Acute Toxicity Test Results

Sample Biosix,
collected February 17, 2020

Final Report

March 3, 2020

Submitted to: **Biocleaner Inc.**
Monterey Park, CA

8664 Commerce Court, Burnaby, BC V5A 4N7

SAMPLE INFORMATION

Sample ID	Dates			Receipt temperature
	Collected	Received	*Daphnia magna* test initiation	
Biosix	17-Feb-20 at 0900h	19-Feb-20 at 1100h	20-Feb-20 at 1355h	11.7°C

TEST

- *Daphnia magna* 48-h single concentration screening test

RESULTS

Toxicity test results

Sample ID	Percent survival in 100% (v/v) sample
Biosix	93

QA/QC

QA/QC summary	*Daphnia magna*
Reference toxicant LC50 (95% CL)	6.3 (5.2 – 7.7) g/L NaCl[1]
Reference toxicant historical mean (2 SD range)	6.1 (4.4 – 8.5) g/L NaCl
Reference toxicant CV	17%
Organism health history	Acceptable
Protocol deviations	None
Water quality range deviations	None
Control performance	Acceptable
Test performance	Valid

[1] Test date: February 11, 2020, LC = Lethal Concentration, SD = Standard Deviation, CL = Confidence Limits, CV = Coefficient of Variation

Report By:
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Laboratory Biologist

Reviewed By:
Edmund Canaria, R.P. Bio.
Senior Analyst

This report has been prepared by Nautilus Environmental Company Inc. based on data and/or samples provided by our client and the results of this study are for their sole benefit. Any reliance on the data by a third party is at the sole and exclusive risk of that party. The results presented here relate only to the samples tested.

APPENDIX A – Summary of test conditions

Table 1. Summary of test conditions: 48-h *Daphnia magna* single concentration test.

Test species	*Daphnia magna*
Organism source	In-house culture
Organism age	<24-hour old neonates
Test type	Static
Test duration	48 hours
Test vessel	250-mL glass beaker
Test volume	200 mL
Test solution depth	6 cm
Test concentrations	100% (undiluted) sample, plus laboratory control
Test replicates	3 per treatment
Number of organisms	10 per replicate
Control/dilution water	Moderately-hard reconstituted water + 2.5 µg/L Se
Test solution renewal	None
Test temperature	20 ± 2°C
Feeding	None
Light intensity	400 to 800 lux
Photoperiod	16 hours light / 8 hours dark
Aeration	None
Test measurements	Temperature, dissolved oxygen and pH measured daily; salinity, hardness and alkalinity measured in the undiluted sample at test initiation; conductivity measured at test initiation and termination; survival checked daily
Test protocol	Environment Canada (2000), EPS 1/RM/14, with 2016 amendments
Test endpoint	Survival
Test acceptability criterion for controls	Survival ≥90%
Reference toxicant	Sodium chloride (NaCl)

APPENDIX B – Toxicity test data

Daphnia magna Summary Sheet

Client: Biocleaner Inc.
Work Order No.: 200274

Start Date/Time: Feb 20/2020 @ 1355 L
Test Species: *Daphnia magna*
Set up by: MK

Sample Information:

Sample ID: MF 01 Biosix
Sample Date: Feb 17/2020
Date Received: Feb 19/2020
Sample Volume: 1 x 2L

Test Validity Criteria:

≥ 90% mean control survival and/or mobility and ≤2 daphnids exibit immobility and/or mortality in any single control replicate.

WQ Ranges:

T (°C) = 20 ± 2; DO (mg/L) = 3.6 to 9.4; pH = 6 to 8.5

Test Organism Information:

Broodstock No.: 020520B
Age of young (Day 0): <24 h
Avg No. young per brood in previous 7 d: 16
Mortality (%) in previous 7 d: 0
Days to first brood: 9

NaCl Reference Toxicant Results:

Reference Toxicant ID: DinDC 46
Stock Solution ID: 20 NaCl
Date Initiated: Feb 11/2020
48-h LC50 (95% CL): 6.3 (5.2-7.7) g/LNaCL

Reference Toxicant Mean and Historical Range: 6.1 (4.4-8.5) g/L NaCL
Reference Toxicant CV (%): 17

Test Results: 93% survival at 48h in the 100% (v/v) undiluted sample.

Reviewed by: [signature]
Date reviewed: Feb. 25, 2020

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Biocleaner Inc.
Sample ID: ~~MF-01~~ Biosix
Work Order No.: 200274

Start Date/Time: Feb 20/2020@1355h
CER #: 5
No. Organisms/volume: 10/200mL
Test Organism: D.magna
Set up by: MWF

Thermometer: CER#5 pH meter/probe: 3 / 3 DO meter/probe: 3 / 3 Cond./Salinity meter/probe: 3 / 3

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	19.5	20.0	20.5	8.9	8.5	8.4	7.7	7.7	7.4	342	351
	B	10	10	0											
	C	10	10	0											
	D								MWF			MWF			
100	A	10	9	0	20.5	20.0	20.0	8.0	~~8.0~~	8.7	7.2	~~6.8~~	7.3	2520	~~2550~~ 2540
	B	10	9	0					8.4			7.3			2510
	C	10	10	0											
	D														
	A														
	B														
	C														
	D														
	A														
	B														
	C														
	D														
	A														
	B														
	C														
	D														
	A														
	B														
	C														
	D														
Technician Initials		MWF	JAD	JAD	MWF	MWF	JAD	MWF	MWF	JAD	MWF	MWF	JAD	MWF	JAD

	Hardness*	Alkalinity*
Concentration	*(mg/L as CaCo3)	
Control (MHW)	100	72
Highest conc.	780	60
Hardness adjusted	—	—

	Initial WQ	Adjustment	Adjusted WQ
Temp (°C)	20.5		
DO (mg/L)	8.0		
pH	7.2		
Cond (µS/cm)	2520		
Salinity (ppt)	1.3		

Comments: (1) one daphnid immobilized

Mortality: Heartbeat checked under microscope ✓

Sample Description: clear pale yellow liquid, slight chemical odour, some particles

Batch#: 020520B 7-d previous # young/brood: 16 Previous 7-d Mortality (%): 0 Day of 1st Brood: 9

Reviewed by: [signature] Date reviewed: Feb. 25, 2020

APPENDIX C – Chain-of-custody form

Nautilus Environmental

Chain of Custody (electronic)

[X] British Columbia: 8664 Commerce Court, Burnaby, BC, V5A 4N7

Date 02-17-20 Page 1 of 1

Sample Collection By:		
	Report to:	Invoice to:
Company	BIOCLEANER Inc.	Same Address
Address	2550 Corporate Pl. Suite C101	
City/Prov/Postal Code	Monterey Park CA 91754 USA	
Contact	Renen Seville	
Phone	323 981 0707	
Email	renen@biocleaner.com	

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	# OF CONTAINERS	COMMENTS	ANALYSES REQUIRED: 48h Daphnia Mag test	Receipt Temperature (°C)
1	~~01~~ Ar	02-17-20	900 am		plasticContainer	1	water sample (fresh)	✓	11.7
2	Biosix								
3									
4									
5									
6									
7									
8								2D02174	
9									
10									

PROJECT INFORMATION	SAMPLE RECEIPT		RELIQUINSHED BY (CLIENT)	RELIQUINSHED BY (COURIER)
Client: Bioclenaer Inc.	Total # Containers:	1	Signature:	Signature:
P.O. No.:	Good Condition?	Y	Print: Renen Seville	Print:
Shipped Via: Fedex	Matches Schedule?	Y	Company: Bioclenaer Inc.	Company:
			Time/Date: 2-17-20 9:00 am	Time/Date:

SPECIAL INSTRUCTIONS/COMMENTS:	RECEIVED BY (COURIER)	RECEIVED BY (LABORATORY)
Client specified pass/fail testing, following Environment Canada protocols. Sample ID changed from 01 to Biosix at request of client.	Signature:	Signature: Tyme
	Print:	Print: TM
	Company:	Company: Nautilus
	Time/Date:	Time/Date: Feb. 19/20 @ 11:00

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

END OF REPORT