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enviroquatics limited

September 11, 2013

Greg Clarkin
Caduceon Environmental Laboratories
2378 Holly Lane
Ottawa, ON K1V 7P1

Dear Greg:

On August 30, 2013, Pollutech EnviroQuatics Limited personnel received a water sample from Caduceon Environmental Laboratories (Test E B13-22724), Ottawa Site. The following acute toxicity tests were performed on this sample observing Environment Canada methods:

- Rainbow trout 96-hour single-concentration toxicity test according to the criteria outlined in the "Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout", Second Edition, Method Development and Applications Centre, Ottawa, ON., Report EPS 1/RM/13, 2000 (with 2007 amendments).

- *Daphnia magna* 48-hour LC50 toxicity test according to the criteria outlined in the "Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*", Environmental Technology Center, Ottawa, Ontario, Report EPS 1/RM/14 Second Edition, December 2000.

The results of the acute toxicity tests are summarized in Table 1.

Table 1 Summary of Acute Toxicity Results for Test E B13-22724 Water Sample Collected August 23, 2013

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
Test E B13-22724 #79911304	*Daphnia magna*	48-hour LC50 (95% Confidence)	Mortality	Non-lethal[2] (Not available)
	Rainbow Trout	96-hour Single-Concentration	Mortality	100% Mortality[2,3]

[1] – Results relate only to the sample tested
[2] – Test invalid as hold time greater than five days. Tested as instructed.
[3] – Most regulations regard ≤50% mortality to be a "pass". Check your applicable regulatory requirements.

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Toxicity Test Endpoint Descriptions

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

The following pages contain the required details for reporting of the acute lethality toxicity tests. If there are any further details which you require, please do not hesitate to contact us.

Sincerely,
Pollutech EnviroQuatics Limited

R. Clay Ferguson, B.Sc. (Hon.)
Laboratory Manager

File ID:\bioassay\2013\7000\7991\7991au2 T, D LC50

Rainbow Trout 96-Hour Single-Concentration Toxicity Test

METHOD: Environment Canada, "Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/13, 2000 (with 2007 amendments). Pollutech Test Method RT-SC-R1.2.

Test Material

Client Name/Location:	Caduceon Environmental Laboratories, Ottawa, ON		
Sample #:	79911304	**Sample Name:**	Test "E" B13-22724
Sample Method:	Grab	**Collected by:**	n/a
Date/Time Collected:	Aug. 23/13, 08:30	**Arrival Temp.:**	25.4°C
Date/Time Received:	Aug. 30/13, 14:30	**Sample Description:**	Cloudy brown
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Road		
Storage:	Overnight at 15 ± 1°C	In dark, no headspace	

n/a – not available

Test Organisms

Species:	Rainbow Trout (*Oncorhynchus mykiss*)				
Source:	Rainbow Springs Hatchery				
Culture Temp.:	15 ± 2°C	**Batch Number:**	RS072413		
Water Source:	Dechlorinated Municipal Drinking Water				
Mean Weight:	0.71 g	**Min:**	0.42 g	**Max:**	1.03 g
Mean Fork Length:	40.8 mm	**Min:**	34 mm	**Max:**	47 mm
Loading Density:	0.36 g/L	**Sample Size:**	10 fish		
Life Stage:	Fry				
Number Dead Daily In Previous 7 Days For Fish Culture:	0+0+2+1+0+4+4=11				
Previous 7-Day Holding Mortalities For Fish Culture:	1.15%				

Sample Number: 79911304 **Sample Name:** Test "E" B13-22724

Test Conditions

Date/Time Started:	Aug. 31, 2013, 13:00		
Test Volume:	20 L/Vessel	**Number of Fish Per Vessel:**	10
# of Vessels Per Conc.:	1	**Test Temperature:**	15 ± 1°C
Pre-aeration:	Yes	**Duration of Pre-aeration:**	120 minutes
Pre-aeration Rate:	6.5 ± 0.26 ml/min·L^{-1}	**Aeration Rate During Test:**	6.5 ± 0.26 ml/min·L^{-1}
Sample Adjustment:	No	**Sample pH Adjustment:**	No
Test Method Deviations:	Yes, sample hold time greater than five days.		

Test Facilities

CALA Testing Accreditation No. A1225

Testing Laboratory: Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4

This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA). The test included in this report is within the scope of this laboratory.

Test Performed By: M. Long/C. Ferguson/C. D'Andrea

Initial Measurement of Variables in Unadjusted Sample

Cond: 420 µmhos **pH:** 6.6 **O_2:** 0.3 mg/L **Temp:** 16.0°C

Test Results

NUMBER OF MORTALITIES

	Rep.No.		Time (hours)			
Conc'n	1	4	24	48	72	96
100%	1	0 10	-	-	-	-
Control	1		0	0	0	0

Number of Control Fish Showing Atypical/Stressed Behaviour: 0

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Sample Number: 79911304 **Sample Name:** Test "E" B13-22724

Test Results

TOXICITY TEST VARIABLES

Conc'n	Rep. No.	Variables	Time (hours) 0	24	48	72	96
100%	1	Cond. (µmhos)	408				n/r
		O_2 (mg/L)	6.2				4.3
		pH (units)	7.1				7.3
		Temp. (°C)	15.8				15.9
Control	1	Cond. (µmhos)	186				n/r
		O_2 (mg/L)	10.2				9.6
		pH (units)	7.5				7.8
		Temp. (°C)	15.6				16.0

n/r = not required

Summary of Test Results

Mean Mortality Rate: 100%[1] **Test Results Verified By:** R. C. Ferguson

[1] - Most regulations regard ≤50% mortality to be a "pass". Check your applicable regulatory requirements.

Reference Toxicant Results

Reference Chemical: Zinc **Date Test Initiated**: 08/20/13

Fish Lot #: RS072413

Method: Spearman-Karber (α = 10%)

96-Hour LC50 (95% Confidence Limits): 0.58 mg/L (0.44 mg/L; 0.78 mg/L)

Historic Geometric Mean LC50: 0.35 mg/L (0.18 mg/L; 0.67 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Daphnia magna 48-Hour Single*Daphnia magna* 48-Hour LC50 Toxicity Test

METHOD: Environment Canada, "Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*", Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/14, Second Edition, December 2000. Pollutech Test Method DM-LC-R10.7.

Test Material

Client Name/Location: Caduceon Environmental Laboratories, Ottawa, ON

Sample #:	79911304	**Sample Name:**	Test "E" B13-22724
Sample Method:	Grab	**Collected by:**	n/a
Date/Time Collected:	Aug. 23/13, 08:30	**Arrival Temp.:**	25.4°C
Date/Time Received:	Aug. 30/13, 14:30	**Sample Description:**	Cloudy brown
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Road		
Storage:	4 ± 2 °C	In dark, no headspace	

n/a not available

Test Organisms

Species:	*Daphnia magna*	**Source:**	Pollutech Culture (MOE/EPA)
Culture Temp.:	20 ± 2°C	**Age:**	< 24-hours old
Water Source:	Reconstituted dechlorinated tap water		
Cultures Used in Testing:	63 SA		
Days to First Brood:	8		
Average # Number of Neonates/Brood:	39		
Previous 7 Days Mortality in Culture:	0%		

Test Facilities

Testing Laboratory: Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4

CALA Testing Accreditation No. A1225

This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA). The test included in this report is within the scope of this laboratory.

Test Performed By: M. Long

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Sample Number: 79911304 **Sample Name:** Test "E" B13-22724

Test Conditions

Date/Time Started:	Sept. 01/13, 09:30	**# of Neonates/Vessel:**	3
Test Volume:	50 mL/Vessel	**mL Solution/Neonate:**	16.7 mL
Reps/Concentration:	4	**Dilution Water:**	DW13-72
Pre-aeration:	Yes	**Pre-aeration Rate:**	50 ± 2 ml/min·L^{-1}
Pre-aeration Duration:	30 minutes	**Test Temperature:**	20 ± 2°C
Sample pH Adjustment:	No	**Sample pH Adjustment Procedure:**	N/A
Sample Hardness Adjustment:		No	
Hardness Before and After Adjustment:		Not Applicable	
Test Method Deviations:		Yes, sample hold time greater than five days.	

Initial Measurement of Variables of Unadjusted, Undiluted Sample

pH: 7.0 **O_2:** 0.3 mg/L **Cond:** 440 µmhos **Temp:** 19.6°C

Test Results

TOXICITY TEST VARIABLES

Concentration (% Volume)	pH		Oxygen mg/L		Cond. µmhos	Hardness mg/L	Temperature C°	
	Initial	Final	Initial	Final	Initial	Initial	Initial	Final
Control	8.1	8.3	8.9	8.5	530	198	20.3	20.5
6.25	7.8	7.9	8.6	7.5	511	--	20.4	20.3
12.5	7.7	7.7	8.4	6.5	510	--	20.3	20.3
25	7.5	7.6	7.5	5.0	503	--	20.3	20.3
50	7.4	7.5	6.0	3.1	486	--	20.3	20.3
100	7.1	7.5	2.0	1.2	438	50	19.8	20.3

Sample Number: 79911304 **Sample Name:** Test "E" B13-22724

Test Results - continued

DAPHNIA OBSERVATIONS

Concentration (% Volume)	Test Vessel	Number of Daphnia Immobile 24 hr.	48 hr.	Number of Daphnia Dead 48 hr.
Control	A	0	0	0
	B	0	0	0
	C	0	0	0
	D	0	0	0
6.25	A	0	0	0
	B	0	0	0
	C	0	0	0
	D	0	0	0
12.5	A	0	0	0
	B	0	0	0
	C	0	0	0
	D	0	0	0
25	A	0	0	0
	B	0	0	0
	C	0	0	0
	D	0	0	0
50	A	0	0	0
	B	0	0	0
	C	0	0	0
	D	0	0	0
100	A	0	0	0
	B	0	0	0
	C	0	0	0
	D	0	0	0

Sample Number: 79911304 **Sample Name:** Test "E" B13-22724

Summary of Test Results

48-Hour LC50: Non-lethal

95% Confidence Limits: Not applicable

Analysis Method: No mortality

Results Verified By: R. C. Ferguson

Reference Toxicant Results

Reference Chemical: Phenol **Date Test Initiated**: 08/27/13

Method: Spearman-Karber (α = 0%)

48-Hour LC50 (95% Confidence Limits): 17.68 mg/L (14.32 mg/L; 21.83 mg/L)

Historic Geometric Mean LC50: 17.44 mg/L (8.97 mg/L; 33.91 mg/L)
(Historic Warning Limits) (± **2 Standard Deviations)**