King County

Water and Land Resources Division
Environmental Laboratory
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TTY Relay: 711

March 31, 2017

Phuong Truong
West Point Treatment Plant
Fort Lawton, Discovery Park
1400 Utah Street
Seattle, WA 98199

Dear Phuong:

Enclosed please find our report on NPDES characterization tests conducted on 3-21-17 with effluent collected at the West Point Treatment Plant.

Detailed findings are in the "Results" section of this report. The following table shows a summary of the results:

Acute Toxicity Tests

Test Organism	LC50 % Effluent	NOEC % Effluent	Percent Survival in 100% Effluent
Fathead Minnow (*Pimephales promelas*)	not applicable	100	93
Water Flea (*Daphnia pulex*)	not applicable	100	100

If you would like additional information, please call Francis Sweeney at 477-7117.

Sincerely,

Gary Yoshida
King County Environmental Laboratory

Enclosures
cc: Jeff Lafer, MS: KSC-NR-0505
Fritz Grothkopp: LAB-NR-0100

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM
BIOLOGICAL MONITORING REPORT FOR THE
WEST POINT TREATMENT PLANT
FORT LAWTON, DISCOVERY PARK
1400 UTAH STREET WEST
SEATTLE, WASHINGTON 98199

FIRST QUARTER, 2017

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

Test Numbers: 8266, 8267
Test Date: March 21, 2017

Report Date: March 31, 2017

METHODS

SAMPLE

One sample (#L67347) of unchlorinated effluent was collected from the West Point Treatment Plant by compositing on ice on a time-paced basis. The sample was delivered to the King County Environmental Laboratory (KCEL) in a 5-gal glass carboy containing 16.5 liters and tested as received. The acute tests with *Daphnia* and with fathead minnows were both initiated within 3.5 hours of the termination of sampling.

The sample was refrigerated in the dark at 4°C between renewals.

	Day 0
Day / Time of Collection	3-20-17 / 1028h to 3-21-17 / 1010h
Ave Flow, mgd	118 mgd
Total Residual Chlorine, mg/L	< 0.01*

* Field measurement with kit

Physical-chemical characteristics of the effluent samples measured upon arrival at KCEL (unless otherwise noted) are listed in the table below:

Parameter	Day 0	Units
pH*	6.98	
pH	7.31	
Temperature	1.5	°C
Dissolved Oxygen	9.0	mg/L
Total Alkalinity	103	mg/L as $CaCO_3$
Total Hardness	95	mg/L as $CaCO_3$
Total NH_4-N	10.8	mg/L
Conductivity	539	µmhos/cm

* Measured at the West Point Treatment Plant before sample transport
NM: not measured

Additional water quality data are listed on the photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report.

CONTROL WATER

The control and dilution water for acute tests was freshwater obtained from a 95 ft. deep well located at the KCEL. The well water (WW) was filtered to 0.45 µm and 60 µm before use with fathead minnows and *Daphnia*, respectively. The WW used in testing was diluted approximately 25% to achieve a hardness of approximately 100 mg/L as $CaCO_3$ (Moderately Hard). *D. pulex* are routinely maintained in static-renewal cultures of well water (WW) at 20 ± 1°C.

Metals are measured monthly (last analysis: 3-17), except for mercury, silver, and organic compounds, which are measured annually (last analyses: 2-17). Hardness, alkalinity, conductivity and pH are measured at the beginning of each test.

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

Parameter	Value	Units
Conductivity	330	µmhos/cm
pH	8.00	
Total Hardness (calc.)	104	mg/L as $CaCO_3$
Total Alkalinity	75	mg/L as $CaCO_3$
Total Ag	< 0.05	µg/L
Total Cd	< 2	µg/L
Total Cr	< 3	µg/L
Total Cu	< 4	µg/L
Total Hg	< 0.05	µg/L
Total Ni	< 5	µg/L
Total Pb	< 20	µg/L
Total Zn	< 5	µg/L
Total Sulfide	< 0.05	mg/L
Volatile Organics	45 cmpds not detected	
Organic Analysis (BNA'S): Bis(2-Ethylhexyl)Phthalate	68 cmpds not detectable 0.82	µg/L
Pesticides & PCB's:	28 cmpds not detected	

ACUTE TOXICITY TESTS

Fathead Minnow - *Pimephales promelas*

The fathead minnow acute toxicity test (#8266) followed the methods of USEPA (2002). Larvae were shipped from Aquatic Biosystems, Fort Collins, Colorado via UPS (overnight) and held in the laboratory for 4 days before testing. Upon receipt the temperature was 21.3°C. Larvae were held in two 1.5-L Pyrex crystallizing dishes containing WW (renewed daily), maintained at 25 ± 1°C in a waterbath and were fed 3-4 mL live *Artemia* nauplii two to three times daily. The larvae were 11 days old at test initiation.

The sample was tested as received and diluted with WW to the concentrations listed below. Four replicates of 10 larvae each were tested at each concentration, including the WW-only control. Test chambers were 250-mL beakers containing 200 mL of test solution. The larvae were loaded directly into the test beakers using nylon screen. Assignment of fish to the test chambers was random, as was placement of the test chambers in the waterbath.

A continuous flow of CO_2-enriched air was supplied to the headspace of a clear acrylic chamber covering the test beakers for the first 48 hours of the test. However, due to low dissolved oxygen (< 4 mg/L) in the 100% effluent concentration, direct aeration of the test beakers became necessary. Aeration of the effluent sample prior to preparation of the test concentrations for the 48 hour renewal did not suffice in raising the dissolved oxygen. The D.O. level that was achieved (7.2 mg/L after 1 hour of aeration) would not sustain the D. O. at acceptable concentrations for the remainder of the test due to the biological oxygen demand of the effluent.

The test was incubated at 25 ± 1°C for 96 hours on a 16:8 hour light:dark cycle. Solutions were renewed (80%) at 48 hours. The fish were fed live *Artemia* nauplii (2 drops per test chamber) approximately 2 hours before renewal. Mortality, temperature, pH and dissolved oxygen measurements were recorded every 24 hours. Temperature was recorded daily in one replicate of each concentration and in replicates from six water bath (or environmental chamber) positions (4 corner and 2 center). In addition the test was monitored at 15-minute intervals using an Onset data logger placed in a beaker of water among the test beakers. The temperature, pH and D.O. values measured

during the test can be found on the photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report.

Test #	Test Start	Test End	% Effluent Concentrations	Larvae Age	# Reps/ Treatment	# Larvae/Rep
8266	3-21-17/1330h	3-25-17/1340h	0, 3.6*, 12.5, 25, 50, 100	11 days	4	10

* ACEC (Acute Critical Effluent Concentration)

Water Flea - *Daphnia pulex*

The *Daphnia* acute toxicity test (#8267) followed the methods of USEPA (2002). Test animals were neonates (<24 h old) taken from an overnight brood board; parent animals were adults isolated from mass cultures. Test chambers were 30-mL glass beakers containing 25 mL of test solution. Individual broods were blocked across treatments, and each replicate contained representatives of five separate broods. 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. Mortality and water quality measurements were recorded every 24 hours. The pH and dissolved oxygen values measured during testing can be found on the photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report.

Test #	Test Start	Test End	% Effluent Concentrations	Larvae Age	# Reps/ Treatment	# Organisms/Rep
8267	3-21-17/1320h	3-23-17/1300h	0, 3.6*, 12.5, 25, 50, 100	< 24 h	4	5

* ACEC

QUALITY CONTROL (Reference Toxicant Tests)

NaCl was used as a reference toxicant in acute tests with fathead minnows and *Daphnia*. 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. Temperature, pH and dissolved oxygen measurements remained within acceptable limits throughout the reference toxicant tests for *Daphnia* (#8269) and fathead minnow (#8268). All tests met acceptability criteria regarding control survival and control limits (USEPA, 2002).

Reference toxicant control and test performance results are summarized in the following table.

Test #:	8268	8269
	Fathead Minnow	*Daphnia*
Control Survival	100	100
Criteria	≥ 90	≥ 90
Acceptable?	**Yes**	**Yes**
Survival LC50	8.14	3.99
Lab Control Limits	7.34 – 9.17	2.34 – 4.67
Acceptable?	**Yes**	**Yes**

WATER QUALITY TESTS

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

Parameter	Method and Method Number
Water Quality Tests	APHA (1992); USEPA (1991).
Temperature	Digital Reference Thermometer (traceable to NBS records) and Onset, Tidbit (v2), UTBI-001 Temperature Logger (KCEL #436)
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 319)
Total Hardness	By calculation (Method #2340 B; KCEL #612)
Conductivity	Orion Model #122 Meter with 012210 conductivity cell (Method 2510B; KCEL #435)
Total Ammonia	Phenate Method (Standard Methods SM 4500 - NH_3-G; KCEL #330)
Unionized Ammonia	Calculated from total ammonia, pH and ionization constants (APHA Method #417 G)
Pesticides and PCBs	Continuous liquid extraction method (EPA Method #608; KCEL #733)
Organic Analysis	Continuous liquid extraction method for BNA's (EPA Method #625; KCEL #731)
Volatile Organic Analysis	Purge and trap method (EPA Method #624; KCEL #732)
Total Metals	ICP-MS for Cd Ref. Tox. (EPA Method #200.8; KCEL #624); ICP for Cd, Cr, Cu, Ni, Pb and Zn (EPA Method #200.7; KCEL #612v4); for Hg analysis (KCEL #604, 601, 605)

RESULTS

Acute Test - Fathead Minnow

The following table shows fathead minnow survival at the end of the 96-hour acute toxicity test (Test #8266):

Sample %	Percent Survival				Replicate Mean	Number of Fish Tested
	% survival in each rep. (n=10 fish/rep**)					
	Rep 1	Rep 2	Rep 3	Rep 4		
0	100	100	100	100	**100**	40
3.6*	100	100	100	100	**100**	40
12.5	100	100	100	100	**100**	40
25	100	100	100	80	**95**	40
50	100	100	90	90	**95**	40
100	100	80	100	91 (10/11)	**93**	41

* ACEC **11 fish in rep 4 100% effluent

Survival was 100% in the control, 3.6% (ACEC) and 12.5% effluent. Survival was 95% in 25% and 50% effluent and 93% in 100% effluent. The No Observed Effect Concentration (NOEC) was 100% effluent. An LC50 could not be calculated due to insufficient mortality. The unionized ammonia level in the 100% effluent reached a maximum of 0.623 mg N/L during the 96 h test.

Acute Test – *Daphnia pulex*

The following table contains survival values for *Daphnia* at the end of the 48-hour acute toxicity test (Test #8267):

Sample %	Percent Survival: % survival in each rep. (n=5 *Daphnia*/rep) Rep 1	Rep 2	Rep 3	Rep 4	Replicate Mean	Number of *Daphnia* Tested
0	100	100	100	100	**100**	20
3.6*	100	100	100	100	**100**	20
12.5	100	100	100	100	**100**	20
25	100	100	100	100	**100**	20
50	100	100	100	100	**100**	20
100	100	100	100	100	**100**	20

* ACEC

Survival was 100% in the control and all effluent concentrations. The NOEC was 100% effluent. An LC50 could not be calculated due to insufficient mortality. The unionized ammonia level in 100% effluent reached a maximum of 0.193 mg N/L during the 48 h test.

Quality Control (Effluent Tests)

Dissolved oxygen, pH and temperature remained within acceptable limits (USEPA, 2002) throughout the effluent acute test for *Daphnia*. The dissolved oxygen for the fathead minnow test fell below the recommended minimum of 4 mg/L; hence, aeration of the test was initiated. Water quality data recorded during testing is shown on the photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report. The tests met acceptability criteria regarding control survival (USEPA, 2002).

Effluent test control performance results are summarized in the following table:

Acute Test	Test #		Criteria	Test		Acceptable? Y	N
Fathead Minnow	8266	Control Survival (%)	≥ 90	100		✓	
				Min	Max		
		Temp °C	25 ± 1; not vary by > 3°C	24.5	25.2	✓	
		D.O. mg/L	≥ 4.0*	3.3	8.6		✓
		pH	6.0 - 9.0	7.16	8.27	✓	

*Approx 50% Saturation

Acute Test	Test #		Criteria	Test		Acceptable? Y	N
Daphnia pulex	8267	Control Survival (%)	≥ 90	100		✓	
				Min	Max		
		Temp °C	20 ± 1; not vary by > 3°C	19.7	20.2	✓	
		D.O. mg/L	≥ 4.0*	4.1	9.9	✓	
		pH	6.0 - 9.0	7.43	8.12	✓	

*Approx 45% Saturation

Both acute effluent tests met acceptability criteria regarding control performance (US EPA, 2002)

Tested By:

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(206) 477-7117

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REFERENCES

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

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

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

Effluent Tests:

Bench Sheets, Calculations, and Statistics

Unionized Ammonia in 100% Effluent

Test #	Test type	pH max	Sample Age	T (°C)	T (°K)	pKa	Total Ammonia (mg/L)	Unionized Ammonia (mg/L)*
8266	Fathead Minnow, acute	8.038	72h	24.8	298.0	9.251	10.8	0.623
8267	*Daphnia pulex*, acute	7.660	48h	20.0	293.2	9.401	10.8	0.193

*Unionized ammonia calculation (APHA Method #417 G):

$$NH_3N = \frac{\text{Total Ammonia}}{1 + 10^{(pKa - pHmax)}}$$

where $pKa = 0.09018 + 2729.92 / T\ (^{o}K)$
and $T\ (^{o}K) = T\ (^{o}C) + 273.2$

West Point NPDES (Project #1033899/001534423) 96-Hour Acute
Fathead Minnow

Test #: 8266
Test Date: 170321

ORGANISMS
Received from ABS as 7 days old (Hatch date: 3-10-17). Shipped via UPS.
Arrived at KCEL at 1000 h on 3-17-17 in 1 double Poly. 8 dead removed.

At Arrival: pH 7.386; D.O. 10.7 mg/L; Temp 21.3 °C. Placed in 2 1.5L crystallizing dishes. Fed 4 mL *Artemia* nauplii/dish at 1040 h. Placed in 23 °C waterbath at 1045 h with light aeration. Replaced 60 % with WW 3-20. ***Acclimation***: WW changed daily (≈ 75%). Fed _____ mL *Artemia* nauplii/dish 3x/day. Waste and dead larvae removed daily. Held 4 days before testing. 1200 WB↑ 25°C

Date	mL per Feeding/Time (h) 1st Feed	2nd Feed	3rd Feed	Analyst
3-17-17	3 / 1040 h	4 / 1410 h	4ml / 1655 h	GY / GY / JA
3-18-17	4 / 0730 h	3 / 1325 h	4ml / 2050 h	GY / JA / JA
3-19-17	4 / 1230 h	— / — h	4ml / 1630 h	JA / — / JA
3-20-17	10dead 4 / 0900 h	4 / 1335 h	4ml / 1635 h	GY / JA / JA
	/ h	/ h	/ h	/ /
	/ h	/ h	/ h	/ /

DILUTION WATER/SAMPLE
1. Well Water (WW) 3-20, filtered to 0.45 µm. Hardness adjusted w/ MilliQ (≈ 20% dilution)
2. West Point Effluent sample

LIMS FMA Sample #: L67347-2 Wkgp #: 150990

Sample Data	Day 0		
Sample #:	L67347-2		
Collect Date:	3-20-17 to 3-21-17		
Collect Time:	1028 h to 1010 h		
Collected by:	MP, DR		
Auto Sample Set:	225 mL 20 min		
Est. Flow/ # Samples	1.8 mgd / 72		
Delv'd to KCEL	1040 h on 3-21-17		
By:	MP, DR		
Rec'd by:	JA		
Vol. (L):	16.5 L		
Containers:	1 x glass carboy		
At Plant: pH, Temp, TRC	6.98	1.7 °C	<0.01 mg/L
At KCEL: pH, Temp., D.O.	7.308	1.5 °C	9.0 mg/L
Storage:	In dark at 4 ± 2°C		

Turbid
Fine Solids on bottom

Treatment Plant under going extensive repair work from damage to plant from flood caused by heavy rain & equipment failure.

West Point NPDES (Project #1033899/001534423) 96-Hour Acute Fathead Minnow

Test #: 8266
Test Date: 170321

DILUTIONS			
Code	**% Sample**	**mL Sample**	**Decant (mL)**
White	100	2000	1000
Red	50	⊆ 2000 w/WW	1000
Orange	25	⊆ 2000 w/WW	1000
Yellow	12.5	⊆ 2000 w/WW	1000
Green	3.6 (ACEC)	Draw down to 576 mL, then ⊆ 2000 w/WW	1000
Blue	0	0 (WW only)	(WW only)

36ml/1L

PROCEDURE

1. Prepare dilutions as above in a 2-L graduated cylinder; decant to 1-L flasks.
2. Add 200 mL each treatment to each of 5 (five) 250-mL beakers (reps A → D for fish, plus 1 additional beaker per treatment for WQ).
3. Place beakers randomly in water bath (Bath # B); bring solutions to 25°C. **Setup** at ___ h.
4. Add 10 fish/beaker directly into solutions using nylon screen; rinse with DW between beakers.
5. Place acrylic cover over beakers. Add air and 5% CO_2 mixture continuously to headspace. Adjust rate to maintain pH during test. Record rates in table.
6. **Start** test at 1330 h on 3-21-17. Start counts verified by GY & JA. Sample for 0h water quality: ✓ Acidify: ✓ Analyst: JA. Place HOBO/Tidbit temp recorder (SN 10680548) in beaker w/DW into water bath B Transfer to EC 1230 3-23 SN 20067700
7. Record # survivors and remove dead larvae daily. Measure temperature daily in 1 rep/treatment and in 6 positions (4 corner + 2 center beakers). Measure pH and D.O. in WQ rep daily for each treatment; return to water bath.
8. Renew solutions at 48h (80% renewal):
   a. Feed larvae 2-3 drops *Artemia* nauplii/beaker at 0640 h on 3-23-17 ≥ 2 hours before renewal. Analyst: GY
   b. Prep solutions as indicated above; bring to 25°C.
   c. Before renewal, measure temperature in 1 rep/treatment and in 6 positions (4 corner + 2 center beakers).
   d. Remove approx. 160 mL of old solution plus waste and dead larvae from each beaker by decant and/or bulb + pipet.
   e. Replace ⊆ 200 mL with new solution by pouring down side of beaker.
   f. Count larvae before and after renewal.
   g. Sample for 48-hour WQ: ✓ Acidify: ✓ Analyst: GP
9. **End** test at 1340 h on 3-25-17 by GY.
10. Record temp and survival. Measure pH and DO. Sample for 96-hour WQ: ✓ Analyst: GY.

Rate Air/CO_2 Headspace		
Day	**Air (L/min)**	**5% CO_2 (L/min)**
0h	2.3	0.125
24h	3.4	0.2
48h		
72h		
96h		

Increased air

West Point NPDES (Project #1033899/001534423) 96-Hour Acute
Fathead Minnow

Test #: 8266
Test Date: 170321

MEASUREMENTS

Code	% Smpl	Cumulative Survival (# Alive/Rep) 24h A	24h B	24h C	24h D	48h A	48h B	48h C	48h D	72h A	72h B	72h C	72h D	96h A	96h B	96h C	96h D	Tot # Alive	% Surv**
BL	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	40	100
GN	3.6*	10	10	10	10	10	10	10 IS	10	10	10	10 IS	10	10	10	10 IS	10	40	100
Y	12.5	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	40	100
OR	25	10	10	10	9	10	10	10	9	10	10	10	9 IS	10	10	10	8	38	95
R	50	10	10	10	10	10	10	10	10	10	10 IS	10 IS	10 INF	10	10	9	9	38	95
WH	100	10	10	10	9a	10	10	10	*10	10	10 2S	10	10	10	8	10	10 IS	38/41	95/93
Analyst:		JA				GY				GY				GY					

s = stressed; *ACEC; **Pass if control survival ≥ 90%

* possibly had 11 to start
a = Assume 11 to start, Ideed = 10 Alive

CHEMISTRY

Code	Sample %	pH 0 h	pH 24 h	pH 48 h	pH 72 h	pH 96 h	D. O. (mg/L) 0 h	D. O. (mg/L) 24 h	D. O. (mg/L) 48 h	D. O. (mg/L) 72 h	D. O. (mg/L) 96 h
Blue	0	8.013	7.276 7.289	7.298	8.159	8.255	8.5	7.3	7.2	8.0	8.4
Green	3.6	8.026	7.285	7.345	8.199	8.266	8.4	7.2	7.3	8.0	8.3
Yellow	12.5	7.914	7.235	7.291	8.112	8.214	8.5	6.4	6.7	7.9	8.2
Orange	25	7.761	7.181	7.234	8.042	8.129	8.6	5.3	6.0	7.9	8.2
Red	50	7.590	7.155	7.216	7.947	7.979	8.6	4.2	4.8	7.6	7.8
White	100	7.338	7.195	7.291	8.038	7.993	8.3	3.3	3.8	7.5	7.5
Analyst:		GY	GY	GY	GY	GY	GY	GY	GY	GY	GY

3-23 (48h) The effluent for renewal: DO = 5.9 mg/L. Aerate Sample
After 40 min of aeration D.O. 6.9 mg/L (½L/min)
After 60 min of aeration D.O. 7.2 mg/L Cease aeration
3-23-17 1230h D.O. 100% 3.1 mg/L. Move Test to EC 8556 (East Bench) with aeration.

West Point NPDES (Project #1033899/001534423) 96-Hour Acute
Fathead Minnow

Test #: 8266
Test Date: 170321

Code	Sample %	Temperature (°C) (SN 160516630)				
		0 h	24 h	48 h	72 h	96 h
Blue	0	25.1	25.2	25.0	24.7	25.0
Green	3.6	25.1	25.1	25.0	24.8	25.0
Yellow	12.5	25.1	25.1	25.0	24.5	24.9
Orange	25	25.1	25.1	24.9	24.8	24.9
Red	50	25.1	25.0	25.0	24.9	25.0
White	100	25.1	GY 25.0 25.1	25.0	24.8	24.8
	Analyst:	JA	GY	GY	GY	GY

Temperature (°C) (4 Corner + 2 Center Positions)						
Code	**Rep**	**0h**	**24h**	**48h**	**72h**	**96h**
Orange	B	25.1	25.1	25.1	Blue D 25.0	25.0
Red	WQ	25.1	25.2	24.9	Blue B 24.7	24.9
Blue	D	25.1	25.2	25.0	Green C 24.8	25.1
White	B	25.1	25.0	24.9	orange C 25.0	25.0
Green	D	25.1	25.2	25.0	Red C 24.8	24.9
Red	A	25.1	25.1	25.0	White C 24.7	24.8

EC

Sample		Sample #	Tot Alkalinity (mg/L as $CaCO_3$)	Tot Hardness (mg/L as $CaCO_3$)	Conductivity (µmhos/cm)	T. NH_4^+ (mg/L)
0% WW	0 h	67347-5	74.7	104	278	
	48 h				258	
	96 h				268	
100% EFF	0 h	67347-4	103	94.6	539	10.8
	48 h				530	
	96 h				535	
				Analyst:	JA	

Survival

	A	B	C	D
DL	10	10	10	10
GM	10	10	10	10
Y	10	10	10	10
O	10	10	10	9
R	10	10	10	10
W	10	10	10	10

72 Hour QC count.
FS
3/24/17

West Point NPDES (Project #1033899/001534423) 96-Hour Acute
Fathead Minnow

Test #: 8266
Test Date: 170321

Random # Beaker Position

Code	Rep	Random #		Code	Rep	Random #	
Blue	A	18	13	Orange	A	23	8
	B	16	11		B	1	28
	C	29	4		C	3	30
	D	26	1		D	9	26
	WQ	17	12		WQ	27	2
Green	A	10	27	Red	A	19	14
	B	14	20		B	28	3
	C	7	24		C	13	19
	D	12	18		D	6	22
	WQ	2	29		WQ	5	15
Yellow	A	21	6	White	A	8	25
	B	20	16		B	30	5
	C	15	21		C	22	7
	D	25	10		D	11	17
	WQ	4	23 ↑ EC		WQ	24	9 ↑ EC

Treatment/Rep (Facing Bath)

(Left Rear)			(Right Rear)
	(Center)	(Center)	
(Left Front)			(Right Front)

NOTES:

Glassware rinsed w/hot tap & DW before use.

CETIS Analytical Report

Fathead Minnow 96-h Acute Survival Test — **King County Metro Services, WQ Lab**

Analysis ID:	10-6033-3489	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	29 Mar-17 9:08	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	16-2058-1002	**Test Type:**	Survival (96h)	**Analyst:**	GY
Start Date:	21 Mar-17 13:30	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Well Water
Ending Date:	25 Mar-17 13:40	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Duration:	4d 0h	**Source:**	Aquatic Biosystems, CO	**Age:**	11d
Sample ID:	12-3051-5756	**Code:**	L67347-2	**Client:**	West Point Treatment Plant, King Co
Sample Date:	21 Mar-17 10:10	**Material:**	POTW Effluent	**Project:**	WET Quarterly Compliance Test (1Q)
Receive Date:	21 Mar-17 10:40	**Source:**	West Point Permit WA002918-1 (WA00291		
Sample Age:	3h (1.5 °C)	**Station:**			

Sample Note: Collect: 3-20-17 to 3-21-17 (1028h to 1010h); Rec'c KCEL on 3-21-17 (1040h). At arrival: Temp = 1.5C, pH = 7.31, D.O. = 9.0 mg/L,

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	9.84%	100	>100	NA	1

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		3.6	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		12.5	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		25	16	10	1	6	0.6105	Asymp	Non-Significant Effect
		50	14	10	1	6	0.3451	Asymp	Non-Significant Effect
		100	14	10	1	6	0.3451	Asymp	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.05206783	0.01041357	5	1.173	0.3605	Non-Significant Effect
Error	0.1598085	0.008878252	18			
Total	0.2118764		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	2.123	4.248	0.1094	Equal Variances
Variances	Levene Equality of Variance	8.017	4.248	0.0004	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8375	0.884	0.0013	Non-normal Distribution

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	1	0	0.0%	0.0%
3.6		4	1	1	1	1	1	1	0	0.0%	0.0%
12.5		4	1	1	1	1	1	1	0	0.0%	0.0%
25		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	5.0%
50		4	0.95	0.8581	1	0.95	0.9	1	0.02887	6.08%	5.0%
100		4	0.9273	0.776	1	0.9545	0.8	1	0.04753	10.25%	7.27%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
3.6		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
12.5		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
25		4	1.336	1.093	1.578	1.412	1.107	1.412	0.07622	11.41%	5.4%
50		4	1.331	1.181	1.48	1.331	1.249	1.412	0.04705	7.07%	5.77%
100		4	1.299	1.067	1.531	1.338	1.107	1.412	0.07277	11.2%	8.01%

Fathead Minnow 96-h Acute Survival Test — King County Metro Services, WQ Lab

Analysis ID: 10-6033-3489 Endpoint: 96h Survival Rate CETIS Version: CETISv1.8.7
Analyzed: 29 Mar-17 9:08 Analysis: Nonparametric-Control vs Treatments Official Results: Yes

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
3.6		1	1	1	1
12.5		1	1	1	1
25		1	1	1	0.8
50		1	1	0.9	0.9
100		1	0.8	1	0.9091

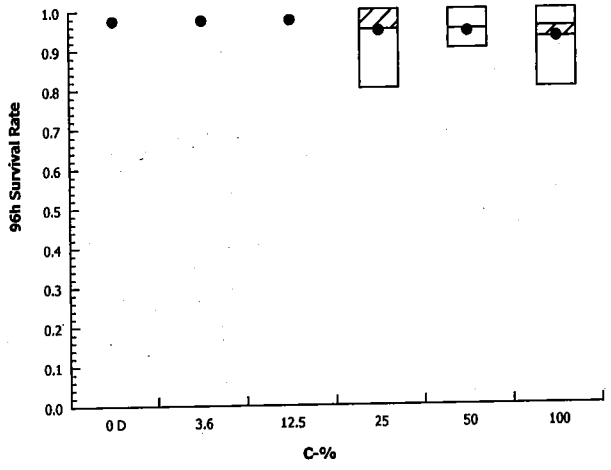
Angular (Corrected) Transformed Detail

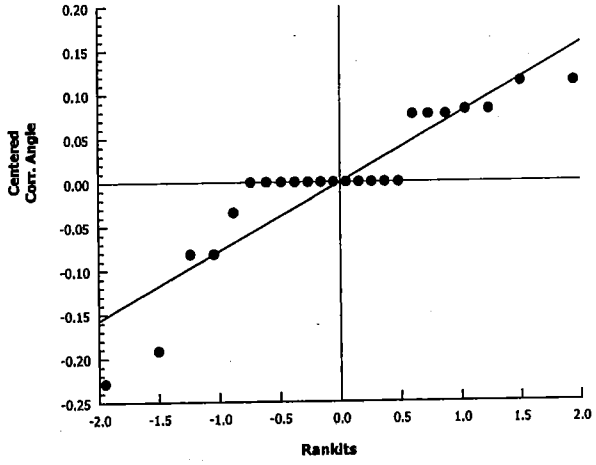
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.412
3.6		1.412	1.412	1.412	1.412
12.5		1.412	1.412	1.412	1.412
25		1.412	1.412	1.412	1.107
50		1.412	1.412	1.249	1.249
100		1.412	1.107	1.412	1.265

96h Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
3.6		10/10	10/10	10/10	10/10
12.5		10/10	10/10	10/10	10/10
25		10/10	10/10	10/10	8/10
50		10/10	10/10	9/10	9/10
100		10/10	8/10	10/10	10/11

Graphics





CETIS Summary Report

Fathead Minnow 96-h Acute Survival Test — **King County Metro Services, WQ Lab**

Batch ID:	16-2058-1002	**Test Type:**	Survival (96h)	**Analyst:**	GY
Start Date:	21 Mar-17 13:30	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Well Water
Ending Date:	25 Mar-17 13:40	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Duration:	4d 0h	**Source:**	Aquatic Biosystems, CO	**Age:**	11d
Sample ID:	12-3051-5756	**Code:**	L67347-2	**Client:**	West Point Treatment Plant, King Co
Sample Date:	21 Mar-17 10:10	**Material:**	POTW Effluent	**Project:**	WET Quarterly Compliance Test (1Q)
Receive Date:	21 Mar-17 10:40	**Source:**	West Point Permit WA002918-1 (WA00291		
Sample Age:	3h (1.5 °C)	**Station:**			

Sample Note: Collect: 3-20-17 to 3-21-17 (1028h to 1010h); Rec'c KCEL on 3-21-17 (1040h). At arrival: Temp = 1.5C, pH = 7.31, D.O. = 9.0 mg/L,

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-6033-3489	96h Survival Rate	100	>100	NA	9.84%	1	Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
10-6033-3489	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
3.6		4	1	1	1	1	1	0	0	0.0%	0.0%
12.5		4	1	1	1	1	1	0	0	0.0%	0.0%
25		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	5.0%
50		4	0.95	0.8581	1	0.9	1	0.02887	0.05774	6.08%	5.0%
100		4	0.9273	0.776	1	0.8	1	0.04753	0.09506	10.25%	7.27%

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
3.6		1	1	1	1
12.5		1	1	1	1
25		1	1	1	0.8
50		1	1	0.9	0.9
100		1	0.8	1	0.9091

96h Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
3.6		10/10	10/10	10/10	10/10
12.5		10/10	10/10	10/10	10/10
25		10/10	10/10	10/10	8/10
50		10/10	10/10	9/10	9/10
100		10/10	8/10	10/10	10/11

King County Environmental Laboratory
Lab Review Report

Reported: 29-Mar-17 10:00 ~ Data Source: ELD

Listtype / Method: AQFATHEAD-ACUTE / EPA821-R-02-012
Run ID / Workgroup: R217484 / WG150990

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
2017/03/21 00:00:00		421187C	LC	SESA02	L67347-2	Survival in 100% Sample	93	%				
2017/03/21 00:00:00		421187C	LC	SESA02		Survival LOEC		%sample	TA			Indeterminate (>highest concentration tested)
2017/03/21 00:00:00		421187C	LC	SESA02		Survival NOEC	100	%sample				
2017/03/21 00:00:00		421187C	LC	SESA02		Test Number						8266
2017/03/21 00:00:00		421187C	LC	SESA02		Date Analyzed		none				21-MAR-17 13:30
2017/03/21 00:00:00		421187C	LC	SESA02		Prep Date		none				21-MAR-17 13:30

No products missing

West Point NPDES (Project #421187), 48-Hour Acute Test
Daphnia pulex

Test # 8267
Test Date: ~~210317~~ 170321

BROODS
Started at 1110 h on 3-20-17 with 35 adults from cultures # D1 → D5. Each into 25 mL WW 3-10 60 µm filt + 0.1 mL Diet #180 @ 3.0 g/L solids (3.7 mL diet + 6.3 mL NWW) + 100 µL *Selenastrum capricornutum* @ 36 x 10^6 cells/mL. Into Incubator # 256828, Shelf 1.

Broods Checked: @ 1645h, 3 broods released (Blue);
3-21-17 1020, 23 broods (Black)

Brood Sizes:

A: 30, 26, 30, 30, 27	C: 26, 19, 10, 29, 28
B: 30, 30, 28, 26, 23	D: 25, 28, 28, >30, 21

Broods sharing superscript letters are split from same brood

DILUTION WATER/ SAMPLE
1. "New" Well Water (NWW) 3-10 filtered to 60 µm. & adjusted ≈ 25‰ with Milli Q
2. West Point Final Effluent: See Test # 8266 Day 0 for sample information.

3. DPA LIMS Sample # L673477-3 **LIMS Wkgp #: WG** 150991

DILUTIONS

Code	% Sample	mL Sample	Decant
White	100	500	250
Red	50	⊆ 500 w/NWW	250
Orange	25	⊆ 500 w/NWW	250
Yellow	12.5	⊆ 500 w/NWW	250
Green	3.6 (ACEC)	Draw down to 144 mL, then ⊆ 500 w/NWW	250
Blue	0	0	NWW only

PROCEDURE
1) Prep solutions as above in 500-mL graduated cylinder; decant to 400-mL beakers.
2) Add 25 mL each trtmt to each of (5) 30-mL glass beakers (Reps A – D for daphnids; WQ for chemistry). Bring to 20°C. ~~**Setup at** ———— h.~~
3) Select 20 broods; add 5 neonates/cup, 1 brood across all trtmts; 5 different broods/cup; randomize cup position according to random number template.
4) **Start** test at 1320 h on 3/21/17. Start counts verified by RR & EF. Into Incubator #256828, Shelf 1. Place HOBO/Tidbit temp recorder (SN 10168448) in beaker w/DW into incubator. Take 0h WQ/Cond samples: ✓ Acidify: ✓ Analyst.: JA
5) Measure pH & D.O. at 0h, 24h & 48h in reps E and F for all trtmts. Record survival at 24h & 48h. Measure temp at 24h & 48h in 6 positions (4 corner + 2 center cups) with digital thermometer (SN 160516630).
6) **End** test at 1300 h on 3/23/17 by FS/JA. Sample for Cond: JA ✓

West Point NPDES (Project #421187), 48-Hour Acute Test
Daphnia pulex

Test # 8267
Test Date: 210317
(170321)

MEASUREMENTS

Code	% Sample	Cumulative Survival (# Alive/Rep) 24h A	24h B	24h C	24h D	48h A	48h B	48h C	48h D	Tot # Alive	% Surv*
Blue	0	5	5	5	5	5/5	5/5	5/5	5/5	20	100
Green	3.6 (ACEC)	5	5	5	5	5/5	5/5	5/5	5/5	20	100
Yellow	12.5	5	5	5	5	5/5	5/5	5/5	5/5	20	100
Orange	25	5	5	5	5	5/5	5/5	5/5	5/5	20	100
Red	50	5	5	5	5	5/5	5/5	5/5	5/5	20	100
White	100	5	5	5	5	5/5	5/5	5/5	5/5	20	100
Analyst:		RR				FS/JA					

a = floating on surface; b = stuck on side; s = stressed
*Pass if control survival ≥ 90%

Chemistry

Code	% Sample	pH 0 h	pH 24 h	pH 48 h	D. O. (mg/L) 0 h	D. O. (mg/L) 24 h	D. O. (mg/L) 48 h
Blue	0	7.968	8.081	8.001	9.9	8.5	8.2
Green	3.6 (ACEC)	8.035	8.118	8.113	9.5	8.5	7.8
Yellow	12.5	7.977	7.936	7.930	9.1	8.0	7.7
Orange	25	7.829	7.730	7.729	8.9	7.0	7.4
Red	50	7.657	7.569	7.728	8.4	5.6	5.8
White	100	7.428	7.543	7.660	7.1	4.1	5.4
Analyst:		RR	RR	FS	RR	RR	FS

Sample	Sample #	Tot. Alkalinity (mg/L as $CaCO_3$)	Tot. Hardness (mg/L as $CaCO_3$)	T. NH_4^+ (mg/L)	Conductivity (µmhos/cm) 0 h	Conductivity (µmhos/cm) 48 h
WW	67347-5	74.7	104		278	257
EFF (100%)	67347-4	103	94.6	10.8	539	518
				Analyst:	JA	JA

West Point NPDES (Project #421187), 48-Hour Acute Test
Daphnia pulex

Test # 8267
Test Date: 210317
(170321)

Position (Code/Rep)	Temperature (°C) SN: 16051663O (digital) 24h	48h
A	20.2	19.9
B	20.2	20.0
C	20.1	20.0
D	20.1	19.9
E	19.8	19.8
F	19.7	20.0
Analyst:	RR	FS

Random # Beaker Position

Code	Rep	Random #	Code	Rep	Random #
Blue	A	3	Orange	A	11
	B	28		B	21
0	C	18	25%	C	29
	D	20		D	19
	WQ	22		WQ	1
Green	A	9	Red	A	2
ACEC	B	8		B	12
3.6%	C	25	50%	C	14
	D	24		D	7
	WQ	27		WQ	5
Yellow	A	23	White	A	15
	B	16		B	4
12.5%	C	6	100%	C	30
	D	26		D	13
	WQ	17		WQ	10

NOTES

Glassware rinsed w/hot tap & DW before use.

CETIS Analytical Report

Daphnia pulex 48-h Acute Survival Test — **King County Metro Services, WQ Lab**

Analysis ID:	03-8082-6506	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	29 Mar-17 7:32	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	14-5878-6731	**Test Type:**	Survival (48h)	**Analyst:**	GY
Start Date:	21 Mar-17 13:20	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Well Water
Ending Date:	23 Mar-17 13:00	**Species:**	Daphnia pulex	**Brine:**	Not Applicable
Duration:	48h	**Source:**	In-House Culture	**Age:**	<24h
Sample ID:	05-2966-7269	**Code:**	L67347-3	**Client:**	West Point Treatment Plant, King Co
Sample Date:	21 Mar-17 10:10	**Material:**	POTW Effluent	**Project:**	WET Quarterly Compliance Test (1Q)
Receive Date:	21 Mar-17 10:40	**Source:**	West Point Permit WA002918-1 (WA00291		
Sample Age:	3h	**Station:**			

Sample Note: Collect: 3-20-17 to 3-21-17 (1028h to 1010h); Rec'c KCEL on 3-21-17 (1040h). At arrival: Temp = 1.5C, pH = 7.31, D.O. = 9.0 mg/L,

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	100	>100	NA	1

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		3.6	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		12.5	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		25	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		50	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		100	18	10	1	6	0.8333	Asymp	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	5	65540	<0.0001	Significant Effect
Error	0	0	18			
Total	0		23			

48h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	1	0	0.0%	0.0%
3.6		4	1	1	1	1	1	1	0	0.0%	0.0%
12.5		4	1	1	1	1	1	1	0	0.0%	0.0%
25		4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	1	1	1	1	1	1	0	0.0%	0.0%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
3.6		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
12.5		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
25		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
50		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
100		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%

Daphnia pulex 48-h Acute Survival Test — King County Metro Services, WQ Lab

Analysis ID:	03-8082-6506	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	29 Mar-17 7:32	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

48h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
3.6		1	1	1	1
12.5		1	1	1	1
25		1	1	1	1
50		1	1	1	1
100		1	1	1	1

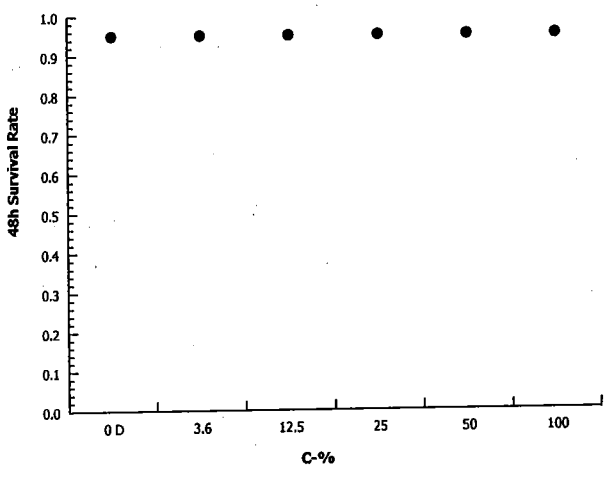
Angular (Corrected) Transformed Detail

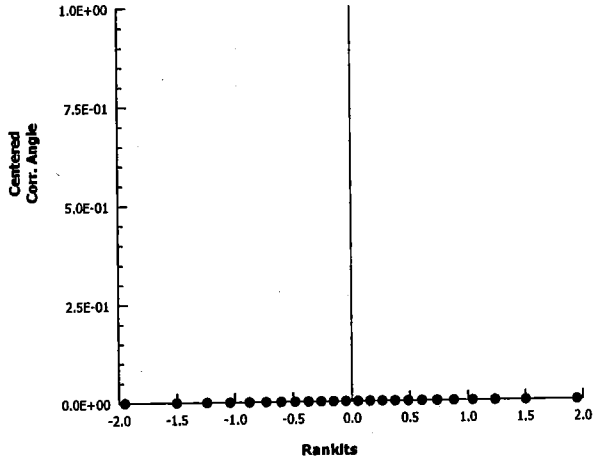
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.345	1.345	1.345	1.345
3.6		1.345	1.345	1.345	1.345
12.5		1.345	1.345	1.345	1.345
25		1.345	1.345	1.345	1.345
50		1.345	1.345	1.345	1.345
100		1.345	1.345	1.345	1.345

48h Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	5/5
3.6		5/5	5/5	5/5	5/5
12.5		5/5	5/5	5/5	5/5
25		5/5	5/5	5/5	5/5
50		5/5	5/5	5/5	5/5
100		5/5	5/5	5/5	5/5

Graphics





CETIS Summary Report

Test Code: 8267DPAWP | 21-2016-5262

Daphnia pulex 48-h Acute Survival Test — **King County Metro Services, WQ Lab**

Batch ID:	14-5878-6731	**Test Type:**	Survival (48h)	**Analyst:**	GY
Start Date:	21 Mar-17 13:20	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Well Water
Ending Date:	23 Mar-17 13:00	**Species:**	Daphnia pulex	**Brine:**	Not Applicable
Duration:	48h	**Source:**	In-House Culture	**Age:**	<24h
Sample ID:	05-2966-7269	**Code:**	L67347-3	**Client:**	West Point Treatment Plant, King Co
Sample Date:	21 Mar-17 10:10	**Material:**	POTW Effluent	**Project:**	WET Quarterly Compliance Test (1Q)
Receive Date:	21 Mar-17 10:40	**Source:**	West Point Permit WA002918-1 (WA00291		
Sample Age:	3h	**Station:**			

Sample Note: Collect: 3-20-17 to 3-21-17 (1028h to 1010h); Rec'c KCEL on 3-21-17 (1040h). At arrival: Temp = 1.5C, pH = 7.31, D.O. = 9.0 mg/L,

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-8082-6506	48h Survival Rate	100	>100	NA	NA	1	Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-8082-6506	48h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

48h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
3.6		4	1	1	1	1	1	0	0	0.0%	0.0%
12.5		4	1	1	1	1	1	0	0	0.0%	0.0%
25		4	1	1	1	1	1	0	0	0.0%	0.0%
50		4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	1	1	1	1	1	0	0	0.0%	0.0%

48h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
3.6		1	1	1	1
12.5		1	1	1	1
25		1	1	1	1
50		1	1	1	1
100		1	1	1	1

48h Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	5/5
3.6		5/5	5/5	5/5	5/5
12.5		5/5	5/5	5/5	5/5
25		5/5	5/5	5/5	5/5
50		5/5	5/5	5/5	5/5
100		5/5	5/5	5/5	5/5

King County Environmental Laboratory
Lab Review Report

Reported: 29-Mar-17 10:00 ~ Data Source: ELD

Listtype / Method: AQDAPHNIA-ACUTE / EPA821-R-02-012
Run ID / Workgroup: R217483 / WG150991

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
2017/03/21 00:00:00		421187C	LC	SESA02	L67347-3	Survival in 100% Sample	100	%				
2017/03/21 00:00:00		421187C	LC	SESA02		Survival NOEC	100	%sample				
2017/03/21 00:00:00		421187C	LC	SESA02		Survival LOEC		%sample	TA			Indeterminate (>highest concentration tested)
2017/03/21 00:00:00		421187C	LC	SESA02		Test Number						8267
2017/03/21 00:00:00		421187C	LC	SESA02		Date Analyzed		none				21-MAR-17 13:20
2017/03/21 00:00:00		421187C	LC	SESA02		Prep Date		none				21-MAR-17 13:20

No products missing

Reference Toxicant Tests:

Bench Sheets
and
Precision Tables

110

Reference Toxicant (NaCl)
Fathead Minnow 96-Hour Acute Static Renewal Test

Test#: 8268
Test Date: 170321

ORGANISMS

Purchased from ABS. Shipped via UPS. Arrived as 7 days old (hatch date: 3-10-17) to KCEL at 1000 h on 3-17-17 in 1 double poly bag. 8 dead removed. ***At Arrival***: pH 7.386; D.O. 10.7 mg/L; Temp 21.3 °C. Placed in 2 x 1.5L crystallizing dishes. Fed *Artemia* nauplii. Placed in 23 °C waterbath at 1045 h with light aeration. Replaced 60 % with WW 3-20. ***Acclimation***: WW changed daily (≈ 75%). Fed *Artemia* nauplii 3x/day. Waste and dead larvae removed daily. Held 4 days before testing.
At 1200 h incr WB temp to 25 °C
At _____ h incr WB temp to _____ °C

	mL/dish per Feeding Time			
Date	**1st Feed**	**2nd Feed**	**3rd Feed**	**Analyst**
3-17-17	3 / 1040 h	4ml / 1410 h	4ml / 1655 h	GY / GY / JA
3-18-17	4 / 0730 h	3 / 1325 h	4ml / 2050 h	GY / JA / JA
3-19-17	4 / 1230 h	— / — h	4ml / 1630 h	JA / — / JA
3-20-17	10ml 4 / 0900 h	4 / 1335 h	4ml / 1635 h	GY / JA / JA
	/ h	/ h	/ h	/ /
	/ h	/ h	/ h	/ /

DILUTION WATER/SAMPLE

1. Well Water (WW) 3-20, filtered to 0.45 µm. adjusted 25% dilution with milliQ
2. NaCl Fishers #S271-3: Rec'd 4-20-16; Opened 8-15-16; Lot# 156697
3. **LIMS FMA Sample #: WG** 150734-1 **-1 Wkgp #: WG** 150734

DILUTIONS

Code	NaCl (g/L)	NaCl (g)	WW (mL)	COND (µmhos/cm) 0h Before	0h After*	48h Before	48h After*	95% PI COND Ranges Based on Test #'s 609, 613, 633 (8/17/1993)*
Blue	0	0	1000	256	---	254	---	---
Green	4.92	3.93	800	9280	9024	9260	9006	7734 – 11009
Yellow	6.14	4.92	800	11430	11174	11420	11166	10456 – 13861
Orange	7.68	6.14	800	13960	13704	13980	13726	12232 – 18523
Red	9.60	7.68	800	17170	16914	17140	16886	16202 – 22689
White	12.0	12.0	1000	25700	25444	—	—	17905 - 27540
			Analyst:	JA		GY /	OK.	

*After subtracting control value

Reference Toxicant (NaCl)
Fathead Minnow 96-Hour Acute Static Renewal Test

Test#: 8268
Test Date: 170321

PROCEDURE

1. Weigh NaCl into 1 L flasks; dissolve in WW by stirring.
2. Measure conductivity in 40 mL sample/treatment. If outside 95% PI, prepare new dilutions and re-measure conductivity.
3. Decant 200 mL each treatment to each of three (3) 250-mL beakers (Reps A → C) per treatment plus 1 additional WQ beaker each for control ("Blue") and high concentration ("White") measurements.
4. Place beakers randomly in water bath # B; bring solutions to 25°C. **Setup** at ——— h.
5. Add 12 fish/beaker including WQ reps directly into solutions using a polyscreen; rinse screen with DW between beakers.
6. **Start** test at 1338 h on 170321. Start counts verified by JA & GY. Place Tidbit temp recorder (SN 10680548) in beaker w/DW into water bath .
7. Record number alive and remove dead larvae daily. Measure temperature daily in 1 rep/treatment and in 6 positions (4 corners + 2 center beakers). Measure pH and D.O. daily in WQ Rep for Blue and White treatments; return to water bath.
8. Renew solutions at 48 h (80% renewal):
   a. Feed larvae 2-3 drops *Artemia* nauplii/beaker at 0640 h on 3-23-17 ≥ 2 hours before renewal. Analyst: GY
   b. Prep solutions as indicated above; bring to 25°C.
   c. Before renewal, measure temperature in 1 rep/treatment and in 6 positions (4 corner + 2 center beakers).
   d. Remove approx. 160 mL of old solution from each beaker by decant or bulb and pipet; also remove waste, excess feed and dead larvae.
   e. Replace ⊆ 200 mL with new solution by pouring down side of beaker.
   f. Count larvae before and after renewal.
9. **End** test at 1400 h on 3-25-17 by GY.
   a. Record temperature and survival.
   b. Measure pH and D.O.

MEASUREMENTS

Code	NaCl	Cumulative Survival (#Alive/ Rep) 24h A	24h B	24h C	48h A	48h B	48h C	72h A	72h B	72h C	96h A	96h B	96h C	Tot # Alive	% Surv*
B	0	12 1S	12	12	12 1S	12	12	GY 12	GY 12	12	12	12	12	36	100
Grn	4.92	12	12	12	12 1S thin	12	12 1 thin	10	12	12	10	12	12	34	94
Y	6.14	12	12 1S	11	12	12	11 1S thin	12	12 1S	11	12	11	11	34	94
Or	7.68	12 1S	11 1S	9	11	10 1S	7	11 1S	9	6	11 2S	9	6	26	72
R	9.60	5	5 1S	5 2S	0	1	1	0	1	0	0	1	0	1	3
W	12.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Analyst:	JA			JA / GY			GY			GY				

s = stressed
*Pass if control survival ≥ 90%

Reference Toxicant (NaCl)
Fathead Minnow 96-Hour Acute Static Renewal Test

Test#: 8268
Test Date: 170321

CHEMISTRY

Code	pH					D. O. (mg/L)				
	0 h	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
Blue	7.985	7.901	7.805	7.955	8.070	8.7	6.9	6.7	7.4	7.8
White	7.837	7.527	—	—	—	8.3	9 6.5 4.6	—	—	—
Analyst:	GY	JA	GY	GY	GY	GY	JA	GY	~~7.3~~ GY	~~7.9~~ GY
Red	7.834	7.847	7.730	8.015	8.105	8.2	6.5	6.0	7.3	7.9

Code	NaCl (g/L)	Temperature (°C) (SN 160516630) digital				
		0 h	24 h	48 h	72 h	96 h
Blue	0	25.1	25.1	25.4	25.3	25.4
Green	4.92	25.1	25.2	25.4	25.3	25.4
Yellow	6.14	25.1	25.2	25.4	25.2	25.4
Orange	7.68	25.1	25.2	25.4	25.3	25.4
Red	9.60	25.1	25.2	25.4	25.3	25.4
White	12.0	25.1	25.1	—	—	—
	Analyst:	JA	JA	JA	GY	GY

Temperature (°C) (4 Corner + 2 Center Positions)						
Code	Rep	0h	24h	48h	72h	96h
Blue	A	25.1	25.1	25.4	25.3	25.5
Green	B	25.1	25.2	25.4	25.3	25.4
Orange	B	25.1	25.2	25.4	25.3	25.5
Green	C	25.1	25.2	25.4	25.3	25.4
Orange	C	25.1	25.1	25.3	25.2	25.5
Red	C	25.1	25.2	25.4	25.2	—
	Analyst:	JA	JA	JA	GY	GY

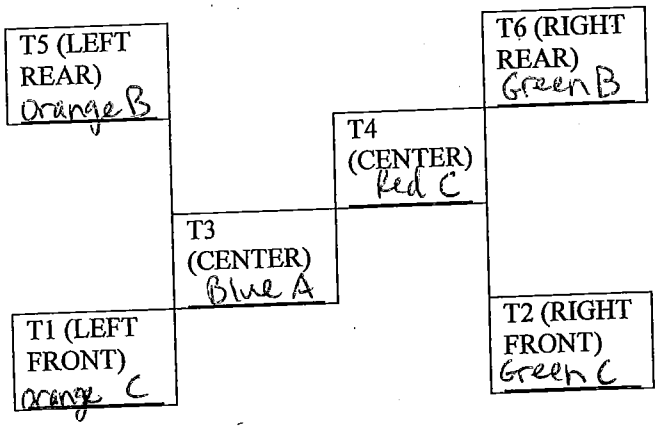
Reference Toxicant (NaCl)
Fathead Minnow 96-Hour Acute Static Renewal Test

Test#: 8268
Test Date: 170321

Random # Beaker Position

Code	Rep	Random #	Code	Rep	Random #
Blue	A	5	Orange	A	7
	B	2		B	16
	C	15		C	1
	WQ	-			
Green	A	9	Red	A	6
	B	18		B	13
	C	3		C	11
Yellow	A	10	White	A	14
	B	8		B	12
	C	17		C	4
				WQ	-

Temperature Positions (Treatment/Rep; Facing Bath)



NOTES:
Glassware rinsed with hot tap and DW before use.

WG150734 8268 FMAQC

CETIS Analytical Report

Test Code: 8268FMAQC | 20-0894-1902

Fathead Minnow 96-h Acute Survival Test

King County Metro Services, WQ Lab

Analysis ID:	01-4205-6573	Endpoint:	96h Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	27 Mar-17 13:41	Analysis:	Linear Regression (MLE)	Official Results:	Yes
Batch ID:	06-4906-7381	Test Type:	Survival (96h)	Analyst:	JA
Start Date:	21 Mar-17 13:38	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Well Water
Ending Date:	25 Mar-17 14:00	Species:	Pimephales promelas	Brine:	Not Applicable
Duration:	4d 0h	Source:	Aquatic Biosystems, CO	Age:	11d
Sample ID:	12-8952-6049	Code:	WG150734-1	Client:	Internal Lab
Sample Date:	21 Mar-17 13:00	Material:	Sodium chloride	Project:	Reference Toxicant
Receive Date:		Source:	Reference Toxicant		
Sample Age:	38m	Station:			

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
15	-42.94	93.59	94.55	0.9107	0.03784	0.8866	1.447	3.49	0.2779	Non-Significant Lack of Fit

Point Estimates

Level	gm/L	95% LCL	95% UCL
EC50	8.141	7.828	8.474

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.03669	0.0182	0.001024	0.07237	2.016	0.0620	Non-Significant Parameter
Slope	26.42	5.108	16.41	36.43	5.173	0.0001	Significant Parameter
Intercept	-24.06	4.647	-33.17	-14.96	-5.178	0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	154.4785	154.4785	1	134.9	<0.0001	Significant
Lack of Fit	4.564223	1.521408	3	1.447	0.2779	Non-Significant
Pure Error	12.6128	1.051067	12			
Residual	17.17702	1.145135	15			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	17.18	25	0.3084	Non-Significant Heterogenity
	Likelihood Ratio GOF	17.39	25	0.2958	Non-Significant Heterogenity
Variances	Mod Levene Equality of Variance	0.7833	4.387	0.5967	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9238	0.8965	0.1511	Normal Distribution
	Anderson-Darling A2 Normality	0.6922	2.492	0.0707	Normal Distribution

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-gm/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	3	1	1	1	0	0	0.0%	0.0%	36	36
4.92		3	0.9444	0.8333	1	0.05556	0.09623	10.19%	5.56%	34	36
6.14		3	0.9444	0.9167	1	0.02778	0.04811	5.09%	5.56%	34	36
7.68		3	0.7222	0.5	0.9167	0.1211	0.2097	29.04%	27.78%	26	36
9.6		3	0.02778	0	0.08333	0.02778	0.04811	173.2%	97.22%	1	36
12		3	0	0	0	0	0		100.0%	0	36

3-27-17

Analyst: QA: GP 3-29-17

CETIS Analytical Report

Fathead Minnow 96-h Acute Survival Test — **King County Metro Services, WQ Lab**

Analysis ID: 01-4205-6573 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 27 Mar-17 13:41 **Analysis:** Linear Regression (MLE) **Official Results:** Yes

96h Survival Rate Detail

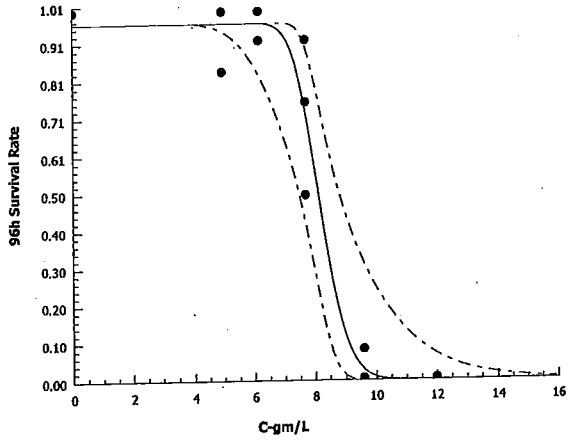
C-gm/L	Control Type	Rep 1	Rep 2	Rep 3
0	Dilution Water	1	1	1
4.92		0.8333	1	1
6.14		1	0.9167	0.9167
7.68		0.9167	0.75	0.5
9.6		0	0.08333	0
12		0	0	0

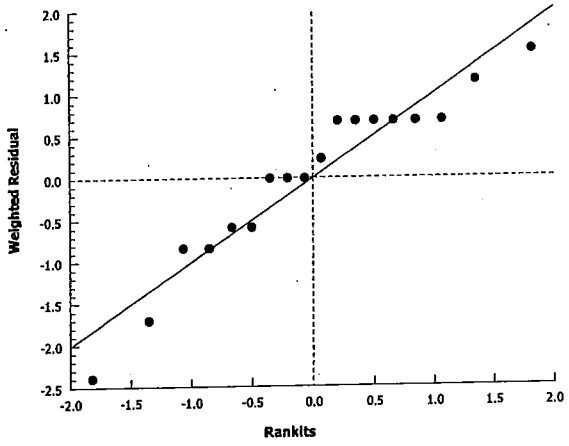
96h Survival Rate Binomials

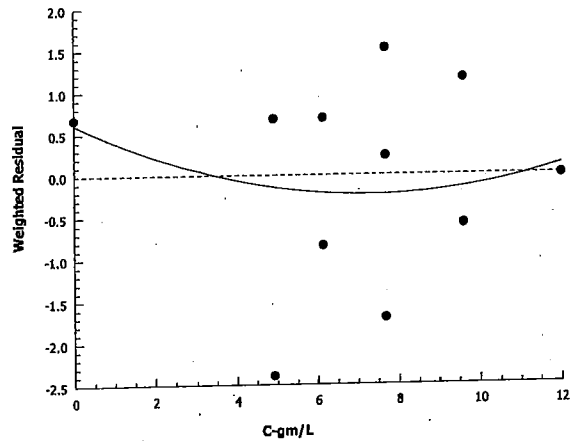
C-gm/L	Control Type	Rep 1	Rep 2	Rep 3
0	Dilution Water	12/12	12/12	12/12
4.92		10/12	12/12	12/12
6.14		12/12	11/12	11/12
7.68		11/12	9/12	6/12
9.6		0/12	1/12	0/12
12		0/12	0/12	0/12

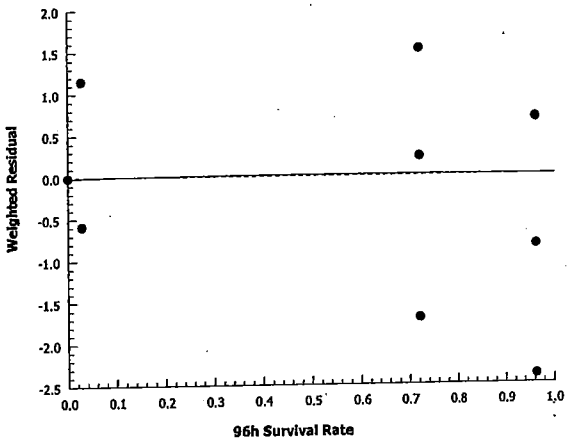
Graphics

Log-Normal [NED=A+B*log(X)]









8268

CETIS Summary Report

Report Date: 27 Mar-17 13:41 (p 1 of 1)
Test Code: 8268FMAQC | 20-0894-1902

Fathead Minnow 96-h Acute Survival Test — **King County Metro Services, WQ Lab**

Batch ID:	06-4906-7381	**Test Type:**	Survival (96h)	**Analyst:**	JA
Start Date:	21 Mar-17 13:38	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Well Water
Ending Date:	25 Mar-17 14:00	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Duration:	4d 0h	**Source:**	Aquatic Biosystems, CO	**Age:**	11d
Sample ID:	12-8952-6049	**Code:**	WG150734-1	**Client:**	Internal Lab
Sample Date:	21 Mar-17 13:00	**Material:**	Sodium chloride	**Project:**	Reference Toxicant
Receive Date:		**Source:**	Reference Toxicant		
Sample Age:	38m	**Station:**			

Point Estimate Summary

Analysis ID	Endpoint	Level	gm/L	95% LCL	95% UCL	TU	Method
01-4205-6573	96h Survival Rate	EC50	8.141	7.828	8.474		Linear Regression (MLE)

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
01-4205-6573	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	3	1	1	1	1	1	0	0	0.0%	0.0%
4.92		3	0.9444	0.7054	1	0.8333	1	0.05556	0.09623	10.19%	5.56%
6.14		3	0.9444	0.8249	1	0.9167	1	0.02778	0.04811	5.09%	5.56%
7.68		3	0.7222	0.2013	1	0.5	0.9167	0.1211	0.2097	29.04%	27.78%
9.6		3	0.02778	0	0.1473	0	0.08333	0.02778	0.04811	173.2%	97.22%
12		3	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

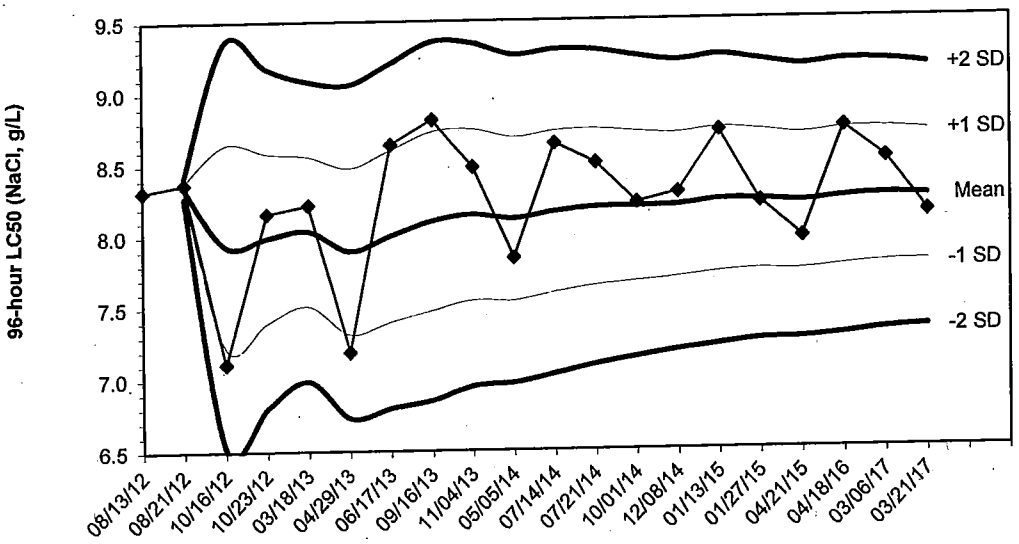
C-gm/L	Control Type	Rep 1	Rep 2	Rep 3
0	Dilution Water	1	1	1
4.92		0.8333	1	1
6.14		1	0.9167	0.9167
7.68		0.9167	0.75	0.5
9.6		0	0.08333	0
12		0	0	0

96h Survival Rate Binomials

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3
0	Dilution Water	12/12	12/12	12/12
4.92		10/12	12/12	12/12
6.14		12/12	11/12	11/12
7.68		11/12	9/12	6/12
9.6		0/12	1/12	0/12
12		0/12	0/12	0/12

Control Chart for Acute Reference Toxicant Tests with Fathead Minnows 96-Hour Survival LC50 (NaCl, g/L)

CV% = 5.5



Test Date

Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
08/13/12	8.32					
08/21/12	8.37	8.3450	8.3096	8.2743	8.3804	8.4157
10/16/12	7.11	7.9333	7.2199	6.5064	8.6468	9.3603
10/23/12	8.16	7.9900	7.3965	6.8031	8.5835	9.1769
03/18/13	8.22	8.0360	7.5119	6.9877	8.5601	9.0843
04/29/13	7.19	7.8950	7.3127	6.7304	8.4773	9.0596
06/17/13	8.64	8.0014	7.3999	6.7984	8.6030	9.2045
09/16/13	8.81	8.1025	7.4765	6.8505	8.7285	9.3545
11/04/13	8.48	8.1444	7.5455	6.9466	8.7434	9.3423
05/05/14	7.84	8.1140	7.5412	6.9683	8.6868	9.2597
07/14/14	8.64	8.1618	7.5957	7.0296	8.7279	9.2940
07/21/14	8.50	8.1901	7.6415	7.0929	8.7387	9.2872
10/01/14	8.22	8.1924	7.6671	7.1418	8.7177	9.2429
12/08/14	8.29	8.1994	7.6940	7.1887	8.7047	9.2101
01/13/15	8.72	8.2341	7.7289	7.2237	8.7392	9.2444
01/27/15	8.22	8.2332	7.7451	7.2571	8.7213	9.2093
04/21/15	7.97	8.2177	7.7408	7.2640	8.6946	9.1714
04/18/16	8.74	8.2467	7.7680	7.2893	8.7254	9.2042
03/06/17	8.52	8.2611	7.7917	7.3222	8.7305	9.2000
03/21/17	8.14	8.2551	7.7973	7.3396	8.7128	9.1705

Fathead Minnow (*Pimephales promelas*), Acute Test Precision

96-Hour Exposure to Reference Toxicant, NaCl, g/L

Table 5 of 5

Date	Test #	Dilution Series, NaCl, g/L	Ref. Tox. Lot #	Water	Control Surv, %	Pass/ Fail	Surv LC50	Stats	Control Limits	% COV
100913	5434	4.92 6.14 7.68 9.60 12.0	096146	NWW	100	P	7.37	TSK	6.94 – 8.83	6.0
110516	5681	4.92 6.14 7.68 9.60 12.0	096146	NWW	100	P	8.64	SK	6.93 – 8.93	6.3
110718	5776	4.92 6.14 7.68 9.60 12.0	096146	NWW	97	P	8.98	SK	6.88 – 9.02	6.7
110808	5834	4.92 6.14 7.68 9.60 12.0	096146	NWW	97	P	8.16	SK	6.90 – 9.04	6.7
120110	6098	4.92 6.14 7.68 9.60 12.0	096146	NWW	100	P	8.40	PA	6.94 – 9.08	6.7
120214	6112	4.92 6.14 7.68 9.60 12.0	096146	NWW	100	P	8.02	SK	6.97 – 9.09	6.6
120402	6142	4.92 6.14 7.68 9.60 12.0	096146	NWW	94	P	8.38	PA	6.97 – 9.09	6.6
120718	6275	4.92 6.14 7.68 9.60 12.0	113666	NWW	100	P	8.68	PA	7.13 – 9.09	6.0
120813	6336	4.92 6.14 7.68 9.60 12.0	113666	NWW	97	P	8.32	SK	7.18 – 9.10	5.9
120821	6348	4.92 6.14 7.68 9.60 12.0	113666	NWW	97	P	8.37	SK	7.20 – 9.13	5.9
121016	6463	4.92 6.14 7.68 9.60 12.0	113666	NWW	100	P	7.11	TSK	7.11 – 9.17	6.3
121023	6480	4.92 6.14 7.68 9.60 12.0	113666	NWW	94	P	8.16	PA	7.16 – 9.17	6.1
130211	6580	4.92 6.14 7.68 9.60 12.0	120348	NWW	86	F	-	-	-	-
130318	6605	4.92 6.14 7.68 9.60 12.0	120348	NWW	100	P	8.22	SK	7.16 – 9.17	6.1
130429	6621	4.92 6.14 7.68 9.60 12.0	120348	NWW	100	P	7.19	PA	7.02 – 9.21	6.7
130617	6684	4.92 6.14 7.68 9.60 12.0	120348	NWW	100	P	8.64	SK	7.07 – 9.26	6.7
130916	6818	4.92 6.14 7.68 9.60 12.0	120348	NWW	97	P	8.81	SK	7.22 – 9.28	6.2
131104	6910	4.92 6.14 7.68 9.60 12.0	130586	NWW	100	P	8.48	PA	7.22 – 9.29	6.3
140505	7049	4.92 6.14 7.68 9.60 12.0	130586	NWW	100	P	7.84	PA	7.07 – 9.28	6.8
140714	7132	4.92 6.14 7.68 9.60 12.0	130586	NWW	100	P	8.64	PA	7.21 – 9.28	6.3
140721	7146	4.92 6.14 7.68 9.60 12.0	130586	NWW	97	P	8.50	TSK	7.21 – 9.28	6.3
141001	7276	4.92 6.14 7.68 9.60 12.0	130586	NWW	100	P	8.22	PA	7.34 – 9.24	5.7
141208	7364	4.92 6.14 7.68 9.60 12.0	142463	NWW	97	P	8.29	PA	7.33 – 9.21	5.7
150113	7412	4.92 6.14 7.68 9.60 12.0	142463	NWW	92	P	8.72	PA	7.34 - 9.19	5.5
150127	7427	4.92 6.14 7.68 9.60 12.0	142463	NWW	97	P	8.22	TSK	7.36 - 9.16	5.5
150421	7498	4.92 6.14 7.68 9.60 12.0	142463	NWW	100	P	7.97	SK	7.33 - 9.15	5.5
160418	7921	4.92 6.14 7.68 9.60 12.0	142463	NWW	97	P	8.74	TSK	7.35 - 9.20	5.6
170306	8263	4.92 6.14 7.68 9.60 12.0	156697	NWW	97	P	8.52	PA	7.35 – 9.21	5.6
170321	8268	4.92 6.14 7.68 9.60 12.0	156697	NWW	100	P	8.14	PA	7.34 – 9.17	5.5

PA = Probit Analysis
MA = Moving Average
(T)SK = (Trimmed) Spearman Karber
GI = Graphical Interpolation

RW = Reconstituted Water
WW = Well Water
* = Value Outside Control Limits

8268

King County Environmental Laboratory
Lab QC Report - 3/27/1713:48
Run ID: R217444 Workgroup: WG150734 (8268 FMA-QC)

RT:WG150734-1 Matrix:FRESH WTR Listtype:AQFATHEAD-ACUTE Method:EPA821-R-02-012 Project: Pkey:STD
(Reference Toxicant)

Parameter	Unit	RT Value	Ctrl-Limits	Mean	Qual	CV
LC50	g/L	8.14	7.64-9.04	8.34		4.2

8268

King County Environmental Laboratory
Lab Review Report

Reported: 27-Mar-17 13:47 ~ Data Source: ELD

Listtype / Method: AQFATHEAD-ACUTE / EPA821-R-02-012
Run ID / Workgroup: R217444 / WG150734

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
			LK	RT	WG150734-1	LC50	8.14	g/L	TA			95% Confidence Interval: 7.83 to 8.47. Reference toxicant is NaCl. 8268
			LK	RT		Test Number		none				21-MAR-17 13:38
			LK	RT		Date Analyzed		none				21-MAR-17 13:38
			LK	RT		Prep Date						

No products missing

REFERENCE TOXICANT TEST, NaCl, 48-HOUR ACUTE TEST
Daphnia pulex

Test# 8269
Test Date 170328

BROODS
Started at ≈ 1130 h on 3-27 with 35 adults from cultures # D1→D5. Each into 25 mL WW 3-14 60-µm/AC filt + 0.1 mL Diet # 120 @ 3.0 g/L solids (3.7 mL diet + 6.3 mL WW) + 100 µL *Selenastrum capricornutum* @ 36 x 10^6 cell/mL. Into Incubator # 2576825, shelf 1.
Broods Checked: On 3-27 @ 1700 h, no (very gravid) broods released (Blue);
On 3-28 @ 1200 h, 10 (+ 3 w/dead neos or ab emb) broods released (Black); Blue
On _____ @ _____ h, _____ broods released (Red);

Brood Size:

A: 15A, 15B, 13A, 14, 16(6)A	**C:** 15A, 20, 13A, 16B, 16(6)A
B: 13B, 20, 13C, 16B, 13(6)D.	**D:** 13B, 15B, No, 13C, 14, 13(6)D

Broods sharing superscript letters are split from same brood

DILUTION WATER/ REFERENCE TOXICANT
New Well Water (NWW) 60-µm filtered on 3-14-17 Diluted to approx 100 mg $CaCO_3$/L w/milliQ
NaCl (Fisher Sci #S271-3: Rec'd 4-20-16; Opened 8-15-16; Lot # 156697).
LIMS DPA Sample #: WG 150735 -1 LIMS Wkgp #: WG 150735

NaCl SOLUTIONS

Code	NaCl (g/L)	NaCl (g)	NWW (mL)	COND (µmhos/cm) Before	COND (µmhos/cm) After*	95% PI COND Ranges: 8-17-1993 (Based on Test #'s 602, 614, 628, 642)
Blue	0	0	300	256	---	----------
Green	0.907	0.272	↓	2040	1784	1681 – 2016
Yellow	1.512	0.454	↓	3200	2944	2729 – 3300
Orange	2.52	0.756	↓	5060	4804	4502 – 5453
Red	4.20	1.260	↓	8080	7824	7067 – 8817
White	7.00	2.100	↓	12900	12644	12136 – 15891
			Analyst:	JA		

*After subtracting control value

PROCEDURE
1) Prep glassware ((24) 30-mL glass beakers; (6) 400-mL glass beakers; and (1) 500-mL glass cylinder):
   a) Soak in 1 g NaCl/L DW overnight.
   b) Rinse with WW; let air dry before use.
2) 24h before test, prep NaCl solns as above in 400-mL beakers; dissolve NaCl by mixing.
3) Measure Conductivity in 40 mL sample/trtmt. If outside 95% PI, prep new soln and re-measure Cond. Place solns in 20°C incubator until test start.
4) On day of test, add 25 mL each trtmt to each of (4) 30-mL beakers/trtmt (Reps A → D). Bring solns to 20°C. **Setup** at _____h.
5) Select 20 broods. Add 5 neonates/beaker – each from a different brood; one brood across all trtmts. Rinse pipet with WW or use new pipet between trtmts.
6) Arrange beakers randomly according to random # template in glass tray; cover.

REFERENCE TOXICANT TEST, NaCl, 48-HOUR ACUTE TEST
Daphnia pulex

Test# 8269
Test Date 170328

7) **Start** test @ 1220 h on 170328. Start counts verified by JA & —. Into incubator # 256828, shelf \. Place Tidbit temp recorder (SN 10468449) into beaker w/DW into incubator.
8) Measure pH & D.O. (adjusted for salinity) in control ("Blue") and highest conc ("White") trtmts at 0h and 48h. Measure Temp at 24h & 48h in 6 positions (4 corner + 2 center cups). Record survival at 0h and 48h.
9) **End** test @ 1350 h on 170330. Analyst: JA.

MEASUREMENTS

Cumulative Survival (# Alive/Rep)

Code	NaCl (g/L)	24 Hours				48 Hours				Tot # Alive	% Surv*
		A	B	C	D	A	B	C	D		
Blue	0	5 2a	5	5	5 3a,1s	5 1a	5	5	5	20	100
Green	0.907	5	5 1a	5	5	5	5	5	5	20	100
Yellow	1.512	5	5	5	5	5	5	5 1a	5	20	100
Orange	2.52	5 1a,1s	5	5 2a,1s	5 2a,2s	5 2a	5 1a	5 3a,1s	5 2a	20	100
Red	4.20	5 2a,1s	4 3a	4 3a	5 2a,2s	0	2 2a,2s	3 2a,3s	3 2s	8	
White	7.00	0 1a	0 4a	0 2a	0 5a	0	0	0	0	0	0
Analyst:		JA				JA					

a = floating on surface; b = stuck on side; s = stressed
*Pass if control survival ≥ 90%

Chemistry

Code	pH		D.O. (mg/L)	
	0h	48h	0h	48h
Blue	8.005	7.727	9.1	8.8
White	7.955	8.027	8.4	8.6
Analyst:	JA	JA	JA	JA

Position	Temperature (°C) SN: 16051663O (digital)	
	24h	48h
T1	20.0	19.8
T2	19.9	19.8
T3	19.9	19.9
T4	19.9	19.8
T5	19.5	19.5
T6	19.6	19.7
Analyst:	JA	JA

REFERENCE TOXICANT TEST, NaCl, 48-HOUR ACUTE TEST
Daphnia pulex

Test# 8269
Test Date 170328

Random Number Assignment (Rep Positions)								
Code	**Rep**	**Random #**	**Code**	**Rep**	**Random #**	**Code**	**Rep**	**Random #**
Blue	A	7	Yellow	A	1	Red	A	10
	B	9		B	12		B	20
	C	4		C	8		C	18
	D	15		D	17		D	24
Green	A	21	Orange	A	14	White	A	11
	B	5		B	23		B	22
	C	19		C	3		C	13
	D	6		D	16		D	2

NOTES

DPA-QC WG-150735 8269

CETIS Analytical Report

Test Code: 8269DPAQC | 21-0917-1277

Daphnia pulex 48-h Acute Survival Test

King County Metro Services, WQ Lab

Analysis ID:	14-7382-7177	Endpoint:	48h Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	30 Mar-17 15:22	Analysis:	Untrimmed Spearman-Kärber	Official Results:	Yes
Batch ID:	03-9689-0620	Test Type:	Survival (48h)	Analyst:	JA
Start Date:	28 Mar-17 12:20	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Well Water
Ending Date:	30 Mar-17 13:50	Species:	Daphnia pulex	Brine:	Not Applicable
Duration:	50h	Source:	In-House Culture	Age:	<24h
Sample ID:	08-1075-9915	Code:	WG150735-1	Client:	Internal Lab
Sample Date:	28 Mar-17 12:00	Material:	Sodium chloride	Project:	Reference Toxicant
Receive Date:		Source:	Reference Toxicant		
Sample Age:	20m	Station:			

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	0.6011	0.0243	3.991	3.568	4.463

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

48h Survival Rate Summary

			Calculated Variate(A/B)								
C-gm/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	20	20
0.907		4	1	1	1	0	0	0.0%	0.0%	20	20
1.512		4	1	1	1	0	0	0.0%	0.0%	20	20
2.52		4	1	1	1	0	0	0.0%	0.0%	20	20
4.2		4	0.4	0	0.6	0.1414	0.2828	70.71%	60.0%	8	20
7		4	0	0	0	0	0		100.0%	0	20

48h Survival Rate Detail

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
0.907		1	1	1	1
1.512		1	1	1	1
2.52		1	1	1	1
4.2		0	0.4	0.6	0.6
7		0	0	0	0

48h Survival Rate Binomials

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	5/5
0.907		5/5	5/5	5/5	5/5
1.512		5/5	5/5	5/5	5/5
2.52		5/5	5/5	5/5	5/5
4.2		0/5	2/5	3/5	3/5
7		0/5	0/5	0/5	0/5

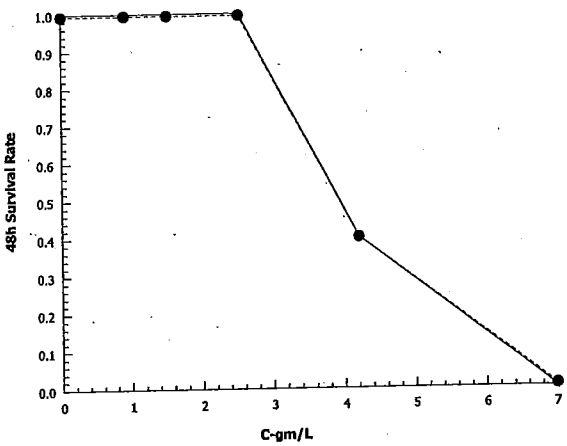
Analyst: [illegible] QA: GY 3-31-17

CETIS Analytical Report

Daphnia pulex 48-h Acute Survival Test — **King County Metro Services, WQ Lab**

Analysis ID:	14-7382-7177	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	30 Mar-17 15:22	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



8269

CETIS Summary Report

Report Date: 30 Mar-17 15:22 (p 1 of 1)
Test Code: 8269DPAQC | 21-0917-1277

Daphnia pulex 48-h Acute Survival Test

King County Metro Services, WQ Lab

Batch ID:	03-9689-0620	Test Type:	Survival (48h)	Analyst:	JA
Start Date:	28 Mar-17 12:20	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Well Water
Ending Date:	30 Mar-17 13:50	Species:	Daphnia pulex	Brine:	Not Applicable
Duration:	50h	Source:	In-House Culture	Age:	<24h
Sample ID:	08-1075-9915	Code:	WG150735-1	Client:	Internal Lab
Sample Date:	28 Mar-17 12:00	Material:	Sodium chloride	Project:	Reference Toxicant
Receive Date:		Source:	Reference Toxicant		
Sample Age:	20m	Station:			

Point Estimate Summary

Analysis ID	Endpoint	Level	gm/L	95% LCL	95% UCL	TU	Method
14-7382-7177	48h Survival Rate	EC50	3.991	3.568	4.463		Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
14-7382-7177	48h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

48h Survival Rate Summary

C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
0.907		4	1	1	1	1	1	0	0	0.0%	0.0%
1.512		4	1	1	1	1	1	0	0	0.0%	0.0%
2.52		4	1	1	1	1	1	0	0	0.0%	0.0%
4.2		4	0.4	0	0.8501	0	0.6	0.1414	0.2828	70.71%	60.0%
7		4	0	0	0	0	0	0	0		100.0%

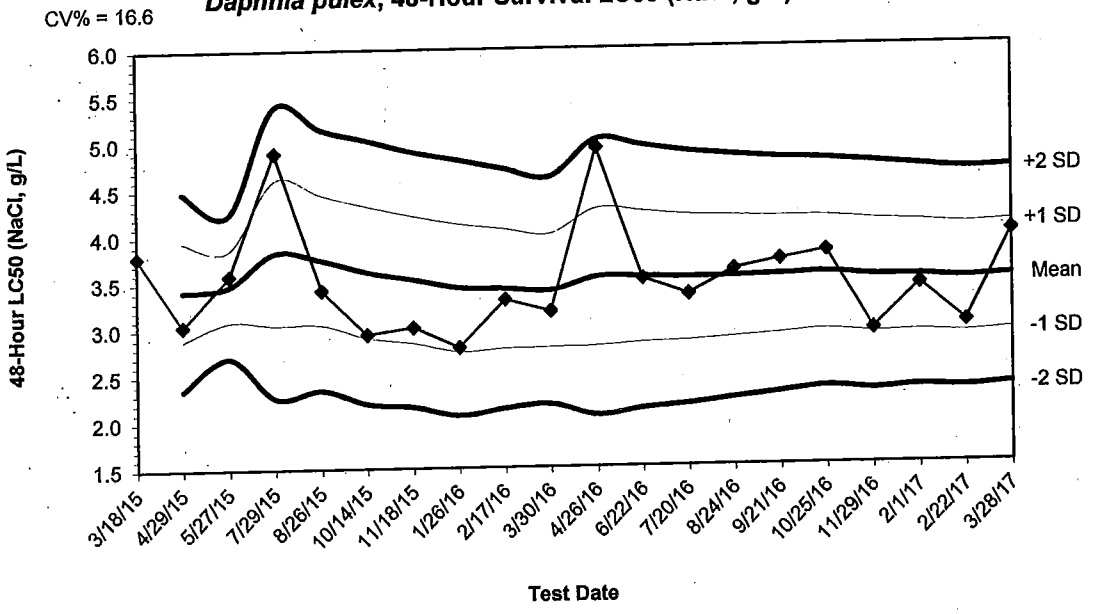
48h Survival Rate Detail

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
0.907		1	1	1	1
1.512		1	1	1	1
2.52		1	1	1	1
4.2		0	0.4	0.6	0.6
7		0	0	0	0

48h Survival Rate Binomials

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	5/5
0.907		5/5	5/5	5/5	5/5
1.512		5/5	5/5	5/5	5/5
2.52		5/5	5/5	5/5	5/5
4.2		0/5	2/5	3/5	3/5
7		0/5	0/5	0/5	0/5

Control Chart for Acute Reference Toxicant Tests with *Daphnia pulex*, 48-Hour Survival LC50 (NaCl, g/L)



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
3/18/15	3.79					
4/29/15	3.04	3.4150	2.8847	2.3543	3.9453	4.4757
5/27/15	3.58	3.4700	3.0831	2.6962	3.8569	4.2438
7/29/15	4.90	3.8275	3.0458	2.2641	4.6092	5.3909
8/26/15	3.42	3.7460	3.0449	2.3439	4.4471	5.1481
10/14/15	2.94	3.6117	2.9035	2.1954	4.3198	5.0279
11/18/15	3.01	3.5257	2.8404	2.1552	4.2110	4.8963
1/26/16	2.79	3.4338	2.7481	2.0624	4.1194	4.8051
2/17/16	3.30	3.4189	2.7759	2.1330	4.0618	4.7048
3/30/16	3.17	3.3940	2.7827	2.1715	4.0053	4.6165
4/26/16	4.92	3.5327	2.7925	2.0522	4.2730	5.0132
6/22/16	3.51	3.5308	2.8250	2.1192	4.2367	4.9425
7/20/16	3.34	3.5159	2.8380	2.1601	4.1938	4.8718
8/24/16	3.60	3.5219	2.8702	2.2185	4.1736	4.8254
9/21/16	3.70	3.5338	2.9041	2.2744	4.1635	4.7932
10/25/16	3.79	3.5498	2.9381	2.3264	4.1615	4.7732
11/29/16	2.94	3.5138	2.9031	2.2925	4.1244	4.7350
2/1/17	3.42	3.5088	2.9160	2.3232	4.1016	4.6944
2/22/17	3.01	3.4825	2.8952	2.3078	4.0699	4.6572
3/28/17	3.99	3.5079	2.9251	2.3422	4.0907	4.6736

CV%
16.6

Daphnia pulex, Acute Test Precision

48-Hour Exposure to Reference Toxicant, NaCl, g/L

Table 11 of 11

Date	Test #	Dilution Series, NaCl, g/L	Ref. Tox. Lot #	Water	Control Survival (%)	Pass/ Fail	Surv LC50	Stats	Control Limits	% COV
160330	7905	0.907 1.512 2.52 4.20 7.00	142463	NWW	100	P	3.17	SK	2.43 – 4.36	14.2
160426	7937	0.907 1.512 2.52 4.20 7.00	142463	NWW	95	P	4.92*	SK	2.36 – 4.64	16.2
160622	7986	0.907 1.512 2.52 4.20 7.00	142463	NWW	100	P	3.51	SK	2.36 – 4.62	16.2
160720	8020	0.907 1.512 2.52 4.20 7.00	142463	NWW	100	P	3.34	SK	2.39 – 4.62	15.9
160824	8057	0.907 1.512 2.52 4.20 7.00	142463	NWW	100	P	3.60	SK	2.39 – 4.63	15.9
160921	8107	0.907 1.512 2.52 4.20 7.00	156697	NWW	100	P	3.70	SK	2.44 – 4.64	15.5
161025	8199	0.907 1.512 2.52 4.20 7.00	156697	NWW	100	P	3.79	SK	2.47 – 4.66	15.4
161129	8230	0.907 1.512 2.52 4.20 7.00	156697	NWW	100	P	2.94	SK	2.39 – 4.65	16.0
170201	8249	0.907 1.512 2.52 4.20 7.00	156697	NWW	100	P	3.42	SK	2.39 - 4.65	16.0
170222	8255	0.907 1.512 2.52 4.20 7.00	156697	NWW	100	P	3.013	SK	2.35 – 4.65	16.5
170328	8269	0.907 1.512 2.52 4.20 7.00	156697	NWW	100	P	3.99	SK	2.34 – 4.67	16.6

PA = Probit Analysis
MA = Moving Average
(T)SK = (Trimmed) Spearman Karber
GI = Graphical Interpolation
* = Value Outside Control Limits

RW = Reconstituted Water
WW = Well Water
NWW = New Well Water

8269

King County Environmental Laboratory
Lab QC Report - 3/30/1715:24
Run ID: R217515 Workgroup: WG150735 (8269 DPA-QC)

RT:WG150735-1 Matrix:FRESH WTR Listtype:AQDAPHNIA-ACUTE Method:EPA821-R-02-012 Project: Pkey:STD
(Reference Toxicant)

Parameter	Unit	RT Value	Ctrl-Limits	Mean	Qual	CV
LC50	g/L	3.99	2.2-4.68	3.44		18.02

9269

King County Environmental Laboratory
Lab Review Report

Reported: 30-Mar-17 15:24 ~ Data Source: ELD

Listtype / Method: AQDAPHNIA-ACUTE / EPA821-R-02-012
Run ID / Workgroup: R217515 / WG150735

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
			LK	RT	WG150735-1	LC50	3.99	g/L	TA			95% Confidence Interval: 3.57 to 4.46. Reference toxicant is NaCl.
			LK	RT		Test Number		none				28-MAR-17 12:20
			LK	RT		Date Analyzed		none				28-MAR-17 12:20
			LK	RT		Prep Date						

No products missing