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ACUTE TOXICITY OF WATERLYNX 494X TO RAINBOW TROUT AND *DAPHNIA MAGNA* (NON-GLP)

Prepared for:
CLEARFLOW Enviro Systems Group
#140- 134 Pembina Road,
Sherwood park, AB
Canada, T8A 0M2

Prepared by:
Maxxam Analytics
Ecotoxicology Group
4606 Canada Way
Burnaby, BC
Canada V5G 1K5

Job #: B2A3552
Project No.: 2-11-0691
January 2013

EXECUTIVE SUMMARY

The acute toxicity of WaterLynx 494x to two aquatic organisms was assessed (non-GLP) using the following methods:

OECD. 1992. Fish, Acute Toxicity Test. OECD Guidelines for Testing of Chemicals, Section 2: Effects on Biotic Systems. Test Method OECD 203.

OECD. 2004. *Daphnia* sp., Acute Immobilisation Test. OECD Guidelines for Testing of Chemicals, Section 2: Effects on Biotic Systems. Test Method OECD 202. (48-hr Immobilisation of *Daphnia magna*)

Test solutions were prepared from stock solutions of dissolved Test Substance in dilution water. The stock solution was prepared using small piece of the test item stir into dilution water for 24 hours. A range of nominal concentrations of the test substance and a negative control of dilution water only were tested with each species. The toxicity values are based on nominal concentrations (mg/L) and are listed below:

Test	**LC50 (95% CI)**	**EC50 (95% CI)**
96-hr Survival of Rainbow Trout	210.2 (170, 259.9)	Not Applicable
48-hr Immobilisation of *Daphnia magna*	Not Applicable	418.4 (336.3, 520.6)

SECTION

1 ACUTE LETHALITY TO RAINBOW TROUT (96-HR STATIC)

Acute lethality tests were conducted with rainbow trout (*Oncorhynchus mykiss*) according to the OECD method, OECD 203, Fish, Acute Toxicity Test (OECD 1992).

A preliminary range-finding test was conducted over the range of 0, 0.1, 1, 10, 100 and 1000 mg test substance/ litre of dilution water. The results showed that there was 100% survival up to 100 mg/L, and 0% survival at 1000 mg/L. Therefore, the definitive test used the following concentrations: 0, 63, 125, 250, 500, and 1000 mg/L.

To prepare the Stock solution, the test item (WaterLynx 494x) was cut into very small pieces with a clean knife and transferred into a 5-L glass vessel with fish lab water. The solution was agitated with a stir bar for ~24h to allow the test item to dissolve into the water. The final solution of 5,000 mg/L was diluted with the dilution water to obtain the final concentrations.

There was one replicate per treatment, which consisted of 8 fish in a total volume of 8 L test solution in a glass vessel. The mean fish length was 4.425 cm with a mean weight of 0.79 g. The loading density was 0.79 g/L. The fish were not fed a minimum of 24 h prior to test initiation or during the tests. The test chambers were covered with a Plexiglass sheet and aeration was provided during the test. The tests were conducted at a daily mean water temperature of 15 ± 2°C, with a photoperiod of 16L:8D.

Test chamber observations and survival checks were conducted and recorded daily. Measurements of dissolved oxygen concentrations, temperature, and pH, were taken at the start and end of the test except for those concentrations that had 100% mortality before the end of the test. For the 500 and 1000 mg/L concentrations, the final water quality was conducted at the time 100% mortality was observed. Conductivity was measured at test initiation.

The 96-hr LC50 was calculated using the Untrimmed Spearman-Karber method in the statistical program, CETIS™ (Version 1.7.0.3) (Tidepool Scientific Software Copyright 2000-2009). The LC50 value was 210.2 mg/L (95% Confidence Interval: 170 – 259.9 mg/L).

The tests were considered valid as none of control neonates died or displayed atypical or stressed behaviour. A reference toxicant (positive control) test was conducted with zinc sulphate within two weeks of this test and the resulting LC50 was within two standard deviations of the mean LC50 of previous tests.

The following range finding and definitive raw data are presented in Appendix: CETIS™ statistical reports, benchsheets, organism information and reference toxicant control chart.

SECTION

2 ACUTE IMMOBILISATION OF *DAPHNIA MAGNA* (48-HR, STATIC)

An acute toxicity test was conducted with the freshwater invertebrate, Daphnia magna, according to the OECD method, OECD 202, Daphnia sp., Acute Immobilisation Test (OECD 2004).

The stock and test solution were prepared in reconstituted water. This water was prepared by adding 1.144 g MgSO4, 1.670 g CaSO4.2H2O, 2.112 g NaHCO3, 0.088 g KCl, 10 mL of a 4 mg/L Vitamin B12 (as cyancobalamin) solution, and 40 mL of a 1 mg/L selenium solution to ~20 L of deionised water. The water was aerated at test temperature at least overnight prior to use in the test. The water hardness of the dilution water was 92 mg/L as CaCO3 (measured by EDTA titration).

To prepare the Stock solution, the test item (WaterLynx 494x) was cut into very small pieces with a clean knife and transferred into a 1-L glass beaker with daphnia dilution water. The solution was agitated with a stir bar for ~24h to allow the test item to dissolve into the water. The final solution of 5,000 mg/L was diluted with the dilution water to obtain concentrations.

A preliminary range-finding test was conducted over the range of 0, 0.1, 1, 10, 100 and 1000 mg test substance/ litre of dilution water. The results showed that there was sporadic immobilisation within the range. Therefore, the definitive test used the following concentrations: 0, 15, 30, 65, 125, 250, 500, and 1000 mg/L to cover the maximum range possible. Test solutions were not renewed during the tests.

There were four replicates per treatment; each replicate consisted of 5 neonates in a total volume of 200 mL test solution in a 250 mL glass beaker. The neonates were <24 h old at test initiation and were collected from a brood that had 5.9% parental mortality (<25% is required) in the 7 days preceding test initiation. The neonates were not fed during the tests, but were fed a mixture of algae (*P. subcapitata* and *C. pyrenoidosa*) prior to use in the tests. The test chambers were covered with a Plexiglass sheet and no aeration was provided during the test. The tests were conducted at a daily mean water temperature of 20 ± 2°C, with a photoperiod of 16L:8D. The test chambers were monitored daily for number of immobilised or floating organisms. At test completion, the number of immobilised organisms was recorded. Measurements of dissolved oxygen concentrations, temperature, and pH, were taken at the start and end of the test. Conductivity was measured at test initiation only.

The OECD 202 test method yields an EC50 based on immobilisation. The Trimmed Spearman-Karber method in the statistical program, CETIS™ (Version 1.7.0.3) (Tidepool

Scientific Software Copyright 2000-2009), was used to calculate the endpoint. The EC50 value was 418.4 mg/L (95% Confidence Interval: 336.3 – 520.6 mg/L).

The tests were considered valid as none of the control neonates died or displayed atypical or stressed behaviour. A reference toxicant (positive control) test was conducted with zinc sulphate within two weeks of this test and the resulting LC50 was within two standard deviations of the mean LC50 of previous tests.

The following range finding and definitive test raw data is presented in Appendix: CETIS™ statistical reports, benchsheets, culture health and reference toxicant control chart.

APPENDIX

A SAMPLE INFORMATION

Client: CLEARFLOW ENVIRO SYSTEMS GROUP
#140 - 134 PEMBINA ROAD
SHERWOOD PARK AB
CANADA T8A0M2

Inv Attn: JESSE MEINTS

Task Order:
Line Item:

Printed: 2013/01/08 Version 6
Reception Date: 2012/11/14
Reception Time: 13:07
Login Date: 2012/11/14
REQUIRED DATE: 2013/01/31, 18:00
Quote Number:

Report: same

Attention: JESSE MEINTS
PHONE: (780) 410 - 1403Ext:
FAX: (780) 464 - 2026
EMAIL: jesse.meints@clearflowgroup.com

P.O. Number: 335-201211
PROJECT NUMBER:
Site Location:
Site #:
Client Number: 6683
Rpt Address #:

Project Coordinator: JLI
Q.C. Samples: No

Maxxam Number	Client Sample ID/Report ID	Cont's	Store Code	Recd. OK	Sampling Date	Matrix	Test Codes
FA6618-01R	WATERLYNX 494X WATERLYNX 494X	1	N/A	Yes		SOLID	DISPOSAL, ECOATTACH, OECD202 OECD203

Remarks: RK8// client did not provide date or time of relinquishment.
IW.

Please note: Client uses minimum billing ($150.00) !

Entered by: RK8
Date: 2012/11/14
Time: 15:10

Approved by:
Date:
Time:

Date of Sample Disposal:
Disposal by:

11/505

61945

Maxxam Analytics

Calgary: 4000 19st St. NE, T2E 6P8 | Ph: (403) 291-3077 | Fax: (403) 735-2240 | Toll free: (800) 386-7247

Edmonton: 9331 – 48 Street, T6B 2R4 | Ph: (780) 465-1212 | Fax: (780) 450-4187 | Toll free: (877) 465-8889

www.maxxamanalytics.com

CHAIN OF CUSTODY

Page: 1 of 1

Invoice To: Require Report? Yes ☒ No ☐

Company Name: Clearflow Enviro Systems Group

Contact Name: Jesse Meints

Address: #140 - 134 Pembina Rd. Sherwood Park

Prov: AB **PC:** T8H 0M2

Contact #s: Ph: 780 410 1403 Fax: 780 410 1406

Report To: Same

Prov: **PC:**

Ph: **Fax:**

PO # / AFE #: 335 - 201211

Quotation #:

Project #:

Project Name:

Location:

Sampler's Initials:

DETECTION LIMIT REQUIREMENTS:

Check the applicable criterion and indicate land use

☐ AT1

☐ CCME

☐ OTHER

REPORT DISTRIBUTION:

EMAIL ADDRESS(S):

SERVICE REQUESTED:

☐ **RUSH** (Please ensure you contact the lab to reserve)

Date Required:

☐ REGULAR Turnaround (5 to 7 Days)

	Sample Identification	Matrix S/W	Date & Time Sampled Year/Month/Day	SOILS (footnotes defined on back): BTEX F1-F4	Sieve (75 micron)	Salinity 4	Regulated Metals (CCME / AT1)[1]	Assessment ICP Metals[2]	☐ Paint Filter ☐ Flashpoint ☐ pH (1:1)	TCLP ☐ BTEX ☐ Metals	96 hr Rainbow Trout Bio Assay		48hr Daphnia magna Bio Assay	WATERS (footnotes defined on back): ☐ BTEX F1 ☐ VOCs	☐ BTEX F1-F2 ☐ BTEX F1-F4	Routine Water Package ☐ Turb ☐ F	REGULATED METALS (CCME / AT1)[2] Total ☐ Preserved ☐ Not Preserved	Dissolved ☐ Preserved ☐ Not Preserved ☐ Filtered ☐ Not Filtered	Mercury ☐ Total ☐ Dissolved	☐ Ammonia ☐ TKN ☐ COD	☐ TOC ☐ DOC	OTHER TEST(S)	*HOLD for 60 Days	# of Containers Submitted
1	WaterLynx 494X FA648										X		X											
2	WaterLynx 494X (pH neutralized) FA649										X		X											
3	WaterLynx 494X and WaterLynx 360 (pH neutralized) FA620										X		X											
4																								
5																								
6																								
7																								
8																								
9																								
10																								
11																								
12																								

B2A3552

All samples are held for 60 calendar days after sample receipt. For long term storage please contact your project manager.

Maxxam Job #: B2A3552

Relinquished By: Jesse Meints **Date/Time:**

Sign and Print:

COMMENTS/SPECIAL INSTRUCTIONS:

See Attached Email for specifics

Relinq. Ches Cumby
CHES CUMBY
2012/11/16 1540

# JARS USED & NOT SUBMITTED	Received By	Temperature			Ice
	Amanda L'Hirondelle 20121114 @ 1307	18	18	18	absent
	CUSTODY SEAL YES / (NO)				

BBY: [illegible] 2012/11/17 11:53 Temp: 9.6 [illegible] C.S. YES - INTACT

APPENDIX

B RAINBOW TROUT

CETIS Analytical Report

Test Code: 05-8654-0913/OM-6683-0112

Rainbow Trout Acute Toxicity Test — Maxxam Analytics

Analysis ID:	06-9543-1612	Endpoint:	96h Survival Rate	CETIS Version:	CETISv1.7.0
Analyzed:	02 Jan-13 15:44	Analysis:	Untrimmed Spearman-Kärber	Official Results:	Yes
Batch ID:	03-0233-1903	Test Type:	Survival (96h)	Analyst:	D. Lai
Start Date:	12 Dec-12 14:47	Protocol:	OECD Method 203 (1992)	Diluent:	Fish House Water
Ending Date:	16 Dec-12 15:10	Species:	Oncorhynchus mykiss	Brine:	Not Applicable
Duration:	4d 0h	Source:	Aqua Farms	Age:	
Sample ID:	10-3659-8769	Code:	Job# B2A3552	Client:	Clearflow Enviro. Systems Group
Sample Date:	14 Nov-12 09:00	Material:	Chemical	Project:	2-11-0691
Receive Date:		Source:	Clearflow		
Sample Age:	28d 6h	Station:	Waterlynx 494X		

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.323	0.04609	210.2	170	259.9

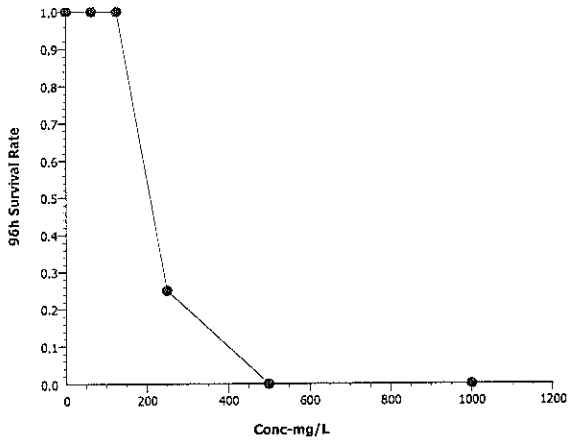
96h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	Diff%	A	B
0	Negative Control	1	1	1	1	0	0	0.0%	0.0%	8	8
63		1	1	1	1	0	0	0.0%	0.0%	8	8
125		1	1	1	1	0	0	0.0%	0.0%	8	8
250		1	0.25	0.25	0.25	0	0	0.0%	75.0%	2	8
500		1	0	0	0	0	0		100.0%	0	8
1000		1	0	0	0	0	0		100.0%	0	8

96h Survival Rate Detail

Conc-mg/L	Control Type	Rep 1
0	Negative Control	1
63		1
125		1
250		0.25
500		0
1000		0

Graphics



2013Jan02 2013Jan02

Analyst: LS QA: KF

CETIS Analytical Report

Test Code: 05-8654-0913/OM-6683-0112

Rainbow Trout Acute Toxicity Test — Maxxam Analytics

Analysis ID:	18-5951-9328	Endpoint:	96h Survival Rate	CETIS Version:	CETISv1.7.0
Analyzed:	02 Jan-13 15:44	Analysis:	STP 2x2 Contingency Tables	Official Results:	Yes
Batch ID:	03-0233-1903	Test Type:	Survival (96h)	Analyst:	D. Lai
Start Date:	12 Dec-12 14:47	Protocol:	OECD Method 203 (1992)	Diluent:	Fish House Water
Ending Date:	16 Dec-12 15:10	Species:	Oncorhynchus mykiss	Brine:	Not Applicable
Duration:	4d 0h	Source:	Aqua Farms	Age:	
Sample ID:	10-3659-8769	Code:	Job# B2A3552	Client:	Clearflow Enviro. Systems Group
Sample Date:	14 Nov-12 09:00	Material:	Chemical	Project:	2-11-0691
Receive Date:		Source:	Clearflow		
Sample Age:	28d 6h	Station:	Waterlynx 494X		

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	125	250	176.8		N/A

Fisher Exact/Bonferroni-Holm Test

Control	vs	Conc-mg/L	Test Stat	P-Value	Decision(0.05)
Negative Control		63	1	1.0000	Non-Significant Effect
		125	1	1.0000	Non-Significant Effect
		250	0.003497	0.0105	Significant Effect
		500	0.0000777	0.0004	Significant Effect
		1000	0.0000777	0.0004	Significant Effect

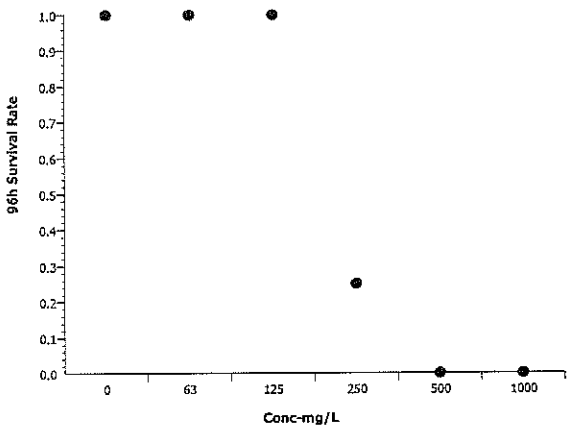
Data Summary

Conc-mg/L	Control Type	No-Resp	Resp	Total
0	Negative Contr	8	0	8
63		8	0	8
125		8	0	8
250		2	6	8
500		0	8	8
1000		0	8	8

96h Survival Rate Detail

Conc-mg/L	Control Type	Rep 1
0	Negative Control	1
63		1
125		1
250		0.25
500		0
1000		0

Graphics



2013Jan02 2013Jan02

Analyst: LS QA: KK

ECOTOXICOLOGY

OECD 203 SURVIVAL TEST – DAILY SURVIVAL

Client # & Name:	Clearflow 6683	**Project Number:**	2-11-0691
Sample ID:	Lynx 49% ∞	**Study Number:**	n/a
Job #/Sample #:	FA6618 B2A3552	**Date & Time Started:**	2012 Dec 12 @ 14:47
Test Species:	Rainbow trout	**Organism Lot #:**	AF121121
Test Volume:	8L	**# Fish/Treatment:**	8

# of Surviving Organisms

	Date & Day			
Date	2012 Dec 13	2012 Dec 14	2012 Dec 15	2012 Dec 16
Treatment	1	2	3	4
CTRL	8	8	8	8
63 mg/L	8	8	8	8
125 mg/L	8	8	8	8
250 mg/L	2	2	2	2
500 mg/L	0	0	0	0
1000 mg/L	0	0	0	0
		JL 2013 Jan 7th		
Analyst	DML	DML	JL	AR
Date	2012 Dec 13	2012 Dec 14	2012 Dec 15	2012 Dec 16

Notes:

2012 Dec 12 at time zero fish are dying in 1000mg/L. Fish are surfacing and inverted in 500mg/L Fish are breathing rapidly in 250mg/L. Fish appear normal in all other concn DML

2012 Dec 12 1000mg/L fish all dead @ 1500. DO: 10.1 @ 16.0°C pH = 7.4 DML

2012 Dec 12 500mg/L fish all dead @ 1700 DO: 10.0 @ 15.6 pH = 7.6 DML

2012 Dec 13 WQ @ 1450, fish normal in all concn DML

2012 Dec 14 WQ @ 1543 fish normal in all concn DML

2012 Dec 15 WQ @ 1530 fish normal in all concn JL

2012 Dec 16 WQ @ 15:10, fish appear normal in all concentrations. AR

JL 2013 Jan 7th

ECOTOXICOLOGY

OECD 203 SURVIVAL TEST – DAILY MEASUREMENTS

Customer # & Name: Clearflow 6683 **Project Number:** 2-11-0691

Sample ID: Lynx 494x **Study Number:** N/A

Job #/Sample #: FA6618 B2A3552 **Date & Time Started:** 2012 Dec 12 @ 14:47

Test Species: Rainbow trout **Organism Lot #:** AF121121

Volume (L): 8 **# of Fish/Replicate:** ~~10~~ 8 **Aeration Rate (mL/min):** 15 mL/min

correction Dec12

0 hours

Treatment (mg/L)	Date	Conductivity (µS/cm)	Temperature (°C)	D.O. (mg/L)	pH
Ctrl	2012 Dec 11 ①	46	15.2	10.2	7.3
63	2012 Dec 11	52	15.3	10.2	7.5
125	2012 Dec 11	57	15.4	10.2	7.5
250	2012 Dec 11	70	15.5	10.1	7.6
500	2012 Dec 11	100	15.8	10.1	7.6
1000	2012 Dec 11	171	16.3	9.9	7.6
Analyst	AR	AR	AR	AR	AR

① Dec 12 2012, not Dec 11 - SL 2012 Dec 12.

24 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
Ctrl	2012 Dec 13	15.0	9.1	6.8
63	2012 Dec 13	15.0	9.6	7.1
125	2012 Dec 13	14.9	9.8	7.3
250	2012 Dec 13	14.9	10.0	7.4
500			n/a DML 2012 Dec 13	
1000				
Analyst		DML	DML	DML

ECOTOXICOLOGY
OECD 203 SURVIVAL TEST – DAILY MEASUREMENTS

48 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 Dec14	15.0	9.9	7.0
63	2012 Dec14	15.0	9.9	7.0
125	2012 Dec14	14.9	9.9	7.2
250	2012 Dec14	14.9	10.1	7.3
500			n/a	
1000			DML 2012 Dec14	
Analyst		DML	DML	DML

72 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
Ctrl	2012Dec15	15.1	9.9	7.3
63	2012Dec15	15.0	9.8	7.5
125	2012Dec15	14.9	9.9	7.6
250	2012Dec15	15.0	9.9	7.7
500	2012Dec15		JL 2012Dec15	
1000	2012Dec15			
Analyst		JL for JG	JL for JG	JL for JG

96 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012Dec16	15.3	9.7	7.4
63	2012Dec16	15.3	9.8	7.4
125	2012 Dec16	15.1	9.9	7.4
250	2012 Dec16	15.2	9.9	7.6
500				
1000		AR 2012 Dec16		
Analyst	AR	AR	AR	AR

ECOTOXICOLOGY

OECD 203 RAINBOW TROUT TEST
FISH LENGTHS AND WEIGHTS AT TEST COMPLETION

Maxxam

Client # & Name: Clearflow # 6683
Sample Name: Lynn 49+x
Start Date & Time: 2012 Dec 12 @ 14:47
End Date: 2012 Dec 16

Study #: 2-11-0691
Project #: n/a
Maxxam ID: FA6618

Fish Number	Length (cm)	Weight (g)
1	4.0	0.57
2	4.7	0.93
3	4.8	1.06
4	4.8	0.78
5	4.2	0.69
6	4.4	0.78
7	3.7	0.43
8	4.8	1.07
9	AR 2012 Dec 16	
10		

Loading Density (g/L): #DIV/0!
Final test volume (L):
Number of fish used:

Mean	#DIV/0!	#DIV/0!
SD	#DIV/0!	#DIV/0!
Minimum	0.0	0.00
Maximum	0.0	0.00

Mean — Length 4.425 — Weight 0.78875 — JL 2013 Jan 7

Entered by:

A. Rakhmangulova 2012 Dec 16
Name Date

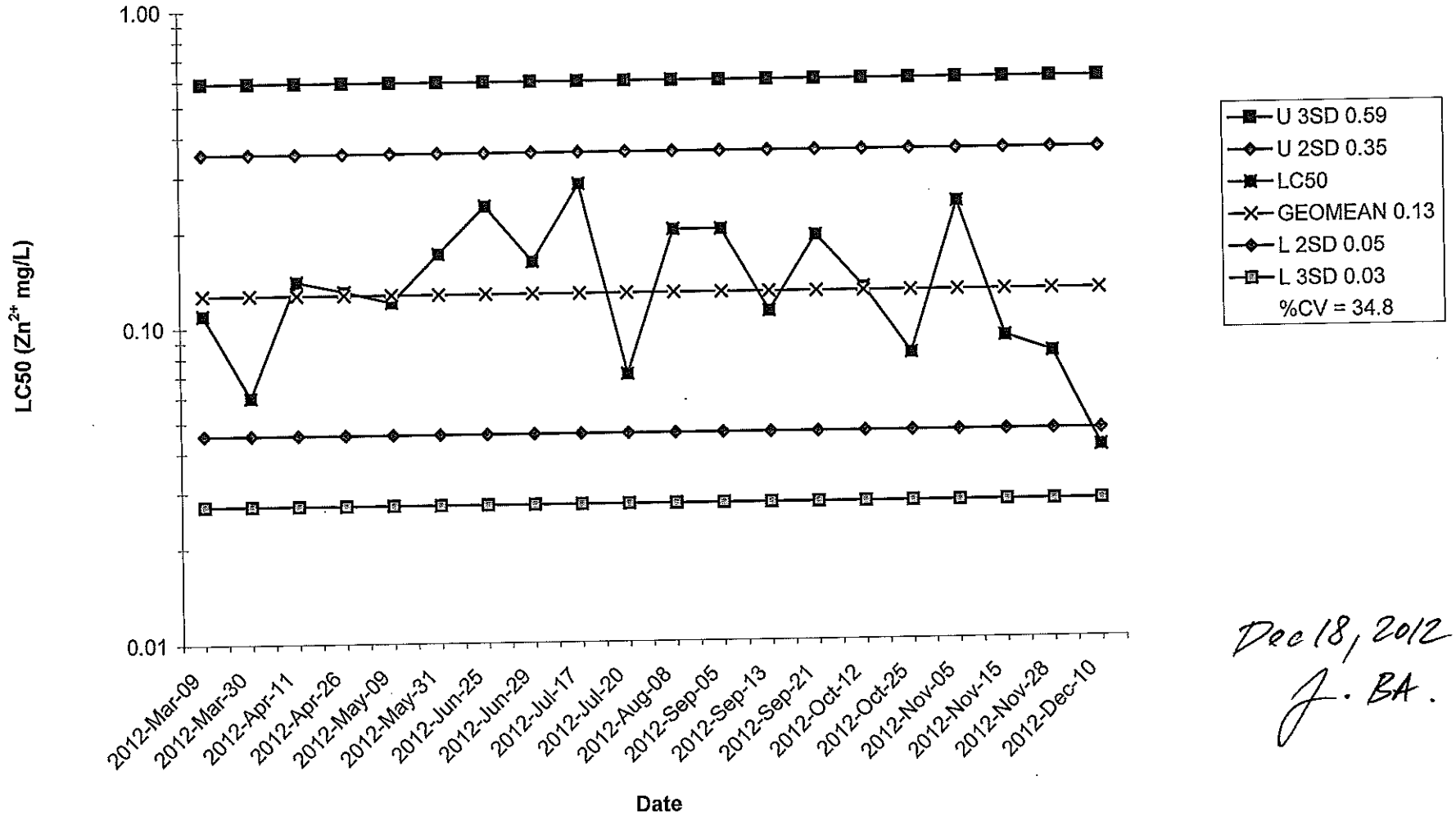
Reviewed by:

JL 2013 Jan 7th
Name Date

Maxxam

Rainbow Trout

96-h Reference Toxicant Control Chart for Zinc



Dec 18, 2012
J. BA.

ECOTOXICOLOGY

STOCK AND TEST SOLUTION PREPARATION

Maxxam

BBY2FCD-00190/1

Client # & Name: Clearflow #618 | Test Species: *Rainbow trout* | Sample ID: Lynx 494X

Project or Study #: 2-11-0691 | Test Protocol: OECD 203 | Maxxam ID: FA6618 (B2A3552)

Solid or Liquid: Solid | Date Received: 2012 Nov 14

Preparation of Stock A from Chemical

Date Prepared	
Volume Prepared	5000 mL
Dilution Water	Fish lab water
Solvent	none
Concentration (mg/L)	5000
Nominal Weight (g)	25.0000
Actual Weight (g)	
Lot Number/Code	
Balance Type	
Analyst	
Verified by	

Preparation of Stock B from Stock A

Date Prepared	
Volume Prepared (mL)	
Dilution Water	
Solvent	
Concentration (mg/L)	
Volume Stock A Added (mL)	
Analyst	
Verified by	

The calculations MUST be verified by another person before solutions are prepared (Formula: $C_1V_1=C_2V_2$)

Final Test Concentration (mg/L) (C_1)	Final Volume (mL) (V_1)	Stock Concentration (mg/L) (C_2)	Stock A or B	Stock Volume Added (mL) (V_2)	Analyst	Date	Verified by	Date
0	0	0	N/A	0.00 ✓	JL	2012 Dec 12	JL	2012 Dec 11
63	8000	5000	A	100.00 ✓				
125	8000	5000	A	200.00 ✓				
250	8000	5000	A	400.00 ✓				
500	8000	5000	A	800.00 ✓				
1000	8000	5000	A	1600.00 ✓				

ECOTOXICOLOGY
WATER ACCOMODATED FRACTION (WAF) AND WATER SOLUBLE FRACTION (WSF) PREPARATION

Customer # & Name:	Clearflow - # 6683	Study & Project #:	2-11-0691
Sample I.D. #:	FA6618 - B2A3552	Sample Name:	Lynx 494X
Date & Time Started:	2012 Dec 11 @ 12h00	Date & Time Ended:	2012 Dec 12 @ 10:30
Volume to Prepare (L):	5	Test Method:	OECD 203
Analyst(s):	JL	Dilution H20:	Fish lab water

Nominal Loading Rate (mg/L)	5000					
Nominal Mass of Test Item to Add (g)	25.0000					
Nominal Mass Verified by:	~~25.0004~~ JL 2012 Dec 11 JL 2012 Dec 11					
Actual Mass Added (g)	25.0004					
Balance ID	BBY2 - 0025					
Method of Addition of Test Item to Vessel, Vessel Size and Type	Test item shaved into small pieces and mixed in a 6-2 glass Erlen meyer with 5L fish lab water.					
Stir Plate ID	BBY2-0050					
Time Started Spinning	12:10					
Height of Vortex	10 cm.					
Analyst/Date	JL 2012 Dec 11					
Time Stopped Spinning	10h30					
Analyst/Date	JL 2012 Dec 12					
Beginning Time for Siphoning						
Siphoned From Middle of Water Column?	☐	☐	☐ JL 24 Dec 12	☐	☐	☐
Type of Tubing Used						
Siphoned Volume Discarded (L)						
Siphoned Volume Collected (L)						
Collection Vessel Size and Type						

~~Nominal~~ Loading Rate (mg/L)	5000					
Analyst/Date						
Filter Apparatus Used: Vessel Size and Type, Filter Type, Brand, and Lot, Vacuum Pump AIN (if used)	JJ 2012 Dec 12					
Collection Vessel Size and Type						
Analyst/Date						

Notes:

Dec 12 2012, Test items well dissolved in water - stop spinning JC

ECOTOXICOLOGY

OECD 203 SURVIVAL TEST – DAILY SURVIVAL

Client # & Name:	#6683 Clearflow	**Project Number:**	WE DML 2012 Dec04 3-11-0691 ~~3-11-0669~~
Sample ID:	Waterlynx 494	**Study Number:**	n/a
Job #/Sample #:	B2A3552 / FA6618-01	**Date & Time Started:**	2012 Dec04 @ 1610
Test Species:	Rainbow Trout	**Organism Lot #:**	AF21107
Test Volume:	10L	**# Fish/Treatment:**	10

# of Surviving Organisms

	Date & Day			
Date	2012 Dec05	2012 Dec06	2012 Dec07	2012 Dec08
Treatment	1	2	3	4
lab ctrl	10	10	10	10
100mg/L	10	10	10	10
177.5mg/L	4	0	0	0
316mg/L	5	3	0	0
561.5mg/L	4	3	0	0
998.5mg/L	0	0	0	0
			n/a DML 2012 Dec04	
Analyst	DML	DML	DML	
Date	2012 Dec05	2012 Dec06	2012 Dec07	2012 Dec08

Notes:

at time zero, are all dying in 998.5mg/L. 5 fish are dying in 561mg/L. 2 fish are dying in 316mg/L. 1 fish quiescent in 177.5mg/L. Rest are normal in ctrl and 100mg/L DML 2012 Dec04

2012 Dec04 @1750, all fish dead in 998.5mg/L. Final DO: 9.2mg/L temp: 16.3°C pH: 7.5 2012 DML

2012 Dec05 WQ@ 1535. normal in ctrl and 100mg/L. 1 dark fish in 177.5mg/L. 1 dark fish in 316mg/L. ~~AF~~ All fish staying near surface in 561mg/L. DML.

2012 Dec06 WQ@ 1430. normal in ctrl and 100mg/L. 2 inverted and one on surface in 316mg/L. 1 fish inverted and two on surface and dark pigment in 561mg/L DML

ECOTOXICOLOGY
TEST OBSERVATIONS

Page 1 of 1

Test Initiation Date: 2012 Dec04
Test Item: Waterlynx
Sponsor: Clearflow
Study Number: 2-11-0691
Test Method: OECD 203
Project Number: n/a

2012 Dec06 @ 1715. all fish dead in 316mg/L.
DO: 3.4 mg/L temp° 15.3°C pH 7.1
all fish dead in 561 mg/L
DO: 3.0 mg/L temp 15.3°C pH = 7.2.
2012 Dec07 @ 1520. all fish normal DML DML
2012 Dec08 - Recorded WQ @ 16:02. CLB
Fish in 100 mg/L appear normal CLB
Weighed CTL fish - recorded in weights/lengths CLB.

Form approved by: J. Pickard
Date: June 30, 2011

ECOTOXICOLOGY

OECD 203 SURVIVAL TEST – DAILY MEASUREMENTS

Customer # & Name: 6683 ClearFlow
Project Number: 2-11-691
Sample ID: Waterlynx 4994
Study Number: N/A
Job #/Sample #: B2A3552 / FA6615-01
Date & Time Started: 2012 Dec 04 1610
Test Species: RBT
Organism Lot #: AFN21107
Volume (L): 10L
# of Fish/Replicate: 10 (Dec 5, 2012 Dec 6)
Aeration Rate (mL/min): 6.5

0 hours

Treatment (mg/L)	Date	Conductivity (µS/cm)	Temperature (°C)	D.O. (mg/L)	pH
labCtrl	2012 Dec 04	46	15.9	9.7	7.4
100	2012 Dec 04	51	15.9	9.7	7.4
177.5	2012 Dec 04	56	15.9	9.7	7.5
316	2012 Dec 04	60	16.0	9.7	7.5
561.5	2012 Dec 04	64	16.2	9.7	7.6
998.5	2012 Dec 04	67	16.4	9.7	7.6
Analyst	NS	NS	NS	NS	NS

24 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
labctrl	2012 Dec 05	15.2	7.8	6.7
100	2012 Dec 05	15.1	6.9	6.8
177.5	2012 Dec 05	15.2	7.4	7.1
316	2012 Dec 05	15.2	8.1	7.1
561.5	2012 Dec 05	15.2	8.3	7.2
998.5	2012 Dec 05	n/a	n/a	n/a
Analyst		DHL	DHL	DHL

ECOTOXICOLOGY
OECD 203 SURVIVAL TEST – DAILY MEASUREMENTS

48 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 Dec06	15.4	7.2	7.0
100	2012 Dec06	15.4	6.2	7.1
177.5	2012 Dec06	15.3	3.0	7.2
316	2012 Dec06	15.4	3.6	7.2
561.5	2012 Dec06	15.4	4.2	7.4
9985	n/a	n/a	n/a	n/a
Analyst		DML	DML	DML

72 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 Dec07	15.3	7.3	6.8
100	2012 Dec07	15.2	8.2	7.0
177.5	2012 Dec07	n/a	n/a	n/a
316	2012 Dec07	n/a	n/a	n/a
561.5	2012 Dec07	n/a	n/a	n/a
9985	n/a	n/a	n/a	n/a
Analyst		DML	DML	DML

96 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 Dec08	15.0	7.4	6.7
100	2012 Dec08	15.0	8.1	6.8
177.5	2012 Dec08	n/a	n/a	n/a
316	2012 Dec08	n/a	n/a	n/a
561.5	2012 Dec08	n/a	n/a	n/a
9985	n/a	n/a	n/a	n/a
Analyst		dB	dB	dB

ECOTOXICOLOGY

STOCK AND TEST SOLUTION PREPARATION

Maxxam

BBY2FCD-00190/1

Client # & Name ClearFlow # 6683 | Test Species *rainbow trout* | Sample ID Lynx Liquid

Project or Study # 2-11-0691 | Test Protocol OECD 203 | Maxxam ID FA 6618 B2A3552

Solid or Liquid solid | Date Received 2012 Nov 14

Preparation of Stock A from Chemical	
Date Prepared	
Volume Prepared	5000 mL
Dilution Water	fish lab water
Solvent	none
Concentration (mg/L)	5000
Nominal Weight (g)	25.0000
Actual Weight (g)	25.0003
Lot Number/Code	N/A
Balance Type	BBY2 - 0025
Analyst	JL
Verified by	JL

Preparation of Stock B from Stock A	
Date Prepared	
Volume Prepared (mL)	
Dilution Water	
Solvent	
Concentration (mg/L)	
Volume Stock A Added (mL)	
Analyst	
Verified by	

2012 Dec 04

The calculations MUST be verified by another person before solutions are prepared (Formula: $C_1V_1=C_2V_2$)

Final Test Concentration (mg/L) (C_1)	Final Volume (mL) (V_1)	Stock Concentration (mg/L) (C_2)	Stock A or B	Stock Volume Added (mL) (V_2)	Analyst	Date	Verified by	Date
0	0	0	N/A	0.00	2012 NSherpil	2012 Dec 04	JL	2012 Dec 4th
100.00	10000	5000	A	200.00				
177.50	10000	5000	A	355.00				
316.00	10000	5000	A	632.00				
561.50	10000	5000	A	1123.00				
998.50	10000	5000	A	1997.00				

JL 2012 Dec 4th

ECOTOXICOLOGY
WATER ACCOMODATED FRACTION (WAF) AND
WATER SOLUBLE FRACTION (WSF) PREPARATION

Customer # & Name: 6683 clearflow
Study & Project #: 2-11-0691
Sample I.D. #: FA6618 B2A3552
Sample Name: Lagoon 4½ x
Date & Time Started: 2012 Dec 3 @ 15:30
Date & Time Ended: 2012 Dec 04 @ 1454
Volume to Prepare (L): 5000 ml
Test Method: OECD 203
Analyst(s): JL
Dilution H20: Fish lab system

Nominal Loading Rate (mg/L)	5000					
Nominal Mass of Test Item to Add (g)	25.0008					
Nominal Mass Verified by:						
Actual Mass Added (g)	25.0003					
Balance ID	BBY2-0025					
Method of Addition of Test Item to Vessel, Vessel Size and Type	6 L glass erlen mayer + stir bar					
Stir Plate ID	BBY2-0050					
Time Started Spinning	15:30					
Height of Vortex	10 %					
Analyst/Date	JL 2012 Dec 3					
Time Stopped Spinning	1455					
Analyst/Date	NS Morgillo 2012 Dec 04					
Beginning Time for Siphoning						
Siphoned From Middle of Water Column?	☐	☐	☑ 2012 Dec 04	☐	☐	☐
Type of Tubing Used						
Siphoned Volume Discarded (L)						
Siphoned Volume Collected (L)						
Collection Vessel Size and Type						

ECOTOXICOLOGY
WATER ACCOMODATED FRACTION (WAF) AND
WATER SOLUBLE FRACTION (WSF) PREPARATION

Nominal Loading Rate (mg/L)						
Analyst/Date						
Filter Apparatus Used: Vessel Size and Type, Filter Type, Brand, and Lot, Vacuum Pump AIN (if used)	WS 2012 Dec 04					
Collection Vessel Size and Type						
Analyst/Date						

Notes:

Client # & Name: Clearflow Enviro #6683 Project Number:

Sample ID: Waterlynx Study Number:

Job #/Sample #: B293552/ FA6618 Date & Time Started: 2012 Nov30 @ 1210

Test Species: Rainbow Trout Organism Lot #: AF121107

Test Volume: 10L # Fish/Treatment: 10

# of Surviving Organisms

	Date & Day			
Date	2012 Dec01	2012 Dec02	2012 Dec03	2012 Dec04
Treatment	1	2	3	4
ctrl	10	10	10	10
0.1mg/L	10	10	10	10
1.0mg/L	10	10	9(A)	8(B)
10mg/L	10	10	10	10
100mg/L	10	10	10	10
1000mg/L	0	0	0	0
		n/a DML	2012 Nov30	
Analyst		CT	DML	DML
Date		2012 Dec 02	2012 Dec 03	2012 Dec 04

Notes:

at time zero, vessel 1000mg/L is extreamly viscous. Other concentrations are normal in viscosity. at time zero fish are inverted and dying in 1000mg/L. Normal in all other concentrations DML 2012 Nov30

At 15:00 all fish in 1000 mg/L are dead. Final WQ DO 9.4 mg/L; Temp 15.7°c; pH 7.3; all fish in other 5 TA's appear normal - cwb 2012 Nov 30 (corrected to 2012 Nov 30)

At 15:15, all fish in all concentrations appear normal. JL 2012 Dec 01

2012 Dec 02 - performed H2O quality & observations @ 12:35. Fish appear normal & solutions remain clear in all concentrations CT

2012 Dec 03 - performed WQ @ 1230. all fish appear normal and solutions are clear in all concentrations. (A) 1 jumper fish. DML

ECOTOXICOLOGY
TEST OBSERVATIONS

Page 1 of 1

Test Initiation Date: 2012 Nov 30 Test Item: Waterflynx

Sponsor: Clearflow Study Number:

Test Method: OECD 203 Project Number:

2012 Dec 04 - performed WQ @ 1230. all fish appear normal and solutions are clear in all concentrations.
(B) 1 jumper fish found outside glass vessel. Took weights and length of control. DML

Form approved by: J. Rickard Date: June 30, 2011

ECOTOXICOLOGY

OECD 203 SURVIVAL TEST – DAILY MEASUREMENTS

Customer # & Name: Clearflow Enviro #6683 | Project Number: n/a
Sample ID: Waterlynx | Study Number: 2-11-0691
Job #/Sample #: BRA3552/FA6618 | Date & Time Started: 2012 Nov30 1210
Test Species: Rainbow Trout | Organism Lot #: AF121107
Volume (L): 10L | # of Fish/Replicate: 10 | Aeration Rate (mL/min): 6.5

0 hours

Treatment (mg/L)	Date	Conductivity (µS/cm)	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 nov30	46	15.4	9.8	7.1
0.1	2012 nov30	45	15.4	9.9	7.3
1.0	2012 nov30	49	15.5	9.9	7.4
10	2012 nov30	49	15.5	9.9	7.5
100	2012 nov30	50	15.6	9.9	7.6
1000	2012 nov30	83	15.7	9.8	7.6
			n/a DML	2012 nov30	
Analyst		DML	DML	DML	DML

24 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 Dec01	15.3	8.7	6.7
0.1	2012 Dec01	15.2	7.6	6.6
1.0	2012 Dec01	15.2	7.3	6.7
10	2012 Dec01	15.2	7.0	6.6
100	2012 Dec01	15.3	6.8	6.7
1000	2012 Dec01		N/A 12/2012 Dec01	
			2012 nov30	
		n/a DML		
Analyst	JL	JL	JL	JL

ECOTOXICOLOGY
OECD 203 SURVIVAL TEST – DAILY MEASUREMENTS

48 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 Dec02	15.4	8.7	6.9
0.1	2012 Dec02	15.3	7.2	6.9
1.0	2012 Dec02	15.3	7.5	6.8
10	2012 Dec02	15.4	7.1	6.7
100	2012 Dec02	15.4	6.6	6.8
1000			n/a 2012 Dec02 CT	
	n/a	DML 2012 nov30		
Analyst	CT	CT	CT	CT

72 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 Dec03	15.5	8.4	6.9
0.1	2012 Dec03	15.4	7.8	6.8
1.0	2012 Dec03	15.5	8.0	6.7
10	2012 Dec03	15.5	7.2	6.7
100	2012 Dec03	15.5	6.7	7.0
1000	2012 Dec03	n/a	n/a	n/a
		n/a DML 2012	nov30	
Analyst		DML	DML	DML

96 hours

Treatment (mg/L)	Date	Temperature (°C)	D.O. (mg/L)	pH
ctrl	2012 Dec04	15.4	7.8	6.8
0.1	2012 Dec04	15.4	7.5	6.7
1.0	2012 Dec04	15.3	8.1	6.7
10	2012 Dec04	15.4	7.3	6.7
100	2012 Dec04	15.5	6.4	7.0
1000	2012 Dec04	n/a	n/a	n/a
		n/a DML 2012	nov30	
Analyst		DML	DML	DML

STOCK AND TEST SOLUTION PREPARATION

Client # & Name ClearFlow 6683 WENS 6882 Nov29 | Test Species *Rainbow trout* | Sample ID

Project or Study # | Test Protocol OECD 203 | Maxxam ID

Solid or Liquid | Date Received

Preparation of Stock A from Chemical

Date Prepared	
Volume Prepared	5000 mL
Dilution Water	Rainbow trout
Solvent	non
Concentration (mg/L)	10000
Nominal Weight (g)	50.0g
Actual Weight (g)	
Lot Number/Code	
Balance Type	
Analyst	
Verified by	J.Keating 2012Nov29

2013 Jun 8 JZ

Preparation of Stock B from Stock A

Date Prepared	
Volume Prepared (mL)	
Dilution Water	
Solvent	
Concentration (mg/L)	
Volume Stock A Added (mL)	0
Analyst	
Verified by	

2013 Jun 8 JZ

The calculations MUST be verified by another person before solutions are prepared (Formula: $C_1V_1=C_2V_2$)

Final Test Concentration (mg/L) (C_1)	Final Volume (mL) (V_1)	Stock Concentration (mg/L) (C_2)	Stock A or B	Stock Volume Added (mL) (V_2)	Analyst	Date	Verified by	Date
0	10000	0	N/A	0.00	JS	2012NOV29		
0.10	10000	10	10	100.00				
1.0	10000	100	100	100.00			J.Keating	2012Nov29
10	10000	1000	1000	100.00				
100	10000	10000	A	100.00				
1000	10000	10000	A	1000.00				

ECOTOXICOLOGY
WATER ACCOMODATED FRACTION (WAF) AND WATER SOLUBLE FRACTION (WSF) PREPARATION

Customer # & Name: 6683 clear flow
Study & Project #: 2-11-0691
Sample I.D. #: FA 6618
Sample Name: WaterLynx 494x
Date & Time Started: 2012 Nov 27 @ 11h50
Date & Time Ended: 2012 Nov 28 @ 15:36
Volume to Prepare (L): 5 L
Test Method: OECD 203
Analyst(s): JL
Dilution H20: Fish Lab water

Nominal Loading Rate (mg/L)	10 000					
Nominal Mass of Test Item to Add (g)	50.0000 ± 0.0005					
Nominal Mass Verified by:						
Actual Mass Added (g)	49.9996					
Balance ID	BBY2-0025					
Method of Addition of Test Item to Vessel, Vessel Size and Type	Test item was chopped to very small pieces and mixed in water into a 6L erlenmeyer (glass) with a stir bar.					
Stir Plate ID	BBY2-0061					
Time Started Spinning	11:50					
Height of Vortex	20 %					
Analyst/Date	JL 2012 Nov 27					
Time Stopped Spinning	15:36					
Analyst/Date	JL 2012 Nov 28					
Beginning Time for Siphoning						
Siphoned From Middle of Water Column?	☐	☐	☐	☐	☐	☐
Type of Tubing Used						
Siphoned Volume Discarded (L)						
Siphoned Volume Collected (L)						
Collection Vessel Size and Type						

JL 2012 Nov 28

Nominal Loading Rate (mg/L)						
Analyst/Date						
Filter Apparatus Used: Vessel Size and Type, Filter Type, Brand, and Lot, Vacuum Pump AIN (if used)	JL 2013 Jan 8th					
Collection Vessel Size and Type						
Analyst/Date						

Notes:

at 12:50 Tat item has swell up and viscosity of the water increased - the vortex is now almost nil - JL 2012 Nov 27

@ 1:56. Viscosity has increased and it looked hard to stir the preparation - JL 2012 Nov 27

at 3 hrs on 2012 Nov 28, Dissolution seems to be completed - dough water is very viscous - JL 2012 Nov 28.

2012 Nov 29 @1235 NSheppard

Prepared test solutions today. Compare WQ today & tomorrow

	Ctrl	0.1 mg/L	1.0 mg/L	10 mg/L	100 mg/L	1000 mg/L
pH	7.2	7.4	7.5	7.4	7.6	7.6
DO (%)	99.2	99.8%	100%	100%	100%	100%
Temp (°C)	15.5°C	15.5	15.5	15.5	15.6	15.8

APPENDIX

C *DAPHNIA MAGNA*

CETIS Analytical Report

Test Code: 13-0503-0069/DM-6683-0112

Daphnia magna 48-hr Immobilization Test — Maxxam Analytics

Analysis ID:	10-4275-6981	Endpoint:	Non- Immobilized 48h	CETIS Version:	CETISv1.7.0
Analyzed:	11 Dec-12 12:09	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes
Batch ID:	01-7108-6068	Test Type:	Survival (48h)	Analyst:	N. Shergill
Start Date:	07 Dec-12 14:10	Protocol:	OECD Method 202	Diluent:	Reconstituted Water
Ending Date:	09 Dec-12 15:30	Species:	Daphnia magna	Brine:	Not Applicable
Duration:	49h	Source:	Aquatic Biosystems, CO	Age:	
Sample ID:	00-9794-3715	Code:	Job# B2A3552	Client:	Clearflow Enviro. Systems Group
Sample Date:	14 Nov-12 09:00	Material:	Chemical	Project:	Chemical Characterization
Receive Date:	14 Nov-12 13:07	Source:	Clearflow		
Sample Age:	23d 5h	Station:	Waterlynx 494X		

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	5.00%	2.622	0.04742	418.4	336.3	520.6

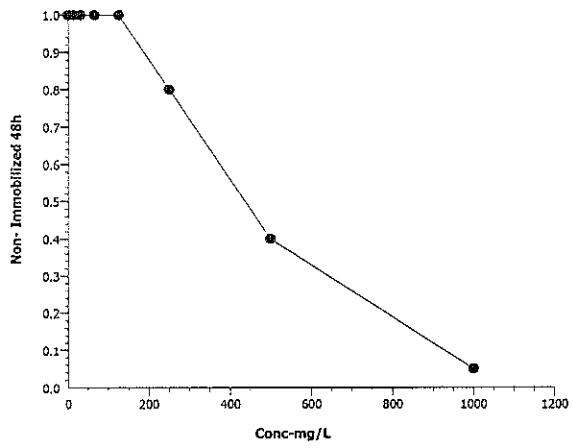
Non- Immobilized 48h Summary

			Calculated Variate(A/B)								
Conc-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	Diff%	A	B
0	Negative Control	4	1	1	1	0	0	0.0%	0.0%	20	20
15		4	1	1	1	0	0	0.0%	0.0%	20	20
30		4	1	1	1	0	0	0.0%	0.0%	20	20
65		4	1	1	1	0	0	0.0%	0.0%	20	20
125		4	1	1	1	0	0	0.0%	0.0%	20	20
250		4	0.8	0.2	1	0.2	0.4	50.0%	20.0%	16	20
500		4	0.4	0	0.8	0.1826	0.3651	91.29%	60.0%	8	20
1000		4	0.05	0	0.2	0.05	0.1	200.0%	95.0%	1	20

Non- Immobilized 48h Detail

Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	1	1
15		1	1	1	1
30		1	1	1	1
65		1	1	1	1
125		1	1	1	1
250		0.2	1	1	1
500		0.8	0.6	0.2	0
1000		0	0.2	0	0

Graphics



Analyst: US 2d2Dec11 QA: JL 2nd Dec 24

CETIS Analytical Report

Test Code: 13-0503-0069/DM-6683-0112

Daphnia magna 48-hr Immobilization Test — Maxxam Analytics

Analysis ID:	18-8594-0429	**Endpoint:**	Non- Immobilized 48h	**CETIS Version:**	CETISv1.7.0
Analyzed:	11 Dec-12 12:08	**Analysis:**	STP 2x2 Contingency Tables	**Official Results:**	Yes
Batch ID:	01-7108-6068	**Test Type:**	Survival (48h)	**Analyst:**	N. Shergill
Start Date:	07 Dec-12 14:10	**Protocol:**	OECD Method 202	**Diluent:**	Reconstituted Water
Ending Date:	09 Dec-12 15:30	**Species:**	Daphnia magna	**Brine:**	Not Applicable
Duration:	49h	**Source:**	Aquatic Biosystems, CO	**Age:**	
Sample ID:	00-9794-3715	**Code:**	Job# B2A3552	**Client:**	Clearflow Enviro. Systems Group
Sample Date:	14 Nov-12 09:00	**Material:**	Chemical	**Project:**	Chemical Characterization
Receive Date:	14 Nov-12 13:07	**Source:**	Clearflow		
Sample Age:	23d 5h	**Station:**	Waterlynx 494X		

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	250	500	353.6		N/A

Fisher Exact/Bonferroni-Holm Test

Control	vs	Conc-mg/L	Test Stat	P-Value	Decision(0.05)
Negative Control		15	1	1.0000	Non-Significant Effect
		30	1	1.0000	Non-Significant Effect
		65	1	1.0000	Non-Significant Effect
		125	1	1.0000	Non-Significant Effect
		250	0.05301	0.2651	Non-Significant Effect
		500	2.255E-05	0.0001	Significant Effect
		1000	0	<0.0001	Significant Effect

Data Summary

Conc-mg/L	Control Type	No-Resp	Resp	Total
0	Negative Contr	20	0	20
15		20	0	20
30		20	0	20
65		20	0	20
125		20	0	20
250		16	4	20
500		8	12	20
1000		1	19	20

Non- Immobilized 48h Detail

Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	1	1
15		1	1	1	1
30		1	1	1	1
65		1	1	1	1
125		1	1	1	1
250		0.2	1	1	1
500		0.8	0.6	0.2	0
1000		0	0.2	0	0

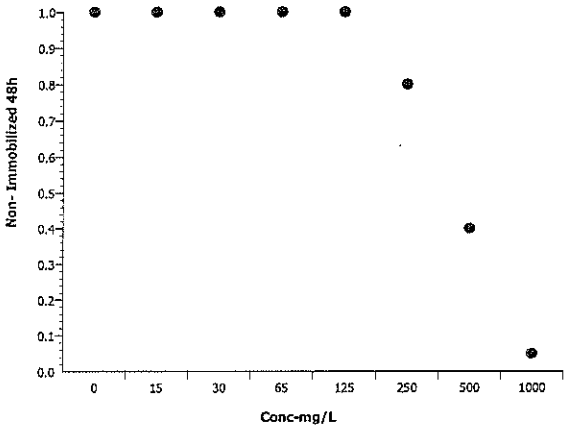
2012Dec11 2012 Dec 24

Analyst: LS QA: JL

Daphnia magna 48-hr Immobilization Test — Maxxam Analytics

Analysis ID:	18-8594-0429	**Endpoint:**	Non- Immobilized 48h	**CETIS Version:**	CETISv1.7.0
Analyzed:	11 Dec-12 12:08	**Analysis:**	STP 2x2 Contingency Tables	**Official Results:**	Yes

Graphics



2012Dec11 2012 Dec 24

Maxxam

BBY2FCD-00038/1

ECOTOXICOLOGY

DAPHNIA MAGNA OECD 202 48 HOUR IMMOBILISATION TEST – STATIC

Customer # & Name: 6683 Clearflow

Study & Project #: 2-11-0691

Maxxam I.D. # / Job #: FA666181 BBA3557

Sample Name: Water lynx 454x

Batch/Lot #: N/A

Date of Sample Receipt: 2012 NOV 14

Date & Time Started: 2012 Dec 04 1410

Analyst(s): NSkergill, D. Gardner

Date & Time Ended: 2012 Dec 06 1530

Stats File ID: 10-4275-6981 WENS 2012 Dec 07

Control Water Date: ~~2012 Dec 04~~ 2012 NOV 30L

Brood Stock: ~~AB1210~~ AB121113

# of Neonates/replicate: 5

Volume/replicate (mL): 200

pH Adjusted: N/A

Concentration	Replicate	Conductivity (µS/cm)	Temperature (°C)		pH		D.O. (mg/L)		# Immobilised	
mg/L		Initial	Initial	Final	Initial	Final	Initial	Final	24 hr	48 hr
Lab Control	A	310	20.6	20.2	8.0	7.8	9.0	9.2	0	0
	B			20.2		7.8		9.1	0	0
	C			20.3		7.8		9.2	0	0
	D			20.3		7.8		9.0	0	0
15	A	330	21.0	20.6	8.0	7.9	9.0	9.0	0	Ⓐ 0
	B			20.3		8.0		9.0	0	0
	C			20.3		8.0		9.0	0	0
	D			20.2		8.0		9.0	0	0
30	A	331	20.9	20.4	8.0	8.0	9.0	9.0	0	0
	B			20.3		8.0		9.0	0	0
	C			20.2		8.0		9.0	0	0
	D			20.1		8.0		9.0	0	0
65	A	326	20.8	20.3	8.0	8.0	8.9	9.0	0	0
	B			20.4		8.0		8.9	0	0
	C			20.2		8.0		8.9	0	0
	D			20.3		8.0		8.9	0	0
Analyst		NS	NS	DML	NS	DML	NS	DML		DML

Ⓐ only see 4 DML 2012 Dec 04

ECOTOXICOLOGY

DAPHNIA MAGNA OECD 202 48 HOUR IMMOBILISATION TEST – STATIC

Customer # & Name: 16883 Clearflow

Study & Project #: 2-11-0691

Concentration	Replicate	Conductivity (µS/cm)	Temperature (°C)		pH		D.O. (mg/L)		# Immobilised	
mg/L		Initial	Initial	Final	Initial	Final	Initial	Final	24 hr	48 hr
125	A	344	20.8	20.3	8.0	8.1	9.0	8.9	1	0
	B			20.4		8.1		8.9	0	0
	C			20.5		8.1		8.9	0	0
	D			20.5		8.1		8.9	0	0
250	A	345	20.9	20.5	8.0	8.1	8.9	8.9	4	4
	B			20.4		8.0		8.8	0	0
	C			20.3		8.0		8.9	0	0
	D			20.3		8.0		8.9	0	0
500	A	340	20.8	20.4	8.0	8.0	8.7	8.9	1	1
	B			20.4		7.9 8.1		8.9 8.5	0	2
	C			20.2		8.1		8.9	3	4
	D			20.2		8.1		7.4	4	5
1000	A	350	21.0	20.2	8.0	7.9	8.7	7.9	5	5
	B			20.3		7.9		7.8	3	4
	C			20.2		7.9		7.8	5	5
	D			20.2		7.9		7.8	5	5
Analyst		NS	NS	DML	NS	DML	NS	DML	LY	DML

Comments/Observations:

Remember to have control/dilution water analysed for Ca, Mg, Na, K and alkalinity.

in 1000mg/L, all daphnia no movement of antenna movement.

in 500mg/L most daphnia, no movement of antenna movement. few daphnia only moving antenna

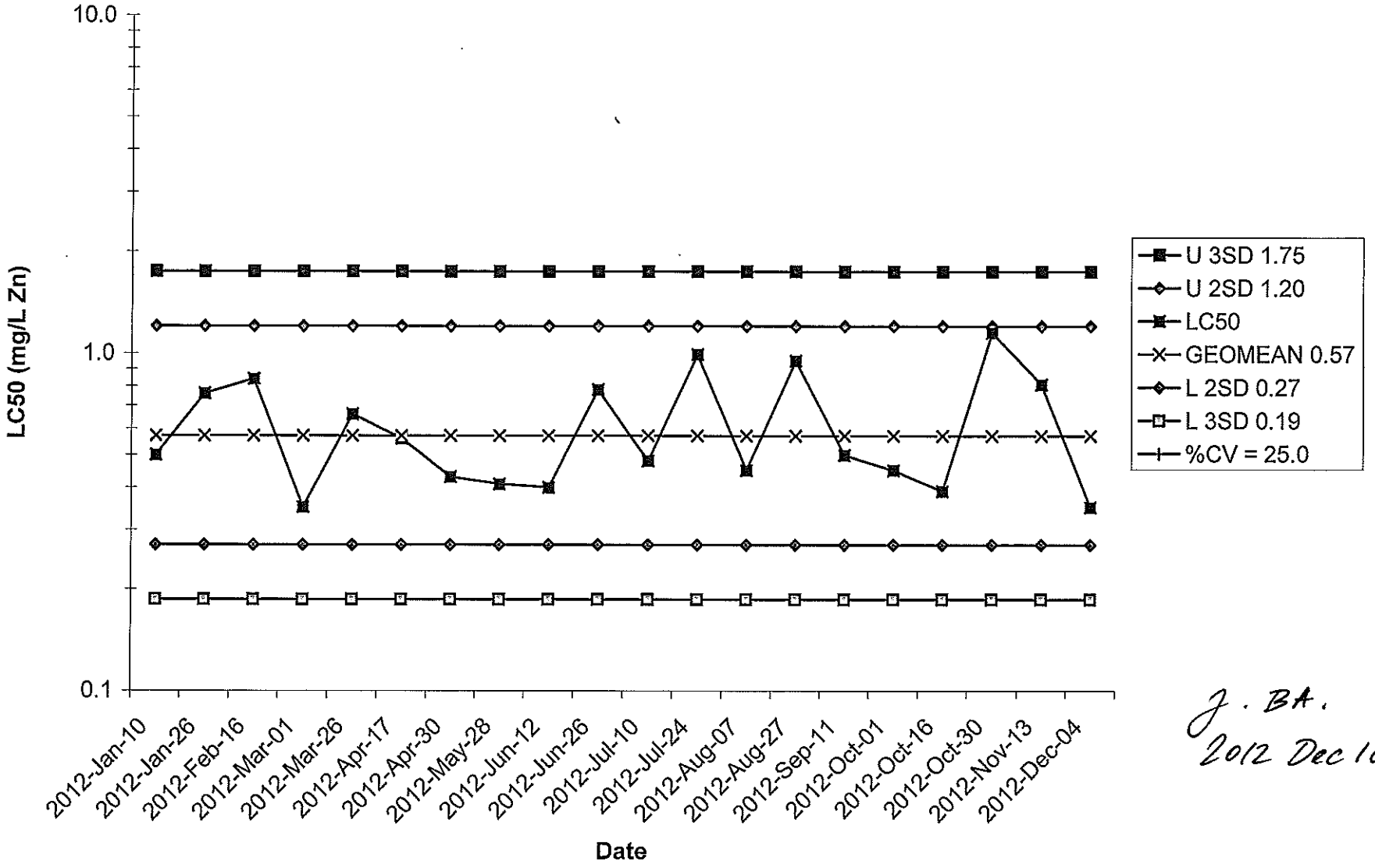
in 250mg/L no movement of antenna. 2012 Dec 09 DML

(A) WE DML 2012 Dec 09

Maxxam

Daphnia magna

48 Hour Reference Toxicant Control Chart - Zinc



J. BA.
2012 Dec 10

ECOTOXICOLOGY

STOCK AND TEST SOLUTION PREPARATION

Maxxam

BBY2FCD-00190/1

Client # & Name ClearFlow 6683 Test Species D. Magna Sample ID FA6618

Project or Study # 2-11-0691 Test Protocol OECD202 Maxxam ID FA6618

Solid or Liquid Solid Date Received 2012 NOV 14

Preparation of Stock A from Chemical	
Date Prepared	
Volume Prepared	
Dilution Water	
Solvent	N
Concentration (mg/L)	
Nominal Weight (g)	2013 Jan 24
Actual Weight (g)	
Lot Number/Code	
Balance Type	
Analyst	
Verified by	

Preparation of Stock B from Stock A	
Date Prepared	
Volume Prepared (mL)	
Dilution Water	
Solvent	Jan 24
Concentration (mg/L)	
Volume Stock A Added (mL)	#DIV/0!
Analyst	
Verified by	

The calculations MUST be verified by another person before solutions are prepared (Formula: $C_1V_1=C_2V_2$)

Final Test Concentration (mg/L) (C_1)	Final Volume (mL) (V_1)	Stock Concentration (mg/L) (C_2)	Stock A or B	Stock Volume Added (mL) (V_2)	Analyst	Date	Verified by	Date
0	0	0	N/A	0.00	MShoggo	2012Dec07	J. Keating	2012Dec06
15	1000	5000	A	3.00				
30	1000	5000	A	6.00				
65	1000	5000	A	13.00				
125	1000	5000	A	25.00				
250	1000	5000	A	50.00				
500	1000	5000	A	100.00				
1000	1000	5000	A	200.00				

ECOTOXICOLOGY
WATER ACCOMODATED FRACTION (WAF) AND WATER SOLUBLE FRACTION (WSF) PREPARATION

Customer # & Name: 6683 Cleanflow
Study & Project #: 2-11-10691
Sample I.D. #: FA 6618 B2A3552
Sample Name: Lynx 394 ss
Date & Time Started: 2012 Dec 3 @ 15:30
Date & Time Ended: 2012 Dec 04 @ 1310
Volume to Prepare (L): 1 (L)
Test Method: OECD202
Analyst(s): JL
Dilution H20: Daphnia culture

Nominal Loading Rate (mg/L)	5000					
Nominal Mass of Test Item to Add (g)	5.0000					
Nominal Mass Verified by:	JL 2012 Dec 3					
Actual Mass Added (g)	5.0004 (g)					
Balance ID	BBY2-0025					
Method of Addition of Test Item to Vessel, Vessel Size and Type	1-L glass coffee mugs + stir bar +					
Stir Plate ID	BBY2-0065					
Time Started Spinning	15:30					
Height of Vortex	10%					
Analyst/Date	JL 2012 Dec 3					
Time Stopped Spinning	1310					
Analyst/Date	Usherjule 2012 Dec 04					
~~Beginning Time for Siphoning~~						
Siphoned From Middle of Water Column?	☐	NS 2012 Dec 04	☐	☐	☐	☐
Type of Tubing Used						
Siphoned Volume Discarded (L)						
Siphoned Volume Collected (L)						
Collection Vessel Size and Type						

ECOTOXICOLOGY
WATER ACCOMODATED FRACTION (WAF) AND
WATER SOLUBLE FRACTION (WSF) PREPARATION

Nominal Loading Rate (mg/L)						
Analyst/Date						
Filter Apparatus Used: Vessel Size and Type, Filter Type, Brand, and Lot, Vacuum Pump AIN (if used)	05 2012 DEC 04					
Collection Vessel Size and Type						
Analyst/Date						

Notes:

@ test end the solution is viscous J.L, 2012 DEC 04

Maxxam

BBY2FCD-00075/1

ECOTOXICOLOGY

***DAPHNIA MAGNA* OECD 202 48HOUR IMMOBILISATION TEST – RANGE FINIDING (STATIC)**

Client # & Name: 6883 Clear Flow

Project #: 2-11-0691

Maxxam I.D. # / Job #: FA6618 B2A3552

Sample Name: Water Lynx 4944

Batch/Lot #: n/a

Date of Sample Receipt: 2012 Nov 17 4 TIME SENSITIVE

Date & Time Started: 2012 Nov 30 @ 13:40

Analyst(s): A. Rakhmangulova, C. TRA

Date & Time Ended: 2012 Dec 02 @ 13:42

Stats File ID: n/a

Control Water Date: 2012 NOV 28 J

Brood Stock: AB 121113

# of Neonates/replicate: 5

Volume/replicate (mL): 200

pH Adjusted: no

Concentration	Replicate	Conductivity (µS/cm)	Temperature (°C)		pH		D.O. (mg/L)		# Immobilised	
%v/v or mg/L			Initial	Final	Initial	Final	Initial	Final	24 hr	48 hr
Lab Control	A	305	20.8	20.4	8.1	8.0	8.9	8.6	0	0
	B			20.3		7.9		8.6	0	0
0.1 mg/L	A	304	20.7	20.4	8.1	8.0	8.9	8.5	0	0
	B			20.6		8.0		8.5	2	2
1.0 mg/L	A	305	20.8	20.7	8.1	8.0	8.8	8.5	0	0
	B			20.7		8.0		8.5	0	1
10 mg/L	A	305	20.8	20.7	8.1	8.1	8.9	8.5	0	0
	B			20.7		8.1		8.5	0	0
100 mg/L	A	315	20.8	20.7	8.1	8.1	8.7	8.5	0	1
	B			20.5		8.1		8.4	0	0
1000 mg/L	A	404	20.8	20.4	(A) 7.7 ~~8.2~~	8.2	8.2	8.3	1	1
	B			20.3		8.2		8.1	1	1
	A			~~20.5~~ 2012 NOV 29						
	B									
Analyst		AR	AR		AR		AR		LA	CT

Comments/Observations: # = floating (A) WE AR 2012 NOV 30

STOCK AND TEST SOLUTION PREPARATION

Client # & Name: ClearFlow | Test Species: *Daphnia water* | Sample ID: Water Lyax 6944
Project or Study #: 2-11-6911 | Test Protocol: OECD 202 | Maxxam ID: FA6618
Solid or Liquid: Solid | Date Received: 2012/11/14

Preparation of Stock A from Chemical	
Date Prepared	2012 Nov 27
Volume Prepared	1000 ml
Dilution Water	daphnia water
Solvent	non
Concentration (mg/L)	10000
Nominal Weight (g)	10.00
Actual Weight (g)	10.0004
Lot Number/Code	N/A
Balance Type	BBY2 - 0025
Analyst	JZ
Verified by	J.Keating 2012Nov29

Preparation of Stock B from Stock A	
Date Prepared	
Volume Prepared (mL)	
Dilution Water	2012 NOV 28
Solvent	Daphnia H2O
Concentration (mg/L)	
Volume Stock A Added (mL)	0
Analyst	
Verified by	

The calculations MUST be verified by another person before solutions are prepared (Formula: $C_1V_1=C_2V_2$)

Final Test Concentration (mg/L) (C_1)	Final Volume (mL) (V_1)	Stock Concentration (mg/L) (C_2)	Stock A or B	Stock Volume Added (mL) (V_2)	Analyst	Date	Verified by	Date
0	1000	0	N/A	0.00	NS	2012 NOV 29	J.Keating	2012Nov29
0.10	1000	10	10	10.00				
1.0	1000	100	100	10.00				
10	1000	1000	1000	10.00				
100	1000	10000	A	10.00				
1000	1000	10000	A	100.00				

ECOTOXICOLOGY
WATER ACCOMODATED FRACTION (WAF) AND WATER SOLUBLE FRACTION (WSF) PREPARATION

Customer # & Name: 6683 Clearflow
Study & Project #:
Sample I.D. #: FA 6618
Sample Name: Water Cyprus 4946
Date & Time Started: 2012 Nov 27 @ 1115 hr
Date & Time Ended:
Volume to Prepare (L): 1 JL 2012 Nov 27 WE
Test Method:
Analyst(s): JL
Dilution H20: Daphnia water

Nominal Loading Rate (mg/L)	10 000					
Nominal Mass of Test Item to Add (g)	10.0000 ± 0.0005					
Nominal Mass Verified by:						
Actual Mass Added (g)	10.0003					
Balance ID	BBY2 - 0025					
Method of Addition of Test Item to Vessel, Vessel Size and Type	Test item was chopped very small and mixed with water into a glass 1L erlen meyer and a stir bar.					
Stir Plate ID	BBY2-0065					
Time Started Spinning	11:50					
Height of Vortex	2cm.					
Analyst/Date	JL 2012 Nov 27					
Time Stopped Spinning	15:36					
Analyst/Date	JL JL 2012 Nov 28					
Beginning Time for Siphoning						
Siphoned From Middle of Water Column?	☐	☐	☐	☐	☐	☐
Type of Tubing Used						
Siphoned Volume Discarded (L)						
Siphoned Volume Collected (L)						
Collection Vessel Size and Type						

JL 2013 Jan 24

ECOTOXICOLOGY
WATER ACCOMODATED FRACTION (WAF) AND
WATER SOLUBLE FRACTION (WSF) PREPARATION

Nominal Loading Rate (mg/L)						
Analyst/Date						
Filter Apparatus Used: Vessel Size and Type, Filter Type, Brand, and Lot, Vacuum Pump AIN (if used)	DB Jan 04 JL					
Collection Vessel Size and Type						
Analyst/Date						

Notes:

at 12:50 = Test item has swelled up and viscosity of the water increased - JL 2012 Nov 27