

AMBIENT TOXICITY TEST REPORT FORM

GENERAL INFORMATION			
PROJECT NAME:	Brewery Creek Ambients	LABORATORY NAME:	Wisconsin State Laboratory of Hygiene
		REPORT NUMBER:	206461002-61004
REPORT TYPE:	Original	If amended, original report number:	

SAMPLE INFORMATION				
SAMPLE NO.	LAB NO.	FIELD NO.	SITE DESCRIPTION	STATION NO. (SWIMS, STORET or LAT/LONG)
1	206461002	Brew-1	Brewery Creek 1	10008015
2	206461003	Brew-2	Brewery Creek 2	253200
3	206461004	Brew-3	Brewery Creek 3	10030385

SAMPLE NO.	SAMPLE COLLECTION			SAMPLE TEMP. °C		pH at LAB	HAND DELIVER? (If Yes, ≤ 4 hr?)	HOLD TIME ≤ 36 HR?	SAMPLE ACCEPTABLE?
	SAMPLE TYPE	SAMPLING DATES	DATE at LAB	COLLECTION	AT LAB				
1	Grab	7/20/2015	7/20/2015	17.1	3.2	8.46	Yes	Yes	Yes
2	Grab	7/20/2015	7/20/2015	17.1	2.6	7.96	Yes	Yes	Yes
3	Grab	7/20/2015	7/20/2015	16.1	3.0	8.20	Yes	Yes	Yes

Describe any unusual conditions during sampling that may influence test results. (see Part 6.1.2 of the Methods Manual for examples.)
COMMENTS:

TEST INFORMATION		
	ACUTE	CHRONIC
Date Test Initiated:	7/21/2015	7/21/2015

QA/QC CONDITIONS		
	ACUTE	CHRONIC
Temperatures maintained during test? (20 ± 1°C or 25 ± 1°C)	Yes	No*
Dissolved oxygen ≥ 4.0 mg/l throughout test?	Yes	Yes
pH maintained within 6.0 - 9.0 s.u. throughout test?	Yes	Yes
Concurrent or monthly reference tests within acceptable limits?	**	**
Tests conducted in a carbon dioxide atmosphere throughout test?	Yes	Yes
Light intensity for <i>Selenastrum</i> maintained throughout test? (4,300 ± 430 lux)		Yes
Were samples modified prior to testing? (ex. filtration, aeration, chem addition)	No	No

COMMENTS: *Temperatures were within spec for chronic FHM and *C. dubia* tests, but the temperature on Day 1 of the *Selenastrum* test was 28.0°C. The test was then moved to another incubator where temperatures were maintained at 25 ± 1°C.
 ***Selenastrum* and FHM monthly reference tests were within acceptable limits. *C. dubia* acute and chronic reference tests were run concurrently with organisms purchased from Aquatic Biosystems because the in-house culture organisms were not performing well.

WATER CHEMISTRY							
(All values reported in mg/L, except pH and Conductivity)							
SAMPLE TYPE	SAMPLE NO.	HARDNESS	ALKALINITY	TOTAL AMMONIA	DISSOLVED OXYGEN	pH (s.u.) After Warming	Conductivity (µS)
SITES	1	420	355	0.019*	9.2	8.35	799
	2	448	290	0.031*	9.1	7.98	1,020
	3	428	300	ND	9.4	8.12	869
LAB WATER	MHW	100	60	NA	8.5	8.46	309
	DC	192	340	NA	7.9	7.68	766

COMMENTS: MHW= Moderately Hard Water was used as the lab control for the *Ceriodaphnia dubia* and the *Selenastrum* tests
 DC = Dechlorinated Madison tap water was used as the lab control for the fathead minnow test.

*Ammonia result is between the LOD (0.015 mg/L) and LOQ (0.0480 mg/L).
 ND= No Detect

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ACUTE TEST CONTROL PERFORMANCE

LAB WATER CONTROLS

Fathead Minnow	<i>Ceriodaphnia dubia</i>
Survival \geq 90% Yes	Survival \geq 90% Yes

COMMENTS:

ACUTE TEST DATA

SPECIES	SITE DESCRIPTION		Percent Survival By Replicate				Mean Percent Survival	Statistical Significance*
			1	2	3	4		
Fathead Minnow	LC	LW Control	100	100	100	100	100.0	A
	1	Brew-1	100	100	100	100	100.0	A
	2	Brew-2	100	100	100	100	100.0	A
	3	Brew-3	100	100	100	100	100.0	A
Age of Organism: 11 Days								

Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

COMMENTS: * Samples with the same letter are not statistically different from each other.

SPECIES	SITE DESCRIPTION		Percent Survival By Replicate				Mean Percent Survival	Statistical Significance*
			1	2	3	4		
<i>Ceriodaphnia dubia</i>	LC	LW Control	100	100	100	100	100.0	A
	1	Brew-1	100	100	60	100	90.0	A
	2	Brew-2	0	0	0	0	0.0	B
	3	Brew-3	0	0	0	0	0.0	B
Age of Organism: < 24 Hours Old								

Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

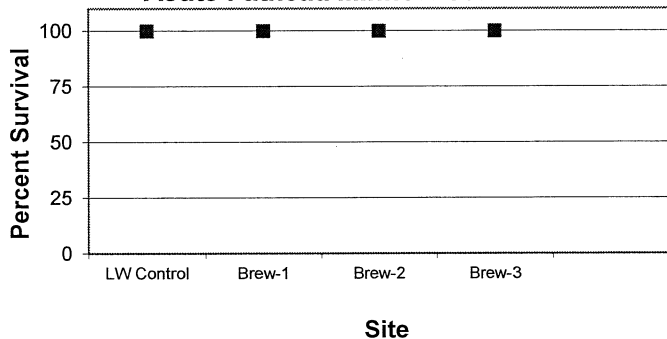
COMMENTS: * Samples with the same letter are not statistically different from each other.

C. dubia acute test was run with organisms purchased from Aquatic Biosystems because the in-house culture organisms were not performing well.

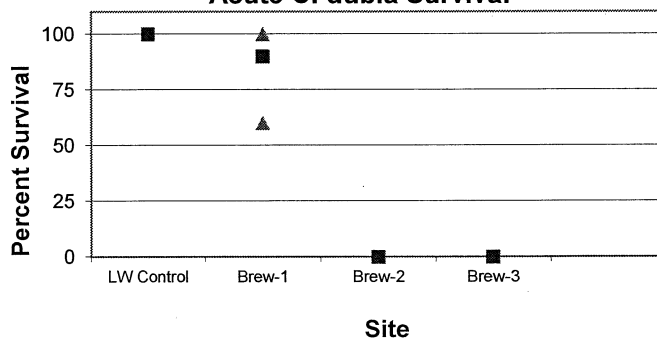
▲ = Individual Data

■ = Mean

Acute Fathead Minnow Survival



Acute *C. dubia* Survival



Project Name : Brewery Creek Ambients

Report # : 206461002-61004

Acute Test Date : 7/21/2015

CHRONIC TEST CONTROL PERFORMANCE

LAB WATER CONTROLS	
Fathead Minnow	<i>Ceriodaphnia dubia</i>
Survival \geq 80% Yes	Survival \geq 80% Yes
\geq 0.25 mg/fish Yes	\geq 15 neonates/female No
Survival Weight CV \leq 40% Yes	Reproduction CV \leq 40% Yes
Survival Weight % CV = 18	Reproduction %CV= 28
	\geq 80% 3rd brood Yes
	< 20% males Yes

COMMENTS:

CHRONIC TEST DATA

SPECIES	SITE DESCRIPTION		MEAN % SURVIVAL	MEAN DRY BIOMASS PER REPLICATE PAIR (mg)					MEAN BIOMASS (mg)	Statistical Significance*
				1	2	3	4	5		
Fathead Minnow Growth & Survival Test	LC	LW Control	95	0.318	0.363	0.395	0.428	0.320	0.365	A
	<i>LW Survival Weight</i>			0.318	0.483	0.395	0.428	0.320		
	1	Brew-1	95	0.448	0.335	0.370	0.398	0.453	0.401	A
	2	Brew-2	95	0.330	0.488	0.348	0.428	0.443	0.407	A
	3	Brew-3	95	0.463	0.430	0.318	0.495	0.348	0.411	A

Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

COMMENTS: * Samples with the same letter are not statistically different from each other. Statistical significance based on biomass.

SPECIES	SITE	NEONATE PRODUCTION BY REPLICATE										MEAN NEONATES	% ADULT SURVIVAL	Statistical Significance*
		1	2	3	4	5	6	7	8	9	10			
<i>C. dubia</i> Reproduction & Survival Test	LC	6	8	6	9	6	0	12	10	11	0	7	80	B
	1	13	16	0	24	18	22	0	20	20	19	15	90	A
	2	0	0	0	0	0	0	0	0	0	0	0	0	C
	3	0	0	0	0	0	0	0	0	0	0	0	0	C

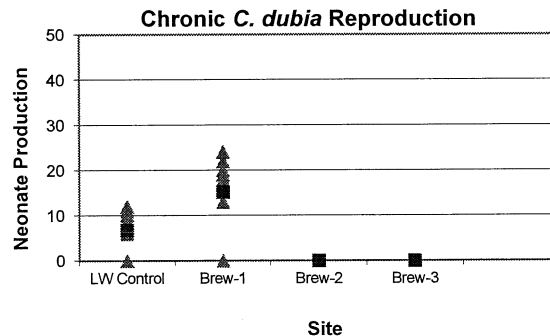
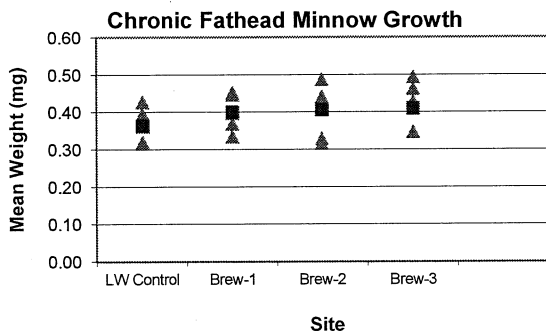
Male Production \leq 20% Over All Treatments? Yes

Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

COMMENTS: * Samples with the same letter are not statistically different from each other. Statistical significance based on reproduction.

C. dubia chronic test was run with organisms purchased from Aquatic Biosystems because the in-house culture organisms were not performing well.

▲ = Individual Data ■ = Mean



CHRONIC TEST CONTROL PERFORMANCE

LAB WATER CONTROLS	
<i>Selenastrum</i>	
≥ 1x10 ⁶ cells/ml	Yes
CV ≤ 20%	Yes
%CV =	8

GROWTH MEASUREMENT PER REPLICATE

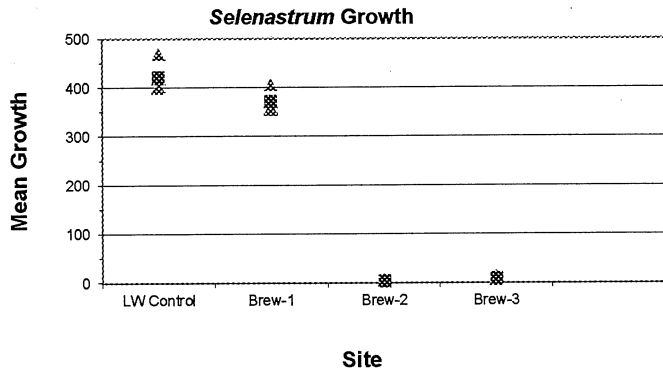
SPECIES	SITE DESCRIPTION		Fluorescence reading minus blank				MEAN GROWTH	%CV	Statistical Significance*
			1	2	3	4			
<i>Selenastrum capricornutum</i> GROWTH TEST	LC	LW Control	470	401	403	420	424	8	A
	1	Brew-1	373	407	357	359	374	6	B
	2	Brew-2	6	5	6	6	6	6	C
	3	Brew-3	10	9	12	8	10	18	C

Test Type: microplate **Endpoint:** fluorescence

Please describe any unusual appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

COMMENTS: * Samples with the same letter are not statistically different from each other.

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Project Name : Brewery Creek Ambients
 Report # : 206461002-61004
 Chronic Test Date : 7/21/2015

I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations. I also certify that these results relate only to these samples.

LAB REPRESENTATIVE:	Camille Danielson	SIGNATURE:	<i>Camille Danielson</i>
DATE:	8/18/2015		
PHONE:	(608) 224-6230	WDNR LAB CERT #:	113133790
LAB ADDRESS:	Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, Madison, WI 53718		
REVIEWED BY:	Dawn Perkins	DATE:	8/19/2015
PERMITTEE	NA	SIGNATURE:	NA
PHONE:	NA	DATE:	NA

Send **all pages** of this form (plus any attachments or additional information which you believe to be relevant to the test) to: Biomonitoring Coordinator, Bureau of Watershed Management, Department of Natural Resources, 101 South Webster St., P.O. Box 7921, Madison, WI 53707-7921.

Copies of the State of Wisconsin Aquatic Life Toxicity Testing Methods Manual (Methods Manual) and the WET Guidance Document can be obtained from the WDNR Biomonitoring Coordinator at the address given above or at: <http://dnr.wi.gov/org/water/wm/www/biomon/>

TO BE COMPLETED BY THE WISCONSIN DEPARTMENT OF NATURAL RESOURCES	
Results Entered Into Database?	
COMMENTS:	
REVIEWED BY:	DATE:
CC:	

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