magna Summary Sheet

Client: Hatfield / Canadian Zinc
Work Order No.: 11201

Start Date/Time: April 28/11 @1415h
Test Species: *D.magna*
Set up by: KLB

Sample Information:

Sample ID: Mine Water
Sample Date: April 28/11
Date Received: April 28/11
Sample Volume: 20L

Test Organism Information:

Broodstock No.: 041311B
Age of young (Day 0): < 24 hours
Avg No. young per brood in previous 7 d: 25
Mortality (%) in previous 7 d: 0
Days to first brood: 10

NaCl Reference Toxicant Results:

Reference Toxicant ID: Dm69
Stock Solution ID: 10NaCl
Date Initiated: April 19/11
48-h LC50 (95% CL): 4.2 (3.7-4.8) g/LNaCL

Reference Toxicant Mean (2SD Range): 4.0 (3.6-4.4) g/L NaCL
Reference Toxicant CV (%): 5

Test Results: The 48-h LC50 is > 100% (v/v)

Reviewed by: A. Tong

Date reviewed: May 5, 2011

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Hatfield / Canadian Zinc
Sample ID: Mine Water
Work Order No.: ~~AHA~~ KLB 11201

Start Date/Time: April 28/11 @ 1415h
No. Organisms/volume: 10/200mL
Test Organism: *D.magna*
Set up by: KLB

DO meter: DO-1 pH meter: pH-1 Conductivity meter: C-1

Concentration	Rep	Number of Live Organisms		No. Immobilized	Temperature (°C)			Dissolved oxygen (mg/L)			pH			Conductivity (µS/cm)	
% v/v		24	48	48	0	24	48	0	24	48	0	24	48	0	48
Control	A	10	10	0	20.0	20.0	19.5	8.5		8.4	7.9		7.9	352	362
	B														
	C														
	D														
6.25	A	10	10	0	20.5	20.0	19.5	8.5		8.5	8.4		8.1	401	418
	B														
	C														
	D														
12.5	A	10	10	0	20.5	20.0	19.5	8.5		8.4	8.6		8.2	454	460
	B														
	C														
	D														
25	A	10	10	0	20.5	20.0	19.5	8.5		8.4	8.8		8.4	549	521
	B														
	C														
	D														
50	A	10	10	0	20.0	20.0	19.5	8.6		8.4	9.1		8.5	735	751
	B														
	C														
	D														
100 ①	A	10	10	0	20.0	20.0	19.5	8.8		8.5	9.2		8.8	1054	1075
	B														
	C														
	D														
Technician Initials		KLB	~	~	KLB	KLB	~	KLB		~	KLB		~	KLB	~

	Hardness*	Alkalinity*
Conc.	*(mg/L as CaCo3)	
Control (MHW)	98	68
Highest conc.	550	140

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	20.0		
DO (mg/L)	8.8		
pH	9.2		
Cond (µS/cm)	1054		

Sample Description: clear ① Tested a pH adjusted sample (pH 8.5) in addition to regular sample. survival @24h = 100% @48h 100% survival

Comments: Batch#: 0413KLB 7-d previous # young/brood: 25 Day of 1st Brood: 9 KLB 10 Previous 7-d % Mortality: 0

Reviewed by: A. Tang Date reviewed: May 5, 2011

Client: Hatfield / Canadian Zinc

W.O.#: 11201

Hardness and Alkalinity Datasheet

	Alkalinity						Hardness			
Sample ID	Sample Date	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
Mine Water	Apr 28/11	10①	1.5	1.6	140		10①	5.5	550	KCB

Notes: ① Diluted to 100mL with DI water

Reviewed by: A. Terry

Date Reviewed: May 5, 2011

Attachment B

April 28, 2011
Whole Effluent Toxicity Testing
C. dubia

Nautilus Environmental

Toxicity Testing on Prepared Effluent Samples

Final Toxicity Test Report

Report date: May 10, 2011

Submitted to:

Hatfield Consultants
North Vancouver, BC

8664 Commerce Court
Burnaby, BC
V5A 4N7

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF APPENDICES

1.0 INTRODUCTION

Nautilus Environmental conducted chronic toxicity tests for Hatfield Consultants on samples identified as Mine Water, Mixture 1 and Mixture 2. The mixtures were prepared in the laboratory on April 28, 2011, from samples identified as Process Water and Mine Water (received on April 28, 2011) and Ditch Water (received in December 2010).

Mixture 1 was a 4:1 combination of Mine Water (80%) and Mill Water (20%) and Mixture 2 was a combination of Mine Water (71.4%), Mill Water (12.4%), and Ditch Water (16.2%). The following toxicity tests were performed on the Mine Water, Mixture 1 and Mixture 2 samples:

- 7-d *Ceriodaphnia dubia* survival and reproduction test

This report describes the results of the toxicity tests. Copies of raw laboratory data sheets and statistical analysis are provided in Appendix A. A copy of 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 for the tests were performed using CETIS (Tidepool Scientific Software, 2009).

2.1 Quality Assurance/Quality Control (QA/QC)

Nautilus follows a comprehensive QA/QC program to ensure that the data generated are of high quality and are scientifically defensible. To meet these objectives, Nautilus has implemented a number of quality control procedures that include the following:

- Negative controls to ensure that appropriate testing performance criteria are met;
- Positive controls to assess the health and sensitivity of the test organisms;
- Use of appropriate species and life stage to meet the study objectives;
- Appropriate number of replicates to allow proper statistical analyses;
- Calibration and proper maintenance of instruments to ensure accurate measurements;
- Proper documentation and recordkeeping to allow traceability of performance;
- Adequate supervision and training of staff to ensure that methods are followed;
- Proper handling and storage of samples to ensure their integrity;
- Procedures in place to address issues that may arise during testing and ensure the implementation of appropriate corrective actions; and
- Rigorous review of data by a Registered Professional Biologist to ensure they are of good quality and scientifically defensible prior to releasing to the client.

Table 1. Summary of test conditions: *Ceriodaphnia dubia* survival and reproduction test.

Test organism	*Ceriodaphnia dubia*
Test organism source	In-house culture
Test organism age	<24 hr old neonates produced within 12 hr
Test type	Static renewal
Test duration	7 ± 1 day
Test chamber	20 mL test tube
Test solution volume	15 mL
Number of replicates	10
Control/dilution water	20% Perrier water (hardness 80-100mg/L $CaCO_3$)
Test solution renewal	Daily
Test temperature	25 ± 1°C
Number of organisms/chamber	1
Feeding	Daily, with 0.1 ml *Pseudokirchneriella subcapitata* and 0.05 mL YCT
Light intensity	100 to 600 lux
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	Environment Canada (2007), EPS 1/RM/21
Test endpoints	Survival and reproduction
Test acceptability criterion for controls	≥80% survival; ≥15 young per surviving control; ≥60% of controls producing three or more broods
Reference Toxicant	Sodium chloride

3.0 RESULTS

There were no effects on survival of *Ceriodaphnia dubia*; the LC50 value for all these samples was >100%. Conversely, effects were observed on reproduction of *C. dubia* in both Mixtures 1 and 2. The IC50 was 28.4% for Mixture 1 and 49.8% for Mixture 2, respectively (Table 2). No adverse effects on reproduction were observed in the toxicity tests using Mine Water.

3.1 Quality Assurance/Quality Control

All the tests reported here met the acceptability criteria for test validity specified in the respective protocol. Water quality parameters measured during the toxicity tests were within acceptable ranges and results of the reference toxicant tests conducted during the testing program were all within the in-house historical mean and range. The reference toxicant test results are summarized in Table 3.

It should be noted that the Ditch water used in Mixture 2 was received in December 2010 and therefore exceeded holding time requirements.

Table 2. Toxicity test results for the *Ceriodaphnia dubia* survival and reproduction tests.

(% v/v)	Mine Water		Mixture 1		Mixture 2	
	Survival (%)	Reproduction (# offspring)	Survival (%)	Reproduction (# offspring)	Survival (%)	Reproduction (# offspring)
Control	100	22.8 ± 3.0	100	26.1 ± 4.3	100	24.2 ± 3.5
5	100	20.9 ± 4.8	100	29.1 ± 5.3	100	29.8 ± 2.7
10	100	25.9 ± 2.8	100	29.3 ± 3.6	100	27.9 ± 4.8
20	90	23.7 ± 9.1	100	28.0 ± 4.7	100	27.6 ± 3.1
40	100	31.3 ± 3.6	100	0.2 ± 0.6	100	27.0 ± 4.6
60	100	22.6 ± 5.5	100	0.0 ± 0.0	100	2.2 ± 2.7
80	100	25.7 ± 3.3	100	0.0 ± 0.0	100	0.6 ± 1.9
100	100	21.9 ± 3.7	90	0.0 ± 0.0	100	0.0 ± 0.0
Test endpoint (% v/v)						
LC50	>100		>100		>100	
IC25 (95% CL)	>100		23.8 (22.4 – 23.9)		44.5 (42.7 – 44.9)	
IC50 (95% CL)	>100		28.4 (27.2 – 28.6)		49.8 (48.3 – 50.4)	

LC = Lethal Concentration.
IC = Inhibition Concentration.
SD = Standard Deviation.
CL = Confidence Limits.

Table 3. Reference toxicant test results.

Test Species	Endpoint	Mean (2SD Range)	CV(%)	Initiation Date
C. dubia	Survival (IC50): 1.7 g/L NaCl	1.8 (1.5-2.3)	11	April 20, 2011
	Reproduction (IC50): 1.1 g/L NaCl	1.2 (0.9-1.4)	12	

4.0 REFERENCES

Environment Canada. 2007. 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. 2009. CETIS comprehensive environmental toxicity information system, version 1.8.0. Tidepool Scientific Software, McKinleyville, CA. 222 pp.

APPENDIX A – *Ceriodaphnia dubia* Toxicity Test Data

Ceriodaphnia dubia Summary Sheet

Client: Hatfield
Work Order No.: 11200

Start Date/Time: April 30, 2011 @ 1130h
Set up by: KLB

Sample Information:

Sample ID: Mine Water
Sample Date: April 28
Date Received: April 28
Sample Volume: 1x20L

Test Organism Information:

Broodstock No.: 041911
Age of young (Day 0): <24-h (within 12-h)
Avg No. young in first 3 broods of previous 7 d: 24
Mortality (%) in previous 7 d: 0.0
Individual female # used ≥8 young on test day: 1, 2, 4, 7, 8, 10, 12, 19, 20

NaCl Reference Toxicant Results:

Reference Toxicant ID: Cd 66
Stock Solution ID: 11NaCl
Date Initiated: April 20/11

7-d LC50 (95% CL): 2.1 (1.7-2.6) g/L NaCL
7-d IC50 (95% CL): 1.2 (1.1-1.5) g/L NaCL

7-d LC50 Reference Toxicant Mean (2SD Range): 1.8 (1.5-2.3) g/L NaCL CV (%): 11
7-d IC50 Reference Toxicant Mean (2SD Range): 1.2 (0.9-1.4) g/L NaCL CV (%): 12

Test Results:

	Survival	Reproduction
LC50 %(v/v) (95% CL)	>100	
IC25 %(v/v) (95% CL)		>100
IC50 %(v/v) (95% CL)		>100

Reviewed by: _____ Date reviewed: _____

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: Hatfield / Canadian Zinc
Sample ID: Mine Water
Work Order #: 11200

Start Date & Time: Apr 30/11 @ 1130h
Stop Date: May 6/11 @ 1400h
Test Species: *Ceriodaphnia dubia*

Concentration: 0% (DJ) / 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)	24.0	24.5	24.0	25.5	24.0	25.5	24.0	25.0	24.0	25.5	24.0	25.5		
DO (mg/L)	8.1	6.8	8.0	7.0	7.9	6.9	7.8	6.7	8.0	6.6	7.8	6.7		
pH	8.1	7.7	8.1	7.9	8.1	7.9	8.1	7.5	8.0	7.7	8.2	7.7		
Cond. (µS/cm)	216	211		216		210		214		210		212		
Initials	~	~		KLB		KLB		KLB		KLB		KLB		

Concentration: 5

	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	24.5	25.0	25.5	24.0	25.5	24.0	25.0	24.0	25.5	24.0	25.5		
DO (mg/L)	8.0	6.9	8.0	7.0	7.7	7.0	8.0	6.7	7.6	6.6	7.8	6.8		
pH	7.9	7.9	8.4	7.9	8.3	7.9	8.3	7.7	8.3	7.7	8.2	7.8		
Cond. (µS/cm)	259	262		264		260		260		265		267		
Initials	~	~		KLB		KLB		KLB		KLB		KLB		

Concentration: 40

	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	24.5	25.0	25.5	24.0	25.5	24.0	25.0	24.0	25.5	24.0	25.5		
DO (mg/L)	8.0	6.8	7.9	7.0	7.8	7.0	7.9	6.7	7.7	6.7	7.9	6.7		
pH	8.6	8.2	8.9	8.1	8.8	8.2	8.7	7.9	8.8	7.9	8.9	7.9		
Cond. (µS/cm)	608	587		582		587		590		588		594		
Initials	~	~		KLB		KLB		KLB		KLB		KLB		

Concentration: 100

	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	24.5	25.0	25.5	24.0	25.5	24.0	25.0	24.0	25.5	24.0	25.5		
DO (mg/L)	7.9	6.9	8.0	6.7	7.9	7.0	7.7	6.9	7.7	6.9	8.2	6.6		
pH	9.1	8.5	9.2	8.3	9.1	8.4	9.0	8.4	8.8	8.1	9.1	8.3		
Cond. (µS/cm)	1047	1053		1021		1041		1036		1033		1043		
Initials	~	~		KLB		KLB		KLB		KLB		KLB		

	Control	100% (v/v)		
Hardness*	100	550		
Alkalinity*	84	140		

* mg/L as CaCO3

Analysts: KLB/AWP
Reviewed by: ART
Date reviewed: May 9/11

Sample Description: 1) Clear

Comments: Broodboard Used: 041911

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: Hatfield / Canadian Zinc
Sample ID: Mine Water
Work Order: 11200

Start Date & Time: April 30 / 11 @ 1130h
Stop Date & Time: May 6 / 11 @ 1400h
Set up by: Amo

0/0 (1/1)

Concentration: Control

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	3	6	5	3	3	✓	5	4	✓	3	KLB
5	10	13	13	10	12	9	11	9	9	10	KLB
6	9	6	8	12	8	7	10	8	14	8	KLB
7											
8											
Total	22	25	26	25	23	16	26	21	23	21	KLB

Concentration: 5

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	4	✓	✓	✓	✓	✓	✓	~	~	✓	KLB
4	✓	3	5	6	✓	6	3	5	3	3	KLB
5	9	10	10	3	5	4	7	4	9	9	KLB
6	14	12	8	8	6	11	9	9	13	11	KLB
7											
8											
Total	27	25	23	17	11	21	19	18	25	23	KLB

Concentration: 10

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	~	~	~	~	~	~	~	KLB
4	5	4	4	4	3	4	3	4	3	3	KLB
5	11	9	8	9	6	12	11	9	10	11	KLB
6	15	13	13	10	13	14	10	14	13	11	KLB
7											
8											
Total	31	26	25	23	22	30	24	27	26	25	KLB

Concentration: 20

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	KLB
4	5	5	4	✓	|	✓	4	3	4	✓	KLB
5	13	11	8	5	|	8	9	9	8	6	KLB
6	15	15	14	15	|	16	14	15	14	17	KLB
7					|						
8					|						
Total	33	31	26	20	0*	24	27	27	26	23	KLB

Concentration: 40

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	5	6	6	6	4	6	6	3	5	5	KLB
5	14	13	10	11	9	10	13	9	9	11	KLB
6	16	17	15	14	15	15	16	13	14	17	KLB
7											
8											
Total	35	36	31	31	28	31	35	25	28	33	KLB

Concentration: 60

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	5	3	3	✓	✓	2	✓	✓	2	4	KLB
5	12	9	7	3	6	11	6	4	9	10	KLB
6	15	12	12	12	10	15	14	14	13	13	KLB
7											
8											
Total	32	24	22	15	16	28	20	18	24	27	KLB

Concentration: 80

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	3	5	2	3	2	6	5	✓	3	4	KLB
5	10	12	8	9	10	9	9	7	10	7	KLB
6	12	13	12	15	14	15	13	12	13	14	KLB
7											
8											
Total	25	30	22	27	26	30	27	19	26	25	KLB

Concentration: 100

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	4	3	4	4	4	4	3	3	3	3	KLB
5	✓	9	7	6	7	11	8	9	9	9	KLB
6	12	13	12	10	12	12	10	12	12	4	KLB
7											
8											
Total	16	25	23	20	23	27	21	24	24	16	KLB

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.

Sample Description: 1)

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

Reviewed by: A. Tang

Date reviewed: May 9, 2011

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID:	10-6497-0093	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:34	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	13-9282-0988	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	
Start Date:	30 Apr-11 11:30	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	
Ending Date:	06 May-11 14:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 3h	**Source:**	In-House Culture	**Age:**	
Sample ID:	02-3579-8115	**Code:**	E0DFE63	**Client:**	Hatfield
Sample Date:	28 Apr-11 16:00	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Apr-11 16:00	**Source:**	Hatfield		
Sample Age:	44h	**Station:**	Mine Water		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.275E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	83.87	47.31	N/A	1.192	N/A	2.114
IC10	94.9	55.93	N/A	1.054	N/A	1.788
IC15	>100	N/A	N/A	<1	N/A	N/A
IC20	>100	N/A	N/A	<1	N/A	N/A
IC25	>100	N/A	N/A	<1	N/A	N/A
IC40	>100	N/A	N/A	<1	N/A	N/A
IC50	>100	N/A	N/A	<1	N/A	N/A

Reproduction Summary

			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	22.8	16	26	0.9638	3.048	13.37%	0.0%
5		10	20.9	11	27	1.509	4.771	22.83%	8.33%
10		10	25.9	22	31	0.9	2.846	10.99%	-13.6%
20		10	23.7	0	33	2.883	9.117	38.47%	-3.95%
40		10	31.3	25	36	1.126	3.561	11.38%	-37.28%
60		10	22.6	15	32	1.733	5.481	24.25%	0.88%
80		10	25.7	19	30	1.055	3.335	12.98%	-12.72%
100		10	21.9	16	27	1.159	3.665	16.74%	3.95%

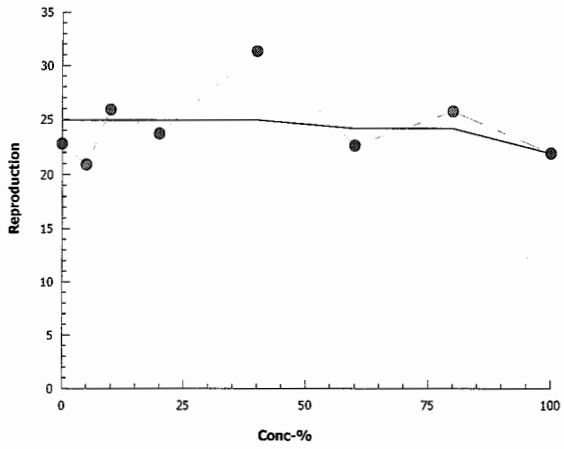
Reproduction Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	22	25	26	25	23	16	26	21	23	21
5		27	25	23	17	11	21	19	18	25	23
10		31	26	25	23	22	30	24	27	26	25
20		33	31	26	20	0	24	27	27	26	23
40		35	36	31	31	28	31	35	25	28	33
60		32	24	22	15	16	28	20	18	24	27
80		25	30	22	27	26	30	27	19	26	25
100		16	25	23	20	23	27	21	24	24	16

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

Analysis ID:	10-6497-0093	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:34	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



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

Analysis ID:	08-0167-1560	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:34	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	13-9282-0988	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	
Start Date:	30 Apr-11 11:30	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	
Ending Date:	06 May-11 14:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 3h	**Source:**	In-House Culture	**Age:**	
Sample ID:	02-3579-8115	**Code:**	E0DFE63	**Client:**	Hatfield
Sample Date:	28 Apr-11 16:00	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Apr-11 16:00	**Source:**	Hatfield		
Sample Age:	44h	**Station:**	Mine Water		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.599E+09	200	Yes	Two-Point Interpolation

Point Estimates

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

6d Survival Rate Summary

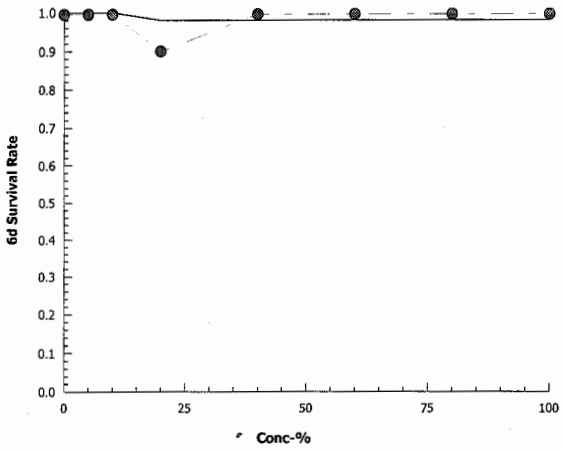
			Calculated Variate(A/B)								
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10
5		10	1	1	1	0	0	0.0%	0.0%	10	10
10		10	1	1	1	0	0	0.0%	0.0%	10	10
20		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10
40		10	1	1	1	0	0	0.0%	0.0%	10	10
60		10	1	1	1	0	0	0.0%	0.0%	10	10
80		10	1	1	1	0	0	0.0%	0.0%	10	10
100		10	1	1	1	0	0	0.0%	0.0%	10	10

6d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	1	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
20		1	1	1	1	0	1	1	1	1	1
40		1	1	1	1	1	1	1	1	1	1
60		1	1	1	1	1	1	1	1	1	1
80		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1

Analysis ID:	08-0167-1560	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:34	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Ceriodaphnia dubia Summary Sheet

Client: Hatfield
Work Order No.: 11200

Start Date/Time: April 29, 2011 @ 1015h
Set up by: KLB

Sample Information:

Sample ID: 4:1 Mixture
Sample Date: April 28, 2011
Date Received: April 28, 2011
Sample Volume: 1x20L

Test Organism Information:

Broodstock No.: 041911
Age of young (Day 0): <24-h (within 12-h)
Avg No. young in first 3 broods of previous 7 d: 24
Mortality (%) in previous 7 d: 0.0
Individual female # used ≥8 young on test day: 2,3,4,5,6,7,8,9,10

NaCl Reference Toxicant Results:

Reference Toxicant ID: Cd66
Stock Solution ID: 11NaCl
Date Initiated: April 20/11

7-d LC50 (95% CL): 2.1 (1.7-2.6) g/L NaCL
7-d IC50 (95% CL): 1.2 (1.1-1.5) g/L NaCL

7-d LC50 Reference Toxicant Mean (2SD Range): 1.8 (1.5-2.3) g/L NaCL CV (%): 11
7-d IC50 Reference Toxicant Mean (2SD Range): 1.2 (0.9-1.4) g/L NaCL CV (%): 12

Test Results:

	Survival	Reproduction
LC50 %(v/v) (95% CL)	>100	
IC25 %(v/v) (95% CL)		23.8 (22.4-23.9)
IC50 %(v/v) (95% CL)		28.4 (27.2-28.6)

Reviewed by: _____ Date reviewed: _____

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: Hatfield
Sample ID: 4:1 Mixture (80% Mine Water, 20% Process Water)
Work Order #: ~~[illegible]~~ 11200 KLB

Start Date & Time: April 29/11 @ 1015h
Stop Date: May 5/11 @ 1400h
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)	24.0	25.0	24.0	25.0	24.0	25.5	24.0	25.0	24.0	25.0	24.0	25.0		
DO (mg/L)	7.8	6.4	8.1	6.6	8.0	7.0	7.9	6.8	7.8	6.7	8.0	6.5		
pH	8.0	7.9	8.1	7.7	8.1	7.8	8.1	7.8	8.1	7.7	8.0	7.9		
Cond. (µS/cm)	215	216		211		210		210		211		223		
Initials	KLB	~		~		KLB		KLB		KLB		KLB		

Concentration: 5	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	25.0	25.0	25.0	25.5	24.0	25.0	24.0	25.0	24.0	25.0		
DO (mg/L)	7.5	6.4	7.5	6.8	7.9	6.5	7.8	6.6	7.9	6.7	7.7	6.3		
pH	8.1	8.2	8.1	7.9	8.5	7.8	8.3	7.8	8.3	7.7	8.0	7.8		
Cond. (µS/cm)	347	355		325		350		350		349		350		
Initials	KLB	~		~		KLB		KLB		KLB		KLB		

Concentration: 40	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	25.0	25.0	25.0	25.5	24.0	25.0	24.0	25.0	24.0	25.0		
DO (mg/L)	7.5	6.5	7.8	6.9	8.0	6.0	7.8	6.3	7.9	6.4	7.8	6.4		
pH	8.7	8.4	8.8	8.1	8.9	8.1	8.7	7.9	8.8	7.9	8.8	8.0		
Cond. (µS/cm)	1274	1300		1273		1277		1302		1301		1304		
Initials	KLB	~		~		KLB		KLB		KLB		KLB		

Concentration: 100	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	25.0	25.0	25.0	25.5	24.0	25.0	24.0	25.0	24.0	25.0		
DO (mg/L)	7.1	6.4	7.8	6.9	8.0	6.6	7.8	6.1	7.9	6.1	7.9	6.4		
pH	9.0	8.6	9.1	8.4	9.1	8.0	9.0	8.3	8.8	8.1	9.1	8.2		
Cond. (µS/cm)	2720	2700		2690		2660		2640		2600		2620		
Initials	KLB	~		~		KLB		KLB		KLB		KLB		

	Control	100% (U/S)		
Hardness*	100	650		
Alkalinity*	84	140		

* mg/L as CaCO3

Analysts: KLB/AWN
Reviewed by: ART
Date reviewed: May 10/11

Sample Description: Clear

Comments: Broodboard Used: 041911

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: Hatfield
Sample ID: 4:1 Mixture (80% Mine Water 20% Process Water)
Work Order: ~~HA~~ KLB 11200

Start Date & Time: April 29/11 @ 1015h
Stop Date & Time: May5/11 @ 1400h
Set up by: KLB

% (v/v)

Days	Concentration: Control										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
3	✓	✓	4	✓	✓	✓	✓	✓	✓	✓	KLB
4	4	3	✓	4	5	6	4	4	6	4	KLB
5	9	7	10	8	9	✓	9	9	10	10	KLB
6	17	13	16	11	13	12	10	13	16	15	KLB
7											
8											
Total	30	23	30	23	27	18	23	26	32	29	KLB

Days	Concentration: 5										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
3	✓	✓	✓	✓	4	✓	✓	✓	✓	✓	KLB
4	4	4	4	5	✓	4	4	5	4	✓	KLB
5	11	10	11	12	10	11	15	10	10	4	KLB
6	13	12	16	16	12	15	17	17	18	13	KLB
7											
8											
Total	28	26	31	33	26	30	36	32	32	17	KLB

Days	Concentration: 10										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
3	✓	✓	✓	✓	3	5	✓	–	✓	–	KLB
4	3	4	4	4	10	✓	4	4	3	4	KLB
5	8	8	6	11	✓	10	9	11	11	8	KLB
6	17	18	12	17	17	20	17	16	16	13	KLB
7											
8											
Total	28	30	22	32	30	35	30	31	30	25	KLB

Days	Concentration: 20										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
3	4	✓	✓	✓	✓	✓	✓	✓	4	✓	KLB
4	✓	4	2	4	5	5	6	4	✓	4	KLB
5	11	11	9	10	14	15	15	8	9	10	KLB
6	15	18	19	16	✓	5	✓	18	18	17	KLB
7											
8											
Total	30	33	30	30	19	25	21	30	31	31	KLB

Days	Concentration: 40										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	✓	2	✓	–	✓	✓	✓	✓	✓	–	KLB
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
7											
8											
Total	0	2	0	0	0	0	0	0	0	0	KLB

Days	Concentration: 60										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
7											
8											
Total	0	0	0	0	0	0	0	0	0	0	KLB

Days	Concentration: 80										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	KLB
4	✓	✓	✓	/	✓	✓	✓	✓	✓	✓	KLB
5	✓	–	–	–	–	–	–	–	–	–	KLB
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
7											
8											
Total	0	0	0	0	0	0	0	0	0	0	KLB

Days	Concentration: 100										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–
3	✓	✓	✓	✓	✓	✓	✓	✓	–	–	KLB
4	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	|	–	–	–	–	–	–	–	–	–	KLB
6	|	✓	✓	✓	–	–	–	–	–	–	KLB
7											
8											
Total	0*	0	0	0	0	0	0	0	0	0	KLB

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

Notes: X = mortality.

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

Reviewed by: L. Tong

Date reviewed: May 10, 2011

Ceriodaphnia 7-d Survival and Reproduction Test

Nautilus Environmental

Analysis ID:	12-4446-6416	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:33	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	10-5584-2752	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	
Start Date:	29 Apr-11 10:15	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	
Ending Date:	05 May-11 14:15	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 4h	**Source:**	In-House Culture	**Age:**	
Sample ID:	20-7896-6862	**Code:**	7BEA844E	**Client:**	Hatfield
Sample Date:	28 Apr-11 16:00	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Apr-11 16:00	**Source:**	Hatfield		
Sample Age:	18h	**Station:**	4:1 Mixture		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2.068E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	20.64	12.68	20.73	4.846	4.824	7.887
IC10	21.38	18.95	21.49	4.677	4.654	5.276
IC15	22.15	20.67	22.27	4.514	4.491	4.838
IC20	22.95	21.5	23.08	4.357	4.333	4.65
IC25	23.78	22.37	23.91	4.205	4.182	4.47
IC40	26.43	25.18	26.6	3.783	3.759	3.972
IC50	28.35	27.24	28.56	3.527	3.502	3.672

Reproduction Summary

			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	26.1	18	32	1.37	4.332	16.6%	0.0%
5		10	29.1	17	36	1.67	5.28	18.14%	-11.49%
10		10	29.3	22	35	1.146	3.622	12.36%	-12.26%
20		10	28	19	33	1.483	4.69	16.75%	-7.28%
40		10	0.2	0	2	0.2	0.6325	316.2%	99.23%
60		10	0	0	0	0	0		100.0%
80		10	0	0	0	0	0		100.0%
100		10	0	0	0	0	0		100.0%

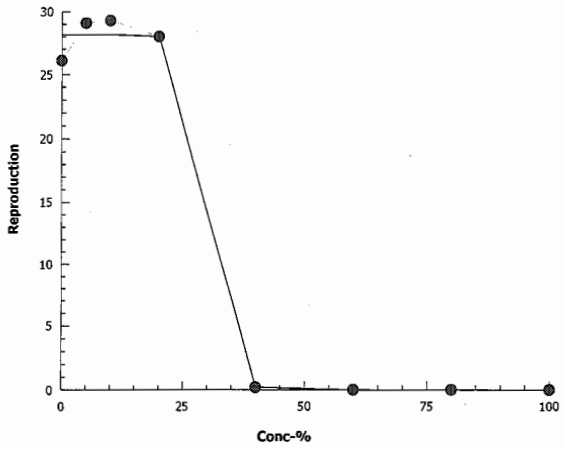
Reproduction Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	30	23	30	23	27	18	23	26	32	29
5		28	26	31	33	26	30	36	32	32	17
10		28	30	22	32	30	35	30	31	30	25
20		30	33	30	30	19	25	21	30	31	31
40		0	2	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

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

Analysis ID:	12-4446-6416	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:33	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



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

Analysis ID:	10-2650-9634	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:33	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	10-5584-2752	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	
Start Date:	29 Apr-11 10:15	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	
Ending Date:	05 May-11 14:15	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 4h	**Source:**	In-House Culture	**Age:**	
Sample ID:	20-7896-6862	**Code:**	7BEA844E	**Client:**	Hatfield
Sample Date:	28 Apr-11 16:00	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Apr-11 16:00	**Source:**	Hatfield		
Sample Age:	18h	**Station:**	4:1 Mixture		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.252E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	89.45	83.03	N/A	1.118	N/A	1.204
EC10	100	86.18	N/A	1	N/A	1.16
EC15	>100	N/A	N/A	<1	N/A	N/A
EC20	>100	N/A	N/A	<1	N/A	N/A
EC25	>100	N/A	N/A	<1	N/A	N/A
EC40	>100	N/A	N/A	<1	N/A	N/A
EC50	>100	N/A	N/A	<1	N/A	N/A

6d Survival Rate Summary

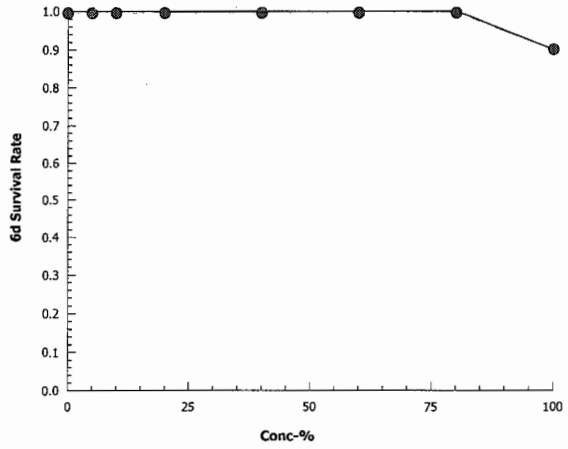
			Calculated Variate(A/B)								
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10
5		10	1	1	1	0	0	0.0%	0.0%	10	10
10		10	1	1	1	0	0	0.0%	0.0%	10	10
20		10	1	1	1	0	0	0.0%	0.0%	10	10
40		10	1	1	1	0	0	0.0%	0.0%	10	10
60		10	1	1	1	0	0	0.0%	0.0%	10	10
80		10	1	1	1	0	0	0.0%	0.0%	10	10
100		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10

6d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	1	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
20		1	1	1	1	1	1	1	1	1	1
40		1	1	1	1	1	1	1	1	1	1
60		1	1	1	1	1	1	1	1	1	1
80		1	1	1	1	1	1	1	1	1	1
100		0	1	1	1	1	1	1	1	1	1

Analysis ID:	10-2650-9634	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:33	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Ceriodaphnia dubia Summary Sheet

Client: Hatfield
Work Order No.: 11200

Start Date/Time: April 29, 2011 @ 1000h
Set up by: KLB

Sample Information:

Sample ID: JAB Mine Water 8:1 Mixture
Sample Date: April 28, 2011
Date Received: April 28, 2011
Sample Volume: 1×20L

Test Organism Information:

Broodstock No.: 041911
Age of young (Day 0): <24-h (within 12-h)
Avg No. young in first 3 broods of previous 7 d: 23
Mortality (%) in previous 7 d: 0.0
Individual female # used ≥8 young on test day: 11, 12, 13, 14, 15, 16, 18, 19, 20

NaCl Reference Toxicant Results:

Reference Toxicant ID: Cd66
Stock Solution ID: 11NaCl
Date Initiated: April 20/11

7-d LC50 (95% CL): 2.1 (1.7-2.6) g/L NaCl
7-d IC50 (95% CL): 1.2 (1.1-1.6) g/L NaCl

7-d LC50 Reference Toxicant Mean (2SD Range): 1.8 (1.5-2.3) g/L NaCl CV (%): 11
7-d IC50 Reference Toxicant Mean (2SD Range): 1.2 (0.9-1.4) g/L NaCl CV (%): 12

Test Results:

	Survival	Reproduction
LC50 %(v/v) (95% CL)	>100	
IC25 %(v/v) (95% CL)		44.5 (42.7-44.9)
IC50 %(v/v) (95% CL)		49.8 (48.2-50.4)

Reviewed by: _____ Date reviewed: _____

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: Hatfield
Start Date & Time: April 29/11 @ ~~1007~~ KLB 1000h
Sample ID: 8:1 mix (71.4% Mine Water 12.4% Process Water 16.2% Ditch Water)
Stop Date: May 5/11 @ 1415h
Work Order #: ~~NTA~~ KLB 11300
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)	24.0	25.0	24.0	25.0	24.0	25.5	24.0	25.5	24.0	25.0	24.0	25.5		
DO (mg/L)	7.8	7.1	8.1	6.7	8.0	7.0	7.9	6.8	7.8	6.5	8.0	6.4		
pH	8.0	7.7	8.1	7.6	8.1	7.9	8.1	7.8	8.1	7.7	8.0	7.7		
Cond. (µS/cm)	215	216		211		210		210		211		210		
Initials	KLB					KLB	KLB	KLB	KLB	KLB	KLB	KLB		

5 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	25.0	25.0	25.0	25.5	24.0	25.5	24.0	25.0	24.0	25.5		
DO (mg/L)	7.8	7.9	7.9	6.8	8.0	7.1	7.8	6.7	8.0	6.6	7.7	6.4		
pH	8.1	8.3	8.3	7.9	8.0	7.9	8.0	7.8	8.0	7.7	8.2	7.7		
Cond. (µS/cm)	320	332		327		310		320		310		309		
Initials	KLB					KLB	KLB	KLB	KLB	KLB	KLB	KLB		

40 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	25.0	25.0	25.0	25.5	24.0	25.5	24.0	25.0	24.0	25.5		
DO (mg/L)	7.8	7.3	7.9	6.8	8.0	6.7	7.9	6.5	8.0	6.4	7.8	6.5		
pH	8.7	8.5	8.6	8.2	8.8	8.0	8.6	8.0	8.7	7.9	8.7	7.9		
Cond. (µS/cm)	993	998		986		977		1010		984		975		
Initials	KLB					KLB	KLB	KLB	KLB	KLB	KLB	KLB		

100 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	25.0	25.0	25.0	25.5	24.0	25.5	24.0	25.0	24.0	25.5		
DO (mg/L)	8.3	7.3	7.9	6.7	8.0	6.8	7.9	6.6	7.9	6.5	7.9	6.6		
pH	9.0	8.6	9.0	8.4	9.1	8.3	8.8	8.2	9.0	8.2	9.0	8.3		
Cond. (µS/cm)	1992	1985		1996		1958		2000		2000		2010		
Initials	KLB					KLB	KLB	KLB	KLB	KLB	KLB	KLB		

	Control	100% (v/v)		
Hardness*	100	560		
Alkalinity*	84	130		

* mg/L as CaCO3

Analysts: KLB/AWD
Reviewed by: ART
Date reviewed: May 10/11

Sample Description: clear

Comments: Broodboard used: 04491

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: Hatfield
Sample ID: (71.4% Mine Water 12.4% Process Water 16.2% Ditch Water) 8:1 Mixture
Work Order: ~~44A~~ 11200 KLB

Start Date & Time: April 29/11 @ 10 00h
Stop Date & Time: May 5/11 @ 1415h
Set up by: KLB

% (v/v)

Days	Concentration: Control										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	2	5	2	6	4	4	4	✓	4	4	KLB
5	6	✓	10	✓	10	9	8	6	8	10	KLB
6	16	15	15	14	16	13	13	13	13	12	KLB
7											
8											
Total	24	20	27	20	30	26	25	19	25	26	KLB

Days	Concentration: 5										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
3	✓	5	3	4	✓	4	4	✓	✓	4	KLB
4	5	✓	✓	8	6	8	✓	3	5	✓	KLB
5	9	10	9	✓	10	✓	10	8	9	10	KLB
6	~~13~~ 16 KLB	16	16	~~18~~ 18 KLB	20	16	13	17	18	14	KLB
7											
8	30 KLB			30 KLB							
Total	27	31	28	28	36	28	27	28	32	28	KLB

Days	Concentration: 10										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
3	✓	✓	6	✓	✓	5	✓	✓	✓	6	KLB
4	2	3	✓	4	4	✓	5	3	4	✓	KLB
5	7	10	10	10	8	7	12	7	10	10	KLB
6	15	18	17	16	14	18	✓	16	16	16	KLB
7											
8											
Total	24	31	33	30	26	30	17	26	30	32	KLB

Days	Concentration: 20										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
3	✓	✓	3	✓	✓	✓	✓	✓	✓	✓	KLB
4	3	4	✓	4	4	4	4	5	3	2	KLB
5	6	10	8	10	8	9	6	10	8	10	KLB
6	13	16	16	16	15	16	13	17	17	16	KLB
7											
8											
Total	22	30	27	30	27	29	23	32	28	28	KLB

Days	Concentration: 40										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
3	✓	✓	✓	✓	4	✓	✓	✓	✓	3	KLB
4	6	✓	2	3	✓	2	9	4	4	✓	KLB
5	7	10	9	10	8	10	✓	11	✓	9	KLB
6	17	15	17	17	15	15	15	16	12	20	KLB
7											
8										32 KLB	
Total	30	25	28	30	27	27	24	31	16	~~28~~ 32	KLB

Days	Concentration: 60										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	7	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
6	7	2	2	2	✓	✓	2	✓	✓	✓	KLB
7											
8											
Total	7	2	2	2	0	0	2	0	7	0	KLB

Days	Concentration: 80										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	6	✓	KLB
5	✓	~	✓	✓	✓	~	✓	~	✓	~	KLB
6	✓	✓	✓	✓	✓	~	~	✓	~	✓	KLB
7											
8											
Total	0	0	0	0	0	0	0	0	6	0	KLB

Days	Concentration: 100										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
7											
8											
Total	0	0	0	0	0	0	0	0	0	0	KLB

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

Notes: X = mortality.

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

Reviewed by: A. Tang
Date reviewed: May 10, 2011

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

Analysis ID:	02-7523-4331	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:32	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	10-2191-8202	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	
Start Date:	29 Apr-11 10:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	
Ending Date:	05 May-11 14:15	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 4h	**Source:**	In-House Culture	**Age:**	
Sample ID:	00-0037-4702	**Code:**	5B7AE	**Client:**	Hatfield
Sample Date:	28 Apr-11 16:00	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Apr-11 16:00	**Source:**	Hatfield		
Sample Age:	18h	**Station:**	8:1 Mixture		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.833E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	40.66	19.88	40.93	2.46	2.443	5.031
IC10	41.58	33.16	41.89	2.405	2.387	3.016
IC15	42.53	40.52	42.86	2.352	2.333	2.468
IC20	43.49	41.66	43.86	2.299	2.28	2.401
IC25	44.48	42.72	44.88	2.248	2.228	2.341
IC40	47.57	45.9	48.11	2.102	2.079	2.179
IC50	49.75	48.25	50.39	2.01	1.985	2.072

Reproduction Summary

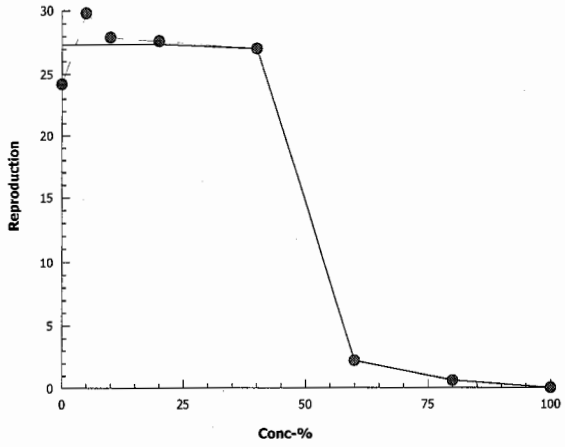
			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	24.2	19	30	1.114	3.521	14.55%	0.0%
5		10	29.8	27	36	0.8537	2.7	9.06%	-23.14%
10		10	27.9	17	33	1.516	4.795	17.19%	-15.29%
20		10	27.6	22	32	0.9798	3.098	11.23%	-14.05%
40		10	27	16	32	1.468	4.643	17.2%	-11.57%
60		10	2.2	0	7	0.8537	2.7	122.7%	90.91%
80		10	0.6	0	6	0.6	1.897	316.2%	97.52%
100		10	0	0	0	0	0		100.0%

Reproduction Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	24	20	27	20	30	26	25	19	25	26
5		30	31	28	30	36	28	27	28	32	28
10		24	31	33	30	26	30	17	26	30	32
20		22	30	27	30	27	29	23	32	28	28
40		30	25	28	30	27	27	24	31	16	32
60		7	2	2	2	0	0	2	0	7	0
80		0	0	0	0	0	0	0	0	6	0
100		0	0	0	0	0	0	0	0	0	0

Analysis ID:	02-7523-4331	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:32	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



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

Analysis ID:	10-8727-1085	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:32	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	10-2191-8202	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	
Start Date:	29 Apr-11 10:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	
Ending Date:	05 May-11 14:15	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 4h	**Source:**	In-House Culture	**Age:**	
Sample ID:	00-0037-4702	**Code:**	5B7AE	**Client:**	Hatfield
Sample Date:	28 Apr-11 16:00	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Apr-11 16:00	**Source:**	Hatfield		
Sample Age:	18h	**Station:**	8:1 Mixture		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.259E+09	200	Yes	Two-Point Interpolation

Point Estimates

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

6d Survival Rate Summary

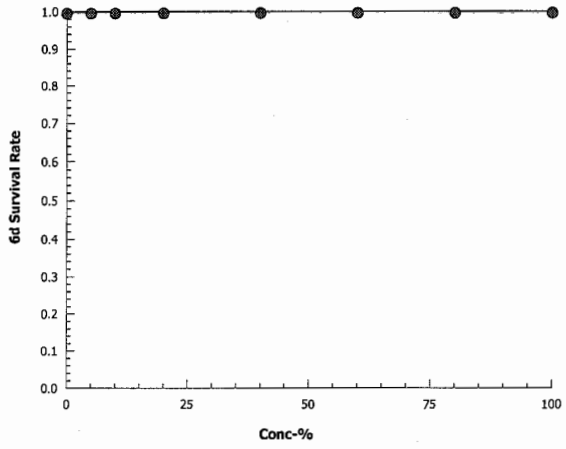
			Calculated Variate(A/B)								
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10
5		10	1	1	1	0	0	0.0%	0.0%	10	10
10		10	1	1	1	0	0	0.0%	0.0%	10	10
20		10	1	1	1	0	0	0.0%	0.0%	10	10
40		10	1	1	1	0	0	0.0%	0.0%	10	10
60		10	1	1	1	0	0	0.0%	0.0%	10	10
80		10	1	1	1	0	0	0.0%	0.0%	10	10
100		10	1	1	1	0	0	0.0%	0.0%	10	10

6d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	1	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
20		1	1	1	1	1	1	1	1	1	1
40		1	1	1	1	1	1	1	1	1	1
60		1	1	1	1	1	1	1	1	1	1
80		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1

Analysis ID:	10-8727-1085	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 May-11 15:32	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Nautilus Environmental

TESTING LOCATION (Please Circle)

California
5550 Morehouse Drive, Suite 150
San Diego, CA 92121
Phone 858.587.7333
Fax 858.587.3961

Washington
5009 Pacific Highway East, Suite 2
Tacoma, WA 98424
Phone 253.922.4296
Fax 253.922.5814

British Columbia (circled)
8664 Commerce Court
Burnaby, British Columbia, Canada V5A 4N3
Phone 604.420.8773
Fax 604.357.1361

Chain of Custody

Date April 28/11 Page 1 of 1

Sample Collection By:

Report to:		**Invoice To:**	
Company	Canadian Zinc	Company	Canadian Zinc
Address		Address	
City/State/Zip		City/State/Zip	
Contact	John Wilcockson (Hatfield)	Contact	
Phone	604-926-3261	Phone	
Email		Email	

ANALYSES REQUIRED

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	7-d C. dubia	48-hr D. magna LC50	Receipt Temperature (°C)
1	Mixture 4:1	April 28/11	1600h	Water	20 L	1	80% Mine + 20% Process	✓	✓	13.5
2	Mixture 8:1	↓	↓	↓	20 L	1	71.4% Mine + 12.4% Process + 16.2% Ditch	✓	✓	13.5
3	Mine Water	↓	↓	↓	↓	1		✓	✓	
4								WO#11200	WO#11201	
5										
6										
7										
8										
9										
10										

PROJECT INFORMATION		SAMPLE RECEIPT	
Client:		Total No. of Containers	6
PO No.:		Received Good Condition?	✓
Shipped Via:		Matches Test Schedule?	✓

RELINQUISHED BY (CLIENT)

(Signature) (Time)

(Printed Name) JO JXB per J. Wilcockson (Date) April 28/11

(Company) SGS (Hatfield)

RELINQUISHED BY (COURIER)

(Signature) (Time)

(Printed Name) (Date)

(Company)

RECEIVED BY (COURIER)

(Signature) (Time)

(Printed Name) (Date)

(Company)

RECEIVED BY (LABORATORY)

(Signature) [signature] (Time) 1405

(Printed Name) John T. (Date) Apr 28/11

(Company) Nautilus Environmental

SPECIAL INSTRUCTIONS/COMMENTS:

Received 4×20L of Mine Water, 2×20L of Process Water.

Also used Ditch Water received previously

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

Attachment C

April 6, 2011
Whole Effluent Toxicity Testing
***D. magna*, *C. dubia*, O. mykiss**
and *L. minor*

Nautilus Environmental

Toxicity Testing on Synthetic Effluent Samples

Final Toxicity Test Report

Report date: April 6, 2011

Submitted to:

Hatfield Consultants
North Vancouver, BC

8664 Commerce Court
Burnaby, BC
V5A 4N7

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF APPENDICES

1.0 INTRODUCTION

Nautilus Environmental conducted acute and chronic toxicity tests for Hatfield Consultants on samples identified as Mixture 1 and Mixture 2, prepared on January 28, 2011. The mixtures were prepared in the laboratory from samples identified as Process Water, Mine Water and Ditch Water.

Mixture 1 was a 4:1 combination of Mine Water (80%) and Mill Water (20%) and Mixture 2 was a combination of Mine Water (77%), Mill Water (11%), and Ditch Water (12%), reflecting a 7:1 ratio of Mine to Mill Water, with a small contribution of Ditch Water. The following toxicity tests were performed on the Mixture 1 and Mixture 2 samples:

- 7-d *Ceriodaphnia dubia* survival and reproduction test
- 7-d *Lemna minor* growth inhibition test
- 96-h rainbow trout (*Oncorhynchus mykiss*) acute toxicity test (conducted with lab water and Prairie Creek water as dilution water)
- 48-h *Daphnia magna* acute toxicity test (conducted with lab water and Prairie Creek water as dilution water)

In addition, a single concentration screening test was conducted with *D. magna* on the full-strength Mine Water sample. Due to a technician error in producing Mixture 2, there was insufficient volume of remaining Mine Water sample available to conduct a 96-h single concentration screening test with rainbow trout.

This report describes the results of these toxicity tests. Copies of raw laboratory data sheets and statistical analysis for each test species are provided in Appendices A through F.

2.0 METHODS

Methods for the toxicity tests are summarized in Tables 1 through 6. Testing was conducted according to procedures described by Environment Canada (2000a, 2000b, 2007a and 2007b).

Statistical analyses for the tests were performed using CETIS (Tidepool Scientific Software, 2009).

2.1 Quality Assurance/Quality Control (QA/QC)

Nautilus follows a comprehensive QA/QC program to ensure that the data generated are of high quality and are scientifically defensible. To meet these objectives, Nautilus has implemented a number of quality control procedures that include the following:

- Negative controls to ensure that appropriate testing performance criteria are met;
- Positive controls to assess the health and sensitivity of the test organisms;
- Use of appropriate species and life stage to meet the study objectives;
- Appropriate number of replicates to allow proper statistical analyses;
- Calibration and proper maintenance of instruments to ensure accurate measurements;
- Proper documentation and recordkeeping to allow traceability of performance;
- Adequate supervision and training of staff to ensure that methods are followed;
- Proper handling and storage of samples to ensure their integrity;
- Procedures in place to address issues that may arise during testing and ensure the implementation of appropriate corrective actions; and
- Rigorous review of data by a Registered Professional Biologist to ensure they are of good quality and scientifically defensible prior to releasing to the client.

Table 1. Summary of test conditions: *Ceriodaphnia dubia* survival and reproduction test.

Test organism	*Ceriodaphnia dubia*
Test organism source	In-house culture
Test organism age	<24 hr old neonates produced within 12 hr
Test type	Static renewal
Test duration	7 ± 1 day
Test chamber	20 mL test tube
Test solution volume	15 mL
Number of replicates	10
Control/dilution water	20% Perrier water (hardness 80-100mg/L $CaCO_3$)
Test solution renewal	Daily
Test temperature	25 ± 1°C
Number of organisms/chamber	1
Feeding	Daily, with 0.1 ml *Pseudokirchneriella subcapitata* and 0.05 mL YCT
Light intensity	100 to 600 lux
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	Environment Canada (2007a), EPS 1/RM/21
Test endpoints	Survival and reproduction
Test acceptability criterion for controls	≥80% survival; ≥15 young per surviving control; ≥60% of controls producing three or more broods
Reference Toxicant	Sodium chloride

Table 2. Summary of test conditions: *Lemna minor* growth inhibition test.

Test organism	*Lemna minor*
Test organism source	In-house culture
Test organism age	7- to 10-day old
Test type	Static
Test duration	7 days
Test chamber	250-mL glass containers
Test solution volume	150 mL
Number of replicates	4
Control/Dilution water	Deionized or distilled water with nutrients added
Test solution renewal	None
Test temperature	25 ± 2°C
Number of organisms/chamber	Two 3-frond plants
Light intensity	3600 to 4400 lux full spectrum light
Photoperiod	Continuous
Aeration	None
Test protocol	Environment Canada (2007b), EPS 1/RM/37
Test endpoint	Number of fronds and dry weight
Test acceptability criteria for controls	≥ 8-fold increase in number of fronds
Reference toxicant	Potassium chloride

Table 3. Summary of test conditions: 96-h rainbow trout test.

Test organism	*Oncorhynchus mykiss*
Test organism source	Commercial hatchery
Test organism age	Juveniles
Test type	Static
Test duration	96 hours
Test chamber	20 L glass aquarium
Test solution volume	10 L
Number of replicates	1
Control/Dilution water	Dechlorinated municipal tapwater; and Prairie Creek Water
Test solution renewal	None
Test temperature	15 ± 1°C
Number of organisms/chamber	Ten
Feeding	None
Light intensity	100 to 500 lux
Photoperiod	16 hours light/8 hours dark
Aeration	6.5 ± 1 mL/min/L
Test protocol	Environment Canada (2000a), EPS 1/RM/13
Test endpoint	96-h LC50
Test acceptability criteria for controls	Survival ≥ 90%
Reference toxicant	Sodium dodecyl sulphate

Table 4. Summary of test conditions: 48-h *Daphnia magna* test.

Test organism	*Daphnia magna*
Test organism source	In-house culture
Test organism age	< 24 h
Test type	Static
Test duration	48 hours
Test chamber	250-mL glass beakers
Test solution volume	200 mL
Number of replicates	Three (Single Concentration Screening), One (LC50)
Control/Dilution water	Moderately-hard reconstituted water (hardness 80-100 mg/L); and Prairie Creek Water
Test solution renewal	None
Test temperature	20 ± 2°C
Number of organisms/chamber	Ten
Feeding	None
Light intensity	400 to 800 lux
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	Environment Canada (2000b), EPS 1/RM/14
Test endpoint	48-h LC50
Test acceptability criteria for controls	Survival ≥ 90%
Reference toxicant	Sodium chloride

3.0 RESULTS

Effects on survival of *Ceriodaphnia dubia* were minimal; the LC50 value for both samples was >100%. Conversely, effects were observed on reproduction of *C. dubia* in all concentrations of both Mixtures 1 and 2. The IC25 was <5% in both samples, and the IC50 was <5% for Mixture 1 and 16.1% for Mixture 2, respectively (Table 5). These values indicate that more than a 25% reduction in reproduction was observed in all test concentrations of both mixtures (the lowest test concentration was 5% sample).

The *Lemna minor* growth inhibition test exhibited enhanced growth in all test concentrations compared to the negative control for both Mixture 1 and Mixture 2 (Table 6). The IC25 and IC50 values for both samples were >97%, indicating that there was no evidence of an adverse toxicological effect associated with either of the samples to this species.

Acute toxicity tests using rainbow trout tests exhibited 100% survival in all concentration tested with Mixtures 1 and 2, using both dechlorinated and Prairie Creek water for dilution (Tables 7 and 8). Therefore the 96-h LC50 results were >100%, and there was no evidence of an adverse toxicological effect to this species.

Acute toxicity tests using *Daphnia magna* resulted in an LC50 value of 89% for Mixture 1 and >100% for Mixture 2 when diluted with laboratory-prepared moderately hard water (Table 9). The samples diluted with Prairie Creek water exhibited an unusual pattern of mortality in both tests; specifically, elevated mortality was observed at the lower concentrations of sample, and not in higher concentrations (Table 10). This is an unusual result, since you would typically anticipate a larger adverse effect associated with a larger dose, and in this case, the opposite occurred. This finding suggests that toxicity occurred as a result of some interaction between the water types. Regardless, the fact that mortalities were not observed in the higher concentrations of sample tends to suggest that this was not indicative of a substantial degree of toxicity in the samples.

It should be noted that the full-strength Mixture 1 elicited a 60% reduction in survival of *Daphnia magna* in one test (the one using moderately hard water) and no reduction in survival in the other (using Prairie Creek water for dilution). Since there is no dilution in the full-strength sample, these two treatments are equivalent to one another, and reflect two, somewhat different measures of effect in the Mixture 1 sample. Based on the partial effect observed in one of the

two tests, and the lack of effect in the second test, it would appear that this mixture contained a toxicant at close to its threshold for toxicity to this species.

In the *Daphnia magna* 48-h single concentration screening test with Mine Water, survival was 100% in the undiluted sample, indicating that the Mine Water did not exhibit an adverse effect on this species (Table 11).

Collectively, the results indicated that rainbow trout and duckweed were not sensitive to the samples. Conversely, *C. dubia* displayed a substantial reduction in reproduction in both mixtures, with a greater adverse effect associated with Mixture 1 than Mixture 2. Consistent with this finding, Mixture 1 exhibited a small degree of adverse effect on survival of *D. magna*, whereas Mixture 2 did not. These results suggest that the toxicity observed to cladocerans was from the Mill Water sample, since Mixture 1 contained a higher concentration of Mill Water than Mixture 2. This conclusion is supported by the fact that the full-strength Mine Water did not have any adverse effect on *D. magna*.

3.1 Quality Assurance/Quality Control

All the tests reported here met the acceptability criteria for test validity specified in the respective protocol. Water quality parameters measured during the toxicity tests were within acceptable ranges and results of the reference toxicant tests conducted during the testing program were all within the in-house historical mean ± two standard deviations. The reference toxicant test results are summarized in Table 12.

It should be noted that the samples produced for this testing project were derived from samples that had been collected previously, and treated in a manner that was similar to that anticipated at the mine site. Consequently, holding times associated with these samples exceeded those specified in the test methods. However, the time period in between preparation of the treated Mine and Mill Water samples and initiation of the toxicity tests fell within the required holding times associated with the various tests.

Table 5. Toxicity test results for the *Ceriodaphnia dubia* survival and reproduction tests.

Concentration (% v/v)	Mean ± SD			
	Mixture 1		Mixture 2	
	Survival (%)	Reproduction (No. of Young/Female)	Survival (%)	Reproduction (No. of Young/Female)
Control	100	16.1 ± 4.5	100	16.1 ± 1.9
5	100	6.3 ± 3.5	100	11.6 ± 2.6
10	100	7.1 ± 3.8	100	11.2 ± 3.2
20	100	9.1 ± 1.4	90	6.6 ± 2.8
40	100	0.0 ± 0.0	80	3.0 ± 3.1
60	90	0.0 ± 0.0	100	0.0 ± 0.0
80	80	0.0 ± 0.0	70	0.0 ± 0.0
100	100	0.0 ± 0.0	100	0.0 ± 0.0
Test endpoint (% v/v)				
LC50	>100	--	>100	--
IC25 (95% CL)	--	< 5%	--	< 5%
IC50 (95% CL)	--	< 5%	--	16.1 (13.1 – 20.2) %

LC = Lethal Concentration.
IC = Inhibition Concentration.
SD = Standard Deviation.
CL = Confidence Limits.

Table 6. Toxicity test results for the *Lemna minor* growth inhibition tests.

Concentration (% v/v)	Mean ± SD			
	Mixture 1		Mixture 2	
	Frond Growth (No. of Fronds)	Dry Weight (mg)	Frond Growth (No. of Fronds)	Dry Weight (mg)
Control	67.8 ± 5.6	6.7 ± 0.4	69.0 ± 6.0	7.0 ± 0.9
1.5	92.2 ± 22.0	9.1 ± 1.6	69.2 ± 7.9	7.2 ± 1.1
3.0	90.5 ± 14.5	8.7 ± 1.5	89.0 ± 16.5	9.3 ± 1.3
6.1	104.0 ± 9.9	10.1 ± 1.0	83.0 ± 10.9	8.7 ± 1.0
12.1	122.8 ± 23.2	12.6 ± 2.6	107.8 ± 25.0	11.1 ± 2.7
24.2	120.3 ± 14.5	11.9 ± 0.9	103.8 ± 16.5	10.9 ± 1.2
48.5	116.5± 12.4	13.9 ± 2.9	111.3± 22.9	12.0 ± 2.7
97	122.8 ± 14.0	14.7 ± 0.5	101.3 ± 32.3	13.9 ± 2.6
Test endpoint (% v/v)				
IC25	>97	>97	>97	>97
IC50	>97	>97	>97	>97

IC = Inhibition Concentration.
SD = Standard Deviation.

Table 7. Acute toxicity test results for rainbow trout using dechlorinated water for dilution.

Concentration (% v/v)	% Survival	
	Mixture 1	Mixture 2
Control	100	100
6.25	100	100
12.5	100	100
25.0	100	100
50.0	100	100
100.0	100	100
Test endpoint		
LC50	>100	>100

Table 8. Acute toxicity test results for rainbow trout using Prairie Creek water for dilution.

Concentration (% v/v)	% Survival	
	Mixture 1	Mixture 2
Control	100	100
6.25	100	100
12.5	100	100
25.0	100	100
50.0	100	100
100.0	100	100
Test endpoint		
LC50	>100	>100

Table 9. Acute toxicity test results for *Daphnia magna* using moderately hard water for dilution.

Concentration (% v/v)	% Survival	
	Mixture 1	Mixture 2
Control	100	100
6.25	90	80
12.5	100	90
25.0	100	90
50.0	100	80
100.0	40	90
Test endpoint		
LC50 (95% CL)	89 (65 and 100)	>100

CL = Confidence Limits.

Table 10 . Acute toxicity test results for *Daphnia magna* using Prairie Creek water for dilution.

Concentration (% v/v)	% Survival	
	Mixture 1	Mixture 2
Control	100	100
6.25	30	50
12.5	40	60
25.0	70	90
50.0	100	100
100.0	100	100
Test endpoint		
LC50	>100 [1]	>100 [1]

[1] See text for discussion of results.

Table 11. Acute toxicity test results for *Daphnia magna* single concentration screening test using Mine Water.

Concentration (% v/v)	Survival (%)
Control	100
100	100

Table 12. Reference toxicant test results.

Test Species	Endpoint	Mean (2SD Range)	CV(%)	Initiation Date
C. dubia	Survival (IC50): 1.7 g/L NaCl	1.8 (1.4 – 2.3)	12	January 27, 2011
	Reproduction (IC50): 1.1 g/L NaCl	1.2 (0.9 – 1.5)	13	
L.minor	Frond Count (IC50): 3.6 mg/L KCL	3.7 (2.8 – 5.0)	15	January 19, 2011
O.mykiss	Survival (LC50): 5.0 mg/L SDS	5.2 ± (4.4 – 6.1)	8	December 16, 2010
D. magna	Survival (LC50): 4.2 g/L NaCl	4.0 (3.6 – 4.3)	5	January 21, 2011

4.0 REFERENCES

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Tidepool Scientific Software. 2009. CETIS comprehensive environmental toxicity information system, version 1.8.0. Tidepool Scientific Software, McKinleyville, CA. 222 pp.

APPENDIX A – *Ceriodaphnia dubia* Toxicity Test Data

Ceriodaphnia dubia Summary Sheet

Client: Hatfield
Work Order No.: 11 066

Start Date/Time: Jan 29/11@ 1100L
Set up by: KLB

Sample Information:

Sample ID: Mixture 1
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2+2 L

Test Organism Information:

Broodstock No.: 011811
Age of young (Day 0): <24-h (within 12-h)
Avg No. young in first 3 broods of previous 7 d: 23
Mortality (%) in previous 7 d: 0
Individual female # used ≥8 young on test day: 1, 2, 6, 7, 8, 10, 12, 15) 16

NaCl Reference Toxicant Results:

Reference Toxicant ID: Cd 63
Stock Solution ID: 10 NaCl
Date Initiated: ~ Jan 29/11

7-d LC50 (95% CL): 1.7 (1.3 - 2.3) g/L NaCL
7-d IC50 (95% CL): 1.1 (0.9 - 1.4) g/L NaCL

7-d LC50 Reference Toxicant Mean (2SD Range): 1.8 (1.4 - 2.3) g/L NaCL CV (%): 12
7-d IC50 Reference Toxicant Mean (2SD Range): 1.2 (0.9 - 1.5) g/L NaCL CV (%): 13

Test Results:

	Survival	Reproduction
LC50 %(v/v) (95% CL)	> 100	
IC25 %(v/v) (95% CL)		1.3 (1.1 - 2.0)
IC50 %(v/v) (95% CL)		4.4 (3.2 - 22.2)

Reviewed by: A. Teng

Date reviewed: March 24, 2011

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: Hatfield
Sample ID: Mixture 1 (4:1)
Work Order: 11066

Start Date & Time: Jan 29 2011 @ ~~1200h~~ KLB 1100h
Stop Date & Time: Feb 4 2011 @ 1200h
Set up by: KLB/AMS

%(v/v)

Days	Concentration: Control										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	3	✓	2	✓	✓	KLB
4	3	4	4	4	4	5	4	3	3	3	KLB
5	5	✓	8	7	7	✓	2	5	4	5	KSL
6	10	7	11	8	10	8	4	10	10	8	KLB
7											
8											
Total	18	11	23	19	21	16	10	10	17	16	KLB

Days	Concentration: 5										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	3	2	✓	✓	✓	KLB
4	6	3	3	6	5	4	4	4	✓	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	4	✓	✓	KSL
6	✓	4	✓	2	4	3	2	2	1	1	KSL
7											
8											
Total	6	7	3	8	9	10	8	10	1	1	KSL

Days	Concentration: 10										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	4	4	2	4	✓	4	4	✓	2	KLB
5	6	✓	5	2	✓	2	2	6	✓	3	KSL
6	✓	2	4	6	2	1	1	1	✓	4	
7											
8											
Total	6	6	13	10	6	3	7	11	0	9	KSL

Days	Concentration: 20										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	32 KLB	2	✓	✓	✓	KLB
4	2	3	3	✓	4	5	5	2	✓	5	KLB
5	3	7	5	4	6	1	✓	4	3	4	KSL
6	4	✓	3	4	✓	5	3	✓	7	✓	
7											
8											
Total	9	10	11	8	10	~~15~~ KLB	10	6	10	9	KSL

Days	Concentration: 40										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KSL
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KSL
7											
8											
Total	0	0	0	0	0	0	0	0	0	0	KSL

Days	Concentration: 60										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	KSL
6	✓	✓	✓	✓	✓	✓	✓	✓		✓	KSL
7											
8											
Total	0	0	0	0	0	0	0	0	0*	0	KSL

Days	Concentration: 80										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	|	✓	✓	✓	✓	✓	X	✓	✓	✓	KSL
6	|	✓	✓	✓	✓	✓	|	✓	✓	✓	KSL
7											
8											
Total	0*	0	0	0	0	0	0*	0	0	0	KSL

Days	Concentration: 100										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KSL
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KSL
7											
8											
Total	0	0	0	0	0	0	0	0	0	0	KSL

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

Notes: X = mortality.

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

Reviewed by: A. Tang
Date reviewed: March 24, 2011

CETIS Analytical Report

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

Analysis ID:	06-7520-1693	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 17:05	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	03-7225-7749	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Krysta Banack
Start Date:	29 Jan-11 11:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	Perrier Water
Ending Date:	04 Feb-11 12:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 1h	**Source:**		**Age:**	
Sample ID:	08-2902-6478	**Code:**	3169F0AE	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Jan-11	**Source:**	Hatfield		
Sample Age:	35h	**Station:**	Mixture 1		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2.08E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	49.01	44.28	N/A	2.04	N/A	2.258
EC10	100	49.01	N/A	1	N/A	2.04
EC15	>100	N/A	N/A	<1	N/A	N/A
EC20	>100	N/A	N/A	<1	N/A	N/A
EC25	>100	N/A	N/A	<1	N/A	N/A
EC40	>100	N/A	N/A	<1	N/A	N/A
EC50	>100	N/A	N/A	<1	N/A	N/A

6d Survival Rate Summary

			Calculated Variate(A/B)								
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10
5		10	1	1	1	0	0	0.0%	0.0%	10	10
10		10	1	1	1	0	0	0.0%	0.0%	10	10
20		10	1	1	1	0	0	0.0%	0.0%	10	10
40		10	1	1	1	0	0	0.0%	0.0%	10	10
60		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10
80		10	0.8	0	1	0.1333	0.4216	52.7%	20.0%	8	10
100		10	1	1	1	0	0	0.0%	0.0%	10	10

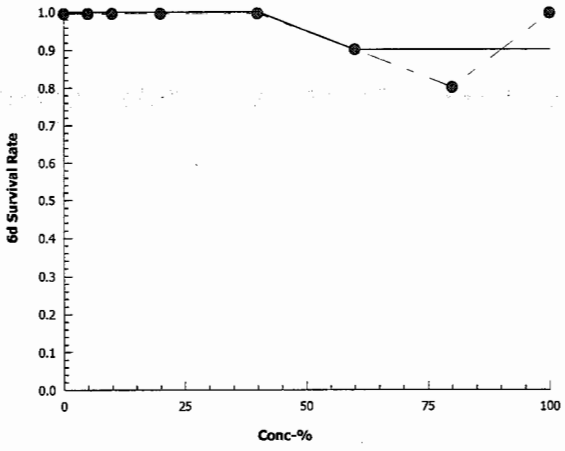
6d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	1	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
20		1	1	1	1	1	1	1	1	1	1
40		1	1	1	1	1	1	1	1	1	1
60		1	1	1	1	1	1	1	1	0	1
80		0	1	1	1	1	1	0	1	1	1
100		1	1	1	1	1	1	1	1	1	1

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

Analysis ID:	06-7520-1693	**Endpoint:**	6d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 17:05	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



CETIS Analytical Report

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

Analysis ID:	20-8319-6722	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 16:58	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	03-7225-7749	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Krysta Banack
Start Date:	29 Jan-11 11:00	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	Perrier Water
Ending Date:	04 Feb-11 12:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 1h	**Source:**		**Age:**	
Sample ID:	08-2902-6478	**Code:**	3169F0AE	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Jan-11	**Source:**	Hatfield		
Sample Age:	35h	**Station:**	Mixture 1		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.639E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	0.1826	0.1546	0.2432	547.6	411.1	647
IC10	0.3985	0.333	0.5456	250.9	183.3	300.3
IC15	0.6539	0.539	0.9216	152.9	108.5	185.5
IC20	0.9559	0.7769	1.389	104.6	72	128.7
IC25	1.313	1.052	1.97	76.16	50.76	95.1
IC40	2.826	2.157	4.707	35.39	21.24	46.35
IC50	4.35	3.209	22.22	22.99	4.5	31.16

Reproduction Summary

			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	16.1	10	23	1.433	4.533	28.15%	0.0%
5		10	6.3	1	10	1.096	3.466	55.01%	60.87%
10		10	7.1	0	13	1.215	3.843	54.12%	55.9%
20		10	9.1	6	11	0.4583	1.449	15.92%	43.48%
40		10	0	0	0	0	0		100.0%
60		10	0	0	0	0	0		100.0%
80		10	0	0	0	0	0		100.0%
100		10	0	0	0	0	0		100.0%

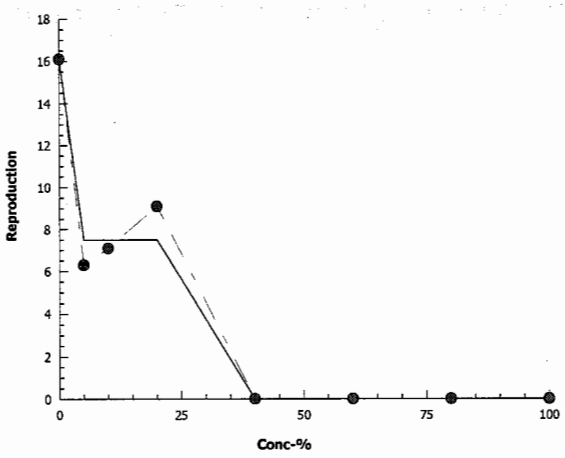
Reproduction Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	18	11	23	19	21	16	10	10	17	16
5		6	7	3	8	9	10	8	10	1	1
10		6	6	13	10	6	3	7	11	0	9
20		9	10	11	8	10	8	10	6	10	9
40		0	0	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

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

Analysis ID:	20-8319-6722	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 16:58	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Ceriodaphnia dubia Summary Sheet

Client: Hatfield
Work Order No.: 11 066

Start Date/Time: Jan 30/11 @ 1115
Set up by: KLB

Sample Information:

Sample ID: Mixture 2
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2 x 2 L

Test Organism Information:

Broodstock No.: 01181
Age of young (Day 0): <24-h (within 12-h)
Avg No. young in first 3 broods of previous 7 d: 28
Mortality (%) in previous 7 d: 0
Individual female # used ≥8 young on test day: 1, 2, 4, 5, 6, 7, 8, 10

NaCl Reference Toxicant Results:

Reference Toxicant ID: Cd 63
Stock Solution ID: 10 NaCl
Date Initiated: ~Jan 29/11

7-d LC50 (95% CL): 1.7 (1.3 - 2.3) g/L NaCL
7-d IC50 (95% CL): 1.1 (0.9 - 1.4) g/L NaCL

7-d LC50 Reference Toxicant Mean (2SD Range): 1.8 (1.4 - 2.3) g/L NaCL CV (%): 12
7-d IC50 Reference Toxicant Mean (2SD Range): 1.2 (0.9 - 1.5) g/L NaCL CV (%): 13

Test Results:

	Survival	Reproduction
LC50 %(v/v) (95% CL)	> 100	
IC25 %(v/v) (95% CL)		~~3.5 (2.2 - 10.5) AC~~ 4.0 (2.4 - 10.8)
IC50 %(v/v) (95% CL)		16.1 (~~13.7~~ 12.3 - 20.8)

Reviewed by: A. Tong

Date reviewed: March 25, 2011

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: Hatfield
Sample ID: mixture 2 (8:1)
Work Order #: 11066

Start Date & Time: Jan 30 2011 @ 1137
Stop Date: Feb 6 2011 @ 1100
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	26.0	24.0	24.0	24.0	26.0	24.0	25.0	24.0	25.0	25.0	25.0	25.0	25.0
DO (mg/L)	8.2	7.9	6.5	7.3	8.0	7.1	8.4	7.1	8.2	7.1	8.1	7.1	7.9	6.7
pH	8.1	8.0	8.1	7.8	8.2	7.9	8.1	8.0	8.2	8.0	8.2	7.9	8.1	7.9
Cond. (µS/cm)	219	228		222		223		232		225		225		230
Initials	~	KLB		KLB		KLB		KLB		KLB		~		~

Concentration 5	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	26.0	24.0	24.0	24.0	26.0	24.0	24.0	24.0	25.0	24.0	25.0	25.0	25.5
DO (mg/L)	7.8	7.8	6.5	7.4	7.8	7.2	8.2	7.0	8.2	7.0	7.1	7.0	7.9	6.5
pH	8.1	8.0	8.2	7.9	8.2	7.9	8.1	7.8	8.2	7.9	8.2	7.9	8.1	7.9
Cond. (µS/cm)	343	340		321		326		353		329		334		348
Initials	~	KLB		KLB		KLB		KLB		KLB		~		~

Concentration 40	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	26.0	24.0	24.0	24.0	26.0	24.5	25.0	24.5	25.0	25.0	25.0	25.0	25.5
DO (mg/L)	7.8	7.6	6.7	7.4	7.8	7.2	8.0	7.1	8.2	7.0	7.1	7.0	7.8	6.3
pH	8.3	8.0	8.5	8.0	8.4	7.9	8.2	7.8	8.6	8.2	8.4	7.9	8.3	7.9
Cond. (µS/cm)	916	940		941		928		897		973		945		915
Initials	~	KLB		KLB		KLB		KLB		KLB		~		~

Concentration 100	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)	25.5	26.0	24.0	24.0	24.0	26.0	24.5	25.5	24.5	25.0	25.5	25.0	25.0	25.5
DO (mg/L)	7.9	7.0	6.8	7.3	7.6	7.2	7.9	7.1	8.2	7.0	7.1	6.5	7.8	5.9
pH	8.5	7.9	8.8	8.1	8.5	8.0	8.3	7.9	8.9	8.1	8.5	8.0	8.4	7.7
Cond. (µS/cm)	1895	1870		1873		1869		1887		1886		1900		1885
Initials	~	KLB		KLB		KLB		KLB		KLB		~		~

	Control	100 %(v/v)		
Hardness*	100	500		
Alkalinity*	86	110		

* mg/L as CaCO3

Analysts: KLB, Am
Reviewed by: ART
Date reviewed: March 24, 2011

Sample Description: (1) light yellow - clear

Comments: **Broodboard Used:** 01811

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: Hatfield
Sample ID: Mixture 2
Work Order: 110610

Start Date & Time: Jan 30 2011 @ ~~1200~~ 1115h KLB
Stop Date & Time: Feb 6 2011 @ 1400h
Set up by: KLB/AMS

%(v/v)

Days	Concentration: Control										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	4	20 ✓	4	KLB
4	2	✓	3 KLB ✓	✓	✓	3	✓	✓	✓	4	KLB
5	4	5	✓	5	✓	✓	2	7	5	7	KLB
6	9	5	4	5	4	6	6	7	9	✓	~
7	✓	8	8	6	8	9	9	✓	✓	✓	~
8											
Total	15	18	15	16	12	18	17	18	17	15	~

Days	Concentration: 5										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	~	✓	✓	✓	✓	✓	✓	3	3	✓	KLB
4	✓	✓	✓	✓	✓	~	~	~	~	4	KLB
5	4	6	4	3	5	6	2	2	2	2	KLB
6	3	4	5	8	5	4	6	✓	✓		~
7	6	4	4	✓	✓	6	✓	5	8	5	~
8								10A			
Total	13	14	13	8	10	16	8	7	13	11	~

Days	Concentration: 10										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	4	4	✓	3	✓	✓	3	4	KLB
5	4	4	✓	6	6	4	4	2	5	2	KLB
6	4	5	4	✓	3	✓	✓	4	✓	✓	~
7	✓	7	4	5	5	✓	5	5	✓	6	~
8											
Total	8	16	12	15	14	7	9	11	8	12	~

Days	Concentration: 20										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	2	✓	✓	KLB
4	✓	2	3	4	✓	✓	✓	3	3	✓	KLB
5	4	✓	✓	X	3	3	2	3	3	4	KLB
6	✓	3	✓	|	✓	✓	2	✓	✓	✓	~
7	5	6	6	|	✓	✓	5	✓	3	✓	~
8											
Total	9	10	9	4 X	3	3	7	8	9	4	~

Days	Concentration: 40										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	X	~	✓	✓	✓	✓	KLB
5	3	2	4	5	|	✓	3	✓	✓	✓	KLB
6	✓	X	✓	5	|	3	3	2	✓	✓	~
7	✓	|	✓	5	|	✓	✓	✓	✓	✓	~
8		|			|						
Total	3	2 X	4	10	0 X	3	6	2	0	0	~

Days	Concentration: 60										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
7	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	~
8											
Total	0	→									~

Days	Concentration: 80										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
2	X	✓	✓	✓	✓	✓	✓	✓	✓	X	KLB
3	|	✓	✓	✓	✓	✓	✓	✓	✓	|	KLB
4	|	✓	✓	✓	✓	X	✓	✓	✓	|	KLB
5	|	✓	✓	✓	✓	|	✓	✓	✓	|	KLB
6	|	~	~	~	~	|	~	~	~	|	~
7	|	~	~	~	~	|	~	~	~	|	~
8	|					|				|	
Total	0 X	0	0	0	0	0 X	0	0	0	0 X	~

Days	Concentration: 100										
	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	KLB
6	~	~	~	~	~	~	~	~	~	~	~
7	~	~	~	~	~	~	~	~	~	~	~
8											
Total	0	→									~

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

Notes: X = mortality.

Sample Description: (1)

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

Reviewed by: A. Tong

Date reviewed: March 24, 2011

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

Analysis ID:	11-6431-9337	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	24 Mar-11 16:46	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	01-6136-4903	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Krysta Banack
Start Date:	30 Jan-11 11:15	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	Perrier Water
Ending Date:	06 Feb-11 14:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	7d 3h	**Source:**		**Age:**	
Sample ID:	11-7008-1972	**Code:**	45BE08B4	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Jan-11	**Source:**	Hatfield		
Sample Age:	59h	**Station:**	Mixture 2		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2.025E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	0.3779	0.2763	0.6173	264.7	162	361.9
IC10	0.8985	0.6289	1.616	111.3	61.89	159
IC15	1.616	1.079	3.231	61.89	30.95	92.68
IC20	2.604	1.653	7.782	38.4	12.85	60.48
IC25	3.966	2.386	10.85	25.21	9.212	41.9
IC40	12.66	8.251	15.71	7.9	6.367	12.12
IC50	16.13	12.3	20.8	6.201	4.809	8.127

Reproduction Summary — **Calculated Variate**

Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	10	16.1	12	18	0.6046	1.912	11.88%	0.0%
5		10	11.6	8	16	0.8327	2.633	22.7%	27.95%
10		10	11.2	7	16	0.9978	3.155	28.17%	30.43%
20		10	6.6	3	10	0.8844	2.797	42.38%	59.01%
40		10	3	0	10	0.9888	3.127	104.2%	81.37%
60		10	0	0	0	0	0		100.0%
80		10	0	0	0	0	0		100.0%
100		10	0	0	0	0	0		100.0%

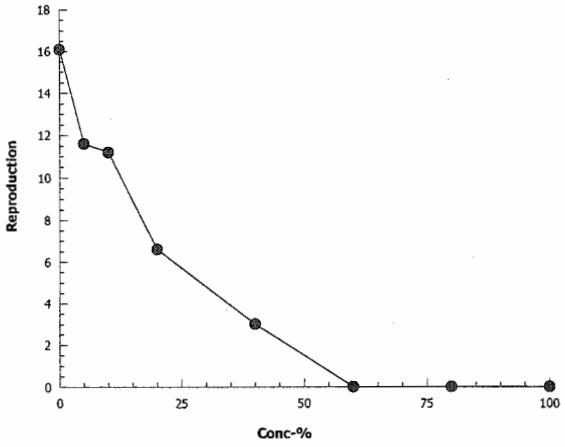
Reproduction Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	15	18	15	16	12	18	17	18	17	15
5		13	14	13	8	10	16	8	10	13	11
10		8	16	12	15	14	7	9	11	8	12
20		9	10	9	4	3	3	7	8	9	4
40		3	2	4	10	0	3	6	2	0	0
60		0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0

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

Analysis ID:	11-6431-9337	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.0
Analyzed:	24 Mar-11 16:46	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



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

Analysis ID:	12-1557-5387	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 17:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	01-6136-4903	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Krysta Banack
Start Date:	30 Jan-11 11:15	**Protocol:**	EC/EPS 1/RM/21	**Diluent:**	Perrier Water
Ending Date:	06 Feb-11 14:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	7d 3h	**Source:**		**Age:**	
Sample ID:	11-7008-1972	**Code:**	45BE08B4	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Jan-11	**Source:**	Hatfield		
Sample Age:	59h	**Station:**	Mixture 2		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.328E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	14.2	12.35	69.29	7.043	1.443	8.094
EC10	60	15.21	N/A	1.667	N/A	6.573
EC15	100	18.69	N/A	1	N/A	5.352
EC20	>100	N/A	N/A	<1	N/A	N/A
EC25	>100	N/A	N/A	<1	N/A	N/A
EC40	>100	N/A	N/A	<1	N/A	N/A
EC50	>100	N/A	N/A	<1	N/A	N/A

7d Survival Rate Summary

			Calculated Variate(A/B)								
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	10	1	1	1	0	0	0.0%	0.0%	10	10
5		10	1	1	1	0	0	0.0%	0.0%	10	10
10		10	1	1	1	0	0	0.0%	0.0%	10	10
20		10	0.9	0	1	0.1	0.3162	35.14%	10.0%	9	10
40		10	0.8	0	1	0.1333	0.4216	52.7%	20.0%	8	10
60		10	1	1	1	0	0	0.0%	0.0%	10	10
80		10	0.7	0	1	0.1528	0.483	69.01%	30.0%	7	10
100		10	1	1	1	0	0	0.0%	0.0%	10	10

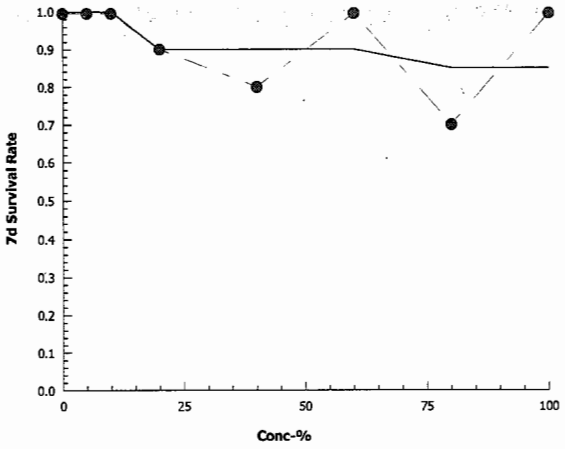
7d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Negative Control	1	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1
20		1	1	1	0	1	1	1	1	1	1
40		1	0	1	1	0	1	1	1	1	1
60		1	1	1	1	1	1	1	1	1	1
80		0	1	1	1	1	0	1	1	1	0
100		1	1	1	1	1	1	1	1	1	1

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

Analysis ID:	12-1557-5387	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 17:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



APPENDIX B - *Lemna minor* Toxicity Test Data

Lemna minor Summary Sheet

Client: Hatfield
Work Order No.: 11067

Start Date: Jan 28, 2011
Set up by: KLB

Sample Information:

Sample ID: Mixture 1
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2 x 20L

Test Organism Information:

Culture Date: Jan 19/11
Age of culture (Day 0): 9
>8X growth in APHA?: yes 27 fronds day 7

KCl Reference Toxicant Results:

Reference Toxicant ID: Ln 60
Date Initiated: Jan 19/11

7-d No. of Fronds IC50 (95% CL): 3.6 (2.6 - 4.5)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 3.7 (2.8-5.0) CV (%): 15.3

		Number of Fronds	Dry Weight
Test Results:	IC25 %(v/v) (95% CL)	>97	>97
	IC50 %(v/v) (95% CL)	>97	>97

Reviewed by: A. Tang

Date reviewed: March 24, 2011

Plant Growth Inhibition Toxicity Test
Water Quality Measurements

Client: Hatfield | Setup by: KLB, AWD

Sample ID: mixture 1 (4:1) | Test Date: Jan 28 2011

Work Order No.: 11067 | Test Species: Lemna minor — red bearers

Culture Source: UTCC # 490

Test Culture Age: 9 days | > 8X Growth? (Y/N): Yes (27 fronds)

Light Intensity Range: 4100 - 4300 | Date Measured: Jan 28 11

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	24.5	25.5	26.0	26.0	26.0	26.0	25.0	27.0
Initials	KLB	~	~	KLB	KLB	KLB	KLB	KLB

Sample Characteristics

		Aeration? 20 min
Temperature (°C)	24.0	25.0
DO (mg/L)	7.8	8.3
pH	8.9	9.1
Conductivity (µS)	2780	3410

Concentration % (v/v)	Temperature (°C)		pH		Conductivity (µS)
	Day 0	Day 7	Day 0	Day 7	0 h
Control	25.5	~~28.5~~ 26.5 KLB	8.3	9.4	861
1.5	24.5	26.5 ~~28.5~~ 26.5 KLB	8.2	9.1	906
3.05	24.5	27.0	8.3	8.9	956
6.1	24.5	26.5	8.4	9.1	1046
12.1	24.5	26.5	8.5	9.2	1224
24.2	24.5	26.5	8.8	9.0	1565
48.5	24.5	27.0	9.0	8.9	2210
97	24.5	27.0	9.1	8.8	3410
Initials	~	KLB	~	KLB	~

Thermometer: Calibrated Thermometer | Cond. Meter: C-1 | pH meter: pH-1

Sample Description: Clear

Comments:

Reviewed: A. Teng | Date Reviewed: March 24, 2011

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: Hatfield
Sample ID: mixture1
Work Order #: 11067

Start Date: Jan 28 2011
Termination Date: Feb 4 2011
Test set up by: KLB, AWD

% (v/v) Concentration	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
Control	A	6	69										KLB
	B	↓	80										↓
	C		77										
	D		69										
1.5	A		84										
	B		129										
	C		99										
	D		81										
3.05	A		99										
	B		77										
	C		112										
	D		98										
6.1	A		121										
	B		111										
	C		97										
	D		111										
12.1	A		139										
	B		142										
	C		94										
	D		140										
24.2	A		127										
	B		132										
	C		140										
	D	↓	106										↓

Comments:

Reviewed by: A. Tang

Date Reviewed: March 24, 2011

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: Hatfield
Sample ID: mixture 1
Work Order #: 11067

Start Date: Jan 28 2011
Termination Date: Feb 4 2011
Test set up by: KLB, AWD

% (JJ) Concentration	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
48.5	A	6	126										KLB
	B		138										
	C		117										
	D		109										
97	A		113										
	B		141										
	C		121										
	D		140										
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments:

Reviewed by: A. Tang

Date Reviewed: March 24, 2011

7-d *Lemna minor* Weight Data Sheet

Client: Hatfield
Sample ID: Mixture 1
Work Order #: 11067 o/o (VJ)

Start Date: Jan 28 2011
Termination Date: Feb 4 2011

Concentration	Rep	Pan No.	Pan weight (mg) (Purple)	Pan + plant (mg)	Initials
Control	A	1	1309.12	1315.52	KLB
	B	2	1304.47	1311.04	
	C	3	1292.60	1299.85	
	D	4	1312.25	1318.65	
1.5	A	5	1304.11 KLB	1310.97 ①	
	B	6	1298.33	1308.95	
	C	7	1310.80	1319.99	
	D	8	1305.17	1314.99	
3.05	A	9	1313.51	1322.93	
	B	10	1308.28	1314.86	
	C	11	1297.28	1307.12	
	D	12	1291.04	1299.94	
6.1	A	13	1312.75	1322.84	
	B	14	1288.14	1298.85	
	C	15	1313.01	1321.74 ②	
	D	16	1291.69	1302.45	
12.1	A	17	1289.81	1304.18	
	B	18	1303.18	1316.85	
	C	19	1288.80	1297.62	
	D	20	1312.10	1325.66	
24.2	A	21	1315.15	1326.85	
	B	22	1308.50	1321.26 ③	
	C	23	1304.06	1316.49	
	D	24	1308.35	1319.02	
48.5	A	25	1305.66	1320.95	
	B	26	1306.97	1324.25	
	C	27	1307.25	1318.44	
	D	28	1305.21	1316.93	

Comments: ① Reweigh = 1310.64 ② Reweigh = 1321.70 ③ Reweigh = 1321.69

Reviewed by: A. Teng

Date Reviewed: March 24, 2011

7-d *Lemna minor* Weight Data Sheet

Client: Hatfield
Sample ID: Mixture 1
Work Order #: 11067
% (J/J)

Start Date: Jan 28 2011
Termination Date: Feb 4 2011

Concentration	Rep	Pan No.	Pan weight (mg) (Purple)	Pan + plant (mg)	Initials
97	A	29	1299.41	1314.72	KLB
	B	30	1307.26	1322.47	↓
	C	31	1297.41	1310.60	↓
	D	32	1298.43	1313.36	↓
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments:

Reviewed by: A. Tang

Date Reviewed: March 24, 2011

CETIS Analytical Report

Test Code: 11067a | 01-3402-0664

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	17-1959-8257	**Endpoint:**	Frond Count	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 15:55	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	14-8278-7197	**Test Type:**	Lemna Growth	**Analyst:**	Krysta Banack
Start Date:	28 Jan-11	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	
Ending Date:	04 Feb-11	**Species:**	Lemna minor	**Brine:**	
Duration:	7d 0h	**Source:**	UTCC #490	**Age:**	9 d
Sample ID:	20-5730-8353	**Code:**	7AA008C1	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Jan-11	**Source:**	Hatfield		
Sample Age:	N/A	**Station:**	Mixture 1		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	241544450	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>97	N/A	N/A	<1.031	N/A	N/A
IC10	>97	N/A	N/A	<1.031	N/A	N/A
IC15	>97	N/A	N/A	<1.031	N/A	N/A
IC20	>97	N/A	N/A	<1.031	N/A	N/A
IC25	>97	N/A	N/A	<1.031	N/A	N/A
IC40	>97	N/A	N/A	<1.031	N/A	N/A
IC50	>97	N/A	N/A	<1.031	N/A	N/A

Frond Count Summary

			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	67.75	63	74	2.81	5.62	8.3%	0.0%
1.5		4	92.25	75	123	10.98	21.96	23.81%	-36.16%
3.05		4	90.5	71	106	7.24	14.48	16.0%	-33.58%
6.1		4	104	91	115	4.933	9.866	9.49%	-53.51%
12.1		4	122.8	88	136	11.6	23.2	18.9%	-81.18%
24.2		4	120.3	100	134	7.261	14.52	12.08%	-77.49%
48.5		4	116.5	103	132	6.225	12.45	10.69%	-71.96%
97		4	122.8	107	135	6.981	13.96	11.37%	-81.18%

Frond Count Detail

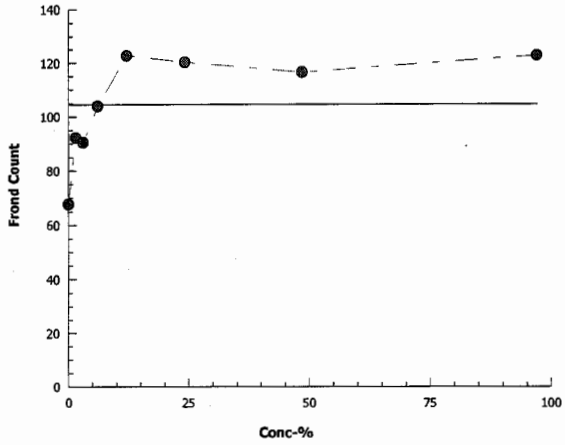
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	63	74	71	63
1.5		78	123	93	75
3.05		93	71	106	92
6.1		115	105	91	105
12.1		133	136	88	134
24.2		121	126	134	100
48.5		120	132	111	103
97		107	135	115	134

Analyst: KLB QA: KRT Mar 24/11

Lemna Growth Inhibition Test **Nautilus Environmental**

Analysis ID:	17-1959-8257	**Endpoint:**	Frond Count	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 15:55	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: KLB QA: Mar 24/11

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	02-8606-1810	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 15:55	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	14-8278-7197	**Test Type:**	Lemna Growth	**Analyst:**	Krysta Banack
Start Date:	28 Jan-11	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	
Ending Date:	04 Feb-11	**Species:**	Lemna minor	**Brine:**	
Duration:	7d 0h	**Source:**	UTCC #490	**Age:**	9 d
Sample ID:	20-5730-8353	**Code:**	7AA008C1	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Water Sample	**Project:**	
Receive Date:	28 Jan-11	**Source:**	Hatfield		
Sample Age:	N/A	**Station:**	Mixture 1		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	2.001E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>97	N/A	N/A	<1.031	N/A	N/A
IC10	>97	N/A	N/A	<1.031	N/A	N/A
IC15	>97	N/A	N/A	<1.031	N/A	N/A
IC20	>97	N/A	N/A	<1.031	N/A	N/A
IC25	>97	N/A	N/A	<1.031	N/A	N/A
IC40	>97	N/A	N/A	<1.031	N/A	N/A
IC50	>97	N/A	N/A	<1.031	N/A	N/A

Total Dry Weight-mg Summary

			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	6.655	6.4	7.25	0.2023	0.4047	6.08%	0.0%
1.5		4	9.122	6.86	10.62	0.8089	1.618	17.73%	-37.08%
3.05		4	8.685	6.58	9.84	0.7275	1.455	16.75%	-30.5%
6.1		4	10.07	8.73	10.76	0.4727	0.9455	9.39%	-51.35%
12.1		4	12.6	8.82	14.37	1.274	2.549	20.22%	-89.41%
24.2		4	11.89	10.67	12.76	0.463	0.9261	7.79%	-78.66%
48.5		4	13.87	11.19	17.28	1.456	2.913	21.0%	-108.4%
97		4	14.66	13.19	15.31	0.4966	0.9931	6.77%	-120.3%

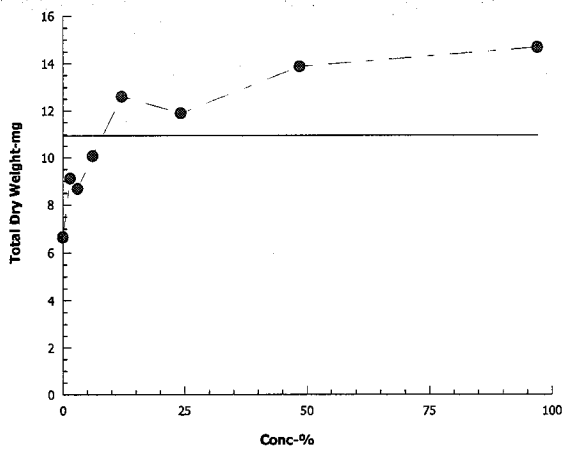
Total Dry Weight-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	6.4	6.57	7.25	6.4
1.5		6.86	10.62	9.19	9.82
3.05		9.42	6.58	9.84	8.9
6.1		10.09	10.71	8.73	10.76
12.1		14.37	13.67	8.82	13.56
24.2		11.7	12.76	12.43	10.67
48.5		15.29	17.28	11.19	11.72
97		15.31	15.21	13.19	14.93

Lemna Growth Inhibition Test | **Nautilus Environmental**

Analysis ID:	02-8606-1810	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 15:55	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: KLB QA: ART Mar 24/11

Lemna minor Summary Sheet

Client: Hatfield
Work Order No.: 11067

Start Date: Jan 28, 2011
Set up by: KLB

Sample Information:

Sample ID: Mixture 2
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2 x 20L

Test Organism Information:

Culture Date: Jan 19/11
Age of culture (Day 0): 9
>8X growth in APHA?: Yes 27 fronds day 7

KCl Reference Toxicant Results:

Reference Toxicant ID: Lm 60
Date Initiated: Jan 19/11

7-d No. of Fronds IC50 (95% CL): 3.6 (2.6 - 4.5)

7-d No. Fronds IC50 Reference Toxicant Mean (2 SD Range): 3.7 (2.8-5.0) CV (%): 15.3

		Number of Fronds	Dry Weight
Test Results:	IC25 %(v/v) (95% CL)	>97	>97
	IC50 %(v/v) (95% CL)	>97	>97

Reviewed by: A. Terry

Date reviewed: March 24, 2011

Plant Growth Inhibition Toxicity Test
Water Quality Measurements

Client: Hatfield | Setup by: KLB, AWD

Sample ID: Mixture 2 (5:1) | Test Date: Jan 28 2011

Work Order No.: 11067 | Test Species: Lemna minor Blue beakers

Culture Source: UTCC # 490

Test Culture Age: 9 days | > 8X Growth? (Y/N): Yes (27 fronds)

Light Intensity Range: 4100 - 4305 | Date Measured: Jan 28/11

Day	0	1	2	3	4	5	6	7
Shelf Temp (°C)	24.5	25.5	~	26.0	26.0	26.0	25.0	27.0
Initials	KLB	~	~	KLB	KLB	KLB	KLB	KLB

Sample Characteristics | **Aeration?** 20 min

Temperature (°C)	24.0	24.0
DO (mg/L)	7.7	8.5
pH	8.5	9.1
Conductivity (µS)	1895	~~219~~ 2410 KLB

Concentration % (v/v)	Temperature (°C)		pH		Conductivity (µS)
	Day 0	Day 7	Day 0	Day 7	0 h
Control	25.5	27.0	8.3	9.3	861
1.5	24.5	27.0	8.2	9.0	895
3.05	24.0	27.0	8.3	9.0	924
6.1	24.0	27.0	8.4	8.8	979
12.1	24.0	27.0	8.5	9.0	1091
24.2	24.0	27.0	8.8	9.0	1301
48.5	24.0	27.0	9.0	9.1	~~2390~~ KLB 1699
97	24.0	27.0	9.1	9.1	2410
Initials	~	KLB	~	KLB	~

Thermometer: Calibrated Thermometer | Cond. Meter: C-1 | pH meter: pH-1

Sample Description: Clear

Comments:

Reviewed: A. Tang | Date Reviewed: March 24, 2011

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: Hatfield
Sample ID: mixture 2
Work Order #: 11067

Start Date: Jan 28 2011
Termination Date: Feb 4 2011
Test set up by: KLB, AWD

% (v/v) Concentration	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
Control	A	6	83										KLB
	B		62										
	C		68										
	D		87										
1.5	A		73										
	B		81										
	C		65										
	D		82 KLB										
3.05	A		~~192~~										
	B		110										
	C		105										
	D		73										
6.1	A		75										
	B		96										
	C		99										
	D		86										
12.1	A		86										
	B		100										
	C		129										
	D		140										
24.2	A		113										
	B		124										
	C		86										
	D	↓	110										↓

Comments:

Reviewed by: A. Tong

Date Reviewed: March 24, 2011

Lemna minor Toxicity Test Data Sheet - 7-d Frond Counts

Client: Hatfield
Sample ID: mixture 2
Work Order #: 11067

Start Date: Jan 28 2011
Termination Date: Feb 4 2011
Test set up by: KLB, AWD

% (v/v) Concentration	Rep	No. of fronds		Chlorosis	Necrosis	Yellow	Abnormal size	Gibbosity	Single fronds	Root destruction	Loss of buoyancy	Comments	Initials
		Day 0	Day 7										
48.5	A	6	131										KLB
	B		106										
	C		141										
	D		91										
97	A		83										
	B		76										
	C		139										
	D		131										
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												
	A												
	B												
	C												
	D												

Comments:

Reviewed by: A. Tong

Date Reviewed: March 24, 2011

7-d *Lemna minor* Weight Data Sheet

Client: Hatfield
Sample ID: Mixture 2
Work Order #: 11067
o/o (VJ)

Start Date: Jan 28 2011
Termination Date: Feb 4 2011

Concentration	Rep	Pan No.	Blank Pan weight (mg)	Pan + plant (mg)	Initials
Control	A	1	1300.81	1308.45	KLB
	B	2	1300.08	1306.25	
	C	3	1306.89	1313.30 ①	
	D	4	1313.07	1320.93	
1.5	A	5	1308.20	1315.36	
	B	6	1312.83	1320.73	
	C	7	1312.06	1317.68	
	D	8	1312.22	1320.34	
3.05	A	9	1321.74	1330.81	
	B	10	1305.14	1315.47	
	C	11	1314.26	1324.57 ②	
	D	12	1310.50	1318.10	
6.1	A	13	1308.81	1316.08	
	B	14	1310.52	1320.23	
	C	15	1308.42	1317.46	
	D	16	1308.33	1317.20	
12.1	A	17	1304.85	1312.73	
	B	18	1313.77	1323.86	
	C	19	1299.23	1311.96	
	D	20	1311.78	1325.57	
24.2	A	21	1308.42	1319.24	
	B	22	1312.34	1324.29	
	C	23	1319.58	1328.80	
	D	24	1307.63	1319.23	
48.5	A	25	1310.21	1324.07	
	B	26	1310.16	1321.19 ③	
	C	27	1312.69	1327.28	
	D	28	1309.48	1318.12	

Comments: ①Reweigh = 1313.65 ②Reweigh = 1324.21 ③1321.09

Reviewed by: A. Teng

Date Reviewed: March 24, 2011

7-d *Lemna minor* Weight Data Sheet

Client: Hatfield
Sample ID: Mixture 2
Work Order #: 11067
% (J/J)

Start Date: Jan 28 2011
Termination Date: Feb 4 2011

Concentration	Rep	Pan No.	Pan weight (mg)	Pan + plant (mg)	Initials
97	A	29	1306.72	1318.45	KLB
	B	30	1308.05	1320.25	↓
	C	31	1306.07	1323.48	↓
	D	32	1305.37	1319.50	↓
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

Comments:

Reviewed by: A. Teng

Date Reviewed: March 24, 2011

CETIS Analytical Report

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	18-8027-6706	**Endpoint:**	Frond Count	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 15:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	07-7819-4315	**Test Type:**	Lemna Growth	**Analyst:**	Krysta Banack
Start Date:	28 Jan-11	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	
Ending Date:	04 Feb-11	**Species:**	Lemna minor	**Brine:**	
Duration:	7d 0h	**Source:**	UTCC #490	**Age:**	9d
Sample ID:	03-5204-2099	**Code:**	14FBBC73	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Water Sample	**Project:**	
Receive Date:	04 Feb-11	**Source:**	Hatfield		
Sample Age:	N/A	**Station:**	Mixture 2		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.034E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>97	N/A	N/A	<1.031	N/A	N/A
IC10	>97	N/A	N/A	<1.031	N/A	N/A
IC15	>97	N/A	N/A	<1.031	N/A	N/A
IC20	>97	N/A	N/A	<1.031	N/A	N/A
IC25	>97	N/A	N/A	<1.031	N/A	N/A
IC40	>97	N/A	N/A	<1.031	N/A	N/A
IC50	>97	N/A	N/A	<1.031	N/A	N/A

Frond Count Summary

			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	69	56	81	5.958	11.92	17.27%	0.0%
1.5		4	69.25	59	76	3.966	7.932	11.45%	-0.36%
3.05		4	89	67	104	8.256	16.51	18.55%	-28.99%
6.1		4	83	69	93	5.431	10.86	13.09%	-20.29%
12.1		4	107.8	80	134	12.52	25.04	23.24%	-56.16%
24.2		4	103.8	80	118	8.25	16.5	15.9%	-50.36%
48.5		4	111.3	85	135	11.43	22.87	20.55%	-61.23%
97		4	101.3	70	133	16.17	32.34	31.94%	-46.74%

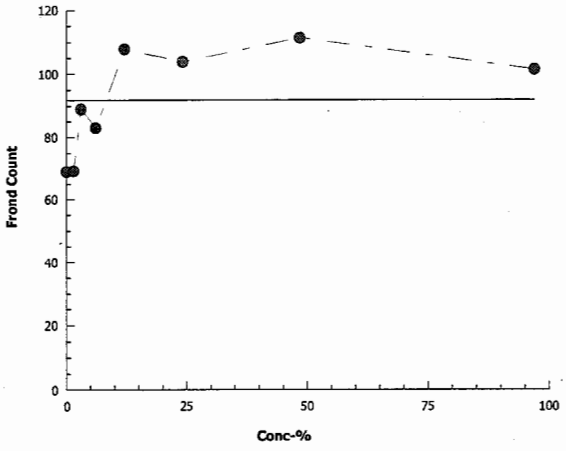
Frond Count Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	77	56	62	81
1.5		67	75	59	76
3.05		86	104	99	67
6.1		69	90	93	80
12.1		80	94	123	134
24.2		107	118	80	110
48.5		125	100	135	85
97		77	70	133	125

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	18-8027-6706	**Endpoint:**	Frond Count	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 15:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: KLB QA: ATR Mar 24/11

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	05-2626-0683	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 15:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	07-7819-4315	**Test Type:**	Lemna Growth	**Analyst:**	Krysta Banack
Start Date:	28 Jan-11	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	
Ending Date:	04 Feb-11	**Species:**	Lemna minor	**Brine:**	
Duration:	7d 0h	**Source:**	UTCC #490	**Age:**	9d
Sample ID:	03-5204-2099	**Code:**	14FBBC73	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Water Sample	**Project:**	
Receive Date:	04 Feb-11	**Source:**	Hatfield		
Sample Age:	N/A	**Station:**	Mixture 2		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.614E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>97	N/A	N/A	<1.031	N/A	N/A
IC10	>97	N/A	N/A	<1.031	N/A	N/A
IC15	>97	N/A	N/A	<1.031	N/A	N/A
IC20	>97	N/A	N/A	<1.031	N/A	N/A
IC25	>97	N/A	N/A	<1.031	N/A	N/A
IC40	>97	N/A	N/A	<1.031	N/A	N/A
IC50	>97	N/A	N/A	<1.031	N/A	N/A

Total Dry Weight-mg Summary

			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	7.02	6.17	7.86	0.4267	0.8533	12.16%	0.0%
1.5		4	7.215	5.68	8.12	0.5513	1.103	15.28%	-2.78%
3.05		4	9.327	7.6	10.33	0.6468	1.294	13.87%	-32.87%
6.1		4	8.722	7.27	9.71	0.517	1.034	11.85%	-24.25%
12.1		4	11.12	7.88	13.79	1.332	2.663	23.94%	-58.44%
24.2		4	10.9	9.22	11.95	0.607	1.214	11.14%	-55.23%
48.5		4	12.03	8.64	14.59	1.366	2.732	22.71%	-71.37%
97		4	13.87	11.73	17.41	1.29	2.58	18.6%	-97.54%

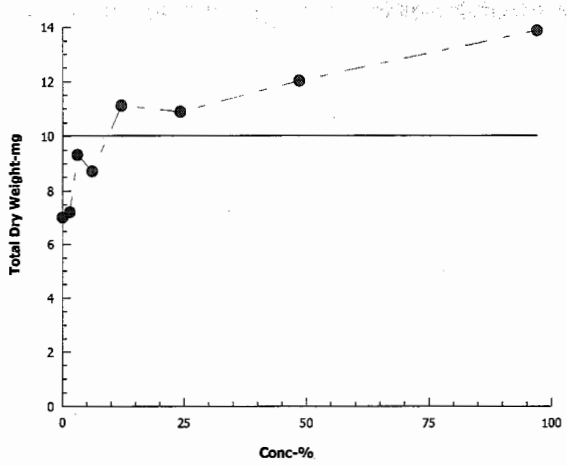
Total Dry Weight-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	7.64	6.17	6.41	7.86
1.5		7.16	7.9	5.68	8.12
3.05		9.07	10.33	10.31	7.6
6.1		7.27	9.71	9.04	8.87
12.1		7.88	10.09	12.73	13.79
24.2		10.82	11.95	9.22	11.6
48.5		13.86	11.03	14.59	8.64
97		11.73	12.2	17.41	14.13

Lemna Growth Inhibition Test — **Nautilus Environmental**

Analysis ID:	05-2626-0683	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETISv1.8.0
Analyzed:	09 Feb-11 15:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: KLB QA: IRT Mar 24/11

APPENDIX C - Rainbow Trout Toxicity Test Data

Rainbow Trout Summary Sheet

Client: Hatfield

Start Date/Time: Jan 31/11 @ 1630h

Work Order No.: 11065

Test Species: *Oncorhynchus mykiss*

Sample Information:

Sample ID: Mixture 1
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2x20L
Other: —

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L $CaCO_3$): 12
Alkalinity (mg/L $CaCO_3$): 6

Test Organism Information:

Batch No.: 111810
Source: Trout Lake Lodge
No. Fish/Volume (L): 10/10L
Loading Density: 0.45
Mean Length ± SD (mm): 38 ± 3 Range: 33-44
Mean Weight ± SD (g): 0.45 ± 0.13 Range: 0.30-0.74

SDS Reference Toxicant Results:

Reference Toxicant ID: RT66
Stock Solution ID: 10510
Date Initiated: Dec 16/10
96-h LC50 (95% CL): 5.0 (4.3-5.6)

Reference Toxicant Mean (2SD Range): 5.2 (4.4-6.1)
Reference Toxicant CV (%): 8.5

Test Results: The 96-hr LC50 >100% v/v

Reviewed by: A. Tang

Date reviewed: March 24, 2011

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: Hatfield
Sample I.D. Mixture 1
W.O. # 11065
RBT Batch #: 111810
Date Collected/Time: Jan 28/11 4PM
Date Setup/Time: Jan 31/11 @1630
Sample Setup By: JAB

D.O. meter: DO-1
pH meter: pH-1
Cond. Meter: C-1

Number Fish/Volume: 10/10L
7-d % Mortality: 0.2%
Total Pre-aeration Time (mins): 30
Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Yes

Undiluted Sample WQ

Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C	14.5		14.5
pH	9.4		9.4
D.O. (mg/L)	9.9		9.9
Cond. (µS/cm)	2710		2710

Concentration % (v/v)	# Survivors							Temperature (°C)					Dissolved Oxygen (mg/L)					pH					Conductivity (µS/cm)	
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	96
Control				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.9	10.0	10.0	9.9	9.9	7.1	6.9	7.1	7.1	7.0	30	34
6.25				10	10	10	10	14.0	14.0	14.0	14.0	14.0	9.8	10.0	9.9	10.1	9.9	8.2	7.1	7.2	7.3	7.2	287	292
12.5				10	10	10	10	14.0	14.0	14.0	14.0	14.0	10.0	9.8	9.9	9.8	9.8	8.8	7.2	7.3	7.4	7.4	432	438
25				10	10	10	10	14.0	14.0	14.0	14.5	14.0	9.9	9.9	9.9	9.8	9.8	9.1	7.4	7.5	7.6	7.5	800	807
50				10	10	10	10	14.5	14.0	14.0	14.5	14.5	9.8	9.9	9.7	9.7	9.6	9.2	7.7	7.7	7.7	7.6	1469	1479
100				10	10	10	10	14.5	14.0	14.0	14.5	14.5	9.9	9.8	9.9	9.8	9.8	9.4	8.0	8.0	7.9	7.8	2710	2740
Initials				JAB	KSL	JAB	JAB	JAB	JAB	KSL	JAB	JAB	JAB	JAB	KSL	JAB	JAB	JAB	JAB	KSL	JAB	JAB	JAB	JAB

Sample Description/Comments: very slight orange tint in highest concentration, clear

Fish Description at 96? All fish appear ok

Other Observations:

Reviewed by: A. Tang Date Reviewed: March 24, 2011

Rainbow Trout Summary Sheet

Client: Hatfield

Start Date/Time: Jan 31/11 @ 1615h

Work Order No.: 11065

Test Species: *Oncorhynchus mykiss*

Sample Information:

Sample ID: Mixture 2
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2+20L
Other: —

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L $CaCO_3$): 12
Alkalinity (mg/L $CaCO_3$): 6

Test Organism Information:

Batch No.: 111810
Source: Trout Lake Lodge
No. Fish/Volume (L): 10/10L
Loading Density: 0.50
Mean Length ± SD (mm): 39 ± 3 Range: 34-43
Mean Weight ± SD (g): 0.50 ± 0.13 Range: 0.31-0.69

SDS Reference Toxicant Results:

Reference Toxicant ID: RT66
Stock Solution ID: 10510
Date Initiated: Dec 16/10
96-h LC50 (95% CL): 5.0 (4.3-5.6)

Reference Toxicant Mean (2SD Range): 5.2 (4.4-6.1)
Reference Toxicant CV (%): 8.5

Test Results: The 96-hr LC50 > 100% v/v

Reviewed by: A. Tang

Date reviewed: March 24, 2011

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: Hatfield
Sample I.D. Mixture 2
W.O. # 11065
RBT Batch #: 111810
Date Collected/Time: Jan 28/11 AM
Date Setup/Time: Jan 31 / 11 @ 1615
Sample Setup By: JAB

D.O. meter: DO-1
pH meter: pH-1
Cond. Meter: C-1

Number Fish/Volume: 10/16L
7-d % Mortality: 0.2 %
Total Pre-aeration Time (mins): 30
Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Yes

Undiluted Sample WQ			
Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C	14.5		14.0
pH	9.2		9.2
D.O. (mg/L)	9.8		9.9
Cond. (µS/cm)	2060		2070

Concentration %v/v	# Survivors							Temperature (°C)					Dissolved Oxygen (mg/L)					pH					Conductivity (µS/cm)	
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	96
Ctrl				10	10	10	10	14.0	14.5	14.5	14.0	14.5	10.0	10.0	10.1	10.0	9.9	7.1	7.0	7.0	7.1	7.0	30	35
6.25				10	10	10	10	14.0	14.5	14.5	14.0	14.5	10.0	10.0	9.9	10.0	9.9	7.6	7.1	7.1	7.2	7.1	197	201
12.5				10	10	10	10	14.0	14.5	14.5	14.0	14.5	10.2	9.9	9.8	9.8	9.4	8.5	7.1	7.1	7.3	7.2	333	339
25				10	10	10	10	14.0	14.5	14.5	14.0	14.5	10.0	10.0	9.8	9.7	9.7	8.9	7.3	7.4	7.5	7.3	610	618
50				10	10	10	10	14.0	14.5	14.5	14.0	14.5	9.9	10.0	9.7	9.9	9.9	9.2	7.6	7.8	7.8	7.7	1106	1117
100				10	10	10	10	14.0	14.5	14.0	14.0	14.5	9.9	10.0	9.8	10.0	9.7	9.2	7.9	8.0	8.0	7.9	2070	2070
Initials				JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB

Sample Description/Comments: Slight orange, clear

Fish Description at 96? All remaining fish appear ok

Other Observations:

Reviewed by: A. Tang

Date Reviewed: March 24, 2011

Rainbow Trout Summary Sheet

Client: Hatfield

Start Date/Time: Jan 31/11 @ 1630h

Work Order No.: 11065

Test Species: *Oncorhynchus mykiss*

Sample Information:

Sample ID: Mixture 1 - Prairie Creek
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2x20L
Other: —

Dilution Water:

Type: Prairie Creek Water ~~Dechlorinated Municipal Tap Water~~ JAB
Hardness (mg/L $CaCO_3$): ~~12~~ JAB 300
Alkalinity (mg/L $CaCO_3$): ~~6~~ JAB 224

Test Organism Information:

Batch No.: 111810
Source: Trout Lake Lodge
No. Fish/Volume (L): 10/10L
Loading Density: 0.48
Mean Length ± SD (mm): 39 ± 3 Range: 33-44
Mean Weight ± SD (g): 0.48 ± 0.13 Range: 0.33-0.72

SDS Reference Toxicant Results:

Reference Toxicant ID: RT66
Stock Solution ID: 10510
Date Initiated: Dec 16/10
96-h LC50 (95% CL): 5.0 (4.3-5.6)

Reference Toxicant Mean (2SD Range): 5.2 (4.4-6.1)
Reference Toxicant CV (%): 8.5

Test Results: The 96-hr LC50 >100% v/v

Reviewed by: A. Tang

Date reviewed: March 24, 2011

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: Hatfield
Sample I.D. Mixture 1 - Prairie Creek
W.O. # 11065
RBT Batch #: 111810
Date Collected/Time: Jan 28/11 @ AM
Date Setup/Time: Jan 31/11 @ 1630h
Sample Setup By: JAB

D.O. meter: DO-1
pH meter: pH-1
Cond. Meter: C-1

Number Fish/Volume: 10/10L
7-d % Mortality: 0.2%
Total Pre-aeration Time (mins): 30 mins
Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Yes

Undiluted Sample WQ			
Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C	14.5		14.5
pH	9.4		9.4
D.O. (mg/L)	9.9		9.9
Cond. (µS/cm)	2710		2710

Concentration %(v/v)	# Survivors							Temperature (°C)					Dissolved Oxygen (mg/L)					pH					Conductivity (µS/cm)	
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	96
Control				10	10	10	10	14.0	14.0	14.0	14.0	14.5	9.9	9.9	10.0	10.1	9.8	8.0	8.1	8.3	8.3	8.3	584	587
6.25				10	10	10	10	14.0	14.0	14.0	14.0	14.5	9.9	10.0	10.0	10.1	9.8	8.1	8.1	8.2	8.2	8.2	731	739
12.5				10	10	10	10	14.0	14.0	14.0	14.0	14.5	9.9	9.9	9.9	9.9	9.8	8.2	8.1	8.2	8.2	8.2	845	854
25				10	10	10	10	14.0	14.0	14.0	14.0	14.5	9.9	9.9	10.0	10.2	9.8	8.5	8.0	8.2	8.2	8.1	1136	1148
50				10	10	10	10	14.5	14.0	14.0	14.0	14.5	9.7	9.7	9.9	10.0	9.8	8.8	8.1	8.2	8.2	8.1	1675	1694
100				10	10	10	10	14.5	14.0	14.0	14.0	14.5	9.9	9.9	9.9	10.0	9.8	9.4	8.0	8.0	7.9	7.8	2710	2740
Initials				JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB

Sample Description/Comments: Clear, very slight orange

Fish Description at 96? All remaining fish appear ok

Other Observations:

Reviewed by: A. Tang

Date Reviewed: March 24, 2011

Rainbow Trout Summary Sheet

Client: Hatfield Start Date/Time: Jan 31/11 @ 1615h

Work Order No.: 11065 Test Species: *Oncorhynchus mykiss*

Sample Information:

Sample ID: Mixture 2-Prairie Creek
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 24 L
Other: —

Dilution Water:

Type: Prairie Creek Water ~~Dechlorinated Municipal Tap Water~~ JAB
Hardness (mg/L $CaCO_3$): ~~12~~ JAB 300
Alkalinity (mg/L $CaCO_3$): ~~6~~ JAB 224

Test Organism Information:

Batch No.: 111810
Source: Trout Lake Lodge
No. Fish/Volume (L): 10/10L
Loading Density: 0.48
Mean Length ± SD (mm): 38 ± 3 Range: 34-43
Mean Weight ± SD (g): 0.48 ± 0.11 Range: 0.34-0.72

SDS Reference Toxicant Results:

Reference Toxicant ID: RT66
Stock Solution ID: 10510
Date Initiated: Dec 16/10
96-h LC50 (95% CL): 5.0 (4.3-5.6)

Reference Toxicant Mean (2SD Range): 5.2 (4.4-6.1)
Reference Toxicant CV (%): 8.5

Test Results: The 96-hr LC50 >100 % (v/v)

Reviewed by: A. Tang Date reviewed: March 24, 2011

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: Hatfield
Sample I.D. Mixture 2 - Prairie Creek
W.O. # 11065
RBT Batch #: 111810
Date Collected/Time: Jan 28/11 @ AM
Date Setup/Time: Jan 31/11 @ 1615h
Sample Setup By: JAB

D.O. meter: DO-1
pH meter: pH-1
Cond. Meter: C-1

Number Fish/Volume: 10/10L
7-d % Mortality: 0.2 %
Total Pre-aeration Time (mins): 30
Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Yes

Undiluted Sample WQ			
Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C	14.5		14.0
pH	9.2		9.2
D.O. (mg/L)	9.8		9.9
Cond. (µS/cm)	2060		2070

Concentration % v/v	# Survivors							Temperature (°C)					Dissolved Oxygen (mg/L)					pH					Conductivity (µS/cm)	
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	96
Ctrl				10	10	10	10	14.0	14.5	14.0	14.0	14.5	9.9	9.8	9.8	10.0	9.8	8.1	8.1	8.2	8.3	8.2	582	587
6.25				10	10	10	10	14.0	14.5	14.0	14.0	14.5	9.8	9.7	9.9	9.9	9.9	8.1	8.0	8.2	8.2	8.1	670	688
12.5				10	10	10	10	14.0	14.5	14.0	14.0	14.5	9.8	9.9	9.9	9.9	9.8	8.2	8.1	8.2	8.2	8.1	760	768
25				10	10	10	10	14.0	14.5	14.0	14.0	14.5	9.7	9.9	9.9	9.9	9.8	8.4	8.0	8.2	8.2	8.1	934	941
50				10	10	10	10	14.0	14.5	14.0	14.0	14.5	9.9	9.9	9.9	9.8	9.7	8.7	8.0	8.1	8.2	8.0	1319	1330
100				10	10	10	10	14.0	14.5	14.0	14.5	14.5	9.9	9.9	9.8	9.9	9.9	9.2	7.9	8.0	7.9	7.9	2070	2070
																					JAB 8.0			
Initials				JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB	KJL	JAB	JAB	JAB	JAB

Sample Description/Comments: Slight orange, clear

Fish Description at 96? All remaining fish appear ok

Other Observations:

Reviewed by: A. Tang

Date Reviewed: March 24, 2011

APPENDIX D - *Daphnia magna* Toxicity Test Data

Daphnia magna Summary Sheet

Client: Hatfield
Work Order No.: 41064

Start Date/Time: Feb 1 / 11@ 1400
Test Species: *D.magna*
Set up by: KB

Sample Information:

Sample ID: Mixture 1
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2 x 2L

Test Organism Information:

Broodstock No.: 011911 A
Age of young (Day 0): < 24 hours
Avg No. young per brood in previous 7 d: 17
Mortality (%) in previous 7 d: 0
Days to first brood: 8

NaCl Reference Toxicant Results:

Reference Toxicant ID: DM 66
Stock Solution ID: 10 NaCl
Date Initiated: Jan 24/11
48-h LC50 (95% CL): 4.2 (3.7 - 4.8) g/LNaCL

Reference Toxicant Mean (2SD Range): 4.0 (3.6 - 4.3) g/L NaCL
Reference Toxicant CV (%): 5

Test Results: The 48-h LC50 no estimated @ 89 % (v/v) w/ 95% C.L @ 6/5 and 100 % (v/v)

Reviewed by: A. Teng

Date reviewed: March 25, 2011

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Yatfield
Sample ID: Mixture 1 (4:1)
Work Order No.: 11064

Start Date/Time: KLB Feb 1 2011 @1400h
No. Organisms/volume: 10/200mL
Test Organism: D.magna
Set up by: KLB

DO meter: DO-1 pH meter: pH-1 Conductivity meter: C-1

Concentration % (v/v)	Rep	Number of Live Organisms 24	48	No. Immobilized 48	Temperature (°C) 0	24	48	Dissolved oxygen (mg/L) 0	24	48	pH 0	24	48	Conductivity (µS/cm) 0	48
Control	A	10	10	0	20.0	20.5	20.0	8.7		8.9	8.0		8.1	359	406
	B														
	C														
	D														
6.25	A	10	9	0	20.0	20.5	20.0	8.7		8.8	8.3		8.0	~~327~~ 321 KLB	571
	B														
	C														
	D														
12.5	A	10	10	0	20.0	20.5	20.0	8.8		8.8	8.5		8.0	688	742
	B														
	C														
	D														
25	A	10	10	0	20.0	20.5	20.0	8.8		8.9	8.5①		8.0	997	1065
	B														
	C														
	D														
50	A	10	10	0	20.0	20.5	20.0	8.8		8.9	8.5①		8.0	1590	1693
	B														
	C														
	D														
100	A	10	4	0	20.0	20.5	20.0	8.8		9.0	8.5①		8.1	2710	2920
	B														
	C														
	D														
Technician Initials		~	KLB	KLB	KLB	~	KJL	KLB		KJL	KLB		KJL	KLB	KJL

	Hardness*	Alkalinity*
Conc.	*(mg/L as CaCo3)	
Control (MHW)	100	74
Highest conc.	470	100

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	20.0		20.0
DO (mg/L)	8.8	~~Added~~ KLB	8.8
pH	9.2	Added 0.1m HCl after dilutions were made	8.5
Cond (µS/cm)	2710		2710

Sample Description: Clear

① Adjusted to pH 8.5 with 0.1m HCl after dilutions were made

Comments: Batch#: 0411A 7-d previous # young/brood: 17 Day of 1st Brood: 8 Previous 7-d % Mortality: 0

Reviewed by: A. Teng Date reviewed: March 24, 2011

CETIS Analytical Report

Daphnia magna 48-h Acute Survival Test — **Nautilus Environmental**

Analysis ID:	17-8561-1589	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.0
Analyzed:	24 Mar-11 16:32	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	00-6731-0545	**Test Type:**	Survival	**Analyst:**	andy diewald
Start Date:	01 Feb-11 14:00	**Protocol:**	EC/EPS 1/RM/14	**Diluent:**	
Ending Date:	03 Feb-11 15:05	**Species:**	Daphnia magna	**Brine:**	
Duration:	49h	**Source:**		**Age:**	
Sample ID:	14-5936-7144	**Code:**	56FC2CE8	**Client:**	Hatfield
Sample Date:	28 Jan-11	**Material:**	Effluent	**Project:**	
Receive Date:	28 Jan-11	**Source:**	Hatfield		
Sample Age:	4d 14h	**Station:**	Mixture 1		

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	40.00%	1.948	0.06715	88.64	65.06	120.8

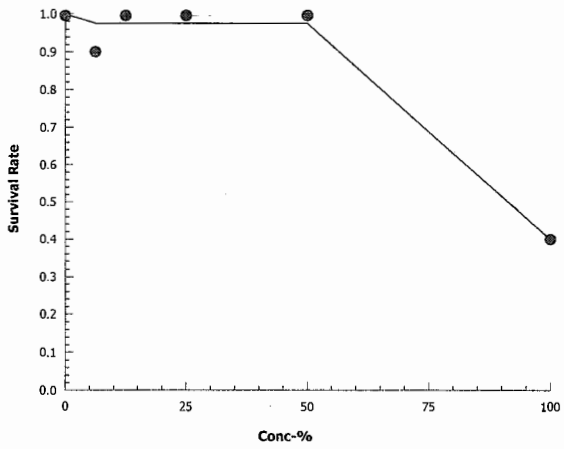
Survival Rate Summary — **Calculated Variate(A/B)**

Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	1	1	1	1	0	0	0.0%	0.0%	10	10
6.25		1	0.9	0.9	0.9	0	0	0.0%	10.0%	9	10
12.5		1	1	1	1	0	0	0.0%	0.0%	10	10
25		1	1	1	1	0	0	0.0%	0.0%	10	10
50		1	1	1	1	0	0	0.0%	0.0%	10	10
100		1	0.4	0.4	0.4	0	0	0.0%	60.0%	4	10

Survival Rate Detail

Conc-%	Control Type	Rep 1
0	Negative Control	1
6.25		0.9
12.5		1
25		1
50		1
100		0.4

Graphics



Daphnia magna Summary Sheet

Client: Hatfield
Work Order No.: 91064

Start Date/Time: Feb 1 / 11@ 14 05L
Test Species: D.magna
Set up by: KB

Sample Information:

Sample ID: Mixture 2
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2 x 20L

Test Organism Information:

Broodstock No.: 011911 A
Age of young (Day 0): < 24 hours
Avg No. young per brood in previous 7 d: 17
Mortality (%) in previous 7 d: 0
Days to first brood: 8

NaCl Reference Toxicant Results:

Reference Toxicant ID: JM 66
Stock Solution ID: 10 $NaCl$
Date Initiated: Jan 24/11
48-h LC50 (95% CL): 4.2 (3.7 - 4.8) g/LNaCL

Reference Toxicant Mean (2SD Range): 4.0 (3.6 - 4.3) g/L NaCL
Reference Toxicant CV (%): 5

Test Results: The 48-h LC50 no estimated @ > 100 % (v/v)

Reviewed by: A. Tang
Date reviewed: March 24, 2011

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Hatfield
Sample ID: Mixture 2 (5:1)
Work Order No.: 11064

Start Date/Time: KLB Feb 1 Jan 2011 1405h
No. Organisms/volume: 10/200mL
Test Organism: D.magna
Set up by: KLB

DO meter: DO-1 pH meter: pH-1 Conductivity meter: C-1

Concentration	Rep	Number of Live Organisms		No. Immobilized	Temperature (°C)			Dissolved oxygen (mg/L)			pH			Conductivity (µS/cm)	
% (v/v)		24	48	48	0	24	48	0	24	48	0	24	48	0	48
Control	A	10	10	0	20.0	20.5	20.0	8.7		8.9	8.0		8.1	359	406
	B														
	C														
	D														
6.25	A	10	8	0	20.0	20.5	20.0	8.7		9.0	8.3		8.0	472	515
	B														
	C														
	D														
12.5	A	10	9	0	20.0	20.5	20.0	8.7		8.9	8.5		8.0	571	621
	B														
	C														
	D														
25	A	10	9	0	20.0	20.5	20.0	8.8		8.9	8.5①		8.0	781	844
	B														
	C														
	D														
50	A	10	8	0	20.0	20.5	20.0	8.8		8.9	8.5①		8.1	1126	1208
	B														
	C														
	D														
100	A	10	9	0	20.0	20.5	20.0	8.8		9.0	8.5①		8.1	1881	2040 KJL
	B														
	C														
	D														
Technician Initials		~	KLB	KLB	KLB	~	KJL	KLB		KJL	KLB		KJL	KLB	KJL

	Hardness*	Alkalinity*
Conc.	*(mg/L as CaCo3)	
Control (MHW)	100	74
Highest conc.	500	110

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	20.0		20.0
DO (mg/L)	8.8		8.8
pH	9.3	added 0.1m HCl after dilutions were made	8.5
Cond (µS/cm)	1881		1881

Sample Description: Clear

① Adjusted to pH 8.5 with 0.1m HCl after dilutions were made

Comments: Batch#:011911A 7-d previous # young/brood: 47 Day of 1st Brood: 8 Previous 7-d % Mortality: 0

Reviewed by: A. Tang Date reviewed: March 24, 2011

Daphnia magna Summary Sheet

Client: Hatfield
Work Order No.: 91064

Start Date/Time: Feb 1 / 11 @ 1330L
Test Species: *D.magna*
Set up by: KLB

Sample Information:

Sample ID: Mixture 1 (diluted w/ Prairie Creek water)
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2 x 2L

Test Organism Information:

Broodstock No.: 01/21/11A
Age of young (Day 0): < 24 hours
Avg No. young per brood in previous 7 d: 20
Mortality (%) in previous 7 d: 0
Days to first brood: 9

NaCl Reference Toxicant Results:

Reference Toxicant ID: DM 66
Stock Solution ID: 10 N_aCl
Date Initiated: ~~Jan 21/11~~
48-h LC50 (95% CL): ~~4.2 (3.6 - 4.3)~~ 4.2 (3.7 - 4.8) g/L NaCL

Reference Toxicant Mean (2SD Range): 4.0 (3.6 - 4.3) g/L NaCL
Reference Toxicant CV (%): 5

Test Results: The 48-h LC50 is estimated @ >100% (J/J)

Reviewed by: L. Tang
Date reviewed: March 24, 2011

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Hatfield
Sample ID: Mixture 1 (4:1)
Work Order No.: 11064

Start Date/Time: KLB 7 Feb 2011 @ 1330h
No. Organisms/volume: 10/200mL
Test Organism: D.magna
Set up by: KLB

DO meter: DO-1 pH meter: pH-1 Conductivity meter: C-1

Concentration (1) % (v/v)	Rep	Number of Live Organisms 24	48	No. Immobilized 48	Temperature (°C) 0	24	48	Dissolved oxygen (mg/L) 0	24	48	pH 0	24	48	Conductivity (µS/cm) 0	48
Control	A	10	10	0	20.0	20.5	20.5	8.9		8.9	8.02 KLB		8.3	~~359~~ 369 KLB	598
	B														
	C														
	D														
6.25	A	10	3	0	20.0	20.5	20.0	8.9		9.0	8.3		8.3	704	747
	B														
	C														
	D														
12.5	A	10	4	0	20.0	20.5	20.0	8.9		9.0	8.4		8.4	826	884
	B														
	C														
	D														
25	A	10	7	0	20.0	20.5	20.0	8.9		9.0	8.5		8.4	1119	1197
	B														
	C														
	D														
50	A	10	10	0	20.0	20.5	20.0	8.9		9.1	8.5 (2)		8.4	1536	1628
	B														
	C														
	D														
100	A	10	10	0	20.0	20.5	20.0	8.9		9.1	8.5 (2)		8.2	2710	2940
	B														
	C														
	D														
Technician Initials		~	KLB	KLB	KLB	~	KJL	KLB		KLB	KLB		KLB	KLB	KLB

	Hardness*	Alkalinity*
Conc.	*(mg/L as CaCo3)	
~~Control (MHW)~~ Prairie Creek KLB	~~100~~ 308 KLB	~~74~~ 224 KLB
Highest conc.	470	100

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	20.0		20.0
DO (mg/L)	8.9		8.9
pH	9.3	added 6 mL HCl after dilutions were made	8.5
Cond (µS/cm)	2710		2710

Sample Description: clear

Comments: Batch#: 011211A 7-d previous # young/brood: 20 Day of 1st Brood: 9 Previous 7-d % Mortality: 0

Reviewed by: A. Tong Date reviewed: March 24, 2011

(1) diluted w/ Prairie Creek water
(2) Adjusted pH to 8.5 with diluted HCl after dilutions were made

Daphnia magna Summary Sheet

Client: Hatfield
Work Order No.: 9106 4

Start Date/Time: Feb 1 / 11 @ 1345 L
Test Species: *D.magna*
Set up by: KLB

Sample Information:

Sample ID: Mixture 2 (diluted w/ Prairie Creek Water)
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2 + 20L

Test Organism Information:

Broodstock No.: 011211A
Age of young (Day 0): < 24 hours
Avg No. young per brood in previous 7 d: 20
Mortality (%) in previous 7 d: 0
Days to first brood: 9

NaCl Reference Toxicant Results:

Reference Toxicant ID: DM 66
Stock Solution ID: 10 N_2Cl
Date Initiated: Jan 21/11
48-h LC50 (95% CL): 4.2 (3.7 - 4.8) ~~g/L NaCL~~ g/L NaCL

Reference Toxicant Mean (2SD Range): 4.0 (3.6 - 4.3) g/L NaCL
Reference Toxicant CV (%): 5

Test Results: The 48h LC50 was estimated @ > 100% (v/v)

Reviewed by: A. Tang

Date reviewed: March 24, 2011

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Hatfield
Sample ID: Mixture 2 (2:1)
Work Order No.: 11064

Start Date/Time: KLB Jan Feb 1 2011 @ 1345h
No. Organisms/volume: 10/200mL
Test Organism: D.magna
Set up by: KLB

DO meter: DO-1 pH meter: pH-1 Conductivity meter: C-1

Concentration % (v/v)	Rep	Number of Live Organisms 24	Number of Live Organisms 48	No. Immobilized 48	Temperature (°C) 0	Temperature (°C) 24	Temperature (°C) 48	Dissolved oxygen (mg/L) 0	Dissolved oxygen (mg/L) 24	Dissolved oxygen (mg/L) 48	pH 0	pH 24	pH 48	Conductivity (µS/cm) 0	Conductivity (µS/cm) 48
Control	A	10	10	0	20.0	20.5	20.5	8.9		8.9	~~8.08~~ 8.2 KLB		8.3	~~3568~~ KLB	598
	B														
	C														
	D														
6.25	A	10	5	0	20.0	20.5	20.5	8.8		9.0	8.3		8.3	652	693
	B														
	C														
	D														
12.5	A	10	6	0	20.0	20.5	20.0	8.9		9.1	8.3		8.4	719	774
	B														
	C														
	D														
25	A	10	~~10~~ 9 KLB	0	20.0	20.5	20.0	8.9		9.1	8.5		8.4	867	924
	B														
	C														
	D														
50	A	10	10	0	20.0	20.5	20.0	8.9		9.2	8.5②		8.4	1186	1278
	B														
	C														
	D														
100	A	10	10	0	20.0	20.5	20.0	8.9		9.1	8.5②		8.2	1867	2090
	B														
	C														
	D														
Technician Initials		~	KLB	KLB	KLB	~	KLB	KLB		KLB	KLB		KLB	KLB	KLB

	Hardness*	Alkalinity*
Conc.	*(mg/L as CaCo3)	
Control (MHW) Prairie Creek KLB	~~100~~ 300 KLB	~~74~~ 224 KLB
Highest conc.	500	110

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	20.0		20.0
DO (mg/L)	8.9		8.9
pH	9.2	Added 0.1mHCl after dilutions were made	8.5
Cond (µS/cm)	1867		1867

Sample Description: clear

Comments: Batch#: 0112LA 7-d previous # young/brood: 30 Day of 1st Brood: 9 Previous 7-d % Mortality: 0

Reviewed by: A. Tong Date reviewed: March ~~25~~ 24, 2011

① diluted w/ Prairie Creek Water
② Adjusted pH to 8.5 with 0.1mHCl after dilutions were made

Daphnia magna Summary Sheet

Client: Hatfield
Work Order No.: 41064

Start Date/Time: Feb 1 / 11 @ 1020h
Test Species: *D.magna*
Set up by: KLB

Sample Information:

Sample ID: Mine Water
Sample Date: Jan 28/11
Date Received: Jan 28/11
Sample Volume: 2 x 2L

Test Organism Information:

Broodstock No.: 011211 A
Age of young (Day 0): < 24 hours
Avg No. young per brood in previous 7 d: 20
Mortality (%) in previous 7 d: 0
Days to first brood: 9

NaCl Reference Toxicant Results:

Reference Toxicant ID: DM 66
Stock Solution ID: 10 NaCl
Date Initiated: Jan 21/11
48-h LC50 (95% CL): 4.2 (3.7 - 4.8) g/LNaCL

Reference Toxicant Mean (2SD Range): 4.0 (3.6 - 4.3) g/L NaCL
Reference Toxicant CV (%): 5

Test Results: 100% survival in the undiluted 100% (v/v) @ 48h

Reviewed by: A. Tang

Date reviewed: March 24, 2011

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Hatfield
Sample ID: Mine Water
Work Order No.: 11064

Start Date/Time: Feb 1/11 @ 1020h
No. Organisms/volume: 10/200mL
Test Organism: D.magna
Set up by: KLB

DO meter: DO-1
pH meter: pH-1
Conductivity meter: C-1

Concentration	Rep	Number of Live Organisms		No. Immobilized	Temperature (°C)			Dissolved oxygen (mg/L)			pH			Conductivity (µS/cm)	
% (V/V)		24	48	48	0	24	48	0	24	48	0	24	48	0	48
Control	A	10	10	0	20.0	20.5	20.5	8.7		8.8	8.0		8.1	359	405
	B	10	10	0											
	C	10	10	0											
	D														
100	A	10	10	0	20.0	20.5	19.5	8.6		8.7	8.1		8.1	~~280~~ 1300	1128
	B	10	10	0											
	C	10	10	0											
	D														
	A														
	B														
	C														
	D														
	A														
	B														
	C														
	D														
	A														
	B														
	C														
	D														
	A														
	B														
	C														
	D														
Technician Initials		~	KLB	KLB	KLB	~	KLB	KLB		KLB	KLB		KLB	KLB	KLB

	Hardness*	Alkalinity*
Conc.	*(mg/L as CaCo3)	
Control (MHW)	100	74
Highest conc.	550	82

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	20.0		
DO (mg/L)	8.6		
pH	8.1		
Cond (µS/cm)	280		

Sample Description: clear

Comments: Batch#: 011211A 7-d previous # young/brood: 20 Day of 1st Brood: 9 Previous 7-d % Mortality: 0

Reviewed by: A. Tang
Date reviewed: March 24, 2011

Client: Hatfield

W.O.#: 11064

Hardness and Alkalinity Datasheet

	Alkalinity						Hardness			
Sample ID	Sample Date	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
Mixture 1 (4:1)	Jan31/11	10①	1.1	1.2	100		10①	4.7	470	KLB
Mixture 2 (8:1)	Jan31/11	10①	1.2	1.3	110		10①	5.0	500	KLB
Mine Water	Feb3/11	50	4.2	4.3	82		10①	5.5	550	KLB
Prairie Creek Water	Jan12/11	50	11.6	12.0	224		50	15.0	300	KLB

Notes: ① Diluted to 100mL with DI water

Reviewed by: A. Tang

Date Reviewed: March 24, 2011

Attachment D

April 8, 2011
Toxicity Identification Evaluation (TIE)

Nautilus Environmental

Toxicity Identification Evaluation of Mill Water Sample

Final Report

Report date:
April 8, 2011

Submitted to:

Hatfield Consultants
North Vancouver, BC

8664 Commerce Court
Burnaby, BC
V5A 4N7

TABLE OF CONTENTS

Page

LIST OF TABLES

1.0 INTRODUCTION

Toxicity tests conducted on two mixtures of Mine Water and Mill Water produced to reflect future anticipated conditions at the Canadian Zinc operation indicated that reproduction of the freshwater cladoceran, *Ceriodaphnia dubia,* was adversely affected by the mixtures. Conversely, rainbow trout and duckweed were not adversely affected in acute and chronic tests, respectively, and only a marginal adverse effect was apparent in one of the mixtures using acute tests with *Daphnia magna*. The results of these tests are provided in a separate test report.

Adverse effects on reproduction of *Ceriodaphnia* appeared to be derived from the Mill Water, since the degree of toxicity observed was related to the proportion of Mill Water in the samples, and the Mine Water tested alone exhibited no adverse effect on *Daphnia*. Consequently, efforts were undertaken to establish the cause of toxicity to *Ceriodaphnia* in the Mill Water using a Toxicity Identification Evaluation. This process involves conducting a series of physico-chemical manipulations on the sample, following by toxicity tests on the treated and untreated samples. Alterations in the degree of toxicity present as a result of the treatments provides an indication of the characteristics of the contaminant that is responsible for toxicity in the sample. The actual identity of the toxicant can then be established through a series of follow-up procedures.

2.0 METHODS

The following treatments were conducted:

EDTA treatment – Chelation of the sample with EDTA was used to identify whether divalent metals, such as copper, cadmium and zinc, were responsible for toxicity. This chemical binds to divalent metals and reduces their bioavailability and, therefore, toxicity. Treatments were conducted at 5 mg/L EDTA.

C18 solid phase extraction – Treatment of the sample through a C18 substrate was utilized to identify whether toxicity was caused by a non-polar organic contaminant. This material binds and removes these materials and, therefore, if toxicity is reduced following treatment with C18, this indicates that organic contaminants are responsible for toxicity.

Anion Exchange – Extraction of the sample through a strong anion exchange column was used to establish whether strong anions were responsible for toxicity. This treatment is similar to the

C18 treatment, except that the substrate contains positively charged amine groups which have an affinity for anions in the sample. Anions that would be expected to be removed include anionic surfactants, but not major anions such as sulphate, carbonate or chloride.

Filtration – Filtration of the sample through a 0.45 µm filter was conducted to remove particulate-bound contaminants.

pH adjustment – Adjustment of the pH of a sample can alter the characteristics of the toxicant, resulting in an alteration in toxicity, or a change in the effectiveness of other TIE procedures. In this case, adjustment of the pH of the sample to 5 and 9 was used in conjunction with C18 and anion exchange in an attempt to establish whether the toxicant exhibited a higher affinity for these materials under different pH conditions. In addition, the sample was filtered after adjustment to pH 10, which would be expected to remove metals, such as zinc.

In order to evaluate the potential contribution to toxicity of a flocculent (Magnafloc 10) that was used in preparation of the sample, a sample of this chemical was obtained from SGS and evaluated for toxicity using *Ceriodaphnia*.

Test procedures used here were consistent with those typically applied for chronic tests using this species, with the exception that the degree of replication was reduced from 10 to 5. This stream-lining of the procedure is appropriate in Toxicity Identification Evaluations, where the purpose is to look for substantial changes in effect as a result of the treatments.

3.0 RESULTS

Initial treatments were conducted on the Mill Water sample diluted to 10%. None of the treatments (filtration, C18 extraction, anion exchange and EDTA) had an appreciable effect on toxicity to *Ceriodaphnia*, indicating that toxicity did not appear to be caused by particulate-bound contaminants, non-polar organic contaminants, strong anions, or divalent metals (Table 1).

These treatments were repeated using a sample diluted to 5% in case there had been too much toxicity present in the 10% sample for the treatments to be effective; however, the results of these treatments were not useful because the reproduction in the untreated sample diluted to 5% (21.2 ± 1.8 offspring per adult) were not significantly lower than the control (22.8 ± 2.9 offspring per adult). Thus, since the 5% sample did not exhibit toxicity, no information with respect to the cause of toxicity could be obtained from these treatments.

Adjusting the pH of the sample to 5 or 9 prior to treatment using C18 and anion exchange did not improve the effectiveness of these treatments at reducing toxicity in the 10% sample. Results of these treatments are also shown in Table 1. These results did not provide further indication as to the cause of toxicity, but are consistent with the initial findings described above.

The results described above are consistent with a number of contaminants, including charged or highly soluble organic contaminants, cations, total dissolved solids, and other chemicals. In order to establish whether one of the process chemicals used in preparation of the samples might have been responsible, the characteristics of the materials were reviewed and Magnafloc 10 was identified as being potentially consistent with the results, and of unknown toxicity to *Ceriodaphnia*. The results of a toxicity test conducted using this chemical are provided in Table 2; in general, this chemical resulted in no adverse effect on reproduction at 1.25 mg/L or less, but reduced reproduction was observed in the 2.5 and 5 mg/L solutions. Since the treatment rate of this material was 14 mg/L in the Mill Water, and most of the material would be expected to be removed during the treatment process, it appears unlikely that this was the cause of toxicity.

Table 1. Results of TIE treatments conducted on 10% Mill Water.

	Survival (%)	Reproduction (offspring per adult)
Control	100	23.6 ± 5.2
Untreated	100	1.2 ± 1.8
Filtered sample	100	1.0 ± 1.4
C18-treated sample	100	0.6 ± 1.3
Anion Exchange-treated sample	100	2.6 ± 1.8
EDTA treated sample	100	0.0 ± 0.0
Control	100	18.0 ± 3.3
Untreated	100	0.0 ± 0.0
pH 5 + anion exchange	100	0.0 ± 0.0
pH 9 + anion exchange	100	0.0 ± 0.0
pH 5 + C18	100	0.0 ± 0.0
pH 9 + C18	100	0.0 ± 0.0
pH 10 + filtration	100	1.4 ± 1.9

Table 2. Results of toxicity test conducted on Magnafloc 10.

Mangafloc 10 (mg/L)	Survival (%)	Reproduction (offspring per adult)
Control	100	21.8 ± 6.6
0.08	100	22.2 ± 10.1
0.16	100	25.0 ± 2.0
0.31	100	20.8 ± 4.2
0.62	100	20.8 ± 6.9
1.25	100	20.2 ± 4.3
2.5	100	14.0 ± 5.7
5.0	100	13.2 ± 2.7

4.0 DISCUSSION

The results of the TIE procedures described here were not conclusive in establishing the cause of toxicity in the Mill Water; however, the results indicate that non-polar organic contaminants, strong anions and divalent metals did not appear to be the primary cause of toxicity in the sample, although it should be noted that these materials may have contributed to toxicity at higher concentrations of sample.

The concentration of sulphate present in the Mill Water would most likely have contributed some portion of the adverse effect observed to *Ceriodaphnia*. For example, Elphick et al. (2011) reported an IC25 value for effects of sulphate for this species of 1212 mg/L sulphate at a hardness of 160 mg/L. Since the Mill Water contained 4500 mg/L sulphate, there was clearly sufficient sulphate present to cause some proportion of the observed effect. Total dissolved solids, in general, which includes sulphate and other major ions, such as calcium, magnesium, sodium, potassium, chloride and carbonate causes effects on this species when elevated as a result of osmotic stress, and so sulphate, or major ions in general, likely explains some of the observed effect. However, the sample diluted to 10% would likely not have contained sufficient major ions to explain the effect observed in the diluted sample.

The Mill Water exhibited toxicity to *Ceriodaphnia* in the sample diluted to 10%, but not when tested at 5%. This result differs somewhat from the initial tests using the Mixtures, in which toxicity was observed in all concentrations tested, as low as 5% sample. Since the Mixtures were comprised of only a portion of Mill Water, the adverse effect observed here with the Mill Water is not consistent with the extent of adverse effect observed in the mixtures. This implies that either: 1) the toxicity of the Mill Water dissipated in between when the original test was conducted and when the TIE treatments were performed; 2) other components of the mixtures (i.e., Mine Water) also contributed to toxicity in the mixtures; or 3) there was some interaction between components in the mixture that exacerbated toxicity. The most likely explanation would be that toxicity dissipated over time in the sample; however, additional investigation would be necessary to fully characterize and identify the cause of toxicity in this sample.

5.0 REFERENCES

Elphick, J.R., Davies, M. Gilron, G., Canaria, E.C., Lo, B. and Bailey, H.C. 2011a. An aquatic toxicological evaluation of sulphate: the case for considering hardness as a modifying factor in setting water quality guidelines. *Environ. Toxicol. Chem.* 30:247-253.