

Appendix F:  
Raw Validated Data and PCB  
Congener Sums

## APPENDIX F

### Contents:

#### Section F1 –Validated KCEL Analytical Data (*Excel spreadsheet*)

**Table F1-1.** Echo Lake Drainage Basin Stormwater BMP Sampling KCEL Analytical Results

**Table F1-2.** Echo Lake Main Stormwater Outfall KCEL

#### Section F2 – Validated PCB Data and Sums (*Excel spreadsheet*)

**Table F2-1.** Echo Lake Drainage Basin Stormwater BMP Sampling PCB Congener Results and Sums

**Table F2-2.** PCB Quality Control Sample Results

#### Section F3 – Toxicity Reports

Echo Lake water quality monitoring data are available online at:

<http://green2.kingcounty.gov/smalllakes/WQData.aspx>

Echo Lake bacteria monitoring data are available online at:

<http://green2.kingcounty.gov/swimbeach/BeachData.aspx?locator=A764SB&CurrentYear=false>

**Sections F1 and F2 available separately as Excel spreadsheets.**

These are available upon request or on the project website:

<http://www.kingcounty.gov/depts/dnrp/wlr/sections-programs/science-section/doing-science/echo-lake-study.aspx>

Section F3 – Toxicity Reports

November 29, 2017

Carly Greyell  
King County Department of Natural Resources & Parks  
Water and Land Resources Division  
Science and Technical Support Section  
King Street Center, KSC-NR-0600  
201 S. Jackson St., Suite 600  
Seattle, WA 98104

Dear Carly:

The summary of a 7-day (*Ceriodaphnia*) test and a 48-hour (*Daphnia*) test conducted on storm water samples collected from an Echo Lake rain garden site on January 21, 2016 is shown in the table below. Tests were initiated January 26 and 27, 2016. Detailed findings and method descriptions are in the "RESULTS" and "METHODS" sections of the attached report. Statistical data analysis was performed in CETIS V1.8.7.16.

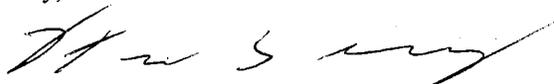
Event # 1 Collect 1/21/16		<i>Ceriodaphnia</i> Survival & Reproduction Test Date 1/27/16			<i>D. pulex</i> Acute Mortality Test Date 1/26/16	
Locator	Sample Information	Test #	% Survival	3rd brood reproduction	Test #	% Survival
LABCON	Standard Control Water	7873	90	34.2	7871	100
ECHO-BP1-IN	L64648-1	7873	50*	23.8**	7871	95
ECHO-BP1-OUT	L64648-3	7873	80	30.6	7871	95
Retest with dilutions 2/4/16 (100 % sample data)						
LABCON	Standard Control Water	7883	100	33.4		
ECHO-BP1-IN	L64648-1	7883	100	27.7**		
ECHO-BP1-OUT	L64648-3	7883	100	33.4		

\*Statistically significant reduction in survival compared to control response ( $p < 0.05$ ; Fisher Exact Test).

\*\*Statistically significant reduction in reproduction compared to control response ( $p < 0.05$ ; 1-tailed, t-Test).

If you would like additional information, please contact me at 477-7117.

Sincerely,



Fran Sweeney  
King County Dept. of Natural Resources and Parks  
Water and Land Resources Division  
Environmental Laboratory Section  
322 West Ewing St.  
Seattle, WA 98119

November 29, 2017

Carly Greuell  
King County Department of Natural Resources & Parks  
Water and Land Resources Division  
Science and Technical Support Section  
King Street Center, KSC-NR-0600  
201 S. Jackson St., Suite 600  
Seattle, WA 98104

Dear Carly:

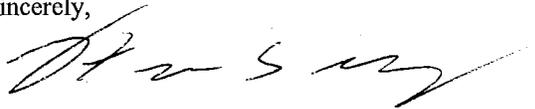
The summary of a 7-day (*Ceriodaphnia*) test and a 48-hour (*Daphnia*) test conducted on storm water samples collected from an Echo Lake rain garden site on March 10, 2016 is shown in the table below. Tests were initiated March 11, 2016. Detailed findings and method descriptions are in the "RESULTS" and "METHODS" sections of the attached report. Statistical data analysis was performed in CETIS V1.8.7.16.

Event # 2 Collect 3/10/16		<i>Ceriodaphnia</i> Survival & Reproduction Test Date 3/11/16			<i>D. pulex</i> Acute Mortality Test Date 3/11/16	
Locator	Sample Information	Test #	% Survival	3rd brood reproduction	Test #	% Survival
LABCON	Standard Control Water	7897	100	17.4	7898	100
ECHO-BP1-IN	L64999-1	7897	100	19.2	7898	85
ECHO-BP1-OUT	L64999-3	7897	90	21.4	7898	100

No statistically significant reduction in survival or reproduction between control and samples, or inlet compared to outlet.

If you would like additional information, please contact me at 477-7117.

Sincerely,



Fran Sweeney  
King County Dept. of Natural Resources and Parks  
Water and Land Resources Division  
Environmental Laboratory Section  
322 West Ewing St.  
Seattle, WA 98119

**BIOLOGICAL MONITORING REPORT FOR THE**

**Echo Lake Rain Garden Storm Water Tests  
Events 1 and 2 (January-March, 2016)**

**Program #421879-250**

**KING COUNTY DEPARTMENT OF NATURAL RESOURCES AND PARKS  
WATER AND LAND RESOURCES DIVISION  
ENVIRONMENTAL LABORATORY SECTION  
322 WEST EWING STREET  
SEATTLE, WASHINGTON 98119**

<b>Test #/Date:</b>	<b>7871 <i>Daphnia</i> Acute</b>	<b>1/26/2016</b>
	<b>7873 <i>Ceriodaphnia</i> Chronic</b>	<b>1/27/2016</b>
	<b>7883 <i>Ceriodaphnia</i> Chronic</b>	<b>2/4/2016</b>
	<b>7897 <i>Ceriodaphnia</i> Chronic</b>	<b>3/11/2016</b>
	<b>7898 <i>Daphnia</i> acute</b>	<b>3/11/2016</b>

**Report Date: July 20, 2016**

## METHODS

### SAMPLES

Two storm water samples were collected at Echo Lake Rain Garden #1 Inlet and Outlet sites on January 21 (Event 1) and March 9 (Event 2), 2016. Approximately 3 to 7L of each sample was delivered to the King County Environmental Laboratory (KCEL) in 2.5 gallon glass screw-cap jars with minimal headspace and tested as received. The samples were stored in the dark at  $4 \pm 2^\circ\text{C}$  and used to initiate the *Daphnia pulex* acute and the *Ceriodaphnia dubia* chronic toxicity tests, as well as for *C. dubia* test renewals.

Collection information and chemical characteristics of the test samples are listed in the table below.

Site:	EVENT 1		EVENT 2	
	Echo Lake Rain Garden #1, INLET	Echo Lake Rain Garden #1, OUTLET	Echo Lake Rain Garden #1, INLET	Echo Lake Rain Garden #1, OUTLET
Station:	ECHO-BP1-IN	ECHO-BP1-OUT	ECHO-BP1-IN	ECHO-BP1-OUT
KCEL Sample #:	L64648-1	L64648-3	L64999-1	L64999-3
Collect Date/Time	1-21-16/ 0845-1110h	1-21-16/ 0911-1122h	3-9-16/ 1440h	3-9-16/ 1445h
Rec'd Date/Time	1-21-16/ 1400h	1-21-16/ 1400h	3-10-16/ 0800h	3-10-16/ 0800h
Volume (L)	6.8	3.4	4.5	4.5
Temp ( $^\circ\text{C}$ )	7.6/ 6.2	6.5	5.5	5.6
pH	7.22/ 7.18	7.14	7.26	7.10
D.O. (mg/L)	11.6/ 12.0	11.8	10.6	11.4
Tot. Alk (mg/L as $\text{CaCO}_3$ )	7.93	10.3	10.8	15.3
Tot. Hard (mg/L as $\text{CaCO}_3$ )	11.2	10.1	13.6	15.3
Cond ( $\mu\text{mhos/cm}$ )	21.2	27.0	28	41
Turbidity (NTU)				
Tot. Susp. Solids (mg/L)	45.4	10.2	56.4	16.8
Ortho-P (mg/L)	0.0125	0.136	0.00218	0.097
$\text{NO}_2 + \text{NO}_3$ (mg/L)	0.031 (<RDL)	0.0885	0.0717	0.202
Tot N (mg/L)	0.324	0.302	0.609	0.503
Tot P (mg/L)	0.07	0.192	0.0736	0.155
Tot $\text{NH}_3$ (mg/L)	0.0651	0.0054 (< RDL)	0.100	0.0064 (< RDL)

### CONTROL WATER

The control water for tests with *Daphnia pulex* is fresh water obtained from a 95 ft. deep well located at the KCEL and filtered to  $60 \mu\text{m}$  with Nitex screen before use. *D. pulex* are routinely maintained in static-renewal cultures of well water (WW) at  $20 \pm 1^\circ\text{C}$ .

Water used for testing and culturing with *Ceriodaphnia* is fresh water obtained monthly from Lake Washington at a site midway between the I-90 and 520 bridges and filtered through  $60 \mu\text{m}$  Nitex screen before use.

Metals by ICP are measured monthly (last analysis: 5-2016); metals by ICP/MS or CVAA and organic compounds are measured annually (last analyses: 02 & 03-2016). Hardness, alkalinity, conductivity and pH are measured at the beginning of each test.

Physical-chemical characteristics of the WW and LWW are listed in the following table:

Parameter	WW Values		LWW Values		Units
	10-16-15	1-18-16	1-21-16	2-29-16	
Temperature	NA	16.9	NA	NA	°C, adjusted as necessary
Conductivity	265	160	98.5	98.2	µmhos/cm
pH	7.96	7.90	7.70	7.71	
Total Hardness (calc.)	109	62	38	39	mg/L as CaCO <sub>3</sub>
Total Alkalinity	80	54	36	37	mg/L as CaCO <sub>3</sub>
Total Cd	< 2		< 2		µg/L
Total Cr	< 3		< 3		µg/L
Total Cu	< 4		< 4		µg/L
Total Ni	< 5		< 5		µg/L
Total Pb	< 20		< 20		µg/L
Total Zn	< 5		< 5		µg/L
Total Mercury	< 0.05		< 0.05		µg/L
Volatile Organics	*		+		
Organic Analysis (BNA'S):	**		++		
Bis(2-Ethylhexyl)Phthalate	7.1		0.56 < RDL		µg/L
Di-N-Butyl Phthalate	< MDL		< MDL		µg/L
Pesticides & PCB's:	***		+++		

\* 45 cmpds not detectable

+ 45 cmpds not detectable

\*\* 68 cmpds not detectable

++ 68 cmpds not detectable

\*\*\* 28 cmpds not detected

+++ 28 cmpds not detected

## ACUTE TOXICITY TESTS

### Water Flea - *Daphnia pulex* – 48-Hour Static Acute Test

#### Events 1 and 2

The *Daphnia* acute toxicity tests followed the methods of US EPA (2002a). Test animals were neonates (< 24-hours old) taken from an overnight brood board; parent animals were adults isolated from in-house mass cultures. Samples were tested as received at one undiluted (100%) concentration along with a WW-only control. Test chambers were 30-mL beakers containing 25 mL of test solution. Individual broods were blocked across treatments such that each replicate contained representatives of five separate broods, with four replicates per treatment. Test chambers were randomized at the start of the test. The test was incubated at 20.0 ± 1.0°C for 48 hours on a 16:8 hour light:dark cycle. Survival and water quality measurements were recorded every 24 hours. Temperature was measured daily by digital thermometer in replicate blanks at six positions of the test tray (4 outer corner + 2 center). In addition, incubator temperature was measured at 15-minute intervals using an Onset Tidbit data logger. Temperature, pH and dissolved oxygen (D.O.) values can be found on the attached photocopied pages from the laboratory notebook in the “Storm Water Tests” section of this report.

Test #	LIMS Sample #	Start Date/ Time	End Date/ Time	Sample Concentrations (%)	Daphnid Age	# Reps/ Trtmt	# Orgs/ Rep
7871 (Event 1)	L64648	1-26-16/ 1400h	1-28-16/ 1455h	0 (WW control), 100%	< 24 hr	4	5
7898 (Event 2)	L64999	3-11-16/ 1255h	3-13-16/ 1300h	0 (WW control), 100%	< 24 hr	4	5

## CHRONIC TOXICITY TESTS

### Water Flea - *Ceriodaphnia dubia* - 7-Day Chronic Static Renewal Test

Event 1 (No Dilutions)

The *Ceriodaphnia dubia* 7-day static renewal chronic toxicity tests were conducted as outlined in US EPA (2002b). Samples were tested as received at one undiluted (100%) concentration. Ten replicates containing one animal each were tested at each treatment, including the control. Test organisms were 3<sup>rd</sup> or 4<sup>th</sup>-brood neonates (< 24 hours old) taken from an in-house individual brood board of adults started from mass culture. Individual broods were blocked across treatments, and each replicate represented a different brood. The test was incubated at 25 ± 1.0°C for 7 days on a 16:8 h light:dark cycle. All test solutions were renewed daily. Reproduction, survival, temperature and water quality measurements were recorded every 24 hours. Temperature was measured daily in six test board temperature blanks (4 outer corner + 2 center) and at 15-minute intervals using an Onset “Tidbit” data logger placed in a beaker of water in the incubator. The pH and D.O. values measured during testing can be found on the attached photocopied pages from the laboratory notebook in the “Storm Water Tests” section of this report.

Test #	LIMS Sample #	Start Date/ Time	End Date/ Time	Sample Concentrations (%)	Daphnid Age	# Reps/ Trtmt	# Orgs/ Rep
7873	L64648-1, -3	1-27-16/ 1330h	2-3-16/ 1300h	0 (LWW control), 100%	< 24 hr	10	1

Event 1 (With Dilutions)

Due to toxicity observed in the Inlet sample in the first set of tests, a second chronic *C. dubia* test was initiated using the original samples diluted with LWW in a dilution series. Five concentrations of each sample were tested: 100, 50, 25, 12.5 and 6.25%, along with a 0% sample (LWW-only) control. Because an insufficient volume of the Outlet sample L64648-3 remained for conducting renewals, the test was run using 5 replicates of the this sample. The test was otherwise run as described above for the Event 1 test.

Test #	LIMS Sample #	Start Date/ Time	End Date/ Time	Sample Concentrations (%)	Daphnid Age	# Reps/ Trtmt	# Orgs/ Rep
7883	L64648-1	2-4-16/ 1450h	2-11-16/ 1443h	0 (LWW control), 6.25, 12.5, 25, 50, 100%	< 24 hr	10	1
"	L64648-3	"	"	"	"	5	"

Event 2 (No Dilutions)

The *C. dubia* chronic test with Event 2 storm water samples was conducted as described above for the Event 1 test, with no sample dilutions.

Test #	LIMS Sample #	Start Date/ Time	End Date/ Time	Sample Concentrations (%)	Daphnid Age	# Reps/ Trtmt	# Orgs/ Rep
7898	L64999-1, -3	3-11-16/ 1255h	3-13-16/ 1300h	0 (LWW control), 100%	< 24 hr	10	1

**QUALITY CONTROL**

Reference toxicant control results are summarized in the following table.

Test Organism:	<i>Daphnia</i>		<i>Ceriodaphnia</i>	
Test #:	7869	7905	7881	7904
Control Survival (%)	100	100	80	100
Criteria	≥ 90	≥ 90	≥ 80	≥ 80
Acceptable?	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>
Survival LC50 (g/L)	2.79	3.17		
Lab Control Limits	2.44 - 4.36	2.43 - 4.36		
Acceptable?	<b>Yes</b>	<b>Yes</b>		
Control Reprod (# neos/adult)			33.8	36.8
Criteria			≥ 15	≥ 15
Acceptable?			<b>Yes</b>	<b>Yes</b>
PMSD for Reproduction (%)*			32.04	24.4
Criteria			13 - 47	13 - 47
Acceptable?			<b>Yes</b>	<b>Yes</b>
Reproduction IC25 (µg/L)			3.09	6.71
Lab Control Limits			0 - 5.29	0 - 6.07
Acceptable?			<b>Yes</b>	<b>No</b>

\*Percent Minimum Significant Difference; determined by Dunnett's Multiple Comparison test (Steels Many-One Rank Test for unequal variance); ( $\alpha = 0.05$ )

NaCl was used as a reference toxicant in acute tests with *Daphnia*. Temperature, pH and dissolved oxygen measurements remained within acceptable limits throughout the reference toxicant tests for *Daphnia* (#7869 & #7905) (US EPA 2002a). Acute positive control tests met acceptability criteria regarding control survival, and the survival LC50 endpoints were within the control limits of the mean  $\pm$  2SD (US EPA, 2002a).

Cadmium nitrate was used as a reference toxicant in chronic toxicity tests with *Ceriodaphnia*. Temperature, pH and dissolved oxygen measurements remained within acceptable limits throughout the reference toxicant tests for *Ceriodaphnia* (#7881 & #7904) (US EPA 2002b). In addition, chronic tests met acceptability criteria regarding control survival and mean control reproduction. For test #7881, the reproduction IC25 fell within the control limits of mean  $\pm$  2SD (US EPA 2002b), whereas for #7904 it slightly exceeded the upper control limit. Because it met all other QC criteria, the test was retained. Reference toxicant tests with *Ceriodaphnia* were re-started in 2015 following a three-year hiatus. It is possible that a change in diet or culture health may have affected the response to the toxicant.

The precision tables located at the end of this report are constructed to monitor the sensitivity of the organisms to the reference toxicant and thereby provide an indication of their overall sensitivity to other compounds.

## **WATER QUALITY MONITORING**

Methods and method numbers for water quality tests are listed in the following table:

Parameter	Method
Water Quality Tests	APHA (1992); US EPA (1991).
Temperature	Standard Mercury Thermometer (calibrated with a certified thermometer traceable to NBS records) and Onset, Tidbit (v2) UTBI-001 Temperature Logger (KCEL #436v1).
Dissolved Oxygen	YSI membrane electrode method (Method #4500-0 G; KCEL #434).
pH	Beckman 690 meter with automatic temperature compensation and Ross combination electrode (Method #4500-H; APHA 1992; KCEL #433).
Total Alkalinity	Potentiometric Method (Method #2320 B; KCEL #319v4).
Total Hardness	By calculation (Method #2340 B; KCEL #612v4).
Conductivity	Orion Model #122 Meter with 012210 conductivity cell (Method 2510B; KCEL #435).
Total Ammonia	Phenate Method (Standard Methods SM 4500 - NH <sub>3</sub> -G; KCEL #330v4).
Unionized Ammonia	Calculated from total ammonia, pH and ionization constants (APHA Method #417 G).
Pesticides and PCB's	Continuous liquid extraction method (EPA Method #608; KCEL #733).
Organic Analysis	Continuous liquid extraction method for BNA's (EPA Method #625; KCEL #731).
Volatile Organics	Purge and trap method (EPA Method #624; KCEL #732).
Total Metals	ICP for Cd, Cr, Cu, Ni, Pb and Zn (EPA Method #200.7; KCEL #612v4); for Hg analysis (KCEL #604v5, 601v4, 605v0).

## RESULTS

### ACUTE TOXICITY TESTS

#### Water Flea - *Daphnia pulex* – 48-Hour Static Acute Test

Event 1 (No Dilutions): KCEL Test #7871

Survival results for the 48-hour *Daphnia* acute test with Event 1 storm water samples are listed in the table below.

Sample #	Station/ Site	% Sample	Percent Survival at 48 Hours					# <i>Daphnia</i> Tested
			% Survival in each rep. (n=5 <i>Daphnia</i> /rep)				Mean	
			Rep 1	Rep 2	Rep 3	Rep 4		
----	Well Water Control	0	100	100	100	100	<b>100</b>	20
L64648-1	ECHO-BP1-IN Rain Garden #1 (Inlet)	100	80	100	100	100	<b>95</b>	20
L64648-3	ECHO-BP1-OUT Rain Garden #1 (Outlet)	100	100	80	100	100	<b>95</b>	20

Survival was 100 % in the well water-only control and 95% in both the Inlet and Outlet storm water samples. Survival in both the Inlet and Outlet samples was not significantly reduced from the control ( $p > 0.05$ ; Wilcoxon Rank Sum Test).

The unionized ammonia level in 100% Inlet and Outlet sample reached a maximum of 0.007 and 0.005 mg N/L, respectively, during the 48-hour test.

Event 2 (KCEL Test #7898)

Results are listed in the table below of the 48-hour acute test with *Daphnia* using Event 2 storm water samples.

Sample #	Station/ Site	% Sample	% Survival at 48 Hours					# <i>Daphnia</i> Tested
			% Survival in each rep. (n=5 <i>Daphnia</i> /rep)				Mean	
			Rep 1	Rep 2	Rep 3	Rep 4		
----	Well Water Control	0	100	100	100	100	<b>100</b>	20
L64999-1	ECHO-BP1-IN Rain Garden #1 (Inlet)	100	80	100	80	80	<b>85<sup>+</sup></b>	20
L64999-3	ECHO-BP1-OUT Rain Garden #1 (Outlet)	100	100	100	100	100	<b>100</b>	20

<sup>†</sup>Different from control at ANOVA, but not significantly ( $p > 0.05$ ; Wilcoxon Rank-Sum test)

Survival was 100% in the well water-only control and Outlet storm sample and 85% in the Inlet storm sample. Survival in the Inlet sample was decreased from the control and Outlet sample (at ANOVA) but not significantly ( $p > 0.05$ ; Wilcoxon Rank-Sum test), whereas survival in the Outlet samples was not reduced from the control.

The unionized ammonia level in both the 100% Inlet and Outlet samples reached a maximum of 0.009 mg NH<sub>3</sub>-N/L during the 48-hour test.

**Water Flea - *Ceriodaphnia dubia* - 7-Day Chronic Static Renewal Test**Event 1 (KCEL Test #7873)

Reproduction and survival results over the 7-day chronic *Ceriodaphnia* test with Event 1 storm water samples are shown in the table below.

Sample #	Station	% Sample	Reproduction (Mean #Neonates/Adult in 7 Days)										Mean Reprod (n = 9)	Mean % Surv
			1	2	3	4	5	6	7	8	9	10		
---	LWW Control	0	28	12	49	48	51	30	16	5 <sup>+</sup>	39	35	34.2 (n = 9)	90
L64648-1	ECHO-BP1-IN	100	23	34	21	14	38	8	34	19	24	23	23.8*	50**
L64648-2	ECHO-BP1-OUT	100	5	45	46	42	24	41	4	41	28	30	30.6	80

<sup>+</sup> Outlier; omitted from analysis

\*Significantly different from control ( $p < 0.05$ ; 1-tailed t-Test)

\*\*Significantly different from control ( $p < 0.05$ ; Fisher's Exact Test)

As shown in the table above, survival was 90% in the LWW-only control, 50% in the Inlet storm sample and 80% in the Outlet storm sample. In the Inlet sample, survival was significantly reduced from the control ( $p < 0.05$ ; Fishers Exact Test), but not significantly ( $p > 0.05$ ; Fishers Exact Test) in the Outlet sample.

Similarly, reproduction in the Inlet storm sample was significantly ( $p < 0.05$ ; homoscedastic 1-tailed t-Test) less than the control but not significantly reduced from the control in the Outlet sample ( $p > 0.05$ ; homoscedastic 1-tailed t-Test). Reproduction in the Inlet and Outlet sample did not differ significantly ( $p > 0.05$ ; Tukey-Kramer and homoscedastic 1-tailed t-Test).

The unionized ammonia level in 100% Inlet and Outlet samples reached a maximum of 0.009 and 0.007 mg NH<sub>3</sub>-N/L, respectively, during the 7-day test.

Event 1, with Dilutions (KCEL Test #7883)

Results of the 7-day *Ceriodaphnia* chronic renewal test run using a dilution series of Event 1 storm water samples are listed in the table below.

Sample #	Station	% Sample	Reproduction (Mean #Neonates/Adult in 7 Days)										Mean Reprod	Mean % Surv
			1	2	3	4	5	6	7	8	9	10		
---	LWW Control	0	36	34	34	34	32	33	28	32	36	35	33.4	100
L64648-1	ECHO-BP1-IN	6.25	38	25	32	9	32	35	29	30	36	36	30.2	100
		12.5	39	37	32	36	35	32	32	34	32	34	34.3	100
		25	32	36	33	37	32	37	32	35	52	27	35.3	100
		50	37	31	29	32	31	33	25	29	31	33	31.1	100
		100	29	23	27	28	27	32	23	32	24	32	27.7*	100
L64648-3	ECHO-BP1-OUT	6.25	31	39	28	35	31						32.8	100
		12.5	37	35	34	36	31						34.6	100
		25	33	34	31	35	36						33.8	100
		50	32	31	31	38	35						33.4	100
		100	33	33	33	34	34						33.4	100

\*Significantly different from control ( $p < 0.05$ ; 1-tailed t-Test)

Survival was 100% in the LWW-only control and all concentrations of both the Inlet and Outlet storm samples.

For the Inlet sample, reproduction was significantly reduced from the control at the 100% sample ( $p < 0.05$ ; Steel Many-One Rank Test); it was also less than the control at the 6.25% and the 50% sample concentrations but not significantly ( $p > 0.05$ ; Steel

Many-One Rank Test). The NOEC was calculated to be 50% sample. Because reproduction was not reduced by more than 25% at any concentration, an IC25 could not be calculated.

For the Outlet sample, reproduction was not reduced from the control at any sample concentration; therefore, an IC25 could not be calculated.

Reproduction in the Outlet sample did not differ significantly from the Inlet sample ( $p > 0.05$ ; heterogeneous 1-tailed t-Test).

The unionized ammonia level in 100% Inlet and Outlet samples reached a maximum of 0.010 and 0.006 mg NH<sub>3</sub>-N/L, respectively, during the 7-day test.

#### Event 2 No Dilutions (KCEL Test #7897)

Average reproduction and survival results at the end of the 7-day *Ceriodaphnia* chronic renewal test with Event 2 storm water samples are listed in the table below.

Sample #	Station	% Sample	Reproduction (Mean #Neonates/Adult in 7 Days)										Mean Reprod	Mean % Surv
			1	2	3	4	5	6	7	8	9	10		
---	LWW Control	0	19	16	20	17	20	18	18	9	19	18	17.4	100
L64999-1	ECHO-BP1-IN	100	8	18	18	20	22	23	19	24	21	19	19.2	100
L64999-3	ECHO-BP1-OUT	100	24	21	18	21	20	22	22	23	22	21	21.4	90

Survival was 100% in the LWW-only control and the Inlet sample and 90% in the Outlet sample. Survival in the Outlet sample was not significantly reduced from the control ( $p > 0.05$ ; Fisher Exact/ Bonferroni t-Test).

Reproduction in both the Inlet and Outlet samples was not reduced from the control.

The unionized ammonia level in 100% Inlet and Outlet sample reached a maximum of 0.016 and 0.013 mg NH<sub>3</sub>-N/L, respectively, during the 7-day test.

#### QUALITY CONTROL

Storm water sample and control performance results are summarized in the following table:

Test Organism:	<i>Ceriodaphnia</i>			<i>Daphnia</i>	
Test #:	7873	7883	7897	7871	7898
Control Survival (%)	90	100	100	100	100
Criteria	≥ 80	≥ 80	≥ 80	≥ 90	≥ 90
Acceptable?	Yes	Yes	Yes	Yes	Yes
Control Reproduction (# neos/adult)	34.2	33.4	17.4		
Criteria	≥ 15	≥ 15	≥ 15		
Acceptable?	Yes	Yes	Yes		
PMSD for Reproduction (%)*	27.7 (IN) 34.9 (OUT)	15.3 (IN)	17.3 (IN)		
Criteria	13-47	13-47	13-47		
Acceptable?	Yes	Yes	Yes		

\*Percent Minimum Significant Difference; determined by Dunnett's Multiple Comparison test ( $\alpha = 0.05$ )

As shown in the table above, both the acute and chronic effluent tests met acceptability criteria regarding control performance and test variability, including survival, reproduction and PMSD (US EPA, 2002a & 2002b).

Dissolved oxygen, pH, temperature and/or salinity remained within acceptable limits throughout both the acute and chronic tests (US EPA, 2002a & 2002b). Water quality data recorded during testing is shown on the photocopied pages from the laboratory notebook in the "Effluent Tests" section of this report.

**Tested By:**

King County Department of Natural Resources & Parks  
Water and Land Resources Division  
Environmental Laboratory Section  
322 West Ewing Street  
Seattle WA 98119  
(206) 477-7123

Julie Alaimo, Gary Yoshida, Robin Revelle, Gabriela Hannach, Elizabeth Frame, Lyndsey Swanson, Fran Sweeney

**REFERENCES**

**APHA. 1992.** Standard Methods for the Examination of Water and Wastewater, 18<sup>th</sup> Edition. American Public Health Association, American Waterworks Association, Water Pollution Control Association, Washington D.C.

**US EPA. 2002a.** Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5<sup>th</sup> edition. EPA-821-02-012, October, 2002. US Environmental Protection Agency, Office of Water (4303T), Washington, DC.

**US EPA. 2002b.** Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 4<sup>th</sup> Edition (EPA-821-R-02-013).

**US EPA. 1991.** Code of Federal Regulations, 40CFR, Appendix A, July 1991. U.S. Environmental Protection Agency, Office of Federal Registry, Washington, D.C.

October 25, 2017

Carly Greyell  
 King County Department of Natural Resources & Parks  
 Water and Land Resources Division  
 Science and Technical Support Section  
 King Street Center, KSC-NR-0600  
 201 S. Jackson St., Suite 600  
 Seattle, WA 98104

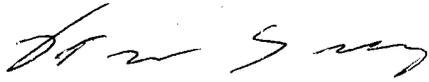
Dear Carly:

The summary of a 7-day (*Ceriodaphnia*) test and a 48-hour (*Daphnia*) test conducted on storm water samples collected from an Echo Lake rain garden site on October 26, 2016 is shown in the table below. The tests were initiated on October 28, 2016 and October 31, 2016 respectively. Detailed findings and method descriptions are in the "RESULTS" and "METHODS" sections of the attached report. Statistical data analysis was performed in CETIS V1.8.7.16.

Event # 3 Collect 10/26/17		<i>Ceriodaphnia</i> Survival & Reproduction Test Date 10/28/16			<i>D. pulex</i> Acute Mortality Test Date 10/31/16	
Locator	Sample Information	Test #	% Survival	3rd brood reproduction	Test #	% Survival
LABCON	Standard Control Water	8174	100	32.8	8175	100
LABCON - LH	Low Hardness Control Water	8174	100	33.9	8175	100
ECHO-BP1-IN	L66498-1	8174	60*†	19.5 **††	8175	100
ECHO-BP1-OUT	L66498-3	8174	100	33.2	8175	100
<b>Retest with dilutions 11/11/16 (100% sample data)</b>						
LABCON	Standard Control Water	8217 8218	NA	NA		
LABCON - LH	Low Hardness Control Water	8217 8218	100	19		
ECHO-BP1-IN	L66498-1	8217	90	22.6		
ECHO-BP1-OUT	L66498-3	8218	90	22.6		
*Statistically significant reduction in survival compared to (low-hardness) control response (p<0.05; Fisher Exact Test).						
** Statistically significant reduction in reproduction compared to (low-hardness) control response (p<0.05; Wilcoxon Rank Sum 2-Sample Test).						
†Statistically significant reduction in survival in Inlet response compared to Outlet response (p<0.05; Fisher Exact Test).						
††Statistically significant reduction in reproduction in the Inlet response compared to Outlet response (p<0.05; Wilcoxon Rank Sum 2-Sample Test).						

If you would like additional information, please contact me at 477-7117.

Sincerely,

A handwritten signature in cursive script, appearing to read "Fran Sweeney".

Fran Sweeney  
**King County Dept. of Natural Resources and Parks**  
**Water and Land Resources Division**  
Environmental Laboratory Section  
322 West Ewing St.  
Seattle, WA 98119

**BIOLOGICAL MONITORING REPORT FOR THE**

**Echo Lake Rain Garden Storm Water Test  
October 26, 2016**

**Program #421879-250**

**KING COUNTY DEPARTMENT OF NATURAL RESOURCES AND PARKS  
WATER AND LAND RESOURCES DIVISION  
ENVIRONMENTAL LABORATORY SECTION  
322 WEST EWING STREET  
SEATTLE, WASHINGTON 98119**

**Test #/Date: 8174 *Ceriodaphnia* Chronic 10/28/16  
8175 *Daphnia* Acute 10/31/16**

**Report Date: March 28, 2017**

## METHODS

### SAMPLES

Storm water samples were collected at Echo Lake Rain Garden #1 Inlet and Outlet sites on October 26, 2016. Approximately 3 to 7L of each sample was delivered to the King County Environmental Laboratory (KCEL) in 9 Liter glass screw-cap jars with minimal headspace and tested as received. The samples were stored in the dark at  $4 \pm 2^\circ\text{C}$  and used to initiate the *Daphnia pulex* acute and the *Ceriodaphnia dubia* chronic toxicity tests, as well as for *C. dubia* test renewals.

Collection information and chemical characteristics of the test samples are listed in the table below.

Site:	Echo Lake Rain Garden #1, INLET	Echo Lake Rain Garden #1, OUTLET
Station:	ECHO-BP1-IN	ECHO-BP1-OUT
KCEL Sample #:	L66498-1	L66498-3
Collect Date/Time	10/26/16 09:12	10/26/16 09:17
Rec'd Date/Time	10/26/16 14:40	10/26/16 14:40
Volume (L)	~ 1.5 gal (~ 6L)*	~ 1.5 gal (~ 6L)*
Temp ( $^\circ\text{C}$ )	5.9	5.4
pH	7.404	7.030
D.O. (mg/L)	10.9	11.0
Total Alkalinity (mg/L as $\text{CaCO}_3$ )	NA	NA
Total Hardness (mg/L as $\text{CaCO}_3$ )	8.99	12
Cond ( $\mu\text{mhos/cm}$ )	15	25.1
Turbidity (NTU)	21.1	18
Tot. Susp. Solids (mg/L)	39.6	14.6
Ortho-P (mg/L)	0.0165	0.134
$\text{NO}_2 + \text{NO}_3$ (mg/L)	0.0424	0.145
Tot N (mg/L)	0.457	0.487
Tot P (mg/L)	0.0714	0.177
Tot $\text{NH}_3$ (mg/L)	0.118	0.0194

\*Split from 9L glass jars in-house

### CONTROL WATER

The control water for tests with *Daphnia pulex* is fresh water obtained from a 95 ft. deep well located at the KCEL and filtered to  $60 \mu\text{m}$  with Nitex screen before use. *D. pulex* are routinely maintained in static-renewal cultures of well water (WW) at  $20 \pm 1^\circ\text{C}$ .

Water used for testing and culturing with *Ceriodaphnia* is fresh water obtained monthly from Lake Washington at a site midway between the I-90 and 520 bridges and filtered through  $60 \mu\text{m}$  Nitex screen before use.

Metals by ICP are measured monthly (last analysis: October 2016); metals by ICP/MS or CVAA and organic compounds are measured annually (last analyses: February and March 2016). Hardness, alkalinity, conductivity and pH are measured at the beginning of each test.

Physical-chemical characteristics of the WW and LWW are listed in the following table:

Parameter	LWW	Low Hardness LWW	WW	Low Hardness WW	Units
Sample Number:	L66510-1	L66510-2	L66510-3	L66510-4	
Temperature:	*	*	*	*	°C, adjusted as necessary
Conductivity:	102.1	27.8	30.1	25.9	µmhos/cm
pH:	7.805	7.536	8.159	7.598	
Total Hardness (calc.):	41.9	10.8	105	10.4	mg/L as CaCO <sub>3</sub>
Total Alkalinity:	37.9	9.87	68.9	7.19	mg/L as CaCO <sub>3</sub>

\*Water held at 0-5°C cooler until needed.

**Metals and Organics:**

Parameter	LWW	WW	Units
Total Cd:	< 2	< 2	µg/L
Total Cr:	< 3	< 3	µg/L
Total Cu:	< 4	< 4	µg/L
Total Ni:	< 5	< 5	µg/L
Total Pb:	< 20	< 20	µg/L
Total Zn:	< 5	< 5	µg/L
Total Mercury:	< 0.05	< 0.05	µg/L
Volatile Organics:	*	+	µg/L
Organic Analysis (BNA'S):	**	++	µg/L
Bis(2-Ethylhexyl)Phthalate:	0.56 <sup>#</sup>	7.1	µg/L
Di-N-Butyl Phthalate:	< 0.47	< 0.47	µg/L
Pesticides & PCB's:	***	+++	µg/L

\* 45 cmpds not detectable

\*\* 68 cmpds not detectable

\*\*\* 28 cmpds not detectable

# < RDL; RDL = 1.89

+ 45 cmpds not detectable

++ 68 cmpds not detectable

+++ 28 cmpds not detectable

**CHRONIC TOXICITY TEST**

**Water Flea - *Ceriodaphnia dubia* - 7-Day Chronic Static Renewal Test (#8174)**

The *Ceriodaphnia dubia* 7-day static renewal chronic toxicity tests were conducted as outlined in US EPA (2002b). Samples were tested as received at one undiluted (100%) concentration. Ten replicates containing one animal each were tested at each treatment, including the control. Test organisms were 3<sup>rd</sup> or 4<sup>th</sup>-brood neonates (< 24 hours old) taken from an in-house individual brood board of adults started from mass culture. Individual broods were blocked across treatments, and each replicate represented a different brood. The test was incubated at 25 ± 1.0°C for 7 days on a 16:8 h light:dark cycle. All test solutions were renewed daily. Reproduction, survival, temperature and water quality measurements were recorded every 24 hours. Temperature was measured daily in six test board temperature blanks (4 outer corner + 2 center) and at 15-minute intervals using an Onset "Tidbit" data logger placed in a beaker of water in the incubator. The pH and D.O. values measured during testing can be found on the attached photocopied pages from the laboratory notebook in the "Storm Water Tests" section of this report.

Test #	LIMS Sample #	Start Date/ Time	End Date/ Time	Sample Concentrations (%)	Daphnid Age	# Reps/ Trtmt	# Orgs/ Rep
8174	L66498	10/28/17 0940	11/04/16 1010	0 (WW control), 0 (Low Hardness control), 100%	< 24 hr	10	1

**RESULTS**

Reproduction and survival results over the 7-day chronic *Ceriodaphnia* test # 8174 are shown in the table below:

Sample #	Station	% Sample	Reproduction (#Neonates/Adult in 7 Days)										Mean Reprod*	Mean % Surv
			1	2	3	4	5	6	7	8	9	10		
----	LWW Control	0	32	35	33	37	18	36	35	28	40	34	32.8	100
----	Low Hardness Control	0	36	30	31	37	36	31	33	35	35	35	33.9	100
L66498-1	ECHO-BP1-IN	100	17	31	5	25	30	27	25	11	0	24	19.5	60
L66498-2	ECHO-BP1-OUT	100	31	35	34	36	31	40	34	36	33	22	33.2	100

\*Numbers based off of 3<sup>rd</sup> brood

As shown in the table above, survival was 100% in all samples except for the Inlet, where survival was 60%.

The Wilcoxon Rank Sum Two-Sample Test (Nonparametric-Two Sample) and an Equal Variance Two-Sample t-test (Parametric) were used to compare the inlet and outlet for both survival and reproduction. A significant reproduction effect was found between pairings for both survival and reproduction.

The unionized ammonia level in 100% Inlet and Outlet samples reached a maximum of 0.003 and <0.001 mg NH<sub>3</sub>-N/L, respectively, during the 7-day test.

**ACUTE TOXICITY TEST**

**Water Flea - *Daphnia pulex* – 48-Hour Static Acute Test (#8175)**

The *Daphnia* acute toxicity test followed the methods of US EPA (2002a). Test animals were neonates (<24-hours old) taken from an overnight brood board; parent animals were adults isolated from in-house mass cultures. Samples were tested as received at one undiluted (100%) concentration along with a WW-only control. Test chambers were 30-mL beakers containing 25 mL of test solution. Individual broods were blocked across treatments such that each replicate contained representatives of five separate broods, with four replicates per treatment. Test chambers were randomized at the start of the test. The test was incubated at 20.0 ± 1.0°C for 48 hours on a 16:8 hour light:dark cycle. Survival and water quality measurements were recorded every 24 hours. Temperature was measured daily by digital thermometer in replicate blanks at six positions of the test tray (4 outer corner + 2 center). In addition, incubator temperature was measured at 15-minute intervals using an Onset Tidbit data logger. Temperature, pH and dissolved oxygen (D.O.) values can be found on the attached photocopied pages from the laboratory notebook in the "Storm Water Tests" section of this report.

Test #	LIMS Sample #	Start Date/ Time	End Date/ Time	Sample Concentrations (%)	Daphnid Age	# Reps/ Trtmt	# Orgs/ Rep
8175	L66498	10/31/16 1040	11/02/16 1050	0 (WW control), 0 (Low Hardness control), 100%	< 24 hr	4	5

**RESULTS**

Survival results for the 48-hour *Daphnia* acute test # 8175 are listed in the table below:

Sample #	Station/ Site	% Sample	Percent Survival at 48 Hours				Mean % Survival	# <i>Daphnia</i> Tested
			% Survival in each rep. (n=5 <i>Daphnia</i> /rep)					
			Rep 1	Rep 2	Rep 3	Rep 4		
----	Well Water Control	0	100	100	100	100	100	20
----	Low Harness Control	0	100	100	100	100	100	20
L66498-1	ECHO-BP1-IN Rain Garden #1 (Inlet)	100	100	100	100	100	100	20
L66498-3	ECHO-BP1-OUT Rain Garden #1 (Outlet)	100	100	100	100	100	100	20

Mean % Survival was 100 % in all samples.

The Wilcoxon Rank Sum Two-Sample Test (Nonparametric-Two Sample) and an Equal Variance Two-Sample t-test (Parametric) were used to compare the inlet and outlet. A non-significant effect was found.

The unionized ammonia level in 100% Inlet and Outlet sample reached a maximum of 0.002 and <0.001 mg N/L, respectively, during the 48-hour test.

**QUALITY CONTROL**

Storm water sample and control performance results are summarized in the following table:

Test Organism:	<i>Ceriodaphnia</i>	<i>Daphnia</i>
Test #:	8174	8175
Control Survival (%)	100	100
Criteria	≥ 80	≥ 90
Acceptable?	Yes	Yes
Control Reproduction (# neos/adult)	32.8 <sup>#</sup>	
Criteria	≥ 15	
Acceptable?	Yes	
PMSD* for Reproduction (%)	NA	
Criteria	NA	
Acceptable?	NA	

# Based on 3<sup>rd</sup> brood.

\*Percent Minimum Significant Difference

As shown in the table above, both the acute and chronic effluent tests met acceptability criteria regarding control, performance and test variability; including survival, and reproduction(US EPA, 2002a & 2002b).

Dissolved oxygen, pH, temperature and/or salinity remained within acceptable limits throughout both the acute and chronic tests (US EPA, 2002a & 2002b). Water quality data recorded during testing is shown on the photocopied pages from the laboratory notebook in the "Effluent Tests" section of this report.

Reference toxicant control results are summarized in the following table.

Test #:	<i>Daphnia</i>	<i>Ceriodaphnia</i>
	8199	8178
Control Survival (%)	100	100
Criteria	≥ 90	≥ 80
Acceptable?	Yes	Yes
Survival LC50 (g/L)	3.8	
Lab Control Limits	3.4 – 4.2	
Acceptable?	Yes	
Control Reprod (# neos/adult)		37.8
Criteria		≥ 15
Acceptable?		Yes
PMSD for Reproduction (%)*		8.58
Criteria		13 - 47
Acceptable?		Yes*
Reproduction IC25 (µg/L)		2.76
Lab Control Limits		0-7.29
Acceptable?		Yes

\*Percent Minimum Significant Difference; PMSD slightly low, however all other QC is acceptable.

NaCl was used as a reference toxicant in the acute test with *Daphnia*. Temperature, pH and dissolved oxygen measurements remained within acceptable limits throughout the reference toxicant test for *Daphnia* (#8199) (US EPA 2002a). The acute positive control test met acceptability criteria regarding control survival, and the survival LC50 endpoint was within the control limits of the mean ± 2SD (US EPA, 2002a).

Cadmium nitrate was used as a reference toxicant in chronic toxicity tests with *Ceriodaphnia*. Temperature, pH and dissolved oxygen measurements remained within acceptable limits throughout the reference toxicant tests for *Ceriodaphnia* (#8178) (US EPA 2002b). In addition, chronic tests met acceptability criteria regarding control survival and mean control reproduction.

The precision tables located at the end of this report are constructed to monitor the sensitivity of the organisms to the reference toxicant and thereby provide an indication of their overall sensitivity to other compounds

**WATER QUALITY MONITORING**

Methods and method numbers for water quality tests are listed in the following table:

Parameter	Method
Water Quality Tests	APHA (1992); US EPA (1991).
Temperature	Standard Mercury Thermometer (calibrated with a certified thermometer traceable to NBS records) and Onset, Tidbit (v2) UTBI-001 Temperature Logger (KCEL #436v1).
Dissolved Oxygen	YSI membrane electrode method (Method #4500-0 G; KCEL #434).
pH	Beckman 690 meter with automatic temperature compensation and Ross combination electrode (Method #4500-H; APHA 1992; KCEL #433).
Total Alkalinity	Potentiometric Method (Method #2320 B; KCEL #319v4).
Total Hardness	By calculation (Method #2340 B; KCEL #612v4).
Conductivity	Orion Model #122 Meter with 012210 conductivity cell (Method 2510B; KCEL #435).
Total Ammonia	Phenate Method (Standard Methods SM 4500 - NH <sub>3</sub> -G; KCEL #330v4).
Unionized Ammonia	Calculated from total ammonia, pH and ionization constants (APHA Method #417 G).
Pesticides and PCB's	Continuous liquid extraction method (EPA Method #608; KCEL #733).
Organic Analysis	Continuous liquid extraction method for BNA's (EPA Method #625; KCEL #731).
Volatile Organics	Purge and trap method (EPA Method #624; KCEL #732).
Total Metals	ICP for Cd, Cr, Cu, Ni, Pb and Zn (EPA Method #200.7; KCEL #612v4); for Hg analysis (KCEL #604v5, 601v4, 605v0).

**Tested By:**

King County Department of Natural Resources & Parks  
Water and Land Resources Division  
Environmental Laboratory Section  
322 West Ewing Street  
Seattle WA 98119  
(206) 477-7123

Julie Alaimo, Gary Yoshida, Robin Revelle, Gabriela Hannach, Elizabeth Frame, Lyndsey Swanson, Fran Sweeney

**REFERENCES**

- APHA. 1992.** Standard Methods for the Examination of Water and Wastewater, 18<sup>th</sup> Edition. American Public Health Association, American Waterworks Association, Water Pollution Control Association, Washington D.C.
- US EPA. 2002a.** Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5<sup>th</sup> edition. EPA-821-02-012, October, 2002. US Environmental Protection Agency, Office of Water (4303T), Washington, DC.
- US EPA. 2002b.** Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 4<sup>th</sup> Edition (EPA-821-R-02-013).
- US EPA. 1991.** Code of Federal Regulations, 40CFR, Appendix A, July 1991. U.S. Environmental Protection Agency, Office of Federal Registry, Washington, D.C.

October 25, 2017

Carly Greyell  
 King County Department of Natural Resources & Parks  
 Water and Land Resources Division/ Scientific and Technical Support  
 Watershed and Ecological Assessment Team  
 King Street Center  
 201 S. Jackson Street, Room 600  
 MS KSC-NR-0600  
 Seattle, WA 98104-3855

Dear Carly:

A summary of 48-hour acute (*Daphnia*) and 7-day chronic (*Ceriodaphnia*) tests conducted with storm water samples collected from Echo Lake Rain Garden #1 Inlet and Outlet sites on January 17-18, 2017 is listed in the following table. The tests were initiated on January 24 and 19, respectively. Detailed findings and method descriptions are in the "RESULTS" and "METHODS" sections of the attached report. Statistical data analysis was performed in CETIS V1.8.7.16.

Event # 4 Collect 1/18/2017		<i>Ceriodaphnia</i> Survival & Reproduction Test Date 1/19/17			<i>D. pulex</i> Acute Mortality Test Date 1/24/17	
Locator	Sample Information	Test #	% Survival	3rd brood reproduction	Test #	% Survival
LABCON	Standard Control Water	8245	100	35.4	8248	95
LABCON - LH	Low Hardness Control Water	8245	100	31	8248	NA
ECHO-BP1-IN	L66938-1	8245	0*†	0 ***††	8248	100
ECHO-BP1-OUT	L66938-3	8245	90	37.9	8248	100
*Statistically significant reduction in survival compared to (low-hardness) control response (p<0.05; Fisher Exact Test).						
** Statistically significant reduction in reproduction compared to (low-hardness) control response (p<0.05; Wilcoxon Rank Sum 2-Sample Test).						
†Statistically significant reduction in survival in Inlet response compared to Outlet response (p<0.05; Fisher Exact Test).						
††Statistically significant reduction in reproduction in the Inlet response compared to Outlet response (p<0.05; Wilcoxon Rank Sum 2-Sample Test).						

If you would like additional information, please contact me at 477-7117.

Sincerely,



Fran Sweeney  
 King County Dept. of Natural Resources and Parks  
 Water and Land Resources Division  
 Environmental Laboratory Section  
 322 West Ewing St.  
 Seattle, WA 98119

**BIOLOGICAL MONITORING REPORT FOR**

**Echo Lake Rain Garden #1 Storm Water Tests  
January 2017**

**Program #421879-250**

**KING COUNTY DEPARTMENT OF NATURAL RESOURCES AND PARKS  
WATER AND LAND RESOURCES DIVISION  
ENVIRONMENTAL LABORATORY SECTION  
322 WEST EWING STREET  
SEATTLE, WASHINGTON 98119**

**Test #/Date: 8248 *Daphnia* Acute 1/24/2017  
8245 *Ceriodaphnia* Chronic 1/19/2017**

**Report Date: March 30, 2017**

## METHODS

### SAMPLES

Two storm water samples were collected by time-paced composite at Echo Lake Rain Garden #1 Inlet and Outlet sites on January 17, 2017. Approximately 2.5-3 L of each sample was split from a larger container and delivered to the King County Environmental Laboratory (KCEL) in 9-L glass jars with Teflon-lined screw-cap lids and tested as-received. The samples were stored in the dark at  $4 \pm 2^\circ\text{C}$  and used to initiate the *Daphnia pulex* acute and the *Ceriodaphnia dubia* chronic toxicity tests, as well as for *C. dubia* test renewals.

Collection information and chemical characteristics of the test samples are listed in the table below.

Site:	Echo Lake Rain Garden #1- Inlet	Echo Lake Rain Garden #1- Outlet
Station:	ECHO-BP1-IN	ECHO-BP1-OUT
KCEL Sample #:	L66938-1	L66938-3
Coll Date/ Time		
From:	1-17-17/ 1435h	1-17-17/ 1433h
to:	1-17-17/ 1527h	1-17-17/ 1530h
Rec'd Date/Time	1-18-17/ 1126h	1-18-17/ 1126h
pH*	7.28	6.93
Tot. Alk (mg/L as CaCO <sub>3</sub> )	---	---
Tot. Hard (mg/L as CaCO <sub>3</sub> )	26	31
Cond (µmhos/cm)	140	235
Turbidity (NTU)	184	49.6
Tot. Susp. Solids (mg/L)	223	23.4
Ortho-P (mg/L)	0.0121	0.203
NO <sub>2</sub> + NO <sub>3</sub> (mg/L)	0.0665	0.508
Tot N (mg/L)	1.31	1.20
Tot P (mg/L)	0.242	0.287
Tot NH <sub>3</sub> (mg/L)	0.324	0.116

\*Measured in field

### CONTROL WATER

The control water for tests with *Daphnia pulex* is fresh water obtained from a 95 ft. deep well located at the KCEL and filtered to 60 µm with Nitex screen before use. *D. pulex* are routinely maintained in static-renewal cultures of well water (WW) at  $20 \pm 1^\circ\text{C}$ . The well water is diluted by approximately a third with MilliQ SuperQ de-ionized water to bring the total hardness to usual levels.

Water used for testing and culturing with *Ceriodaphnia* is fresh water obtained monthly from Lake Washington (LWW) at a site midway between the I-90 and 520 bridges and filtered through 60 µm Nitex screen before use.

For LWW, low-hardness controls were prepared by diluting 1:10 with MilliQ water to approximate the hardness of the storm samples and receiving water.

Metals by ICP are measured monthly (last analysis: 1-2017); metals by ICP/MS or CVAA and organic compounds are measured annually (last analyses: 2-2017). Hardness, alkalinity, conductivity and pH are measured at the beginning of each test.

Physical-chemical characteristics of the WW and LWW are listed in the following table:

Parameter	WW	WW	LWW	Units
	1-18-17	(adjusted TH)	1-9-17	
Temperature	13.8	---	NA	°C, adjusted as necessary
Conductivity	328	242	98.6	µmhos/cm
pH	8.05	8.08	7.72	
Total Hardness (calc.)	131	94.6	36	mg/L as CaCO <sub>3</sub>
Total Alkalinity	72	45	100	mg/L as CaCO <sub>3</sub>
Total Cd	< 2		< 2	µg/L
Total Cr	< 3		< 3	µg/L
Total Cu	< 4		< 4	µg/L
Total Ni	< 5		< 5	µg/L
Total Pb	< 20		< 20	µg/L
Total Se	< 0.05		< 0.05	µg/L
Total Zn	< 5		< 5	µg/L
Total Mercury	< 0.05		< 0.05	µg/L
Volatile Organics	*		+	
Organic Analysis (BNA'S):	**		++	
Bis(2-Ethylhexyl)Phthalate	7.1		0.56 < 1.89 (RDL)	µg/L
Di-N-Butyl Phthalate	< 0.47		< 0.47	µg/L
Pesticides & PCB's:	***		+++	

\* 45 cmpds not detectable

\*\* 68 cmpds not detectable

\*\*\* 28 cmpds not detected

+ 45 cmpds not detectable

++ 68 cmpds not detectable

+++ 28 cmpds not detected

## ACUTE TOXICITY TEST

### *Daphnia pulex* – 48-Hour Static Acute Test #8248

The water flea *Daphnia* acute toxicity test #8248 followed the methods of US EPA (2002a). Test animals were neonates (< 24-hours old) taken from an overnight brood board; parent animals were adults isolated from in-house mass cultures. Because neonates from broods released on 1/21/17 were used in another test, the current test was started the following work day in order to have sufficient numbers of neonates. Samples were diluted with WW to the concentrations listed below, along with a WW-only control. Test chambers were 30-mL beakers containing 25 mL of test solution. Individual broods were blocked across treatments such that each replicate contained representatives of five separate broods, with four replicates per treatment. Test chambers were randomized at the start of the test. The test was incubated at 20.0 ± 1.0°C for 48 hours on a 16:8 hour light:dark cycle. Survival and water quality measurements were recorded every 24 hours. Temperature was measured daily by digital thermometer in replicate blanks at six positions of the test tray (4 outer corner + 2 center). In addition, incubator temperature was measured at 15-minute intervals using an Onset Tidbit data logger. Temperature, pH and dissolved oxygen (D.O.) values can be found on the attached photocopied pages from the laboratory notebook in the "Storm Water Tests" section of this report.

Test #	LIMS Sample #	Start Date/Time	End Date/Time	Sample Concentrations (%)	Daphnid Age	# Reps/Trtmt	# Orgs/Rep
8248	L66938-1,-3	1-24-17/ 1435h	1-26-17/ 1405h	0 (WW controls), 6.25, 12.5, 25, 50, 100	< 24 hr	4	5

**CHRONIC TOXICITY TEST**

***Ceriodaphnia dubia* - 7-Day Chronic Static Renewal Test #8245**

The water flea *Ceriodaphnia dubia* 7-day static renewal chronic toxicity test #8245 was conducted as outlined in US EPA (2002b). Samples were tested as received at one undiluted (100%) concentration. Ten replicates containing one animal each were tested at each treatment, including a LWW-only control and a low-hardness LWW control. Test organisms were 3<sup>rd</sup> or 4<sup>th</sup>-brood neonates (< 24 hours old) taken from an in-house individual brood board of adults started from mass culture. Individual broods were blocked across treatments, and each replicate represented a different brood. The test was incubated at 25 ± 1.0°C for 7 days on a 16:8 h light:dark cycle. All test solutions were renewed daily. Reproduction, survival, temperature and water quality measurements were recorded every 24 hours. Temperature was measured daily in six test board temperature blanks (4 outer corner + 2 center) and at 15-minute intervals using an Onset "Tidbit" data logger placed in a beaker of water in the incubator. The pH and D.O. values measured during testing can be found on the attached photocopied pages from the laboratory notebook in the "Storm Water Tests" section of this report.

Test #	LIMS Sample #	Start Date/ Time	End Date/ Time	Sample Concentrations (%)	Daphnid Age	# Reps/ Trtmt	# Orgs/ Rep
8245	L66938-1, -3	1-19-17/ 1435h	1-26-17/ 1510h	0 (LWW controls), 100%	< 24 hr	10	1

**QUALITY CONTROL**

Reference toxicant control results are summarized in the following table.

	<i>Daphnia</i>	<i>Ceriodaphnia</i>
Test #:	8249	8250
Test Date:	2-1-17	2-3-17
Control Survival (%)	100	90
Criteria	≥ 90	≥ 80
Acceptable?	Yes	Yes
Survival LC50 (g/L)	3.42	
Lab Control Limits	2.39 - 4.65	
Acceptable?	Yes	
Control Reprod (# neos/adult)		39.8
Criteria		≥ 15
Acceptable?		Yes
PMSD for Reproduction (%)*		18.1
Criteria		13 - 47
Acceptable?		Yes
Reproduction IC25 (µg/L)		1.97
Lab Control Limits		0 - 7.25
Acceptable?		Yes

\*Percent Minimum Significant Difference; determined by Dunnett's Multiple Comparison test (Steels Many-One Rank Test for unequal variance); (α = 0.05)

NaCl was used as a reference toxicant in the acute test with *Daphnia*. Temperature, pH and dissolved oxygen measurements remained within acceptable limits throughout the reference toxicant test for *Daphnia* (#8249) (US EPA 2002a). The acute positive control test met acceptability criteria regarding control survival, and the survival LC50 endpoint was within the control limits of the mean ± 2SD (US EPA, 2002a).

Cadmium nitrate was used as a reference toxicant in the chronic toxicity test with *Ceriodaphnia*. Temperature, pH and dissolved oxygen measurements remained within acceptable limits throughout the reference toxicant test for *Ceriodaphnia*



Survival was 95% in the WW-only control and 100% in all concentrations of both Inlet and Outlet samples. Survival in both the 100% Inlet and Outlet samples did not differ significantly from the WW-only control ( $p > 0.05$ ; Wilcoxon Rank Sum 2-Sample Test). The NOEC for survival was 100% for both samples ( $p > 0.05$ ; Steels Many-One Rank Sum Test). An LC50 could not be calculated for either the Inlet or Outlet sample due to insufficient mortality.

The maximum un-ionized ammonia levels in the 100% storm samples during the 48-hour test are listed in the table below.

Station:	ECHO-BP1-IN	ECHO-BP1-OUT
KCEL Sample #:	L66938-1	L66938-3
NH <sub>3</sub> -N (mg/L)	0.006	0.002

***Ceriodaphnia dubia* - 7-Day Chronic Static Renewal Test #8245**

Reproduction and survival results over the 7-day chronic *Ceriodaphnia* test #8245 with 100% Echo Lake storm water samples are shown in the table below. Reproduction was calculated using the 6-day data after 60% or greater of control organisms produced 3 or more broods (US EPA 2002b; WA DOE 2016).

Sample #	Station	% Sample	Reproduction (#Neonates/Adult in 6 Days)										Mean Reprod	Mean 7d % Surv
			1	2	3	4	5	6	7	8	9	10		
---	LWW Control (Low-Hardness)	0	30	30	31	30	30	33	33	34	29	30	31.0	100
---	LWW (unadjusted)	0	37	36	37	38	31	31	37	35	36	36	35.4	100
L66938-1	ECHO-BP1-IN	100	0	0	0	0	0	0	0	0	0	0	0	0
-3	ECHO-BP1-OUT	100	39	14*	40	42	43	33	40	37	45	46	37.9*	90**

\*Significantly greater than in Inlet sample ( $p < 0.05$ ; Wilcoxon Rank Sum 2-Sample Test)

\*\*Significantly greater than in Inlet sample ( $p < 0.05$ ; Fisher Exact Test)

\*Mortality on Day 6

As shown in the table above, survival was 90% in the Outlet sample and 100% in both the hardness-adjusted and non-adjusted LWW-only controls. Survival was 0% in the Inlet sample.

Reproduction in the Outlet sample was significantly greater than in the Inlet sample ( $p < 0.05$ ; Wilcoxon Rank Sum 2-Sample Test).

The maximum un-ionized ammonia levels reached in the 100% storm samples during the 7-day chronic test are listed in the table below.

Station:	ECHO-BP1-IN	ECHO-BP1-OUT
KCEL Sample #:	L66938-1	L66938-3
NH <sub>3</sub> -N (mg/L)	0.005	0.003

**QUALITY CONTROL**

Storm water sample and control performance results are summarized in the following table:

Test Organism:	<i>Ceriodaphnia</i>	<i>Daphnia</i>
Test #:	8245	8248
Control Survival (%)	100	95
Criteria	≥ 80	≥ 90
Acceptable?	Yes	Yes
Control Reproduction (# neos/adult)	31.0	
Criteria	≥ 15	
Acceptable?	Yes	

As shown in the table above, both the acute and chronic effluent tests met acceptability criteria regarding control performance, including survival and reproduction (US EPA, 2002a & 2002b).

Dissolved oxygen, pH, temperature and/or salinity remained within acceptable limits throughout both the acute and chronic tests (US EPA, 2002a & 2002b). Water quality data recorded during testing is shown on the photocopied pages from the laboratory notebook in the "Effluent Tests" section of this report.

**Tested By:**

King County Department of Natural Resources & Parks  
Water and Land Resources Division  
Environmental Laboratory Section  
322 West Ewing Street  
Seattle WA 98119  
(206) 477-7123

Julie Alaimo, Gary Yoshida, Robin Revelle, Gabriela Hannach, Elizabeth Frame, Lyndsey Swanson, Fran Sweeney

**REFERENCES**

- APHA. 1992.** Standard Methods for the Examination of Water and Wastewater, 18<sup>th</sup> Edition. American Public Health Association, American Waterworks Association, Water Pollution Control Association, Washington D.C.
- US EPA. 2002a.** Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5<sup>th</sup> edition. EPA-821-02-012, October, 2002. US Environmental Protection Agency, Office of Water (4303T), Washington, DC.
- US EPA. 2002b.** Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 4<sup>th</sup> Edition (EPA-821-R-02-013).
- US EPA. 1991.** Code of Federal Regulations, 40CFR, Appendix A, July 1991. U.S. Environmental Protection Agency, Office of Federal Registry, Washington, D.C.
- WA DOE. 2016.** Whole Effluent Toxicity Testing Guidance and Test Review Criteria. DOE Pub. #WQ-R-95-80, revised June 2016. Washington State Department of Ecology, Water Quality Program, Olympia, WA.